COMPARISON OF CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS IN BEEF HEIFERS

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by
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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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DEDICATION

This thesis is dedicated to my parents, Homer and Pat Leitman, who provided me the opportunity to experience production agriculture first-hand, instilled in me the values of hard-work and responsibility, and always encouraged me to pursue my dreams; to my siblings, Michelle, Noel, Scott, Cliff, and their families, for their friendship, inspiration, and continuous support; and to Dr. David Patterson for not only providing me the opportunity to continue my education, but allowing me to realize and achieve my true potential. All of you have played a pivotal role in making me the person I am today and in helping me reach this important point in life—for that I will forever be grateful.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
C	Estrous cycling
CL	Corpus luteum/Corpora lutea
cm	Centimeter(s)
CIDR	Controlled Internal Drug Release
d	Day(s)
E_2	Estradiol-17β
FSH	Follicle stimulating hormone
FTAI	Fixed-time artificial insemination
g	Gram(s)
GnRH	Gonadotropin releasing hormone
h	Hour(s)
hd	Head
i.m.	Intramuscular
kg	Kilogram(s)
LH	Luteinizing hormone
MAP	Medroxyprogesterone acetate
MGA	Melengestrol acetate
mg	Milligram(s)

mL	Milliliter(s)
mm	Millimeter(s)
mo	Month(s)
ng	Nanogram(s)
P	Prepubertal
P_4	Progesterone
pg	Picogram(s)
PG	Prostaglandin $F_{2\alpha}$
RIA	Radioimmunoassay
RTS	Reproductive tract score
s	Second(s)
SAS	Statistical Analysis System
T	Treatment(s)
µg	Microgram
U.S.	United States
wk	Week(s)
yr	Year(s)

COMPARISON OF CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS IN BEEF HEIFERS

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ABSTRACT

Estrus synchronization and artificial insemination (AI) are reproductive management techniques that allow beef producers to enhance the reproductive efficiency and genetic composition of their cow herd. However, U.S. beef producers have been reluctant to adopt these reproductive management tools, primarily due to the time and labor associated with implementation of protocols. Recent research to synchronize estrus, therefore, has focused on the development of estrus synchronization protocols that facilitate fixed-time AI (FTAI). Although protocols have been developed that allow the successful use of FTAI in beef cows, the same degree of success in beef heifers has not been realized. Additionally, no published research has focused on characterizing the physiological responses to long- and short-term CIDR-based protocols in beef heifers.

Experiment 1 evaluated ovulatory response to GnRH and synchrony of estrus and ovulation after $PGF_{2\alpha}$ (PG) in beef heifers. The CIDR Select and Select Synch + CIDR protocols were evaluated among estrous cycling and prepubertal beef heifers and the CIDR-PG and Select Synch protocols were evaluated among estrous cycling beef heifers. A reduced variance for the intervals to estrus and ovulation was detected for estrous

cycling heifers treated with the CIDR Select protocol in comparison to the other 3 treatments. The combined results of the estrous cycling and prepubertal heifers revealed an increased ovulatory response to GnRH for heifers treated with the CIDR Select protocol compared to the Select Synch + CIDR protocol, which ultimately resulted in a reduced variance for interval to estrus and ovulation after PG. Furthermore, no differences within treatment were detected in the variance for interval to estrus or ovulation among estrous cycling and prepubertal heifers treated with the CIDR Select protocol. These results suggest that the CIDR Select protocol may facilitate FTAI more effectively in mixed groups of estrous cycling and prepubertal beef heifers.

Experiments 2 and 3 evaluated modifications to the CIDR Select protocol. Estrous cycling beef heifers were used in Exp. 2. The hypothesis tested was that reducing the interval from CIDR removal to GnRH administration by 2 d would facilitate an improvement in the synchrony of estrus after PG. Although a larger number of heifers that were assigned to the 28 d protocol were on d 5 and 6 of the estrous cycle at the time GnRH was administered, response to GnRH was not improved and synchrony of estrus was not enhanced following PG.

In Exp. 3, a second modification to the CIDR Select protocol was evaluated using estrous cycling and prepubertal beef heifers. The hypotheses tested were: 1) reducing the interval from CIDR removal to GnRH may facilitate an improvement in the synchrony of estrus after PG, and 2) the addition of GnRH following CIDR removal is required to improve the synchrony of estrus after PG. Although no difference in estrous response was detected, mean intervals to estrus and variance for interval to estrus differed based on the interaction of treatment length, GnRH, and estrous cyclicity status. The results from

Exp. 3 clearly suggest that further evaluation of long-term CIDR-based protocols is required with and without the addition of GnRH among estrous cycling and prepubertal beef heifers to determine the efficacy of modifying the CIDR Select protocol to facilitate FTAI.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Reproductive performance is recognized as the most important economic trait in a beef cow-calf operation (Wiltbank, 1990; Trenkle and Willham, 1977). Failure of cows to become pregnant and calf losses at or shortly following birth are the primary reasons for reproductive failure (Wiltbank, 1990; Bellows and Short, 1990). In order to maximize reproductive performance and lifetime production efficiency, heifers must conceive by 15 mo and calve by 24 mo of age. Management recommendations suggest that heifers should be bred prior to mature cows to allow for additional time to return to estrus following calving, which translates into a potential increased first service conception rate (Wiltbank, 1970). However, breeding heifers prior to the mature cow herd often results in heifers being bred on their pubertal estrus. Byerley et al. (1987) reported a 21 % reduction in pregnancy rates when heifers were bred on their pubertal versus third estrus.

Therefore, timing of the onset of puberty is a critical factor for determining reproductive efficiency in beef heifers. Estrus synchronization may be used as a means to effectively induce puberty in heifers (Gonzalez-Padilla et al., 1975; Patterson et al., 1990) as well as increase the proportion of females that become pregnant early during the breeding season, resulting in shorter breeding/calving periods and more uniform calf

crops (Dziuk and Bellows, 1983). Females that conceived to a synchronized estrus calved earlier in the calving period and weaned calves that averaged 13 d older and 9.5 kg heavier than calves originating from non-synchronized females (Schafer et al., 1990).

Artificial insemination (**AI**) is an underutilized management tool that maximizes reproductive potential by facilitating use of genetically superior proven sires, without having a bull physically present on the farm or ranch. Through AI, new and improved genetics may be easily incorporated into a cow herd, providing an opportunity to increase the number of pounds of calf a cow produces in a given year (Trenkle and Willham, 1997; Dziuk and Bellows, 1983). Adding value to a pregnancy includes reducing the cost of establishing the pregnancy. Estrus synchronization and AI may reduce the cost of obtaining a pregnancy, which supports the added investment in genetics (Collier, 2000). For these reasons, estrus synchronization and AI are considered to be the most important and widely applicable reproductive biotechnologies currently available to beef producers (Seidel, 1995).

Despite the apparent benefits of increased reproductive efficiency and profitability associated with estrus synchronization and AI, cow-calf producers in the U.S. are reluctant to implement these technologies in their operations. The most current study from the United States Department of Agriculture reports that only 4.3 and 3% of beef producers use any form of estrus synchronization in cows and heifers, respectively. Slightly higher adoption rates for AI are reported, with 5.4 and 3.3% of operations using AI in cows and heifers, respectively (NAHMS, 1994). Lack of time and labor, the view that procedures are too complicated to implement effectively, and perceived high cost of

treatment are the most frequently cited reasons for why these technologies have not been readily adopted by U.S. beef producers (NAHMS, 1998).

Current research programs are focused on addressing these issues. Estrus synchronization protocols that result in a highly synchronized estrus and ovulation will reduce or eliminate the time and labor associated with estrus detection, thereby making estrus synchronization and AI more feasible to a broader range of producers. The development of economical methods to synchronize estrus and ovulation to facilitate fixed-time AI (**FTAI**) with resulting high fertility will increase the adoption of these technologies in U.S. beef herds (Patterson et al., 2003b).

A REVIEW OF THE BOVINE ESTROUS CYCLE

Physiology of the Onset of Puberty

Traditional definitions of puberty include the process of acquiring reproductive competence and/or the ability to accomplish reproduction successfully (Senger, 2003). More specifically, the physiological onset of puberty may be defined as the stage of development when the female first expresses estrus and ovulates (Short, 1984). The primary component of the reproductive system that regulates ovarian function and the time of onset of puberty in heifers is the hypothalamus (Schams et al., 1981; Kinder et al., 1987). However, research has shown that the hypothalamic-pituitary axis and other components of the reproductive endocrine system are functional prior to the actual onset of puberty.

The number and distribution of gonadotropin releasing hormone (**GnRH**) neurons within the hypothalamus (Senger, 2003) and the number of GnRH receptors in the pituitary (Day et al., 1987) are established prior to puberty. Prepubertal primates treated with *N*-methyl-D-aspartic acid were able to respond to treatment by releasing GnRH (Plant et al., 1989). Additionally, preovulatory gonadotropin release may be elicited in heifers through exogenous GnRH administration (Schams et al., 1981), and ovulation of fertile ova may be induced by exogenous gonadotropin treatment (Seidel et al., 1971). Therefore, if stimulated appropriately, the hypothalamic-pituitary axis and the ovary of the prepubertal female are capable of functioning in an adult-like manner.

Pulsatile release of luteinizing hormone (**LH**) from the anterior pituitary has been shown to occur in heifers as early as 2 wk of age (Evans et al., 1994). Secretion of LH and follicle stimulating hormone (**FSH**) transiently increases from 6 to 24 wk of age, and then declines (Evans et al., 1992, 1994; Honaramooz et al., 1999) and remains low until the peripubertal transitional period leading to puberty (Day et al., 1987; Melvin et al., 1999). The pulse frequency of LH is increased postpubertally as compared to prepubertally in both ewes (Foster and Ryan, 1979) and heifers (Day et al., 1984, 1987; Wolfe et al., 1989). The increase in frequency of LH pulses is the major factor responsible for ovulation and the resulting onset of puberty.

Changes in ovarian follicular development are reflected in early fluctuations of gonadotropin concentrations. No ovarian follicles were detected macroscopically at birth (Desjardins and Hafs, 1969), but follicles were present by 2 wk of age in heifers (Evans et al., 1992, 1994). The number of follicles and the maximum diameter of the dominant and largest subordinate follicles increased from 2 to 14 wk of age, then declined and

remained static until the peripubertal period (Evans et al., 1994). Serum concentrations of estradiol tended to increase with age (Evans et al., 1994) as would be expected with increased diameter of the dominant follicle. The number of follicles < 5 mm in diameter decreased between 5 and 1 mo prepuberty, while the number of follicles ≥ 5 mm in diameter increased between 8 and 3 mo prepuberty and remained static until puberty (Melvin et al., 1999). Maximum size of the dominant follicle was increased during the 30 d preceding puberty compared to 60, 90, and 120 d preceding puberty (Bergfeld et al., 1994), perhaps resulting from an increase in LH pulse frequency occurring between 3 and 1 mo prepuberty (Melvin et al., 1999). Serum estradiol concentrations also increased between 3 and 1 mo prepuberty (Melvin et al., 1999).

Ovarian follicular development occurs in distinct, wave-like patterns in prepubertal females (Hopper et al., 1993; Evans et al., 1994) as is seen in mature cows (Pierson and Ginther, 1988; Savio et al., 1988; Sirois and Fortune, 1988), with dominant follicle turnover occurring every 7 to 8 d in prepubertal heifers (Hopper et al., 1993; Adams et al., 1994). Plasma FSH concentrations increase prior to the emergence of follicular waves (Evans et al., 1994).

Since the hypothalamic-pituitary axis is functional and ovarian follicular development occurs prior to puberty, the question arises as to what specific inhibitory mechanism regulates the timing of the onset of puberty in females. Ramirez and McCann (1963) proposed the 'gonadostat theory' which suggests that puberty is delayed due to the negative feedback of estradiol of ovarian origin on the release of LH from the hypothalamic-pituitary axis. The hypothalamus is responsible for secretion of the gonadotropins LH and FSH through pulsatile release of GnRH. Research originally

conducted by Knobil (1981) in primates suggested that pulsatile secretion of LH is necessary for initiation and maintenance of ovarian cycles. The required stimulus for the onset of puberty, therefore, is an increased pulsatile secretion of LH through release of GnRH. Transient increases in serum progesterone (**P_4**) concentrations have been detected in prepubertal heifers. Berardinelli et al. (1979) determined that the rise in P_4 prior to puberty in heifers originated from the ovaries, as ovariectomy resulted in a decline in P_4 to basal levels. Luteal tissue was also found in the ovaries, even though corpora luteua (**CL**) were not palpable.

A conceptual model for the onset of puberty in heifers is shown in Figure 1.1 (adapted from Day and Anderson, 1998). Reduction in the negative feedback of estradiol on the secretion of gonadotropins was shown to occur as puberty approaches in heifers (Schillo et al., 1982; Day et al., 1984) and ewe lambs (Foster and Ryan, 1979). Increased pulsatile release of LH associated with the decline in estradiol negative feedback was observed in heifers (Day et al., 1984). Concentrations of receptors for estradiol in the hypothalamus and pituitary decline preceding puberty, reducing the number of sites at which estradiol can bind and exert its negative feedback effects on secretion of GnRH and hence LH. Pulses of LH are low in frequency during the prepubertal phase, with only 1 to 4 pulses occurring per 24 h period. However, during the peripubertal phase, LH pulse frequency continues to increase and approaches 24 pulses per 24 h period during the few days prior to puberty onset (Day et al., 1987). Increased gonadotropin secretion results in increased follicle growth and estradiol secretion by dominant ovarian follicles (Bergfeld et al., 1994). Estradiol concentrations will eventually reach levels that are

adequate to elicit the preovulatory LH surge and subsequent ovulation through a positive feedback effect (Day et al., 1987).

Endocrinology of the Estrous Cycle

The Estrous Cycle. Following the series of events that culminate in the first ovulatory estrus, the now pubertal female enters into a milieu of reproductive cyclicity—a series of physiologic events characterized by recurring periods of expression of estrus and ovulation. The mean length of the bovine estrous cycle is 21 d and normally ranges between 17 and 24 d (Table 1.1; Senger, 2003; Wishart, 1972). The estrous cycle consists of the follicular and luteal phases, throughout which gonadotropins influence ovarian folliculogenesis, ovulation, and subsequent CL function. The follicular phase encompasses approximately 20% of the cycle length and includes the events leading from CL regression to ovulation. The remainder of the estrous cycle is comprised of the luteal phase, which includes events from ovulation to CL regression (Senger, 2003).

Estrus. Estrus is defined as the period of sexual receptivity in the female (Senger, 2003) and is characterized by behavioral expression of estrus. The duration of estrus ranges from 12 to 21 h (Hammond, 1927; Nalbandov and Casida, 1942; Wishart, 1972; Wiltbank et al., 1967). The steroid hormone estradiol-17β ($\mathbf{E_2}$) secreted by growing follicles on the ovary is the primary hormone responsible for expression of estrus. In addition, E_2 prepares the uterine and vaginal environments for mating and initiates the preovulatory surge of LH (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Rhodes et al., 1995). Frequent, high amplitude pulses of E_2 originating from the preovulatory follicle result in attainment of threshold concentrations of E_2 (Walters and Schallenberger, 1984). This triggers a positive feedback effect on the hypothalamus,

resulting in increased secretion of GnRH that culminates in a GnRH surge, and resulting release of the preovulatory LH surge from the anterior pituitary (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Hansel and Convey, 1983; Spicer and Echternkamp, 1986; Stumpf et al., 1989; Cupp et al., 1995; Moenter et al., 1991). Lesser amounts of FSH are secreted in response to GnRH. The LH surge, characterized by frequent high amplitude pulses of LH (Rahe et al., 1980), ends despite continued elevation of GnRH, suggesting that mechanisms other than lack of GnRH govern the termination of the LH surge, such as pituitary desensitization and/or depletion of LH pools (Moenter et al., 1991). The preovulatory LH surge is necessary for final maturation and ovulation of the oocyte (Schallenberger et al., 1984; Garverick and Smith, 1986; Ginther et al., 2000).

Metestrus. The preovulatory surge of LH induces ovulation of the dominant follicle, and LH concentrations subsequently decline (Garverick et al., 1971; Hansel and Convey, 1983; Schallenberger et al., 1984; Walters and Schallenberger, 1984; Stumpf et al., 1989; Cupp et al., 1995). Ovulation occurs 24 to 32 h after the onset of estrus (Senger, 2003; Wiltbank et al., 1967; Christenson et al., 1975; Bernard et al., 1983). The ovulated follicle undergoes luteinization, or structural and cellular remodeling, resulting in the formation of a CL which produces the hormone progesterone (Figure 1.2; Hansel and Convey, 1983; Garverick et al., 1992). Luteinizing hormone assists in transforming the follicular theca and granulosa cells into small and large luteal cells, respectively (Garverick and Smith, 1986; Garverick et al., 1988; Niswender et al., 1986; Milvae et al., 1991; Garverick et al., 1992; Smith et al., 1994). The majority of P_4 produced by the CL originates from large luteal cells (Niswender et al., 1986, 2007).

Diestrus. Prior to ovulation, the primary ovarian structures are follicles comprised of theca and granulosa cells that secrete E_2. Following ovulation, however, these follicular cells differentiate to form a CL, a transient endocrine gland which secretes the steroid hormone P_4 (Hansel and Convey, 1983; Garverick et al., 1992; Smith et al., 1994; Wiltbank, 1994; Pate, 1994; Niswender, 2002). Progesterone is an important regulator of the bovine estrous cycle. Progesterone functions by suppressing the secretion of other hormones, inhibiting expression of behavioral estrus, and maintaining pregnancy (Hansel and Convey, 1983; Garverick et al., 1992; Smith et al., 1994; Wiltbank, 1994; Driancourt, 2001). Progesterone concentrations begin to increase by d 4 of the estrous cycle, reach maximal concentrations by d 7 to 12, and remain elevated until d 18 or 19 when luteolysis occurs (Hansel and Convey, 1983; Donaldson and Hansel, 1965). Concentrations of P_4 decline after luteolysis and remain low throughout the subsequent follicular phase (Wettemann et al., 1972; Echternkamp and Hansel, 1973). Together, metestrus and diestrus comprise the luteal phase of the bovine estrous cycle.

Proestrus. During the luteal phase of the estrous cycle, P_4 blocks the ability of E_2 to enhance expression of uterine endometrial oxytocin receptors. However, P_4 eventually down-regulates its own receptor toward the end of the luteal phase, removing the inhibitory effect of P_4. This allows E_2 to induce the formation of endometrial oxytocin receptors and stimulate the release of oxytocin from the pituitary, which in turn stimulates the uterus to release low levels of prostaglandin $F_{2\alpha}$ (**PG**). These low levels of PG then cause release of luteal oxytocin, which amplifies the release of PG from the uterus (McCracken et al., 1999). In cattle, PG of uterine origin exerts its effect on the CL by means of vascular countercurrent exchange (Senger, 2003). Luteolysis occurs as a

result of reduced blood flow to the CL which deprives the gland of nutrients, substrates for steriodogenesis, and luteotropic support (Phariss et al., 1970). Luteolysis, or regression of the CL, occurs at the end of the luteal phase around d 18 of the estrous cycle.

Regression of the CL causes a significant decrease in P_4 concentrations that end the negative feedback of P_4 on the preovulatory surge center of the hypothalamus (Hansel and Convey, 1983; Savio et al., 1990). High frequency pulses of GnRH are then secreted from the hypothalamus, which signal the anterior pituitary to release the gonadotropins LH and FSH (Hansel and Convey, 1983; Schallenberger et al., 1984; Spicer and Echternkamp, 1986). The "two-cell, two-gonadotropin model" states that the binding of LH to its receptors on follicular theca cells initiates a series of events leading to the production of testosterone. Testosterone diffuses across the basement membrane into the granulosa cell layer, which contains receptors for FSH. Once FSH binds to its receptors, testosterone is converted to E_2 (Fortune and Quirk, 1988; Hansel and Convey, 1983). Increasing production and secretion of E_2 by ovarian follicles elicits a positive feedback effect on the hypothalamus and anterior pituitary (Schallenberger et al., 1984; Walters and Schallenberger, 1984; Spicer and Echternkamp, 1986; Jainudeen and Hafez, 1987; Stumpf et al., 1989), which stimulates an increase in gonadotropin release. Three days following the decrease in P_4, E_2 reaches peak threshold concentrations (Savio et al., 1990; Garverick et al., 1992; Cupp et al., 1995). Secretion of E_2 and the gonadotropins promote continued growth and development of preovulatory follicles. Together, proestrus and estrus comprise the follicular phase of the bovine estrus cycle.

Folliculogenesis. Folliculogenesis is defined as the formation of mature or preovulatory Graafian follicles from a pool of primordial follicles (Kojima, 2003). The number of primordial follicles present on the ovaries is fixed near birth. The cow has approximately 150,000 primordial follicles at birth with only 3,000 remaining at 15 to 20 yr of age (Webb et al., 1992; Erickson, 1966). During the lifetime of a female, primordial follicles enter a pool of growing follicles in a continuous manner (Hansel and Convey, 1983). Development of large follicles on the ovary is a dynamic process, with 2 to 3 waves of follicular growth occurring per estrous cycle, during which a single follicle is selected per wave (Sirois and Fortune, 1990). Follicular waves occur in three distinct phases: recruitment, selection, and dominance (Fortune et al., 1988; Ginther et al., 2001).

Recruitment. During recruitment, follicles begin to mature in an environment of sufficient gonadotropin stimulation to permit progress toward ovulation (Hodgen, 1982). A cohort of growing follicles, typically consisting of 1 to 6 follicles 4 to 5 mm in diameter, emerges from a pool of primordial follicles formed during fetal development (Bao and Garverick, 1998; Fortune, 1994). Mechanisms regulating recruitment of primordial follicles into the growing cohort are not fully understood, but are likely to involve locally produced growth and differentiation factors (Elvin et al., 2000). Approximately 2.5 d preceding the emergence of each follicular wave, FSH concentrations transiently rise and subsequently decline with the emergence of a new cohort (Adams et al., 1994; Hamilton et al., 1995). Follicles in a cohort are FSH dependent (Roche et al., 1998; Adams, 1999; Ginther et al., 2000; Driancourt, 2001) until they experience atresia or achieve dominance. In cows with two follicular waves, recruitment occurs around d 2 and 11 of the estrous cycle. In three-wave cows,

recruitment occurs around d 2, 9, and 16 (Sirois and Fortune, 1988; Pierson and Ginther, 1987a; 1987b; Ginther et al., 1989).

Selection. Selection is the process by which a single follicle of a cohort is chosen to avoid atresia and progress toward ovulation (Hodgen, 1982). Selection occurs 36 to 48 h following initiation of the follicular wave (Bao et al., 1997) when the largest follicle reaches approximately 8.5 mm in diameter (Ginther et al., 1997). During this phase of the follicular wave, the growing follicle continues to secrete increasing amounts of E_2 and the hormone inhibin. Inhibin exerts a negative feedback effect on the pituitary which decreases the secretion of FSH (Knight and Glister, 2001). Declining FSH concentrations may prevent the other follicles in the cohort from continued growth, and the selected follicle may shift from FSH to LH dependency (Ginther et al., 1996). Selection occurs around d 3 and 12 in two-wave cows, and d 3, 10, and 17 in three-wave cows.

Dominance. Dominance is the phase of the follicular wave in which the selected follicle achieves and maintains its eminence over other follicles and inhibits the emergence of a new follicular wave (Hodgen, 1982; Ginther et al., 1996). The dominant follicle reaches a maximum size of 10 to 15 mm (Webb et al., 1992) and will either undergo atresia or proceed to ovulation. Dominance occurs around d 6 and 15 in two-wave cows, and around d 7, 14, and 21 in three-wave cows (Pierson and Ginther, 1984, 1986; Savio et al., 1990; Lucy et al., 1992; Stock and Fortune, 1993; Driancourt, 2001).

Luteinizing hormone plays an important role in controlling the growth of the dominant follicle. Once a follicle reaches dominance, LH receptors are expressed in the granulosa cell layer and there is an increased expression of LH receptors in the theca cell

layer. Dominant follicles then become LH dependent (Bao and Garverick, 1998; Webb et al., 1999). Luteinizing hormone stimulates androgen precursor production in thecal cells, which controls the synthesis of E_2 and determines the health of the dominant follicle (Garverick and Smith, 1986; Garverick et al., 1988; 1992). The fate of the dominant follicle is dependent on the phase of the estrous cycle when dominance is achieved. A follicle that achieves dominance during the luteal phase of the estrous cycle will maintain its maximum size for 3 to 6 d before regressing (Ginther et al., 1989; Knopf et al., 1989), whereas dominant follicles that are in the growth phase during luteal regression will proceed to ovulate (Kastelic et al.,1990).

USE OF PROGESTINS IN ESTRUS SYNCHRONIZATION SYSTEMS

The discovery by Ulberg et al. (1951) that P_4 inhibited ovulation and maturation of mature preovulatory ovarian follicles (Nellor and Cole, 1956; Hansel et al., 1961; Lamond, 1964) led to the development of estrus synchronization systems to control the estrous cycle of the cow. The steroid hormone P_4 produced by the CL is a potent regulator of the bovine estrous cycle. Progesterone inhibits follicular maturation and ovulation by suppressing the hypothalamic release of GnRH and release of LH from the anterior pituitary (Hansel and Convey, 1983; Jainudeen and Hafez, 1987). A preovulatory surge of LH is necessary for final maturation and ovulation of the oocyte (Schallenberger et al., 1984; Garverick and Smith, 1986; Ginther et al., 2000). Since P_4 levels increase prior to puberty in heifers (Berardinelli et al., 1979) and prior to the resumption of estrous cyclicity in postpartum cows (Short et al., 1974; Rawlings et al.,

1980), exposure to increased concentrations of P_4 is thought to be a prerequisite for establishment of normal estrous cycles.

The development of methods to synchronize estrus occurred in six distinct phases (Table 1.2; Thimonier et al., 1975; Patterson et al., 2003a). Regulation of the estrous cycle was originally believed to be associated with control of the CL, whose lifespan and secretory activity are regulated by trophic and lytic mechanisms (Thimonier et al., 1975). Early estrus synchronization methods involved administration of exogenous progestins alone or, in later years, in combination with estrogens or PG to control the lifespan of the CL. Studies using transrectal ultrasonography to monitor changes in ovarian follicular dynamics throughout the estrous cycle revealed that precise and effective means of estrus synchronization involves the manipulation of both luteal lifespan and follicular waves (Savio et al., 1990).

Induction of Puberty with Progestins. Byerly et al. (1987) reported a decrease in pregnancy rates when heifers were bred on their pubertal versus third estrus, making timing of the onset of puberty a critical factor when heifers are bred to calve as 2-yr-olds. Estrus synchronization programs utilizing progestins were shown to effectively induce puberty in heifers (Gonzalez-Padilla et al., 1975; Patterson et al., 1990). Treatment with a progestin results in an increased frequency of LH pulses with the greatest pulse increase occurring after progestin removal (Anderson et al., 1996; Hall et al., 1997; Smith and Day, 1990; Imwalle et al., 1996). Progestin treatment was also associated with an increase in the diameter of the largest follicle (Imwalle et al., 1996; Hall et al., 1997) and increased follicular growth, resulting in increased E_2 production by ovarian follicles (Hendricks et al., 1973; Wettemann and Hafs, 1973; Sheffel et al., 1982; Garcia-Winder

et al., 1986). Additionally, following a 9 d treatment with a norgestomet implant, a decrease in the number of E_2 receptors in the hypothalamus was observed, with secretion of LH and the number of E_2 receptors being negatively correlated (Anderson and Day, 1994; Anderson et al., 1996). A decrease in E_2 receptors was associated with a decline in the negative feedback of E_2 on GnRH and subsequent LH release from the hypothalamic-pituitary axis (Day and Anderson, 1998).

The predominant exogenous progestins used in current estrus synchronization systems are the feed additive melengestrol acetate (**MGA**) and Controlled Internal Drug Release (**CIDR**) inserts.

Melengestrol Acetate. The orally active progestational steroid MGA (6-methyl-17-alpha-acetoxy-16-methylene-preg-4,6-diene-3, 20-dione; Figure 1.2) was developed in 1962 and marketed for use in feedlot heifers as a means of improving feed efficiency and rate of gain by allowing ovarian follicular development but preventing estrus and ovulation (Zimbelman and Smith, 1966). Melengestrol acetate was developed as an alternative to another orally active progestational compound, medroxyprogesterone acetate (**MAP**, 6-alpha-methyl-17-alpha-acetoxy-pregn-4-ene-3, 20-dione; Figure 1.2), which was shown to have a decreased potency in comparison to MGA for inhibiting estrus and ovulation (Zimbelman and Smith, 1963). When fed to ovariectomized heifers at a dose of 4.0 $mg^{\bullet}hd^{-1\bullet}d^{-1}$, MGA was able to maintain pregnancy (Zimbelman and Smith, 1963), which is eight to twenty times greater than the dose required to suppress estrus and ovulation (Zimbelman and Smith, 1963; 1966). When fed at the recommended level of 0.5 $mg^{\bullet}hd^{-1\bullet}d^{-1}$, MGA may be used successfully in estrus synchronization systems to suppress estrus, prevent the preovulatory surge of LH, inhibit ovulation

(Zimbelman et al., 1966; 1970; Imwalle et al., 2002) and induce estrous cyclicity in prepubertal heifers (Patterson et al., 1990; Imwalle et al., 1998). Low circulating concentrations of MGA are utilized successfully in estrus synchronization systems due to the fact that MGA has an 11.1-fold higher binding affinity for the progesterone receptor than progesterone (Perry et al., 2005).

Researchers have suggested that when synthetic progestins such as MGA are fed at the recommended level, estrus is not suppressed by means of negative feedback of E_2 blocking pulsatile LH release, since development of non-ovulatory persistent dominant follicles (Anderson and Day, 1994; Custer et al., 1994; Yelich et al.,1997) and pulsatile release of LH (Kojima et al., 1995) have been detected during MGA treatment. A much larger dose (1.5 to 5.0 mg) of MGA must be administered to prevent pulsatile secretion of LH (Kojima et al., 1995; Hageleit et al., 2000). However, the preovulatory surge of LH and ovulation are effectively inhibited when administered at 0.5 mg, suggesting that neuroendocrine mechanisms controlling the LH surge are much more sensitive to MGA than those controlling pulsatile release of LH (Imwalle et al., 2002).

To achieve optimal results, MGA must be consumed at a rate of 0.5 $mg^{\bullet}hd^{-1\bullet}d^{-1}$ in a single feeding delivered in a grain carrier. Feeding should take place at approximately the same time each day, and adequate bunk space must be provided to ensure proper intake (Patterson et al., 2003b). Since many beef producers lack the time, labor, and facilities required to facilitate successful use of MGA, other technologies were developed (CIDR) to provide alternative management strategies for estrus synchronization.

Controlled Internal Drug Release Inserts. Recently, progesterone intravaginal inserts were approved by the FDA (2002). Formal approval includes: synchronization of estrus in suckled beef cows and replacement beef and dairy heifers, for advancement of first postpartum estrus in suckled beef cows, and for advancement of first pubertal estrus in replacement beef heifers (FDA, 2002). The EAZI-BREED^{TM} Controlled Internal Drug Release (CIDR) cattle insert is a "T"-shaped nylon device with collapsible, flexible wings that is inserted into the vagina of the heifer or cow to facilitate estrus synchronization. The backbone of the CIDR consists of a layer of silicone impregnated with 1.38 g of P_4 which is continuously secreted and absorbed through the vaginal walls. Blood P_4 concentrations rise rapidly to peak concentrations approximately 1 h after CIDR insertion. These concentrations decline rapidly over a 12 to 24 h period upon CIDR removal (Lamb and Larson, 2006; Perry et al., 2004). In ovariectomized beef heifers, plasma P_4 concentrations peaked at 8.7 ng ml^{-1} 6 h after CIDR insertion and declined to 2.5 ng ml^{-1} following CIDR removal, with an average concentration of 5.7 ng $^{-1}$ during the 15 d treatment period (Macmillian et al., 1991). Average CIDR retention rates of 96 to 99% were reported in heifers treated for 4 to 15 d (Lucy et al., 2001; Macmillian et al., 1988; 1991).

Studies in beef heifers have shown improvements in synchrony of estrus following treatment with CIDR- compared to MGA-based estrus synchronization systems (Kojima et al., 2004; Tauck et al., 2007). The improvements were attributed to the CIDR's ability to deliver a known, consistent dose of P_4. Additionally, clearance rates of P_4 are faster following CIDR removal compared with those following MGA withdrawal from feed (Tauck et al., 2007). Finally, reports by Perry et al. (2004) and Wheaton and

Lamb (2007) indicate that CIDRs may be used to initiate cyclicity in postpartum anestrous beef cows.

DEVELOPMENT OF PROGESTIN-BASED METHODS TO SYNCHRONIZE ESTRUS IN BEEF HEIFERS

Development of the MGA-PG Protocol. Zimbelman and Smith (1966) reported that when fed orally, MGA successfully suppresses estrus and ovulation. Feeding MGA for 10 to 18 d resulted in a similar proportion of MGA treated heifers expressing estrus over a 6 d period following treatment as the proportion of control animals expressing estrus over a 20 d period. There was, however, a reduction in first service conception rate for the MGA treated animals (Zimbelman et al., 1970). When the progestin, norgestomet, was combined with estradiol valerate in the Syncro-Mate B treatment, studies revealed a high estrous response following treatment but variable first service conception rates (Odde, 1990).

Following the discovery of the luteolytic properties of PG and its analogues in cattle (Lauderdale, 1972; Rowson et al., 1972; Louis et al., 1972), prostaglandins were combined with progestins as a means to better control the estrous cycle and obtain improved results from estrus synchronization. Heersche et al. (1979) treated beef heifers with a 7 d norgestomet implant with an injection of PG given 6 or 7 d after implant insertion. Over a 5 d period, 93% of the heifers displayed estrus and 62% of those detected in estrus conceived. However, a disadvantage of this treatment was the additional labor and handling of animals that was required. Beal and Good (1986) and

Patterson et al. (1989) designed a treatment involving short term feeding of MGA with PG administered at the end of the feeding period. A reduction in fertility was reported, however, among heifers assigned to the short-term MGA-PG protocol that began treatment late in their estrous cycles. Reduced conception rate at first service may have occurred among these heifers because of extended interovulatory intervals and the resulting formation of persistent follicles (Beal et al., 1988; Patterson et al., 1989).

In an attempt to address the concerns of reduced fertility, Brown et al. (1988) developed a treatment that was designed to place heifers in the late luteal phase of the estrous cycle when PG was administered. Previous studies had shown that PG is more effective at inducing expression of estrus when given during the late luteal phase (d 10 to 15 of the estrous cycle; King et al., 1982; Tanabe and Hann, 1984; Watts and Fuquay, 1985). In the MGA-PG protocol, MGA is fed for 14 d with an injection of PG given 16 to 18 d after the last day of MGA feeding (Figure 1.3; Brown et al., 1988). A comparison of MGA-PG to Syncro-Mate B (Brown et al., 1988) revealed greater synchronized conception and pregnancy rates for MGA-PG treated heifers. The MGA-PG protocol also resulted in a higher estrous response and increased synchronized conception and pregnancy rates when compared to PG treatment alone (Patterson et al., 1995). Studies conducted in heifers revealed that estrous response and synchrony of estrus are improved when the MGA-PG protocol is modified to administer PG on d 19 versus d 17 following MGA withdrawal (Figure 1.3; Nix et al., 1998; Deutscher, 2000; Lamb et al., 2000).

Development of MGA Select. Recently, Wood et al. (2001) modified the MGA-PG protocol to determine whether the addition of GnRH would increase the synchrony of estrus and ovulation in beef heifers. This protocol, referred to as MGA Select, consists of

feeding MGA for 14 d with GnRH and PG administered 12 and 19 d following MGA feeding, respectively (Figure 1.4). All of the heifers assigned to the MGA Select treatment in this experiment ovulated in response to GnRH and initiated a new follicular wave 2 d following the GnRH injection. Additionally, the synchronized period was reduced for MGA Select compared to MGA-PG treated heifers, which resulted from the improved synchronized development of follicular waves among MGA Select treated heifers (Wood et al., 2001). The degree of synchrony following administration of the MGA Select protocol, however, may be influenced by the pubertal status of heifers prior treatment initiation (Wood-Follis et al., 2004).

Development of Select Synch and CO-Synch. An injection of GnRH given to cows at random stages of the estrous cycle elicits a preovulatory-like surge of LH and is capable of inducing ovulation of follicles $\geq$ 10 mm in diameter, leading to formation of a CL and production of P_4 (Pursley et al., 1995; Garverick et al., 1980; Bao and Garverick, 1998; Sartori et al., 2001). This newly formed luteal tissue is able to undergo PG induced regression 6 to 7 d later (Twagiramungu et al., 1995).

These findings formed the basis of the CO-Synch and Select Synch protocols, in which an injection of GnRH is administered, followed 7 d later with administration of PG. Cows are either inseminated 48 h following PG with a concurrent injection of GnRH (CO-Synch; Figure 1.5; Geary et al., 2001) or inseminated based on observed estrus (Select Synch; Figure 1.5; Geary et al., 1998). Stevenson et al. (1999) determined that Select Synch is less effective than MGA-PG for synchronizing estrus in beef heifers. The decreased success rate of the CO-Synch and Select Synch protocols was attributed to asynchrony among cows. A proportion of cows (approximately 5 to 15%) that are in the

late luteal phase of the estrous cycle when GnRH is administered will exhibit estrus prior to PG (Downing et al., 1998; Kojima et al., 2000). Therefore, when using Select Synch, it is recommended that detection of estrus begin 4 d following the administration of GnRH and that estrus detection continue for 6 d following PG (Kojima et al., 2000) which increases time and labor inputs.

Development of Short-Term CIDR-Based Protocols. In a series of experiments conducted by New Zealand researchers, heifers either received a 7 d CIDR with PG administered at CIDR removal, a 14 d CIDR, or a 21 d CIDR. Heifers that received a 21 d CIDR exhibited an increased synchrony of estrus following CIDR removal as compared to the other two treatments. However, a reduced calving rate was associated with the 21 d CIDR treatment, with highest calving rates associated with those heifers treated with the 7 d CIDR and PG (Macmillian and Peterson, 1993). Another experiment was conducted in which estrous response was evaluated in heifers treated with a 10 d CIDR with PG administered on varying days relative to CIDR treatment. Animals that received PG 2 d prior to CIDR removal exhibited a greater estrous response within 48 h after CIDR removal compared to heifers that received PG at CIDR removal. No difference in pregnancy rate was detected between these two groups (Macmillian and Peterson, 1993). This supports the work by other researchers (Smith et al., 1984) in demonstrating that administering PG 24 to 48 h prior to CIDR removal results in improved synchrony of estrus.

Experiments conducted in the U.S. by Lucy et al. (2001) evaluated a CIDR-PG protocol for synchronizing estrus in beef heifers. Results from this study facilitated the approval by FDA for CIDR use in beef heifers in the U.S. Heifers in the experiment

received one of 3 treatments: 1) 7 d CIDR with PG administered 6 d after CIDR insertion (CIDR-PG; Figure 1.6); 2) PG; and 3) untreated controls. An improvement in the 3 d synchrony of estrus rate was reported for both prepubertal and estrous cycling CIDR-PG treated heifers compared with PG and control treatment groups. Additionally, the CIDR-PG treatment resulted in enhanced pregnancy rates. This study also pointed to the effectiveness of progestin use in prepubertal beef heifers. A large proportion of the prepubertal CIDR-PG treated heifers exhibited estrus following treatment and maintained this advantage throughout the breeding period in comparison to prepubertal heifers in the other two treatments (Lucy et al., 2001). A drawback to the CIDR-PG protocol was that additional animal handling and labor were required since PG was administered on d 6, one day prior to CIDR removal.

A major limitation to the preceding estrus synchronization systems are the time and labor inputs required to facilitate protocol implementation as well as detection of estrus. Development of protocols that eliminate the need for estrus detection and that minimize animal handling with resulting high fertility should increase the adoption of estrus synchronization and AI (Patterson et al., 2003b).

Recently, Lamb et al. (2006) conducted a multi-state study involving 12 locations in 6 states which addressed these issues. The objectives of the multi-state trial were to determine whether: 1) administration of an estrus synchronization protocol followed by FTAI could yield pregnancy rates similar to a protocol requiring detection of estrus; and 2) whether an injection of GnRH at CIDR insertion enhanced pregnancy rates in beef heifers.

Four treatments were involved in the study (Figure 1.7). Heifers in treatment 1 (CO-Synch+CIDR) were observed for signs of behavioral estrus and inseminated on the basis of observed estrus through 72 h after PG. Eighty-four hours following the administration of PG all heifers that failed to exhibit estrus to that point were inseminated by appointment with GnRH administered at AI. Heifers in treatment 2 (CIDR-PG) were handled in the same way as heifers in treatment 1, however all heifers in treatment 2 received an injection of GnRH at CIDR insertion. Heifers in treatments 3 (CO-Synch+CIDR FTAI) and 4 (CIDR-PG FTAI) received the same treatment schedules as heifers in treatments 1 and 2, respectively, however heifers in both treatments 3 and 4 were inseminated by appointment 60 h after PG with GnRH administered at AI.

Although no differences in AI pregnancy rates were detected among treatments, heifers assigned to the estrus detection treatments had numerically higher AI pregnancy rates compared to heifers in the FTAI treatments. For heifers assigned to the two estrus detection treatments, no difference in estrous response, synchrony of estrus, or pregnancy rate was reported; suggesting that the addition of GnRH at CIDR insertion may be of limited value in estrus detection systems. Furthermore, the addition of GnRH in the CO-Synch+CIDR FTAI protocol did not substantially improve pregnancy rates compared to the CIDR-PG FTAI protocol (Table 1.3; 53 versus 49%, respectively). There was a greater variance among locations, however, in regard to CIDR-PG FTAI pregnancy rates when compared to CO-Synch+CIDR FTAI. Collectively, these results indicate that the addition of GnRH at CIDR insertion may be of limited value in heifers except when FTAI is employed. Additionally, the CO-Synch+CIDR FTAI protocol may be used to yield similar results to protocols requiring estrus detection (Lamb et al., 2006).

Comparable pregnancy rates utilizing FTAI with the CO-Synch+CIDR protocol are reported (Table 1.3; Martinez et al., 2000; Colazo et al., 2004; Walker et al., 2005; Busch et al., 2007).

Three summary conclusions may be drawn from this experiment (Lamb et al., 2006): 1) GnRH at CIDR insertion did not improve pregnancy rates after AI; 2) GnRH at CIDR insertion did not alter the percentage of heifers detected in estrus or the distribution of estrus after PG; and 3) a combination of detecting estrus and AI before clean-up AI enhanced pregnancy rates over FTAI.

Development of CIDR Select. Despite the recent development of estrus synchronization protocols that facilitate the successful use of FTAI in beef cows (Bader et al., 2005; Larson et al., 2006; Patterson et al., 2006; Schafer et al., 2007), the same degree of success in beef heifers has not been realized (Martinez et al., 2000; Dahlen et al., 2003; Colazo et al., 2004; Walker et al., 2005; Lamb et al., 2006; Busch et al., 2007). The reason for FTAI failure in heifers has been attributed to the inability of synchronizing follicular waves with the same degree of success that is achievable in beef cows. In response to an injection of GnRH, 64 to 75% of beef and dairy cows ovulated dominant follicles (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004) whereas only 48 to 60% of beef and dairy heifers responded to a similar treatment (Macmillian and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000). Atkins et al. (2007) and Schafer et al. (2006) reported that ovulatory response to GnRH in heifers may be influenced by the day of the estrous cycle on which GnRH is administered and that presynchronization with a progestin prior to a GnRH-PG regimen may be of importance.

Kojima et al. (2004) designed a study to compare estrous response, timing of AI, and pregnancy rate in beef heifers that were presynchronized with MGA (MGA Select) or CIDRs (14 d-CIDR; Figure 1.8; CIDR inserted d 1 and removed on d 14 followed 9 d with an injection of GnRH and PG 7 d after GnRH). Although no difference in estrous response was detected, 14 d-CIDR treated heifers exhibited an improvement in synchrony of estrus as well as higher conception and pregnancy rates during the synchronized period. Improved results with the 14 d-CIDR may be due to a reduced interval to estrus (Macmillian and Peterson, 1993) and improved synchronization of follicular waves after CIDR removal as compared to the end of MGA feeding.

Tauck et al. (2007) conducted a similar experiment comparing estrous response and AI pregnancy rates in beef heifers. Heifers were fed MGA for 14 d with PG administered 19 d later, and CIDR treated heifers received a CIDR for 14 d followed by an injection of PG 17 d later. All heifers were observed for estrus for 60 h following PG, and those not detected in estrus by 60 h were FTAI at 72 h with an injection of GnRH. The CIDR treated heifers had an increased estrous response and tended to exhibit shorter intervals to estrus following PG administration. Overall pregnancy rates did not differ between the two treatments. Together, the results from Kojima et al. (2004) and Tauck et al. (2007) suggest that use of a CIDR as a progestin source is equally as effective as MGA in synchronizing estrus in beef heifers.

Recently, Busch et al. (2007) compared short-term (CO-Synch+CIDR) and long-term (CIDR Select; Figure 1.8) CIDR-based protocols on the basis of synchrony of estrus and FTAI pregnancy rates. Presynchronization utilizing the long-term CIDR Select

protocol resulted in increased estrous response, improved synchrony of estrus, and greater FTAI pregnancy rates (Table 1.3) compared to the CO-Synch+CIDR protocol.

SUMMARY

Over the past thirty years, research and advances in science and technology have led to the development of estrus synchronization protocols that enable beef producers to incorporate superior genetics into the cow herd through the use of AI. However, cow-calf producers in the U.S. have been reluctant to implement these technologies in their operations due to time and labor constraints. Therefore, recent research has focused on the development of CIDR-based protocols which eliminate the need for estrus detection.

Although acceptable pregnancy rates to FTAI are attainable in beef cows, estrus synchronization protocols designed to facilitate FTAI in beef heifers have yielded lower and more inconsistent results. To date, no comprehensive studies have been conducted in prepubertal and estrous cycling beef heifers comparing physiological responses to long- and short-term CIDR-based protocols and their potential for facilitating the successful use of FTAI. These considerations form the basis for the studies reported in this thesis.

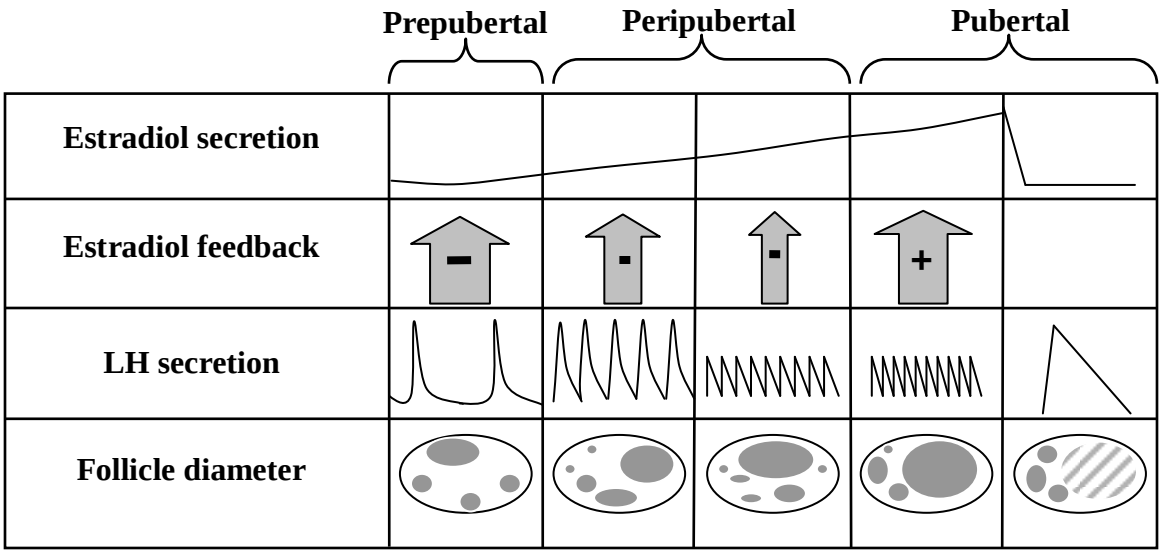


Figure 1.1. A conceptual model for the endocrine changes responsible for the onset of puberty in heifers (Day and Anderson, 1998).

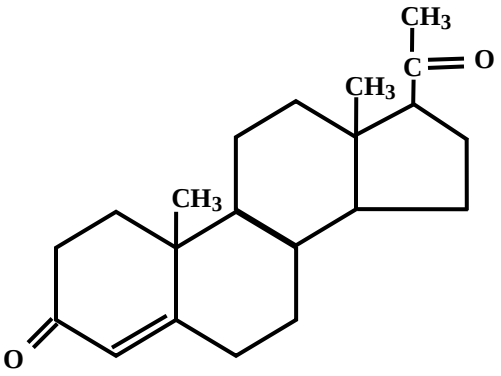
Table 1.1. Characteristics of the bovine estrous cycle.

Stage	Day of the estrous cycle	Characteristics
Estrus	0	Behavioral estrus (heat)
Metestrus	1 to 4	Ovulation and CL formation
Diestrus	5 to 16	CL growth and maintenance, progesterone secretion
Proestrus	17 to 21	Luteolysis and rapid follicular growth

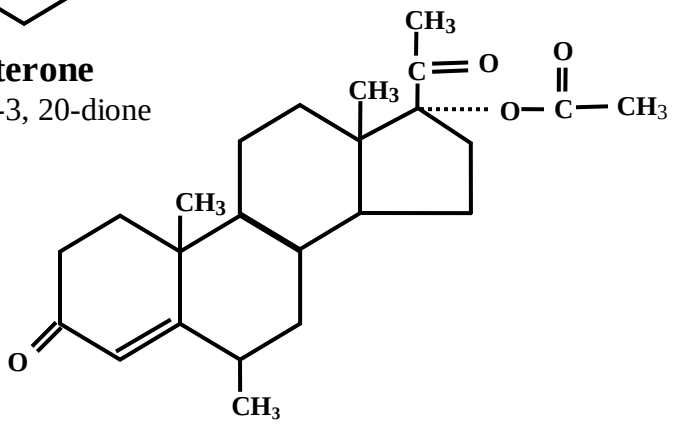
Table 1.2. Development of methods to synchronize estrus[a].

Phase	Method
I (Progesterone phase)	Exogenous progesterone was administered to prolong the luteal phase or establish an artificial luteal phase
II (Progesterone-estrogen phase)	Progestins were combined with estrogens or gonadotropins
III (Prostaglandin $F_{2\alpha}$ (PG) phase)	PG and its analogues were used as luteolytic agents
IV (Progesterone-PG phase)	Progestational agents were combined with PG
V (GnRH-PG phase)	GnRH and PG were used to control the follicular and luteal lifespan
VI (Progesterone-GnRH-PG phase)	Progestins were combined with gonadotropins and PG to more precisely control the interval and timing of estrus

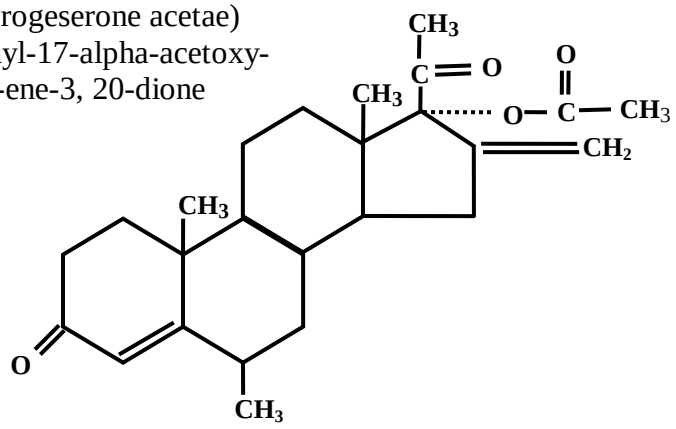
[a] Adapted from Thimonier et al., 1975; Patterson et al., 2003a



Progesterone
Pregn-4-ene-3, 20-dione



MAP
(medroxyprogeserone acetae)
6-alpha-methyl-17-alpha-acetoxy-
pregn-4-ene-3, 20-dione



MGA
(melengestrol acetate)
6-methyl-17-alpha-acetoxy-16-
methylene-pregn-4, 6-diene-3, 20-dione

Figure 1.2. Chemical structures of progesterone (P_4), medroxyprogesterone acetate (MAP), and melengestrol acetate (MGA).

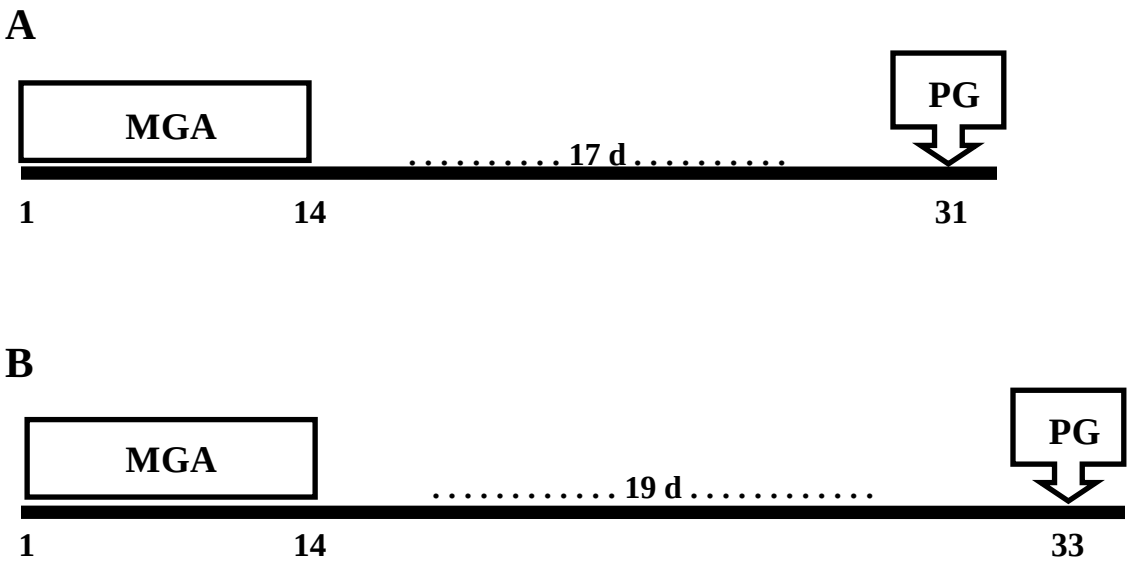


Figure 1.3. Treatment schedule for the14-17 d MGA-PG (A; from Brown et al., 1988) and the 14-19 d MGA-PG (B; from Lamb et al., 2000) protocol.

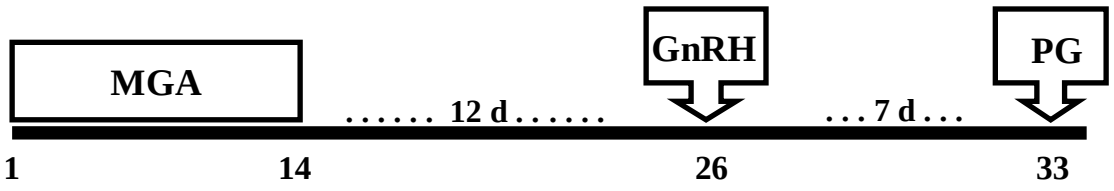


Figure 1.4. Treatment schedule for the MGA Select protocol (from Wood et al., 2001).

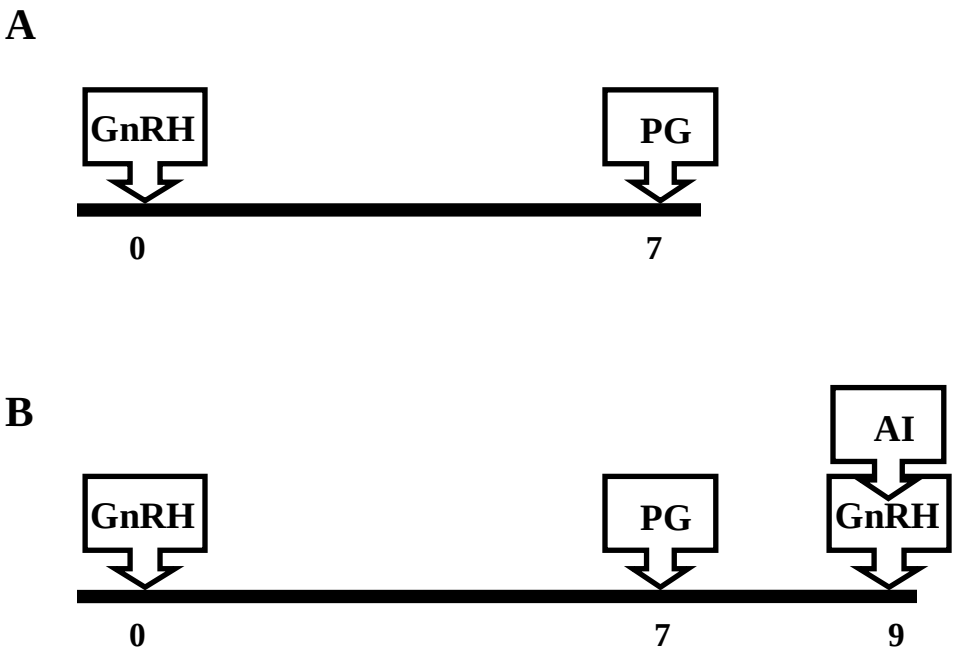


Figure 1.5. Treatment schedule for the Select Synch (A; from Geary et al., 1998) and CO-Synch (B; from Geary et al., 2001) protocols.

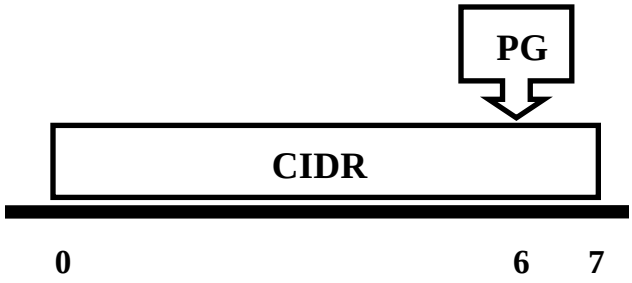


Figure 1.6. Treatment schedule for the original CIDR-PG protocol (from Lucy et al., 2001).

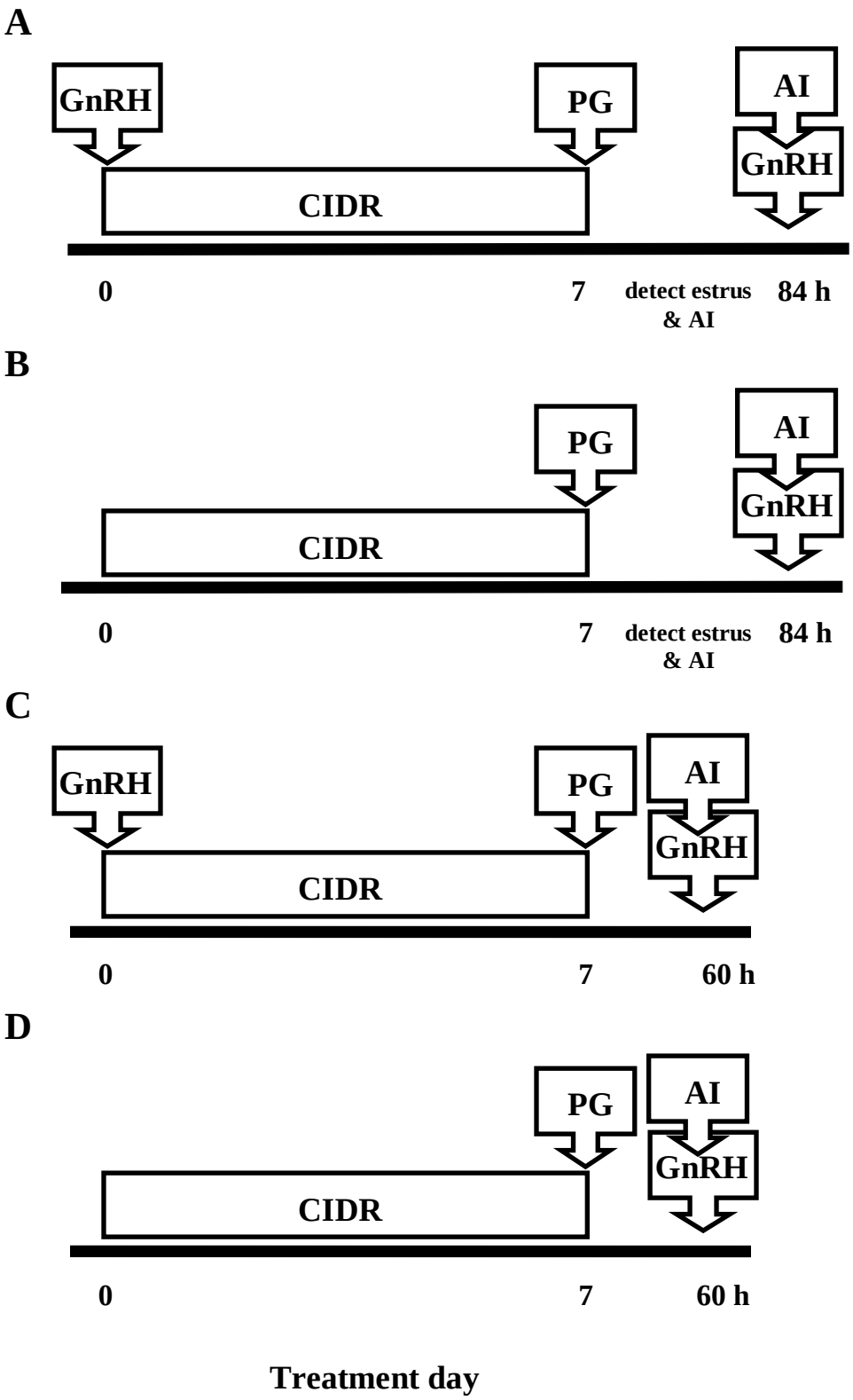


Figure 1.7. Treatment schedules for CO-Synch+CIDR with estrus detection and cleanup AI (A), CIDR-PG with estrus detection and cleanup AI (B), CO-Synch+CIDR with FTAI (C), and CIDR-PG with FTAI (D; from Lamb et al., 2006).

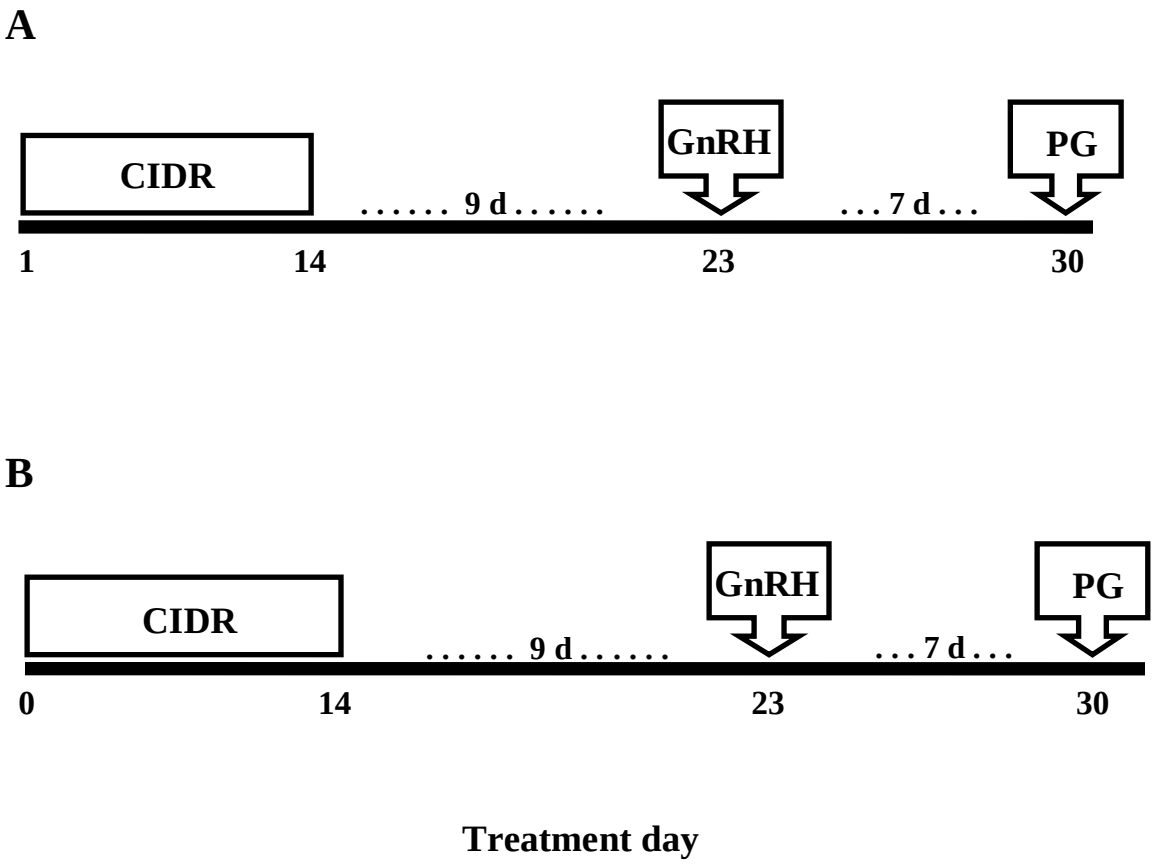


Figure 1.8. Treatment schedule for the 14-d CIDR (A; from Kojima et al., 2004) and the CIDR Select (B; from Busch et al., 2007) protocol.

Table 1.3. A compilation of pregnancy rates from studies in beef heifers inseminated at fixed-times following treatment.

Treatment	Time of AI (h)	No.	PR (%)
Syncro-Mate B[1]	60	44	39
Syncro-Mate B[1]	60	21	62
2-Injection PG 10 to 12 d apart[2]	80	469	36
Syncro-Mate B[3]	45	61	66
Syncro-Mate B[3]	48	50	54
Syncro-Mate B[3]	48 and 60	47	45
Syncro-Mate B[3]	50	65	66
Syncro-Mate B[3]	54	48	50
Syncro-Mate B[3]	54	35	69
Syncro-Mate B[3]	55	59	55
14-17 d MGA-PG[4]	72	51	43
14-17 d MGA-PG[5]	72	289	36
CO-Synch+CIDR[6]	54	42	48
CO-Synch[7]	48	23	39
CO-Synch+CIDR[7]	48	25	68
CO-Synch+CIDR[8]	48	103	65
Syncro-Mate B[9]	48 to 54	N/A[18]	45
Modified Select Synch[10]	48	172	22
CO-Synch+CIDR[11]	52 to 54	165	54
MGA Select[12]	48	178	47
14-19 d MGA-PG[12]	60	151	49
CO-Synch+CIDR[13]	54	189	55
Modified CO-Synch+CIDR[13]	54	186	46
CIDR-PG[14]	60	525	49
CO-Synch+CIDR[14]	60	531	53
14-d CIDR[15]	72	853	61
CO-Synch+CIDR[16]	48	89	39
CO-Synch+CIDR[17]	54	109	47
CIDR Select[17]	72	108	62

[1] Miksch et al., 1978; [2] Lauderdale, 1979; [3] Spitzer et al., 1981; [4] Kesler et al., 1996; [5] Larson et al., 1996; [6] Martinez et al., 2000; [7] Martinez et al., 2002a; [8] Martinez et al., 2002b; [9] Williams et al., 2002; [10] Dahlen et al., 2003; [11] Colazo et al., 2004; [12] Johnson and Day, 2004; [13] Walker et al., 2005; [14] Lamb et al., 2006; [15] Patterson et al., 2006; [16] Saldarriaga et al., 2007; [17] Busch et al., 2007; [18] Information not available.

CHAPTER 2

COMPARISON OF PROTOCOLS TO SYNCHRONIZE ESTRUS AND OVULATION IN ESTROUS CYCLING AND PREPUBERTAL BEEF HEIFERS

ABSTRACT

The objective of the experiment was to evaluate estrus synchronization protocols and compare differences in their ability to facilitate fixed-time AI in beef heifers. The experiment was designed to compare follicular dynamics, estrous response, and synchrony of estrus and ovulation following treatment. Estrous cycling beef heifers were randomly assigned to 1 of 4 treatments and prepubertal beef heifers were randomly assigned to 1 of 2 treatments by age and BW. Blood samples were taken 10 and 1 d prior to treatment initiation to confirm estrous cyclicity status (progesterone $\geq$ 0.5 ng/mL estrous cycling). CIDR Select (C1, estrous cycling heifers, n = 12; P1, prepubertal heifers, n = 14) treated heifers received a controlled internal drug release (CIDR) insert (1.38 g progesterone) from d 0 to 14 followed by GnRH (100 µg, i.m.) on d 23 and $PGF_{2\alpha}$ (PG; 25 mg, i.m.) on d 30. Select Synch + CIDR (C2, estrous cycling heifers, n = 12; P2, prepubertal heifers, n = 11) treated heifers received a CIDR insert and GnRH on d 23 and PG at CIDR removal on d 30. CIDR-PG (C3, estrous cycling heifers, n = 12) treated heifers received a CIDR insert on d 23 and PG at CIDR removal on d 30. Select Synch (C4, estrous cycling heifers, n = 12) treated heifers received GnRH on d 23 and PG on d 30. Heifers were fitted with HeatWatch transmitters at the time of CIDR removal (C1,

C2, C3, P1, and P2) or at GnRH (C4) for continuous estrus detection. Ovaries were scanned by ultrasonography on d 22, 23, and 25 to determine response to GnRH, and daily from d 30 to estrus. Beginning 20 h after the onset of estrus, ovaries were scanned every 4 h until ovulation. There was no difference ($P > 0.05$) in ovulatory response to GnRH (75, 42, and 75%; C1, C2, and C4, respectively) or estrous response (92, 100, 100, and 75%; C1, C2, C3, and C4, respectively) among the estrous cycling heifers. Among the prepubertal heifers, more ($P = 0.02$) P1 heifers responded to GnRH than P2 heifers (86% P1; 36% P2), but there was no difference ($P > 0.05$) in estrous response (86% P1; 64% P2). Among the estrous cycling heifers, variance for interval to estrus after PG differed ($P < 0.05$) between C1 and each of the other treatments and between C2 and C3. Mean intervals to estrus were 53 ± 3.7, 49 ± 3.5, 51 ± 3.5, and 48 ± 4.1 h for C1, C2, C3, and C4, respectively. Variance for interval to ovulation after PG differed ($P < 0.05$) among C1 and each of the other treatments. Mean intervals to ovulation were 83 ± 3.9, 80 ± 3.9, 81 ± 3.8, and 77 ± 4.3 h for C1, C2, C3, and C4, respectively. Among the prepubertal heifers, there was no difference ($P > 0.05$) in variance for interval to estrus or ovulation. Results from C1 and P1 (T1) and C2 and P2 (T2) were combined to compare T1 and T2 among mixed groups of estrous cycling and prepubertal heifers. Response to GnRH ($P < 0.01$; 81% T1 and 39% T2), and the variances for interval to estrus and ovulation were smaller ($P < 0.01$) for T1 than T2. In summary, CIDR Select improved ($P < 0.01$) synchrony of estrus and ovulation compared with Select Synch + CIDR, suggesting the CIDR Select protocol may facilitate fixed-time AI more effectively in mixed groups of prepubertal and estrous cycling beef heifers.

INTRODUCTION

Estrus synchronization and artificial insemination (**AI**) provide beef producers with effective management tools to maximize the reproductive potential of their cow herd by incorporating superior genetics into their operations. However, United States cow-calf producers have been reluctant to implement these technologies due to time and labor constraints. Therefore, development of economical methods to synchronize estrus and ovulation to facilitate fixed-time AI (**FTAI**) with resulting high fertility would likely increase the adoption of these technologies in United States beef herds (Patterson et al., 2003b).

Although acceptable pregnancy rates to FTAI are attainable in beef cows (Bader et al., 2005; Larson et al., 2006; Schafer et al., 2007), estrus synchronization protocols that facilitate FTAI in beef heifers have yielded less desirable and inconsistent results (Dahlen et al., 2003; Lamb et al., 2006; Busch et al., 2007). The reason FTAI often fails in heifers is largely attributed to an inability to successfully synchronize follicular waves with the same degree of success that is achievable in beef cows (Lamb et al., 2006). Research indicates that ovulatory response to GnRH in heifers is influenced by the day of the estrous cycle at GnRH administration and that presynchronization with a progestin [controlled internal drug-releasing (**CIDR**) inserts] prior to a GnRH-$PGF_{2\alpha}$ (**PG**) regimen may increase the proportion of dominant follicles that respond to GnRH and thereby improve the synchronization of follicular waves (Kojima et al., 2004; Schafer et al., 2006; Atkins et al., 2007). Other studies indicate that the addition of GnRH at CIDR insertion may be of limited value (Lamb et al., 2006). Recently, Busch et al. (2007)

reported an increased estrous response, improved synchrony of estrus, and greater FTAI pregnancy rates in beef heifers that were presynchronized with a long-term CIDR protocol compared to a short-term CIDR protocol.

To date, no comprehensive studies have been conducted in beef heifers comparing physiological responses to long- and short-term CIDR-based protocols and their potential for facilitating the successful use of FTAI. The hypothesis tested was that presynchronization with a progestin prior to GnRH and PG will facilitate an improvement in synchrony of estrus and ovulation in beef heifers compared to short-term CIDR-based or GnRH-PG estrus synchronization protocols. The objective of this experiment was to compare follicular dynamics, response to GnRH, and synchrony of estrus and ovulation among estrous cycling and prepubertal beef heifers synchronized with long- or short-term CIDR-based protocols.

MATERIALS AND METHODS

The experimental procedures were approved by the University of Missouri, Columbia Animal Care and Use Committee.

Experimental Design. Crossbred estrous cycling (n = 48) beef heifers were randomly assigned to 1 of 4 treatments and crossbred prepubertal (n = 25) beef heifers were randomly assigned to 1 of 2 treatments by age and BW. Initial treatment assignments were based on blood samples collected 10 and 1 d prior to treatment initiation to confirm estrous cyclicity status (progesterone $\geq$ 0.5 ng/mL, estrous cycling). Prepubertal heifers assigned to the Select-Synch + CIDR protocol were re-sampled 2 d

prior to CIDR insertion to re-confirm estrous cyclicity status. The authors acknowledge the potential for misclassification of heifers by cyclicity determined from 2 blood samples before treatment initiation and the use of progesterone values $\geq$ 0.5 ng/mL to confirm cyclicity. However, the potential for committing a Type II error is greatly minimized if not negated in describing heifers as prepubertal using a 0.5 ng/mL cutoff. Heifers assigned to the CIDR Select protocol (**C1**, estrous cycling heifers, n = 12; **P1**, prepubertal heifers, n = 14) received an EAZI-Breed CIDR insert (1.38 g progesterone; Pfizer Animal Health, New York, NY) from d 0 to 14 followed by GnRH (100 µg, i.m.; Cystorelin, Merial, Athens, GA) on d 23 and PG (25 mg, i.m.; Lutalyse, Pfizer Animal Health) on d 30. Select Synch + CIDR (**C2**, estrous cycling heifers, n = 12; **P2**, prepubertal heifers, n = 11) treated heifers received a CIDR insert and GnRH on d 23 and PG at CIDR removal on d 30. The CIDR-PG (**C3**, estrous cycling heifers, n = 12) treated heifers received a CIDR insert on d 23 and PG at CIDR removal on d 30. Heifers assigned to Select Synch (**C4**, estrous cycling, n = 12) received GnRH on d 23 and PG on d 30 (Figure 2.1). Results from C1 and P1 (**T1**) and C2 and P2 (**T2**) were combined and analyzed to compare T1 and T2 among mixed groups of estrous cycling and prepubertal beef heifers.

Estrus Detection. All heifers were fitted with a HeatWatch estrus detection transmitter (DDx Inc., Denver, CO) at the time of CIDR removal (C1, C2, C3, P1, and P2) or at GnRH (C4) for continuous estrus detection. Estrus was defined as heifers receiving $\geq$ 3 mounts, each of which were $\geq$ 2 s in duration, within a period of 4 h. The synchronized period was designated as 0 to 144 h following PG, and transmitters were maintained on the heifers until ovulation was confirmed.

Ultrasonography. Ovaries were scanned by transrectal ultrasonography (Aloka 500V equipped with a 7.5-MHz linear array transducer, Aloka, Wallingford, CT) on d 22 and 23 to characterize follicular dynamics. Follicles $\geq$ 5 mm and the presence of corpora lutea (**CL**) were recorded. On d 25, ovaries were scanned to determine response to GnRH (defined as disappearance of the dominant follicle). Ovaries were scanned daily from d 30 to estrus to monitor follicular dynamics. Ultrasonography was performed every 4 h until ovulation was confirmed (designated as disappearance of the dominant follicle) beginning 20 h after the onset of estrus. Time of ovulation was considered to be the mean time of ultrasound scans when the dominant follicle was last observed and no longer present.

Blood Collection and RIA. Blood samples were collected via jugular venipuncture 10 and 1 d prior to treatment initiation to confirm estrous cyclicity status. Prepubertal heifers assigned to the Select-Synch + CIDR protocol were re-sampled 2 d prior to CIDR insertion to re-confirm estrous cyclicity status. Heifers were considered to be estrous cycling if their progesterone concentrations were $\geq$ 0.5 ng/mL at any one of the sampling times. Additionally, blood samples were collected daily from PG through estrus. Blood samples were allowed to clot and were stored at 4ºC for 24 h. Serum was collected by centrifugation and was stored at -20ºC until hormone assays were performed. Serum concentrations of estradiol 17-β (**E_2**) and progesterone (**P_4**) were determined by RIA. Serum E_2 concentrations were determined by validated extraction assay (Kirby et al., 1997). Intra- and inter-assay coefficients of variation were 12.5 and 10.48%, respectively, with an assay sensitivity of 0.83 pg/mL. Serum P_4 concentrations were determined with a Coat-A-Count kit (Diagnostic Products Corp., Los Angeles, CA; Kirby

et al., 1997) with intra- and inter-assay coefficients of variation of 2.28 and 8.81%, respectively, and an assay sensitivity of 0.1 ng/mL.

Statistical Analyses. Differences in age, BW, intervals to estrus and ovulation, follicle size at GnRH and 20 h after estrus, and serum P_4 concentrations among treatments were analyzed by one-way ANOVA (PROC GLM; SAS Inst. Inc. Cary, NC). Variances associated with intervals to estrus and ovulation were compared by performing a F-test (greater variance divided by the smaller variance; Snedecor and Cochran, 1989). Response to GnRH was analyzed using the generalized linear models procedure of SAS (PROC GENMOD of SAS). Due to select treatments containing all positive or negative values, estrous responses were analyzed using a contingency χ^2 analysis (PROC FREQ of SAS). Follicle size and serum E_2 concentrations at the time of PG injection, 24 and 48 h following PG were analyzed by using repeated measures over time using the mixed model procedures of SAS (PROC MIXED) as outlined by Littell et al. (1998). Since variances for serum E_2 concentrations were heterogeneous among treatments, a log10 transformation was done for testing differences. However, all tables and figures are presented with actual values.

RESULTS

The number, age, and BW of heifers before the initiation of treatments are shown in Table 2.1. There was no difference among treatments for age or BW among the estrous cycling heifers and the prepubertal heifers. However, the prepubertal heifers weighed less ($P < 0.05$) than the estrous cycling heifers.

Synchrony of estrus following CIDR removal. There was no difference in estrous response following CIDR removal for the estrous cycling or prepubertal heifers treated with the CIDR Select protocol ($P = 0.09$). Figure 2.2 illustrates the distribution of estrus following CIDR removal. Although the mean interval to estrus was shorter for the estrous cycling heifers compared to the prepubertal heifers ($P = 0.02$), there was no difference in the variance for interval to estrus ($P > 0.10$; Table 2.2).

Follicular dynamics. Among the estrous cycling heifers, there was no difference ($P > 0.05$) in response to GnRH, but more P1 heifers responded to GnRH than P2 heifers ($P = 0.02$; Table 2.3). Mean follicle diameter did not differ ($P > 0.10$) among treatments at GnRH, PG, 24 h after PG, 48 h after PG, or 20 h after the onset of estrus. Table 2.4 summarizes the response to GnRH based on the day of the estrous cycle when GnRH was administered for heifers treated with the CIDR Select protocol. The majority of heifers were on d 8 of the estrous cycle at GnRH administration.

Synchrony of estrus and ovulation following PG. There was no difference among treatments for the proportion of heifers exhibiting estrus during the synchronized period ($P > 0.05$) or for mean intervals to estrus or ovulation ($P > 0.20$). Variance for interval to estrus was less for C1 compared to C2 ($P < 0.01$), C3 ($P < 0.05$), and C4 ($P < 0.05$). Variance for interval to estrus was also less for C3 compared to C2 ($P < 0.05$) and tended to be less for P2 compared to C2 ($P = 0.06$). Variance for interval to ovulation was less for C1 compared to C2 ($P < 0.01$), C3 ($P < 0.05$), and C4 ($P < 0.01$). Variance for interval to ovulation was also less for P2 compared to C2 ($P < 0.05$; Table 2.5).

Serum steroid hormone concentrations. Serum concentrations of P_4 at GnRH administration were less for P2 compared to all other treatments ($P < 0.02$). This was

expected, since heifers assigned to P2 were prepubertal and had no exogenous exposure to P_4 prior to GnRH administration. At PG administration, P2 heifers had lower serum P_4 concentrations than P1 ($P < 0.01$), C1 ($P < 0.02$), C2 ($P < 0.01$), and C3 ($P < 0.03$). Heifers assigned to C4 had lower serum P_4 concentrations than C2 ($P < 0.04$). There was a significant treatment x day interaction ($P < 0.05$) for serum E_2 concentrations. At PG administration, P2 treated heifers tended to have greater ($P = 0.06$; Table 2.6) serum E_2 concentrations than P1. Forty-eight hours following PG, P1 treated heifers had greater ($P < 0.02$; Table 2.6) serum E_2 concentrations than P2.

Combined results for CIDR Select and Select Synch + CIDR. Results from C1 and P1 (T1) and C2 and P2 (T2) were combined and analyzed collectively to compare the CIDR Select and Select-Synch + CIDR protocols among mixed groups of estrous cycling and prepubertal heifers. More T1 heifers responded to GnRH than T2 heifers ($P < 0.01$; Table 2.7). There was no difference ($P > 0.10$) between treatments in estrous response during the synchronized period or in mean intervals to estrus or ovulation (Table 2.7). Figures 2.3 and 2.4 illustrate the distribution of estrus and ovulation following PG, respectively. Variance for interval to estrus ($P < 0.01$) and ovulation ($P < 0.01$) was less for T1 compared to T2 (Table 2.7). It is important to note that there were no within treatment differences between estrous cycling and prepubertal heifers in variance for interval to estrus or ovulation for T1 ($P > 0.10$; Table 2.5) but within treatment differences in variance for interval to estrus ($P = 0.06$; Table 2.5) and ovulation ($P < 0.05$; Table 2.5) were detected for T2. Figure 2.5 shows follicle diameters at PG, 24 h, and 48 h following PG for T1 and T2. There were no significant differences in follicle diameter between the two treatments ($P > 0.10$). Figure 2.6 illustrates differences in

serum concentrations of E_2. It is important to note that despite a lack of difference in follicle diameter between treatments, there were differences in serum E_2 profiles between the two treatments as indicated by the significant ($P < 0.05$) treatment x day interaction. Serum concentrations of E_2 tended to be greater ($P = 0.06$) for T2 than T1 at PG. However, 48 h after PG, T1 had greater ($P < 0.01$) serum E_2 concentrations than T2. Serum concentrations of P_4 were significantly greater for T1 than T2 ($P < 0.05$; data not shown) at GnRH and PG injections.

DISCUSSION

Estrus synchronization and AI are reproductive management techniques that have been commercially available to beef producers for over 30 yr. However, United States beef producers have been reluctant to implement these technologies in their operations despite the potential benefits of shortening the calving season, increasing calf uniformity, and incorporating proven superior genetics into the cow herd. The development of economical methods to synchronize estrus and ovulation that reduce time and labor inputs with resulting high fertility may increase the adoption of these technologies in U.S. beef herds (Patterson et al., 2003b). Recent research has focused on the development of estrus synchronization systems that facilitate FTAI, thereby eliminating the time and labor associated with estrus detection (Larson et al., 2006; Busch et al., 2007; Schafer et al., 2007).

However, estrus synchronization protocols recently developed for beef heifers that facilitate FTAI have produced inconsistent results (Dahlen et al., 2003; Lamb et al.,

2006; Busch et al., 2007), which has been attributed to the inability to successfully synchronize follicular waves following administration of GnRH. Reports indicate that only 43 to 60% of beef and dairy heifers ovulated in response to GnRH (Macmillian and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000; Atkins et al., 2007), whereas 64 to 75% of beef and dairy cows ovulated in response to a similar treatment (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004). Lucy and Stevenson (1986) reported a reduced magnitude of GnRH-induced LH release in heifers compared with cows; however, among heifers on d 15 and 18 of the estrous cycle, magnitude of GnRH-induced LH secretion was not associated with ovulatory response (Atkins et al., 2007). Schafer et al. (2006) reported that presynchronization with a 14-d CIDR yielded a higher response to GnRH (86%) in beef heifers.

In the present study, a greater proportion of prepubertal heifers that were presynchronized utilizing P1 (86%) ovulated in response to GnRH than the prepubertal heifers synchronized with P2 (36%), and the combined results of T1 and T2 indicate that presynchronization with the long-term CIDR resulted in a greater ovulatory response (81% T1; 39% T2) among mixed groups of estrous cycling and prepubertal heifers. The greater response to GnRH among heifers treated with T1 may be explained by the greater degree of synchrony that resulted from presynchronization with P_4 prior to the GnRH injection. Atkins et al. (2007) reported a relationship between response to GnRH and day of the estrous cycle, with the greatest response observed when heifers were administered GnRH on d 5 of the estrous cycle followed by d 15, 10, 18, and 2. Schafer et al. (2006) reported similar results in that the highest ovulatory response was observed when GnRH was administered on d 5 to 8 of the estrous cycle when heifers were presynchronized with

a 14-d CIDR. Eighty-eight percent of T1 treated heifers were on d 7 or 8 of the estrous cycle at the time of the GnRH injection with an overall 78% response. No difference in diameter of the dominant follicle was detected among treatments at the GnRH injection.

Characterization of follicular diameters and steroid hormone profiles revealed no differences in diameter of the dominant follicle among the individual treatments at the time of PG administration or 24 or 48 h following PG. This is reflected in similar serum E_2 concentrations among treatments at PG and 24 h following PG. Although there was no difference in follicle diameter 48 h following PG between P1 and P2, the P1 treated heifers had higher concentrations of E_2 than P2 at this time.

When considering the results for T1 and T2, there was no difference in diameter of the dominant follicle at PG, but T2 tended to have greater serum E_2 and had lower serum P_4 concentrations than T1. We assume that follicles in T2 grow and develop under low-circulating P_4 concentrations and thus create higher circulating concentrations of E_2. The lower P_4 concentrations at the time of the GnRH and PG injections for T2 may have allowed for a high pulse frequency and low pulse amplitude release of LH. Increased LH pulse frequency apparently stimulates E_2 secretion by the follicle. At 48 h following the injection of PG, follicle diameters between T1 and T2 remained similar, but T1 had greater serum E_2 concentrations. Stegner et al. (2004) also reported differences in E_2 concentrations when follicular diameters were similar and suggested that the maturation rate of follicles depends on the hormonal milieu under which they develop, independent of measurable differences in follicle diameter.

Another management factor that beef producers must consider at the start of the breeding season is the existence of two physiologically distinct groups of heifers: estrous

cycling and prepubertal heifers. Studies indicate that on average, 11 to 33% of heifers are prepubertal at the start of the breeding season (Lynch et al., 1997; Lammoglia et al., 2000; Lamb et al., 2006). Additionally, Wood-Follis et al. (2004) suggested that the degree of synchrony following administration of an estrus synchronization protocol may be influenced by the pubertal status of heifers prior to treatment initiation. Therefore, efficacy of estrus synchronization protocols must be evaluated on the basis of inducing estrous cyclicity and the resulting synchrony of estrus among mixed populations of estrous cycling and prepubertal heifers.

Various studies have reported successful induction of estrus and ovulation in prepubertal heifers through the use of a progestin (Patterson et al., 1990; Lucy et al., 2001). Among prepubertal heifers in this study, there was no difference in estrous response during the synchronized period following PG.

Estrus synchronization protocols that facilitate FTAI must be capable of synchronizing follicular development and luteal regression, resulting in a highly synchronized estrus and ovulation. Although mean intervals to estrus and ovulation following PG administration did not differ among treatments, differences in the variances for interval to estrus and ovulation were detected. Heifers treated with C1 had less variance associated with the interval from PG to estrus and ovulation when compared to C2, C3, and C4, suggesting that C1 may facilitate FTAI more effectively among estrous cycling beef heifers. Less variance was associated with the interval to estrus for C3 than C2, but no difference in the variance for interval to ovulation was detected.

These findings provide a probable explanation for differences in FTAI pregnancy rate reported by Busch et al. (2007) when beef heifers were FTAI following treatment

with the CIDR Select and CO-Synch + CIDR protocols. Busch et al. (2007) reported a higher FTAI pregnancy rate for estrous cycling heifers assigned to the CIDR Select (62%) compared to the CO-Synch + CIDR protocol (47%). Accordingly, in the current experiment, C1 treated heifers had a reduced variance for interval to estrus and ovulation compared to heifers treated with C2.

Lamb et al. (2006) reported no difference in the distribution of estrus for heifers treated with CO-Synch + CIDR and CIDR-PG. However, estrus detection in the present study was conducted until 144 h following PG injection; whereas estrus detection ended 72 h following PG in the study by Lamb et al. (2006). This may explain the discrepancy in distribution of estrus between the two studies. When FTAI was employed utilizing CO- Synch + CIDR and CIDR-PG, Lamb et al. (2006) reported that the addition of GnRH in CO-Synch + CIDR did not substantially improve pregnancy rates when compared to CIDR-PG (53 versus 49%, respectively). However, there was a greater variance among locations in regard to FTAI pregnancy rates for heifers assigned to CIDR-PG. Collectively, these results indicate that the addition of GnRH at CIDR insertion may be of limited value in heifers except when FTAI is employed (Lamb et al., 2006).

Although no difference in the variance for interval to estrus during the synchronized period was detected between C4 and the two other short-term protocols in the present study, C4 is generally not used to facilitate FTAI due to unacceptable pregnancy rates. Dahlen et al. (2003) reported a 22% pregnancy rate to FTAI in beef heifers utilizing a 6 d GnRH-PG regimen. In the present study, 25% (3/12) of heifers synchronized with C4 displayed estrus prior to PG. Approximately 5 to 15% of cows and

heifers that are in the late luteal phase of the estrous cycle when GnRH is administered will display estrus prior to PG (Downing et al., 1998; Kojima et al., 2000; Atkins et al., 2007), which precludes the use of C4 in FTAI systems. In the present study, 1 of the 3 heifers that displayed estrus prior to PG ovulated in response to GnRH. Atkins et al. (2007) recently reported that a proportion of heifers that were on d 15 of the estrous cycle ovulated and formed an accessory CL in response to GnRH but displayed estrus before PG, which may result from inadequate P_4 production by the accessory CL and (or) premature regression of the accessory CL (Keisler and Keisler, 1989; Atkins et al., 2007).

Busch et al. (2007) reported a greater synchrony of estrus and greater FTAI pregnancy rates for heifers synchronized with CIDR Select in comparison to the CO-Synch + CIDR protocol. The combined results of estrous cycling and prepubertal heifers in T1 and T2 support and provide further explanation of these observations. In the present study, smaller variances for intervals to estrus and ovulation were observed for T1 when compared to T2, which supports the work by Busch et al. (2007). The improved synchrony of estrus and ovulation for T1 treated heifers appears to be associated with an increased response to GnRH (81%, T1; 39%, T2) and more effective control of the emerging follicular wave resulting from presynchronization with the long-term CIDR treatment. This improved synchrony of estrus and ovulation is apparently responsible for the significantly higher FTAI pregnancy rates for CIDR Select (62%) compared to CO-Synch + CIDR (47%) treated heifers (Busch et al., 2007).

Perhaps the most important finding of the current experiment is that the variances for interval to estrus and ovulation did not differ between the estrous cycling and prepubertal heifers assigned to T1, but within treatment differences in variances for

interval to estrus were detected in T2. Busch et al. (2007) also reported more variance associated with the interval from PG to estrus between prepubertal and estrous cycling heifers synchronized with the CO-Synch + CIDR protocol compared with the CIDR Select protocol. It is important to note that in the experiment by Busch et al. (2007) FTAI was performed 54 and 72 h after PG (CO-Synch + CIDR and CIDR Select, respectively) with a concurrent injection of GnRH, which may have influenced the distribution of estrus during the remaining synchronized period. Collectively, these results indicate that the CIDR Select protocol may yield higher FTAI pregnancy rates in mixed groups of estrous cycling and prepubertal heifers compared to the CO-Synch + CIDR protocol, due to the increased synchrony of estrus associated with the progestin presynchronization. However, no within treatment differences for FTAI pregnancy rate between the estrous cycling and prepubertal heifers was observed (CIDR Select, 62% estrous cycling, 62% prepubertal; CO-Synch + CIDR, 47% estrous cycling 48% prepubertal, Busch et al., 2007). The lack of within treatment differences for FTAI pregnancy rate reported by Busch et al. (2007) may be attributed to the low number of prepubertal compared to estrous cycling heifers used in the experiment (CIDR Select, 87 estrous cycling, 21 prepubertal; CO-Synch + CIDR, 86 estrous cycling, 23 prepubertal).

In summary, the CIDR Select protocol improved the synchrony of estrus and ovulation compared to Select Synch + CIDR, CIDR-PG, and Select Synch among estrous cycling beef heifers. There was more variance associated with the interval from PG to estrus and ovulation between prepubertal and estrous cycling beef heifers synchronized with the Select Synch + CIDR protocol compared to the CIDR Select protocol. These data provide a physiological explanation for differences in synchrony of estrus following

treatment administration based on long- versus short-term CIDR-based protocols. Furthermore, these data support the findings of Busch et al. (2007) which clearly demonstrate improvements in FTAI pregnancy rates in beef heifers following treatment with a long-term CIDR-based protocol compared to the 7 d CO-Synch + CIDR protocol.

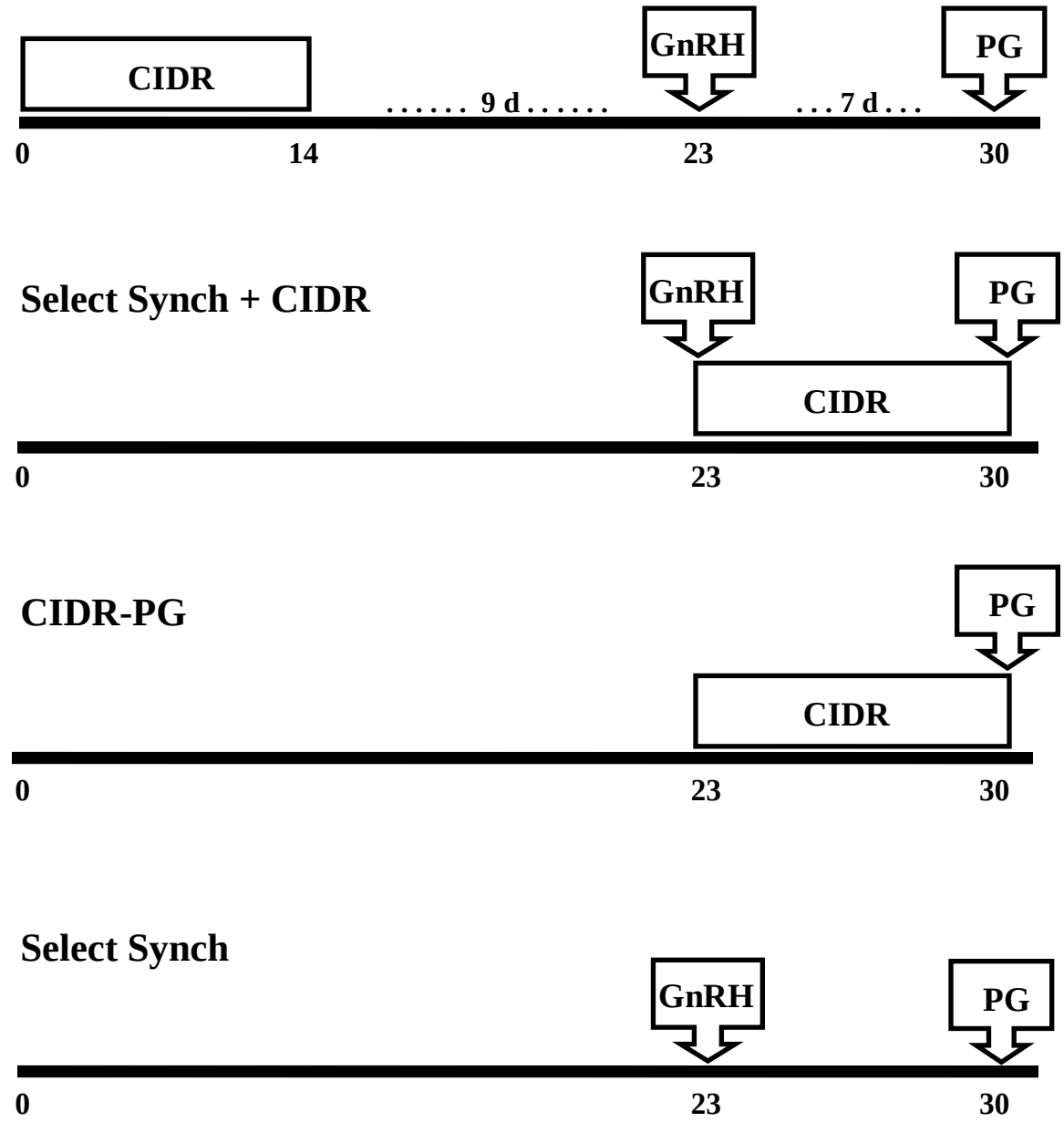


Figure 2.1. Treatment schedule for heifers assigned to the CIDR Select (C1, P1; T1 denotes combined results from C1 and P1), Select Synch + CIDR (C2, P2; T2 denotes combined results from C2 and P2), CIDR-PG (C3), and Select Synch (C4) protocols. Heifers assigned to C1 or P1 were equipped with an EAZI-Breed controlled internal drug release (CIDR; 1.38 g of progesterone) insert from d 0 to 14, GnRH (Cystorelin; 100 µg, i.m.) on d 23, and $PGF_{2\alpha}$ (PG; 25 mg, i.m.; Lutalyse) on d 30. Heifers assigned to C2 or P2 were administered GnRH and received a CIDR insert on d 23 and PG at CIDR removal on d 30. Heifers assigned to C3 received a CIDR insert on d 23 and PG at CIDR removal on d 30. Heifers assigned to C4 received GnRH on d 23 and PG on d 30. "C" denotes estrous cycling and "P" denotes prepubertal heifers.

Table 2.1. Number, age, and BW of heifers prior to treatment initiation (mean ± SE).

	Treatment[1]					
	C1	C2	C3	C4	P1	P2
No. of heifers	12	12	12	12	14	11
Age,[2] d	386 ± 6.2	382 ± 6.2	379 ± 6.2	383 ± 6.2	371 ± 5.8	373 ± 6.5
BW,[3] kg	361 ± 9.9[a]	363 ± 9.9[a]	363 ± 9.9[a]	366 ± 9.9[a]	328 ± 9.2[b]	325 ± 10.4[b]

[1] See Figure 2.1 for a description of treatment protocols.
[2] Age (d) of the heifers at the initiation of treatments.
[3] Body weight (kg) of the heifers at the initiation of treatments.
[a,b] Means within rows with different superscripts differ ($P < 0.02$).

Table 2.2. Estrous response following CIDR[1] removal for estrous cycling (C) and prepubertal (P) heifers assigned to the CIDR Select treatment.

	Treatment[2]	
	C1	P1
Estrous response		
Prop.	12/12	11/14
%	100	79
Interval to estrus, h		
Mean ± SE	27.8 ± 1.9[a]	34.8 ± 2.0[b]
Variance for interval to estrus	53.40	32.78

[1] CIDR = EAZI-Breed CIDR insert (1.38 g progesterone; Pfizer Animal Health, New York, NY).
[2] See Figure 2.1 for a description of treatment protocols.
[a,b] Means within rows with different superscripts differ ($P = 0.02$).

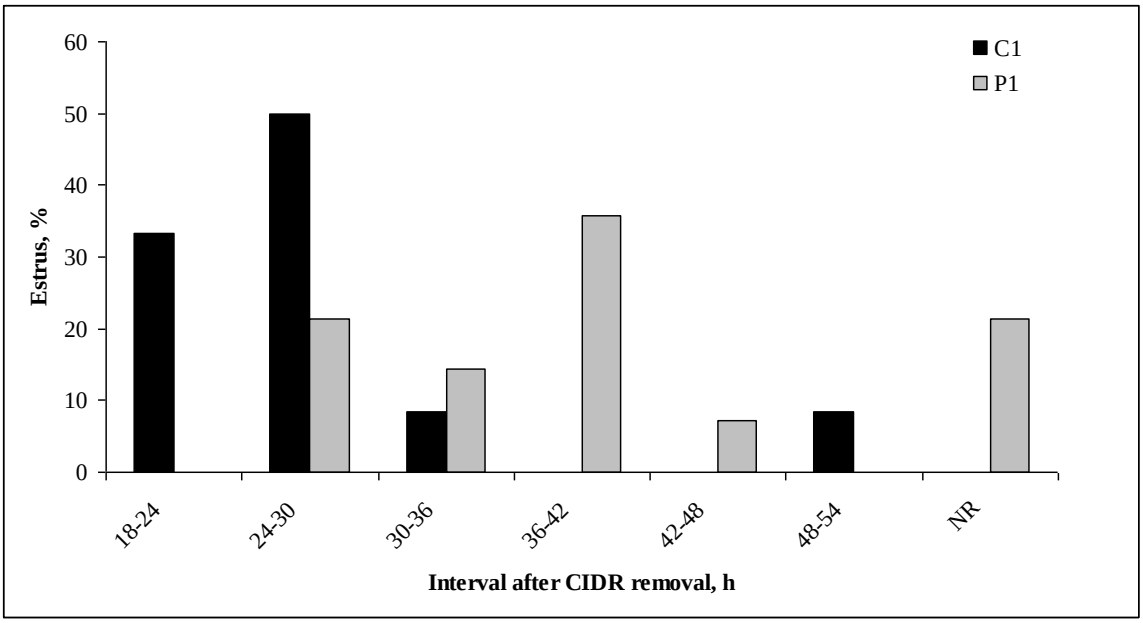


Figure 2.2. Percentage of estrous cycling (C) and prepubertal (P) heifers assigned to the CIDR Select treatment in estrus after CIDR removal; C1 (black bar) and P1 (gray bar); NR = no response (no estrous response). See Figure 2.1 for a description of treatment protocols.

Table 2.3. Response to GnRH and diameter of the dominant follicle among estrous cycling (C) and prepubertal (P) heifers assigned to the various treatments.

	Treatment[1]					
	C1	C2	C3	C4	P1	P2
Response to GnRH[2]						
Prop.	9/12[a,b]	5/12[b]	N/A	9/12[a,b]	12/14[a]	4/11[b]
%	75	42		75	86	36
Follicle diameter at GnRH injection, mm						
Mean ± SE	12.3 ± 0.57	11.0 ± 0.57	10.3 ± 0.57	11.5 ± 0.57	11.0 ± 0.52	10.7 ± 0.59
Follicle diameter at PG[3] injection, mm						
Mean ± SE	11.3 ± 0.54	10.8 ± 0.54	10.3 ± 0.54	11.2 ± 0.54	9.9 ± 0.50	9.8 ± 0.56
Follicle diameter 24 h after PG injection, mm						
Mean ± SE	12.4 ± 0.54	11.5 ± 0.54	11.4 ± 0.54	12.2 ± 0.57	10.6 ± 0.50	11.1 ± 0.56
Follicle diameter 48 h after PG injection, mm						
Mean ± SE	13.3 ± 0.55	12.1 ± 0.55	12.1 ± 0.54	12.9 ± 0.57	11.5 ± 0.50	11.1 ± 0.56
Follicle diameter 20 h after estrus onset, mm						
Mean ± SE	13.7 ± 0.50	13.2 ± 0.48	12.7 ± 0.48	13.8 ± 0.55	12.0 ± 0.48	12.9 ± 0.67

[1] See Figure 2.1 for a description of treatment protocols.
[2] GnRH = gonadotropin releasing hormone (100 μg i.m., Cystorelin; Merial, Athens, GA).
[3] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[4] N/A = not applicable; Heifers assigned to C3 did not receive GnRH.
[a,b] Means within rows with different superscripts differ ($P < 0.05$).

Table 2.4. Response to GnRH based on day of the estrous cycle for estrous cycling (C) and prepubertal (P) heifers assigned to the CIDR Select treatment.

	Treatment[1]							
	C1				P1			
		Follicle diameter	Response to GnRH[2]			Follicle diameter	Response to GnRH	
Day of cycle	No.	Mean ± SE	Prop.	%	No.	Mean ± SE	Prop.	%
7	1	12.5 ± 1.3	1/1	100	3	10.8 ± 0.75	2/3	67
8	11	12.3 ± 0.39	8/11	73	8	11.3 ± 0.46	7/8	88
Unknown[3]	0				3	10.3 ± 0.75	3/3	100

[1] See Figure 2.1 for a description of treatment protocols.
[2] GnRH = gonadotropin releasing hormone (100 µg i.m., Cystorelin; Merial, Athens, GA).
[3] Represents heifers that failed to exhibit estrus following CIDR removal.

Table 2.5. Estrous response and interval from $PGF_{2\alpha}$ to estrus and ovulation among estrous cycling (C) and prepubertal (P) heifers assigned to the various treatments.

	Treatment[1]					
	C1	C2	C3	C4	P1	P2
Estrous response after PG[2]						
Prop.	11/12	12/12	12/12	9/12	12/14	7/11
%	92	100	100	75	86	64
Interval from PG to estrus, h						
Mean ± SE	53.0 ± 3.7	49.5 ± 3.5	51.0 ± 3.5	47.7 ± 4.1	51.8 ± 3.5	42.3 ± 4.6
Variance for interval from PG to estrus	38.86^{a}	390.79^{b}	119.78^{c}	$151.24^{b,c}$	$61.17^{a,c}$	$102.18^{a,b,c}$
Interval from PG to ovulation, h						
Mean ± SE	83.1 ± 3.91	80.0 ± 3.91	81.0 ± 3.75	77.2 ± 4.32	80.2 ± 3.75	67.2 ± 4.9
Variance for interval from PG to ovulation	35.27^{a}	435.39^{b}	$165.33^{b,c}$	$178.75^{b,c}$	$79.25^{a,c}$	$99.78^{a,c}$

[1] See Figure 2.1 for a description of treatment protocols.
[2] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[a,b,c] Means within rows with different superscripts differ ($P < 0.05$).

Table 2.6. Serum steroid hormone concentrations among estrous cycling (C) and prepubertal (P) heifers assigned to the various treatments.

	Treatment[1]					
	C1	C2	C3	C4	P1	P2
Serum concentrations of progesterone at GnRH[2] injection (ng/mL)	$2.32 \pm 0.38^{a,b}$	$1.71 \pm 0.38^{a,b}$	$2.09 \pm 0.38^{a,b}$	1.47 ± 0.38^{a}	2.63 ± 0.35^{b}	0.12 ± 0.40^{c}
Serum concentrations of progesterone at PG[3] injection (ng/mL)	$3.89 \pm 0.67^{a,b,c}$	4.27 ± 0.67^{a}	$3.71 \pm 0.67^{a,b,c}$	$2.18 \pm 0.67^{b,d}$	4.86 ± 0.62^{a}	1.56 ± 0.70^{d}
Serum concentrations of estradiol-17β at PG injection (pg/mL)	$1.13 \pm 0.49^{a,b}$	$1.74 \pm 0.49^{a,b}$	$1.36 \pm 0.49^{a,b}$	2.02 ± 0.49^{a}	1.07 ± 0.46^{b}	$1.68 \pm 0.51^{a,b}$
Serum concentrations of estradiol-17β 24 h after PG injection (pg/mL)	3.78 ± 0.49	3.97 ± 0.49	4.59 ± 0.49	4.26 ± 0.49	3.43 ± 0.46	3.89 ± 0.51
Serum concentrations of estradiol-17β 48 h after PG injection (pg/mL)	5.11 ± 0.49^{a}	$3.55 \pm 0.49^{a,b}$	4.37 ± 0.49^{a}	5.07 ± 0.56^{a}	4.66 ± 0.46^{a}	2.90 ± 0.51^{b}

[1] See Figure 2.1 for a description of treatment protocols.
[2] GnRH = gonadotropin releasing hormone (100 μg i.m., Cystorelin; Merial, Athens, GA).
[3] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[a,b,c,d]Means within rows with different superscripts differ ($P < 0.05$).

Table 2.7. Response to GnRH, estrous response, interval from $PGF_{2\alpha}$ to estrus and ovulation for estrous cycling and prepubertal heifers assigned to the CIDR Select (T1) and Select Synch + CIDR (T2) treatments.

	Treatment[1]	
	T1	T2
Response to GnRH[2]		
Prop.	21/26[a]	9/23[b]
%	81	39
Estrous response after PG[3]		
Prop.	23/26	19/23
%	88	83
Interval to estrus after PG, h		
Mean ± SE	51.9 ± 2.6	46.8 ± 2.9
Variance for interval to estrus after PG	48.27[a]	285.55[b]
Interval to ovulation after PG, h		
Mean ± SE	81.6 ± 2.8	75.0 ± 3.1
Variance for interval to ovulation after PG	57.92[a]	332.29[b]

[1] See Figure 2.1 for a description of treatment protocols.
[2] GnRH = gonadotropin releasing hormone (100 µg i.m., Cystorelin; Merial, Athens, GA).
[3] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[a,b] Means within rows with different superscripts differ ($P < 0.01$).

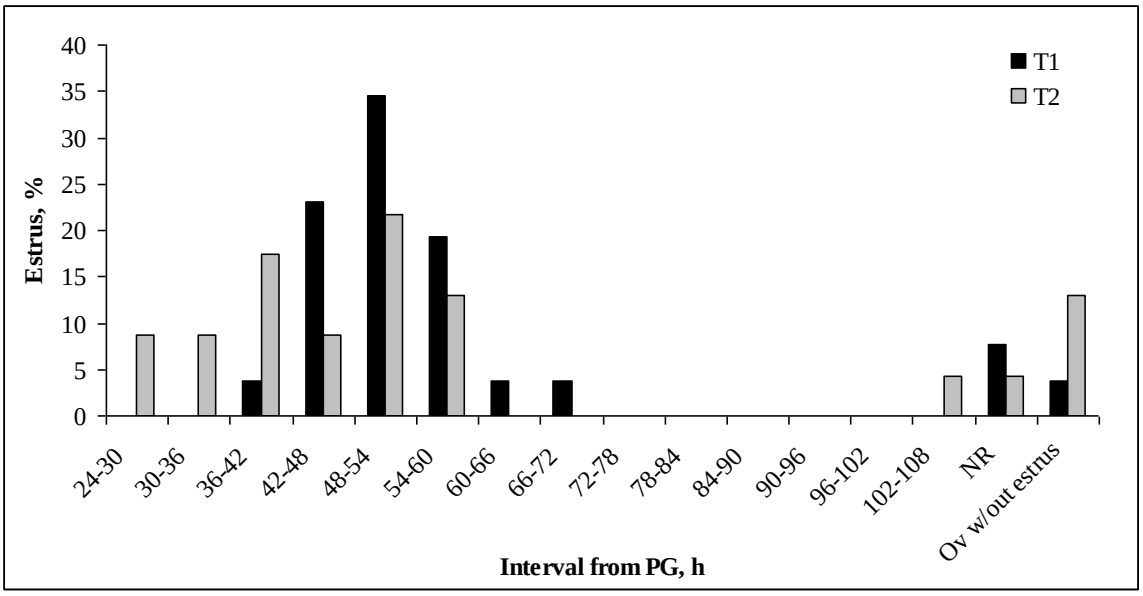


Figure 2.3. Percentage of estrous cycling and prepubertal heifers in CIDR Select (T1) and Select Synch + CIDR (T2) treatments that exhibited estrus after $PGF_{2\alpha}$ (PG); T1 (black bar) and T2 (gray bar); NR = no response (no estrous response); Ov w/out estrus = ovulated without expressing standing estrus. See Figure 2.1 for a description of treatment protocols.

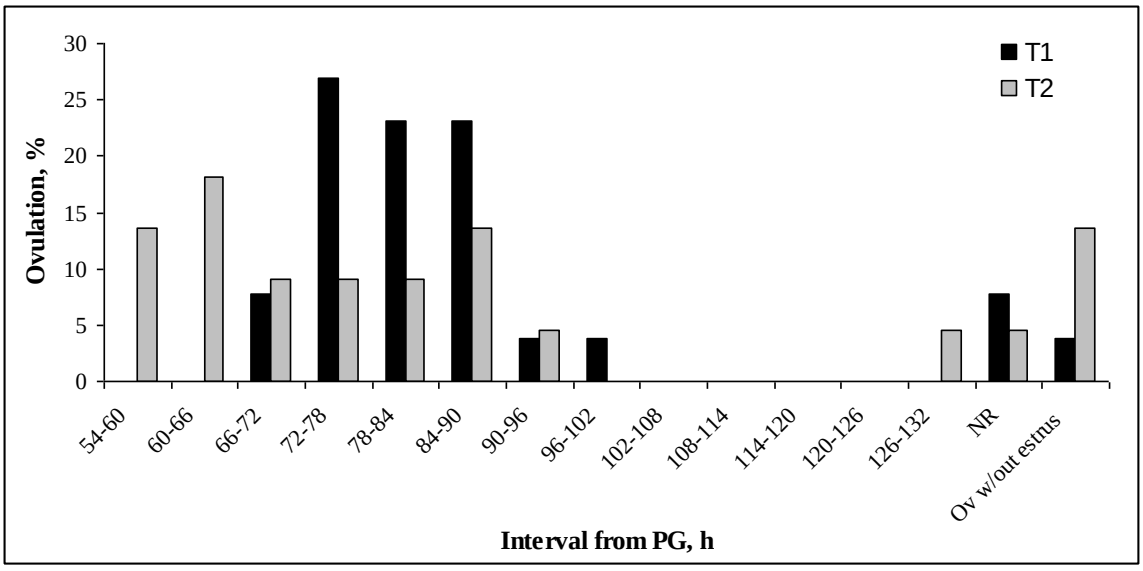


Figure 2.4. Percentage of estrous cycling and prepubertal heifers in CIDR Select (T1) and Select Synch + CIDR (T2) treatments that ovulated after $PGF_{2\alpha}$ (PG); T1 (black bar) and T2 (gray bar); NR = no response (no ovulatory response); Ov w/out estrus = ovulated without expressing standing estrus. See Figure 2.1 for a description of treatment protocols.

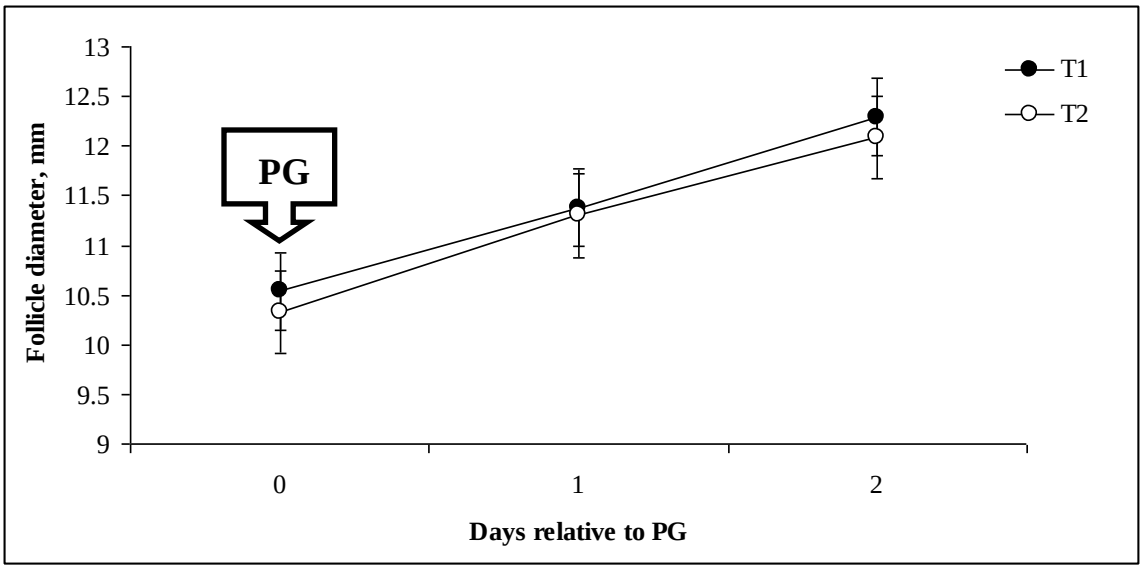


Figure 2.5. Mean dominant follicle diameter (mm) on days relative to $PGF_{2\alpha}$ (PG) for estrous cycling and prepubertal heifers assigned to the CIDR Select (T1) and Select Synch + CIDR (T2) treatments. There was no significant difference detected between treatments. See Figure 2.1 for a description of treatment protocols.

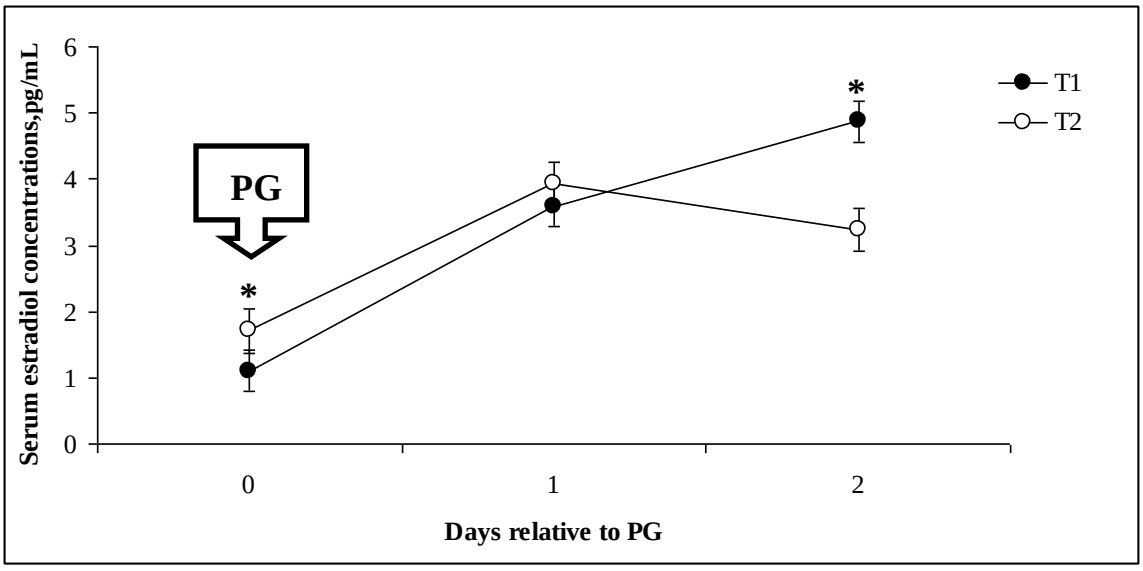


Figure 2.6. Mean serum estradiol concentrations (pg/mL) on days relative to $PGF_{2\alpha}$ (PG) for estrous cycling and prepubertal heifers assigned to the CIDR Select (T1) and Select Synch + CIDR (T2) treatments. The asterisks indicate differences between means (d 0, $P = 0.06$; d 2, $P < 0.01$). See Figure 2.1 for a description of treatment protocols.

CHAPTER 3

COMPARISON OF LONG-TERM CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS IN BEEF HEIFERS

ABSTRACT

Two experiments were conducted to evaluate modifications to a long-term progestin-based (controlled internal drug release [CIDR] insert) protocol to synchronize estrus and compare differences in their ability to facilitate fixed-time AI in beef heifers. In Exp. 1 estrous cycling Angus beef heifers (n = 85) were assigned to 1 of 2 treatments by age and BW. Blood samples were taken 15 and 5 d prior to treatment initiation to confirm estrous cyclicity status (progesterone $\geq$ 0.5 ng/mL estrous cycling). Heifers assigned to T1 (CIDR Select; n = 42) received a CIDR insert (1.38 g progesterone) from d 0 to 14 followed by GnRH (100 µg, i.m.) on d 23 and $PGF_{2\alpha}$ (PG; 25 mg, i.m.) on d 30. Heifers assigned to T2 (n = 43) received a CIDR insert from d 2 to 16 followed by GnRH on d 23 and PG on d 30. Heifers were fitted with HeatWatch transmitters at CIDR removal and at PG for estrus detection. Ovaries were scanned by ultrasonography on d 23 and 25 to determine response to GnRH. Experiment 2 was designed with a 2 x 2 x 2 factorial arrangement of treatments involving the main effects of treatment length, GnRH, and estrous cyclicity status. Angus beef heifers (n = 353) were assigned within reproductive tract scores (RTS; 1 = immature to 5 = cycling) by age and BW to 1 of 4 treatments. Evaluations of RTS were made 5 d prior to treatment initiation, and heifers

with RTS of 2 or 3 were designated as prepubertal (P) and heifers with RTS of 4 or 5 were designated as estrous cycling (C). Heifers treated with T1 (P, n = 25; C, n = 64) and T2 (P, n = 25; C, n = 64) received the same treatment protocols as described in Exp. 1. Heifers assigned to T3 (P, n = 24; C, n = 66) and T4 (P, n = 26; C, n = 63) were placed on the same schedules as heifers in T1 and T2, respectively, minus the addition of GnRH. Heifers were fitted with HeatWatch transmitters at PG for estrus detection. In Exp. 1 and 2 AI was performed approximately 12 h after onset of estrus. In Exp. 1, more ($P < 0.05$) T1 heifers displayed estrus after CIDR removal than T2 heifers (100 vs. 91%, respectively) but mean interval to estrus and variance for interval to estrus after CIDR removal did not differ ($P > 0.10$). Heifers assigned to T1 had larger dominant follicles at GnRH compared to T2 ($P < 0.01$; 10.9 vs. 9.5 mm, respectively) but response to GnRH ($P > 0.10$; 71 vs. 58%, respectively), estrous response after PG ($P > 0.90$; 88 vs. 88%, respectively), mean interval to estrus and variance for interval to estrus after PG did not differ ($P > 0.05$). Conception rate to AI ($P > 0.60$; 65%, T1; 61%, T2) and final pregnancy rate ($P > 0.70$; 93%, T1; 91%, T2) were similar. In Exp. 2 estrous response after PG did not differ ($P > 0.10$) among treatments (90, 93, 91, and 87% for T1, T2, T3, and T4, respectively). However, differences in mean interval to estrus and variance for interval to estrus ($P < 0.05$) differed on the basis of the 3-way interaction of treatment length, GnRH, and estrous cyclicity status. No difference in AI conception rates ($P > 0.60$; 61, 62, 59, and 61% for T1, T2, T3, and T4, respectively) or final pregnancy rates ($P > 0.80$; 90, 92, 91, and 83% for T1, T2, T3, and T4, respectively) were detected among the 4 treatments. In summary, the 2 d schedule modification in the long-term CIDR protocol failed to improve synchrony of estrus over the current schedule. In

addition, no differences in estrous response or synchrony of estrus were detected between T1 and T2 treated heifers in Exp. 1. The 3-way interaction involving treatment length, GnRH, and estrous cyclicity status in Exp. 2 clearly suggests that further evaluation of long-term CIDR-based protocols is required with and without the addition of GnRH to determine the efficacy of these protocols for use in facilitating fixed-time AI.

INTRODUCTION

Estrus synchronization and artificial insemination (**AI**) provide beef producers with effective management tools to maximize the reproductive potential of their cow herd by incorporating superior genetics into their operations. However, United States cow-calf producers have been reluctant to implement these technologies due to time and labor constraints. Therefore, the development of estrus synchronization protocols which limit time and labor inputs may increase the adoption of these technologies.

The CIDR Select protocol has been successfully used to facilitate fixed-time AI (**FTAI**) in beef heifers (Busch et al., 2007). Drawbacks to the CIDR Select protocol include the length of time required to implement the protocol (33 d) and the number of times heifers must be handled (5 trips through the chute). However, the CIDR Select protocol yields significantly higher FTAI pregnancy rates compared to the short-term CO-Synch + CIDR protocol (Busch et al., 2007).

Recently, Atkins et al. (2007) reported that response to GnRH in beef heifers may be influenced by the day of the estrous cycle when GnRH is administered. The highest ovulatory response to GnRH was observed when GnRH was administered on d 5 of the

estrous cycle, followed by d 15, 10, 18, and 2 (Atkins et al., 2007). When beef heifers were presynchronized utilizing a 14 d controlled internal drug release (**CIDR**) insert with GnRH administered 9 d after CIDR removal, the highest ovulatory response was observed for those heifers that were on d 5 or 6 of the estrous cycle at the time GnRH was administered (Schafer et al., 2006). However, the majority of heifers were on d 7 and 8 when GnRH was administered (Schafer et al., 2006). Leitman et al. (2007a, b) reported similar results when beef heifers were synchronized with the CIDR Select protocol, in that the majority of heifers were on d 7 and 8 of the estrous cycle when GnRH was administered.

Given these considerations, two experiments were conducted to determine whether modification of the current treatment schedule for the CIDR Select protocol would result in a more highly synchronized estrus to facilitate FTAI. The hypotheses tested were: 1) reducing the interval from CIDR removal to GnRH by 2 days (to place heifers on d 5 and 6 versus d 7 and 8 of the estrous cycle at GnRH administration) may improve synchrony of estrus following $PGF_{2\alpha}$ (**PG**; Exp. 1 and 2); and 2) the addition of GnRH after CIDR removal is required to improve synchrony of estrus following PG (Exp. 2).

MATERIALS AND METHODS

The experimental procedures were approved by the University of Missouri, Columbia Animal Care and Use Committee.

Experiment 1

Experimental Design. Estrous cycling (n = 85) Angus beef heifers were randomly assigned to 1 of 2 treatments by age and BW. Blood samples were collected 15 and 5 d prior to treatment initiation to confirm estrous cyclicity status (progesterone $\geq$ 0.5 ng/mL estrous cycling). The authors acknowledge the potential for misclassification of heifers by cyclicity determined from 2 blood samples before treatment initiation and the use of progesterone values $\geq$ 0.5 ng/mL to confirm cyclicity. However, the potential for committing a Type II error is greatly minimized if not negated in describing heifers as estrous cycling using a 0.5 ng/mL cutoff. Heifers assigned to T1 (n = 42) received an EAZI-Breed CIDR insert (1.38 g progesterone; Pfizer Animal Health, New York, NY) from d 0 to 14 followed by GnRH (100 μg, i.m.; Cystorelin, Merial, Athens, GA) on d 23 and PG (25 mg, i.m.; Lutalyse, Pfizer Animal Health) on d 30. Heifers assigned to T2 (n = 43) received a CIDR insert from d 2 to 16 followed by GnRH on d 23 and PG on d 30 (Figure 3.1).

Estrus Detection and Artificial Insemination. All heifers were fitted with a HeatWatch estrus detection transmitter (DDx Inc., Denver, CO) at the time of CIDR removal to characterize estrous response (estrus was defined as heifers receiving $\geq$ 3 mounts, each of which were $\geq$ 2 s in duration, within a period of 4 h). Transmitters were removed at the time of GnRH administration. All heifers were re-fitted with a transmitter at the time of PG administration to characterize estrous response during the synchronized period (0 to 144 h following PG) and transmitters were removed at the time of AI. Artificial insemination was performed by 1 of 2 experienced technicians approximately 12 h following the onset of standing estrus with semen from a single sire. Heifers were

exposed to fertile bulls 11 d after the end of the synchronized period for the remainder of the 70 d breeding season.

Ultrasonography and Pregnancy Diagnosis. Ovaries were scanned by transrectal ultrasonography (Aloka 500V equipped with a 7.5-MHz linear array transducer, Aloka, Wallingford, CT) on d 23 to characterize follicular dynamics. Follicles $\geq$ 5 mm and the presence of corpora lutea were recorded. On d 25 ovaries were scanned to determine response to GnRH (defined as disappearance of the dominant follicle). Conception rate to AI was determined by transrectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear array transducer) 68 d after the end of the synchronized period. Final pregnancy rates were determined by rectal palpation 119 d after the end of the breeding season.

Blood Collection and RIA. Blood samples were collected via jugular venipuncture 15 and 5 d prior to treatment initiation to confirm estrous cyclicity status. Blood samples were allowed to clot and were stored at 4ºC for 24 h. Serum was collected by centrifugation and was stored at -20ºC until hormone assays were performed. Serum concentrations of progesterone were determined by RIA using a Coat-A-Count kit (Diagnostic Products Corp., Los Angeles, CA; Kirby et al., 1997) in one assay. The intra-assay coefficient of variation was 1.67 and the assay sensitivity was 0.1 ng/mL. Heifers were considered estrous cycling if their progesterone concentrations were $\geq$ 0.5 ng/mL in one or both blood samples before treatment initiation.

Statistical Analyses. Differences in age, BW, intervals to estrus, and follicle size at GnRH between treatments were analyzed by one-way ANOVA (PROC GLM; SAS Inst. Inc. Cary, NC). Variances associated with intervals to estrus were compared by

performing a F-test (greater variance divided by the smaller variance; Snedecor and Cochran, 1989). Response to GnRH, estrous response after PG, and pregnancy rates were analyzed using a generalized linear models method (PROC GENMOD of SAS). Conception rates to AI were analyzed using a generalized linear models method (PROC GENMOD of SAS) using the model of treatment, technician, and the interaction of treatment x technician. Due to select treatments containing all positive or negative values, estrous response after CIDR removal was analyzed using a contingency χ^2 analysis (PROC FREQ of SAS).

Experiment 2

Experimental Design. Experiment 2 was designed as a 2 x 2 x 2 factorial, with the main effects of treatment length (30 or 28 d), GnRH (with or without the addition of GnRH), and estrous cyclicity status (prepubertal or estrous cycling). Angus beef heifers (n = 357) were assigned to 1 of 4 treatments within reproductive tract score (**RTS**; 1 = immature to 5 = luteal phase; Anderson et al., 1991; Rosenkrans and Hardin, 2003) by age and BW. Evaluations of RTS were made 5 d prior to treatment initiation. Heifers with RTS of 2 or 3 were designated as prepubertal (**P**) and heifers with RTS of 4 or 5 were designated as estrous cycling (**C**). The authors acknowledge the potential for misclassification of heifers by cyclicity determined from the subjective evaluation of RTS. Heifers treated with T1 (P, n = 25; C, n = 64) and T2 (P, n = 25; C, n = 64) received the same treatment protocols described in Exp. 1. Heifers assigned to T3 (P, n = 24; C, n = 66) received a CIDR insert from d 0 to 14 and PG on d 30. Heifers assigned to T4 (P, n = 26; C, n = 63) received a CIDR insert from d 2 to 16 and PG on d 30 (Figure 3.1).

Estrus Detection and Artificial Insemination. All heifers were fitted with a HeatWatch estrus detection transmitter to detect estrus (DDx Inc.) at the time of PG administration to characterize estrous response (estrus was defined as heifers receiving $\geq$ 3 mounts, each of which were $\geq$ 2 s in duration, within a period of 4 h) during the synchronized period (0 to 144 h following PG). Artificial insemination was performed by one experienced technician approximately 12 h following the onset of standing estrus. The heifers were exposed to fertile bulls 8 d after the end of the synchronized period for the remainder of the 63 d breeding season.

Pregnancy Diagnosis. Conception rate to AI and final pregnancy rates were determined by transrectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear array transducer) 30 d after the end of the breeding season.

Statistical Analyses. The experiment was analyzed as a 2 x 2 x 2 factorial, with the main effects of treatment length, GnRH, and estrous cyclicity status. Differences in age, BW, and RTS among treatments were analyzed by a one-way ANOVA (PROC GLM of SAS). Differences in interval to estrus among treatments were analyzed by ANOVA using the linear statistical model of treatment length, GnRH, estrous cyclicity status, and all 2- and 3-way interactions (PROC GLM of SAS). Variances associated with intervals to estrus were compared by performing a F-test (greater variance divided by the smaller variance; Snedecor and Cochran, 1989). Estrous response after PG, AI conception rates, and pregnancy rates were analyzed using a generalized linear models method (PROC GENMOD of SAS). The model includes the main effects of treatment length, GnRH, estrous cyclicity status, and all 2- and 3-way interactions.

RESULTS

Experiment 1

The number, age, and BW of heifers before the initiation of treatments are shown in Table 3.1. Mean age and BW did not differ ($P > 0.80$) between the two treatments.

Synchrony of estrus after CIDR removal. Figure 3.2 illustrates the distribution of estrus following CIDR removal. Although more T1 heifers displayed estrus following CIDR removal compared to T2 heifers ($P < 0.05$), the mean interval to estrus and variance for interval to estrus did not differ ($P > 0.10$; Table 3.2). During the peak response period (24 to 36 h after CIDR removal), 43 and 39% of heifers in T1 and T2, exhibited estrus, respectively.

Follicular dynamics and response to GnRH. Heifers assigned to T1 had larger ($P < 0.01$) dominant follicles than heifers assigned to T2 at the time of GnRH administration. However, the response to GnRH did not differ between the two treatment groups ($P > 0.10$; Table 3.3). Table 3.4 summarizes the response to GnRH based on the day of the estrous cycle when GnRH was administered. As was expected, the majority of T1 heifers were on d 7 and 8 of the estrous cycle at GnRH administration, and the majority of T2 heifers were on d 5 and 6.

Synchrony of estrus after PG. Figure 3.3 illustrates the distribution of estrus following PG administration. There was no difference ($P > 0.90$) in the proportion of T1 and T2 heifers displaying estrus. The mean interval to estrus ($P > 0.40$) and variance for interval to estrus ($P > 0.20$) did not differ between T1 and T2 (Table 3.3).

Conception and pregnancy rates. The AI conception rates and pregnancy rates at the end of the breeding season for T1 and T2 are shown in Table 3.5. There was no effect of treatment ($P > 0.50$) or technician ($P > 0.70$) on conception rate to AI. Final pregnancy rates at the end of the breeding season did not differ ($P > 0.70$) between T1 and T2.

Experiment 2

The number, age, BW, and RTS of heifers before the initiation of treatments are shown in Table 3.6. Mean age, BW, and RTS did not differ ($P > 0.90$) among the four treatments.

Synchrony of estrus after PG. Table 3.7 shows the proportion of heifers displaying estrus during the synchronized period. Estrous response was not affected by treatment length ($P > 0.90$), GnRH ($P > 0.70$), or estrous cyclicity status ($P > 0.70$). The distribution of estrus following PG administration is shown in Figures 3.4 and 3.5. The mean interval to estrus was affected ($P < 0.03$) by the interaction of treatment length x GnRH x estrous cyclicity status (Table 3.8). Among P heifers which received GnRH, those assigned to the 30 d protocol displayed estrus earlier than those assigned to the 28 d protocol ($P < 0.02$). Among the C heifers assigned to the 30 d protocol, those receiving GnRH displayed estrus later than those not receiving GnRH ($P < 0.01$). Among the C heifers not receiving GnRH, those assigned to the 30 d protocol displayed estrus earlier than those assigned to the 28 d protocol ($P < 0.01$; Table 3.9). For a complete comparison of differences in mean interval to estrus based on the 3-way interaction, refer to Table 3.9.

For purposes of the current experiment, variance for interval to estrus is a more descriptive parameter for use in comparing treatments, as it more accurately describes differences in synchrony of estrus. Differences in the variance for interval to estrus were also detected based on the 3-way interaction of treatment length, GnRH, and estrous cyclicity status. A complete summary of differences among treatments for variance in the interval to estrus after PG is shown in Table 3.10.

Conception and pregnancy rates. Conception rates resulting from AI and pregnancy rates at the end of the breeding season are summarized in Table 3.11. There was no affect of treatment length ($P > 0.40$), GnRH ($P > 0.80$), or estrous cyclicity status ($P > 0.60$) on conception rates to AI. Final pregnancy rates at the end of the breeding season were not affected by treatment length ($P > 0.90$), GnRH ($P > 0.10$), or estrous cyclicity status ($P > 0.40$).

DISCUSSION

We conducted two experiments to determine whether a schedule modification of the CIDR Select protocol would result in a more highly synchronized estrus to facilitate FTAI. The first modification in Exp. 1 and 2 reduced the interval from CIDR removal to GnRH administration by 2. Reducing the protocol length by 2 d would conceivably make implementation of the protocol and scheduling easier since all treatments would be administered on the same day of the week except for FTAI. Perhaps more importantly, however, this alteration should place heifers on d 5 and 6 versus d 7 and 8 of the estrous cycle at GnRH administration; a time when previous studies reported a higher ovulatory

response to GnRH (Schafer et al., 2006; Atkins et al., 2007). The potential benefit from the anticipated increase in response would be an overall improvement in synchrony of estrus following PG, and an associated improvement in pregnancy rates resulting from FTAI.

Although heifers assigned to T1 and T2 in Exp. 1 exhibited estrous response rates exceeding 90% following CIDR removal, a significant difference was detected in the proportion of heifers that exhibited estrus. For heifers assigned to T2, 9% fewer heifers displayed estrus compared to T1. These differences may be attributed to random variation in stage of the estrous cycle at treatment initiation. Perhaps more heifers assigned to T2 recently expressed estrus and ovulated prior to treatment initiation, placing them in the metestrus phase of the estrous cycle when CIDRs were inserted. This may have delayed estrous response during the period in which estrous was detected following CIDR removal. However, the mean interval to estrus and variance for interval to estrus between treatments did not differ.

At the time of GnRH administration, 81% of T1 heifers were on d 7 or 8 of the estrous cycle, which supports observations previously reported by Schafer et al. (2006) and Leitman et al. (2007a,b). Of those heifers assigned to T2 that displayed estrus following CIDR removal, 74% were on d 5 or 6 of the estrous cycle when GnRH was administered. These observations point to the importance of presynchronization with a progestin to synchronize luteal regression and follicular wave emergence.

Although a larger number of heifers assigned to T2 were on d 5 or 6 of the estrous cycle when GnRH was administered, overall response to GnRH was not improved compared to T1. Atkins et al. (2007) reported that heifers on d 5 of the estrous cycle had

the greatest response to GnRH compared to d 15, 10, 18, and 2. Additionally, Schafer et al. (2006) reported a 100% response when GnRH was administered on d 5 and 6 of the estrous cycle compared to 85 and 89% response rates on d 7 and 8, respectively. The lack of improvement in response to GnRH in the current experiment may be attributed to differences in mean follicle diameter between T1 and T2. Heifers assigned to T1 had larger dominant follicles at GnRH, which is expected since T1 heifers received GnRH 2 days later than T2 heifers. Response to GnRH among heifers assigned to T2 was perhaps limited due to smaller follicle size.

A highly synchronized estrus following treatment implementation is an important factor to consider when evaluating estrus synchronization protocols on the basis of their ability to facilitate FTAI. No difference in estrous response or synchrony of estrus following PG was detected between T1 and T2. The lack of difference is not unexpected given the similar ovulatory response to GnRH between T1 and T2. The observations from Exp. 1 suggest that T1 and T2 may both facilitate FTAI effectively in estrous cycling beef heifers.

It is important to consider that Exp. 1 evaluated T1 and T2 using only estrous cycling beef heifers. Wood-Follis et al. (2004) and Busch et al. (2007) suggested that the degree of synchrony following administration of an estrus synchronization protocol may be influenced by the pubertal status of heifers prior to treatment initiation. Therefore, efficacy of estrus synchronization protocols must be evaluated on the basis of synchronizing estrus among mixed populations of estrous cycling and prepubertal heifers.

Experiment 2 provided the opportunity to evaluate T1 and T2 among prepubertal and estrous cycling beef heifers. Additionally, Exp. 2 evaluated a second modification to

the CIDR Select protocol to determine whether GnRH administration following CIDR removal is required to maintain a highly synchronized estrus. No difference in estrous response following PG was detected among T1, T2, T3, or T4. However, differences in mean interval to estrus and variance for interval to estrus were detected for the interaction of treatment length, GnRH, and estrous cyclicity status.

It is important to consider several of the relationships related to this interaction. Among prepubertal heifers treated with the 30 d protocol, variance for interval to estrus was reduced for those heifers receiving GnRH compared to those that did not receive GnRH. Enhanced synchrony of estrus among these prepubertal heifers may be attributed to a higher proportion of heifers ovulating to GnRH and the resulting synchronized development of the subsequent emerging follicular wave. These heifers would then be expected to exhibit estrus more synchronously following PG.

Among the estrous cycling heifers treated with the 30 d protocol, variance for interval to estrus was reduced for heifers that did not receive GnRH compared to those that did. This difference may be attributed to random variation in stage of the estrous cycle at treatment initiation. Perhaps more heifers assigned to this treatment that received GnRH were in the very early luteal phase of the estrous cycle at the time of CIDR insertion, which may have delayed estrous response following CIDR removal. This may have reduced the proportion of heifers with dominant follicles capable of responding to GnRH.

The reduced variance for interval to estrus among prepubertal heifers that received GnRH that were assigned to the 30 d protocol may have resulted from enhanced synchrony of follicular waves following GnRH compared to prepubertal heifers assigned

to the 28 d protocol. Heifers assigned to the 28 d protocol received GnRH 2 days earlier than those assigned to the 30 d protocol. As we learned in Exp. 1, this difference resulted in smaller dominant follicles that were perhaps physiologically less mature, which may have reduced their likelihood of being capable of responding to GnRH.

It is important to note that the variance for interval to estrus differed between the prepubertal and estrous cycling heifers assigned to T1, but variances for interval to estrus did not differ between prepubertal and estrous cycling heifers assigned to T2, T3, or T4. The reduced variance for interval to estrus among the prepubertal heifers assigned to T1 compared to the estrous cycling heifers conflicts with previous findings from our laboratory. Busch et al. (2007) and Leitman et al. (2007a, b) reported no difference in the variance for interval to estrus among prepubertal and estrous cycling heifers treated with the CIDR Select protocol. Several factors may account for this discrepancy. In Exp. 2, designation of estrous cyclicity status was based on RTS, whereas Busch et al. (2007) and Leitman et al. (2007a, b) used serum progesterone concentrations to determine estrous cyclicity status.

The RTS system is a subjective estimate of sexual maturity, based on ovarian follicular development and palpable size of the uterus. Heifers assigned a RTS of 1 or 2 are likely the furthest from cycling, whereas those assigned a RTS of 3 are thought to be on the verge of cycling. Heifers assigned a RTS of 4 or 5 are presumed cycling (Anderson et al., 1991; Patterson et al., 1999). However, Rosenkrans and Hardin (2003) reported that the RTS system provides repeatable and accurate evaluation of pubertal status in heifers, and furthermore, is comparable to determining pubertal status based on serum progesterone levels. A second potential explanation for the discrepancy is that

Busch et al. (2007) performed FTAI with a concurrent injection of GnRH 72 h after PG, which in all likelihood influenced the distribution of estrus during the synchronized period. A possible explanation for the differences in results reported by Leitman et al. (2007a, b) and Exp. 2 may be the number of heifers used in each experiment. Leitman et al. (2007a, b) used only 14 prepubertal and 12 estrous cycling beef heifers, whereas 25 prepubertal and 64 estrous cycling beef heifers were used in Exp. 2.

In summary, the high estrous response following PG and resulting AI conception and final pregnancy rates reported for heifers assigned to the two treatments in Exp. 1 and among the four treatments in Exp. 2 suggest that each of these long-term CIDR-based protocols were highly effective in synchronizing estrus in prepubertal and estrous cycling beef heifers. Modification of the CIDR Select protocol to reduce the interval from CIDR removal to GnRH by 2 d failed to improve synchrony of estrus among estrous cycling heifers in Exp. 1. However, the 3-way interaction involving treatment length, GnRH, and estrous cyclicity status in Exp. 2 clearly suggests that further evaluation of long-term CIDR-based protocols is required with and without the addition of GnRH and on the basis of estrous cyclicity status to determine the efficacy of these protocols for use in facilitating FTAI.

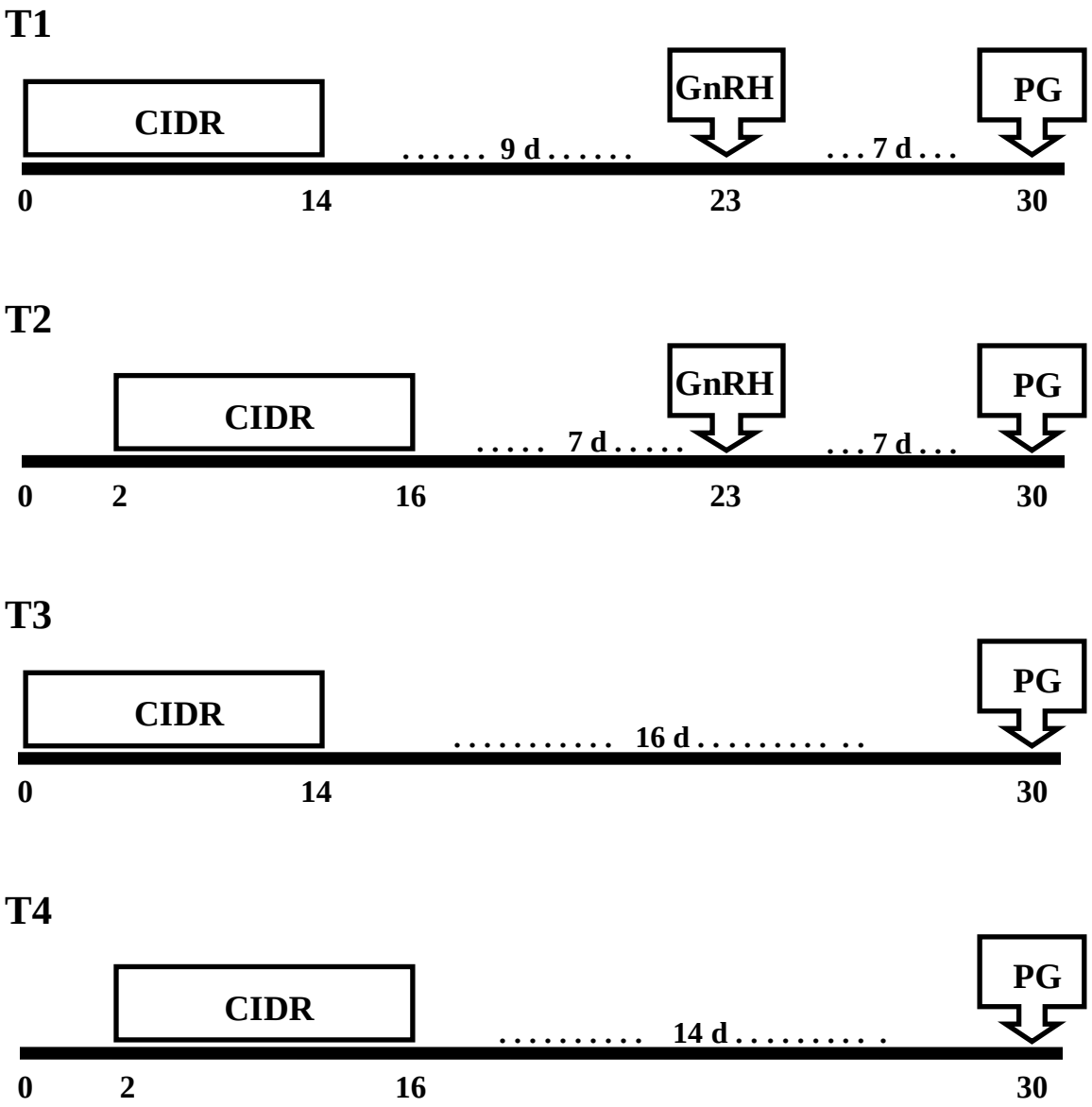


Figure 3.1. Treatment schedule for heifers assigned to T1 and T2 (Exp. 1) and T1, T2, T3, and T4 (Exp. 2). Heifers assigned to T1 were equipped with an EAZI-Breed controlled internal drug release (CIDR; 1.38 g of progesterone) insert from d 0 to 14, GnRH (Cystorelin; 100 µg, i.m.) on d 23, and $PGF_{2\alpha}$ (PG; 25 mg, i.m.; Lutalyse) on d 30. Heifers assigned to T2 received a CIDR insert from d 2 to 16, GnRH on d 23, and PG on d 30. Heifers assigned to T3 received a CIDR insert from d 0 to 14, GnRH on d 23, and PG on d 30. Heifers assigned to T4 received a CIDR insert from d 2 to 14, GnRH on d 23, and PG on d 30.

Table 3.1. Exp.1. Number, age, and BW of heifers prior to treatment initiation (mean ± SE).

	Treatment[1]	
	T1	T2
No. of heifers	42	43
Age,[2] d	396 ± 1.9	395 ± 1.8
BW,[3] kg	363 ± 4.4	364 ± 4.3

[1] See Figure 3.1 for a description of treatment protocols.
[2] Age (d) of heifers at the initiation of treatments.
[3] Body weight (kg) of heifers at the initiation of treatments.

Table 3.2. Exp.1. Estrous response after CIDR[2] removal.

	Treatment[1]	
	T1	T2
Estrous response		
Prop.	42/42[a]	39/43[b]
%	100	90.7
Interval to estrus, h		
Mean ± SE	50.1 ± 4.7	51.8 ± 4.9
Variance for interval to estrus	1128.96	710.34

[1] See Figure 3.1 for a description of each treatment protocol.
[2] CIDR = EAZI-Breed CIDR insert (1.38 g progesterone; Pfizer Animal Health, New York, NY).
[a,b] Means within rows with different superscripts differ ($P < 0.05$).

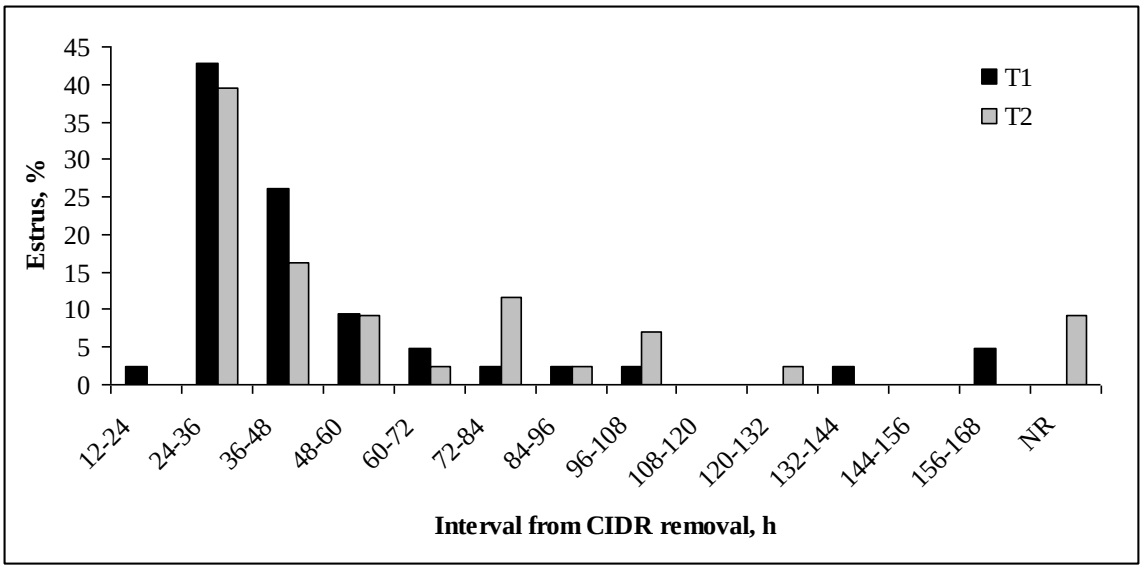


Figure 3.2. Exp. 1. Percentage of heifers in T1 and T2 in estrus after CIDR removal; T1 (black bar) and T2 (gray bar); NR = no response (no estrous response). See Figure 3.1 for a description of the treatment protocols.

Table 3.3. Exp.1. Response to GnRH, mean follicle diameter at GnRH, and estrous response after $PGF_{2\alpha}$.

	Treatment[1]	
	T1	T2
Response to GnRH[2]		
Prop.	30/42	25/43
%	71.4	58.1
Follicle size at GnRH, mm		
Mean ± SE	10.94 ± 0.35[a]	9.48 ± 0.34[b]
Estrous response after PG[3]		
Prop.	37/42	38/43
%	88.1	88.4
Interval to estrus after PG, h		
Mean ± SE	67.78 ± 3.3	71.63 ± 3.3
Variance for interval to estrus after PG	320.26	481.00

[1] See Figure 3.1 for a description of treatment protocols.
[2] GnRH = gonadotropin releasing hormone (100 µg i.m., Cystorelin; Merial, Athens, GA).
[3] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[a,b] Means within rows with different superscripts differ ($P < 0.01$).

Table 3.4. Exp.1. Response to GnRH based on day of the estrous cycle.

	Treatment[1]							
	T1				T2			
		Follicle diameter	Response to GnRH[2]			Follicle diameter	Response to GnRH	
Day of cycle	No.	Mean ± SE	Prop.	%	No.	Mean ± SE	Prop.	%
2	1	5.5 ± 2.2	0/1	0	1	8.4 ± 2.2	1/1	100
3	2	10.6 ± 1.6	0/2	0	2	12.0 ± 1.6	2/2	100
4	0				7	9.3 ± 0.83	1/7	14
5	2	10.4 ± 1.6	2/5	40	11	9.4 ± 0.67	9/11	82
6	3	9.0 ± 1.3	1/3	33	18	9.6 ± 0.52	9/18	50
7	9	11.1 ± 0.74	7/9	78	0			
8	25	11.4 ± 0.44	20/25	80	0			
Unknown[3]	0				4	8.7 ± 1.1	3/4	75

[1] See Figure 2.1 for a description of treatment protocols.
[2] GnRH = gonadotropin releasing hormone (100 µg i.m., Cystorelin; Merial, Athens, GA).
[3] Represents heifers that failed to exhibit estrus after CIDR removal.

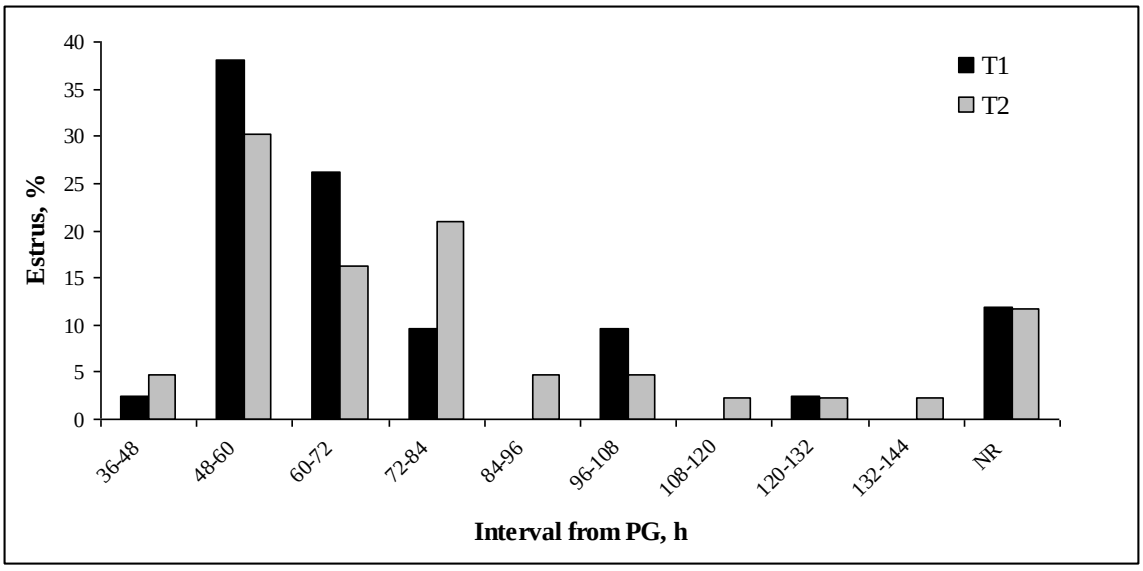


Figure 3.3. Exp.1. Percentage of heifers in T1 and T2 in estrus after $PGF_{2\alpha}$ (PG); T1 (black bar) and T2 (gray bar); NR = no response (no estrous response). See Figure 3.1 for a description of the treatment protocols.

Table 3.5. Exp.1. Conception rate to AI and pregnancy rate at the end of the breeding season.

	Treatment[1]	
	T1	T2
AI conception rate[2]		
Prop.	24/37	23/38
%	64.9	60.5
Pregnancy rate at the end of the breeding season[3]		
Prop.	39/42	39/43
%	92.9	90.7

[1] See Figure 3.1 for a description of treatment protocols.
[2] Conception rate to AI determined by ultrasound 68 d after the end of the synchronized period.
[3] Pregnancy rate determined by rectal palpation 119 d after the end of the 70 d breeding season.

Table 3.6. Exp.2. Number, age, BW, and reproductive tract score (RTS) of heifers prior to treatment initiation (LS mean ± SE).

	Treatment[1]			
	T1	T2	T3	T4
No. of heifers[2]	88	89	87	89
Prepubertal[3]	25	25	23	26
Estrous cycling[4]	63	64	64	63
Age,[5] d	446 ± 2.2	446 ± 2.2	446 ± 2.2	445 ± 2.2
BW,[6] kg	345 ± 3.5	345 ± 3.5	346 ± 3.5	345 ± 3.5
RTS[7]	4 ± 0.10	4 ± 0.10	4 ± 0.10	4 ± 0.10

[1] See Figure 3.1 for a description of treatment protocols.
[2] Total number of heifers per treatment.
[3] Number of prepubertal heifers (RTS 2 and 3 combined) per treatment.
[4] Number of estrous cycling heifers (RTS 4 and 5 combined) per treatment.
[5] Age (d) of heifers at the initiation of treatments.
[6] Body weight (kg) of heifers at the initiation of treatments.
[7] Reproductive tract scores of heifers 5 d prior to treatment initiation (1 to 5 scale, where 1 = immature and 5 = luteal phase).

Table 3.7. Exp.2. Estrous response after $PGF_{2\alpha}$.

	Treatment[1]			
	T1	T2	T3	T4
Estrous response after PG[2] (Prop., %)[3]	79/88 (90)	83/89 (93)	79/87 (91)	77/89 (87)
Prepubertal[4]	20/25 (80)	24/25 (96)	22/23 (96)	23/26 (88)
Estrous cycling[5]	59/63 (94)	59/64 (92)	57/64 (89)	54/63 (86)

[1] See Figure 3.1 for a description of treatment protocols.
[2] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[3] Total response per treatment.
[4] Prepubertal = RTS 2 and 3.
[5] Estrous cycling = RTS 4 and 5.

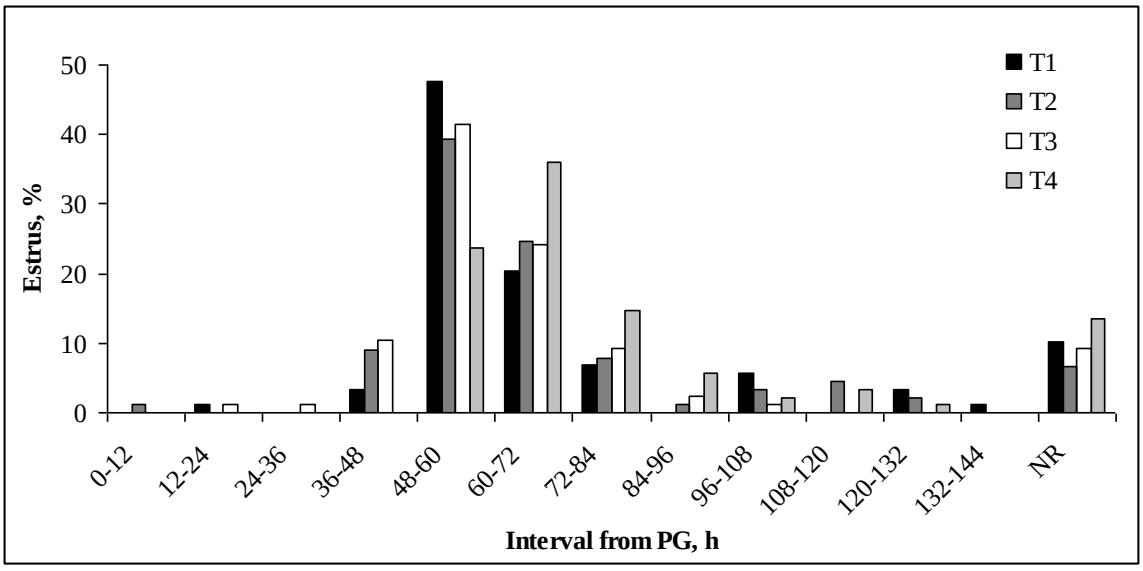


Figure 3.4. Exp.2. Percentage of heifers in T1, T2, T3, and T4 in estrus after $PGF_{2\alpha}$ (PG); T1 (black bar), T2 (gray bar), T3 (black diagonal bar), T4 (gray checkered bar); NR = no response (no estrous response). See Figure 3.1 for a description of treatment protocols.

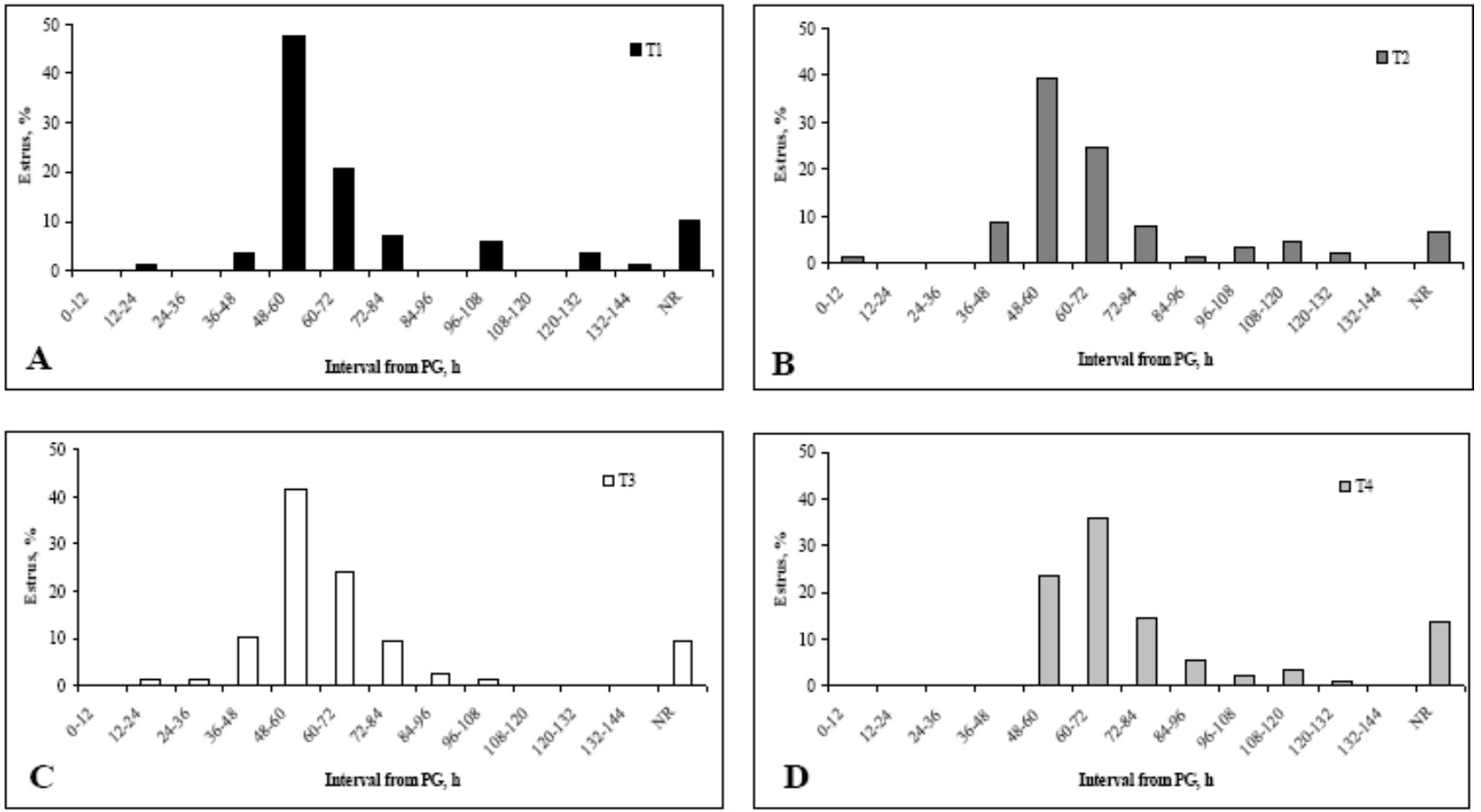


Figure 3.5. Exp. 2. Individual graphs illustrating the percentage of heifers in T1 (A), T2 (B), T3 (C), and T4 (D) in estrus after $PGF_{2\alpha}$ (PG); NR = no response (no estrous response). See Figure 3.1 for a description of treatment protocols.

Table 3.8. Exp.2. Analysis of variance for mean interval to estrus after $PGF_{2\alpha}$[1].

Effect	*P*-value
Treatment length	0.0032
GnRH	0.7925
Estrous cyclicity status	0.7704
Treatment length x GnRH	0.2244
Treatment length x estrous cyclicity status	0.0399
GnRH x estrous cyclicity status	0.3160
Treatment length x GnRH x estrous cyclicity status	0.0232

[1] $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).

Table 3.9. Exp.2. Interval to estrus after $PGF_{2\alpha}$[1] for the interaction of treatment length x GnRH x estrous cyclicity status[2] (LS mean ± SE).

	Prepubertal[3]		Estrous cycling[4]	
	GnRH[5]	No GnRH	GnRH	No GnRH
30 d	$56.6 \pm 4.0^{x,a}$	$61.8 \pm 3.8^{a,b}$	67.9 ± 2.3^{b}	$58.5 \pm 2.4^{x,a}$
28 d	$70.2 \pm 3.6^{y,a,b}$	70.7 ± 3.7^{a}	62.1 ± 2.3^{b}	$68.3 \pm 2.4^{y,a,b}$

[1] $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[2] Treatments based on the interaction: T1 = 30 d, GnRH; T2 = 28 d, GnRH; T3 = 30 d, no GnRH; T4 = 28 d, no GnRH. See Figure 3.1 for a description of treatment protocols.
[3] Prepubertal = RTS 2 and 3.
[4] Estrous cycling = RTS 4 and 5.
[5] GnRH = gonadotropin releasing hormone (100 µg i.m., Cystorelin; Merial, Athens, GA).
[a,b] Means within rows with different superscripts differ ($P < 0.05$).
[x,y] Means within columns with different superscripts differ ($P < 0.02$).

Table 3.10. Exp.2. Variance for interval to estrus after $PGF_{2\alpha}$[1] for the interaction of treatment length x GnRH x estrous cyclicity status[2].

	Prepubertal[3]		Estrous cycling[4]	
	GnRH[5]	No GnRH	GnRH	No GnRH
30 d	$59.94^{x,a}$	229.82^{b}	555.72^{c}	$144.19^{x,b}$
28 d	$556.11^{y,a}$	209.74^{b}	$360.09^{a,b}$	$255.20^{y,b}$

[1] $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).
[2] Treatments based on the interaction: T1 = 30 d, GnRH; T2 = 28 d, GnRH; T3 = 30 d, no GnRH; T4 = 28 d, no GnRH. See Figure 3.1 for a description of treatment protocols.
[3] Prepubertal = RTS 2 and 3.
[4] Estrous cycling = RTS 4 and 5.
[5] GnRH = gonadotropin releasing hormone (100 µg i.m., Cystorelin; Merial, Athens, GA).
[a,b] Means within rows with different superscripts differ ($P < 0.05$).
[x,y] Means within columns with different superscripts differ ($P < 0.05$).

Table 3.11. Exp.2. Conception rate to AI and pregnancy rate at the end of the breeding season (Prop., %).

	Treatment[1]			
	T1	T2	T3	T4
AI Conception rate[2,3]	48/79 (61)	51/82 (62)	47/79 (59)	47/77 (61)
Prepubertal[4]	12/20 (60)	16/24 (67)	12/22 (55)	16/23 (70)
Estrous cycling[5]	36/59 (61)	35/58 (60)	35/57 (61)	31/54 (57)
Pregnancy rate at the end of the breeding season[6,7]	79/88 (90)	81/88 (92)	79/87 (91)	74/89 (83)
Prepubertal	22/25 (88)	24/25 (96)	18/23 (78)	21/26 (81)
Estrous cycling	57/63 (90)	57/63 (90)	61/64 (95)	53/63 (84)

[1] See Figure 3.1 for a description of treatment protocols.
[2] Conception rate to AI determined by ultrasound 30 d after the end of the 63 d breeding season.
[3] Total AI conception rate per treatment.
[4] Prepubertal = RTS 2 and 3.
[5] Estrous cycling = RTS 4 and 5.
[6] Pregnancy rate determined 30 d after the end of the 63 d breeding season.
[7] Total final pregnancy rate per treatment.

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VITA

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