

EVALUATING PROGESTIN-BASED PROTOCOLS TO CONTROL
ESTROUS CYCLES OF BEEF HEIFERS PRIOR TO TIMED
ARTIFICIAL INSEMINATION

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By

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DEDICATION

This thesis is dedicated to my family, who were always sending their love, support, and prayers. Thank you for encouraging me to take opportunities, pursue passions, and enjoy the ride.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
CAP	Chlormadinone acetate
CIDR	Controlled internal drug release
CL	Corpus luteum/corpora lutea
d	Day(s)
DHPA	Dihydroxyprogesterone acetophenide
E ₂	Estradiol-17 β
E	Estrous
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
FTAI	Fixed-time artificial insemination
g	Gram(s)
g	G-force
GnRH	Gonadotropin-releasing hormone
h	Hour(s)
hd	Head
im	Intramuscular
kg	Kilogram(s)
LH	Luteinizing hormone
MAP	Medroxyprogesterone acetate
mg	Milligram(s)

MGA	Melengestrol acetate
mL	Milliliter(s)
mo	Month(s)
ng	Nanogram(s)
NE	Non-estrous
P ₄	Progesterone
PG	Prostaglandin F _{2α}
pg	Picogram(s)
PRID	Progesterone-releasing intravaginal device
RIA	Radioimmunoassay
RTS	Reproductive tract score
SAS	Statistical Analysis System
SE	Standard error
SMB	Syncro-Mate-B
STAI	Split-time artificial insemination
TAI	Timed artificial insemination
US	United States
wk	Week(s)
yr	Year(s)
μg	Microgram(s)

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ABSTRACT

The recent development of split-time artificial insemination as a breeding strategy improved reproductive outcomes following treatment with the 14d CIDR[®]-PG protocol; however, STAI has not been evaluated with other protocols designed to synchronize estrus in beef heifers. Two experiments (Chapter 2) were designed to evaluate estrous response and pregnancy rates resulting from fixed-time (FTAI) or split-time (STAI) artificial insemination among beef heifers. Experiment 1 (Chapter 2) evaluated FTAI and STAI following administration of the melengestrol acetate (MGA[®]) prostaglandin F_{2α} protocol. Experiment 2 (Chapter 2) evaluated FTAI and STAI following administration of the 7d CO-Synch + controlled internal drug release (CIDR[®]) estrus synchronization protocol. Heifers (n = 524) in Experiment 1 (Chapter 2) were managed in 10 pens and assigned within pen to balanced treatments based on weight and reproductive tract score (RTS; Scale 1-5). Heifers were fed MGA[®] (0.5 mg·animal⁻¹·d⁻¹) in a 1.8 kg grain carrier for 14 d. Prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) was administered 19 d

after MGA[®] withdrawal. Estrus detection aids (Estroject[®]) were applied at PG. Estrous status was recorded at 72 h after PG for heifers assigned to FTAI, and 72 and 96 h after PG for heifers assigned to STAI. Estrus was defined as removal of $\geq 50\%$ of the coating from the Estroject[®] patch. Heifers assigned to FTAI were inseminated 72 h after PG and gonadotropin-releasing hormone (GnRH; 100 μg im gonadorelin acetate) was administered at AI. Heifers in the STAI treatment that exhibited estrus by 72 h after PG were inseminated at 72 h; however, AI was postponed until 96 h for heifers that were non-estrous at 72 h. Only heifers that failed to exhibit estrus by 96 h received GnRH at AI. Estrous response 72 h after PG did not differ between treatments; however, total estrous response was increased ($P < 0.001$) among heifers assigned to STAI (88%, STAI; 72%, FTAI). Pregnancy rates resulting from AI were greater ($P < 0.04$) for heifers assigned to STAI compared to FTAI (55% vs 46%, respectively), and were enhanced ($P < 0.05$) among heifers that exhibited estrus. Heifers ($n=456$) in Experiment 2 (Chapter 2) were managed at one location in three pens and assigned within pen to one of two balanced treatments based on weight and reproductive tract score (RTS; Scale 1-5). All heifers were subject to the 7d CO-Synch + controlled internal drug release (CIDR[®]) estrus synchronization protocol and managed in two synchronization groups. Gonadotropin-releasing hormone (100 μg im gonadorelin acetate) was administered and an EAZI-Breed CIDR[®] [1.38g progesterone (P_4); Zoetis, Madison, NJ] inserted in heifers at the start of the protocol. The CIDR[®] inserts were removed after 7 d. Prostaglandin $F_{2\alpha}$ (PG; 250 μg im cloprostenol sodium) was administered and estrus detection aids (Estroject[®]) were applied concurrent with CIDR[®] removal. Estrous status was recorded at 54 h after PG for heifers assigned to FTAI, and 54 and 78 h after PG for heifers assigned

to STAI. Estrous was defined as removal of $\geq 50\%$ of the grey coating from the Estroject[®] patch. Heifers assigned to FTAI were inseminated 54 h after PG and GnRH (100 μg im gonadorelin acetate) was administered at AI. Heifers in the STAI treatment were inseminated when considered estrous by 54 h after PG; however, AI was postponed until 78 h for heifers that were non-estrous at 54 h. Only heifers that failed to exhibit estrus by 78 h received GnRH at AI. Estrous response 54 h after PG did not differ between treatments; however, total estrous response was increased ($P < 0.001$) among heifers assigned to STAI (74%, STAI; 47%, FTAI). Pregnancy rates resulting from AI were not different for heifers assigned to STAI compared to FTAI (48% and 46%, respectively; $P=0.6$), and were enhanced ($P < 0.05$) among heifers in the STAI treatment that exhibited estrus. In summary of Experiment 1 (Chapter 2), STAI has potential to improve estrous response and pregnancy rate to AI following the MGA[®]-PG protocol. When compared to FTAI in Experiment 2 (Chapter 2), STAI enhanced estrous response following the 7d CO-Synch + CIDR[®], however, this did not result in an increase in pregnancy rate to AI.

The 14d CIDR[®]-PG protocol is one of the more widely used protocols designed to synchronize estrus in beef heifers; however, the degree of flexibility in length of progestin treatment as it relates to reproductive outcomes has not been evaluated. A third experiment (Chapter 3) was therefore designed to characterize these differences. Heifers were assigned to either an 18d (Day 0 to 18) or 14d (Day 4 to Day 18) CIDR[®] treatment (1.38 g progesterone controlled internal drug release insert; Zoetis, Madison, NJ), with prostaglandin F_{2 α} (PG; 250 μg im cloprostenol sodium) administered to heifers in both treatments 16 days after CIDR[®] removal (Day 34). Heifers ($n = 193$) at location one were

evaluated over three consecutive years, and heifers at location two (n=649) were managed as two groups during the same year. Heifers at each location were assigned to treatment based on reproductive tract score (RTS; Scale 1-5) and weight. Heifers that were assigned a RTS of one were not retained for the trial (n=6). At location one, radiotelemetric pressure-sensitive devices (Heatwatch[®]) were applied to characterize timing of estrus onset following PG administration. In addition, estrus detection aids (Estrotect[®]) were applied at PG to heifers at both locations. Estrous status was recorded at 66 and 90 h after PG. Estrous was defined as removal of $\geq 50\%$ of the grey coating from the Estrotect[®] patch, and split-time artificial insemination (STAI) was performed at either 66 or 90 h after PG based on timing of estrus onset (Heatwatch[®]; location one) or activation of the Estrotect[®] patch. Only heifers that were non-estrous at 90 h received GnRH (100 μ g im gonadorelin acetate), which was administered at the time AI was performed. At location one, blood samples were collected from all heifers in both treatments at PG and AI, and transrectal ovarian ultrasound was performed to detail ovarian structures on a subset of heifers (n=73) at both time points. Estrous response 66 h after PG did not differ between treatments (P = 0.3; 60%, 14d CIDR[®]-PG; 60%, 18d CIDR[®]-PG), nor were any differences observed during the 24 h delay period from 66 to 90 h after PG (P=0.4; 60%, 14d CIDR[®]-PG; 60%, 18d CIDR[®]-PG). Pregnancy rate to STAI did not differ between treatments (P = 0.3; 52%, 14d CIDR[®]-PG; 50%, 18d CIDR[®]-PG), or at the end of the 60 d breeding season (P=0.2; 86%, 14d CIDR[®]-PG; 82%, 18d CIDR[®]-PG). There were no differences between treatments in mean dominant follicle diameters at PG (P=0.6; 10.9 \pm 0.4mm, 14d CIDR[®]-PG; 11.0 \pm 0.4mm, 18d CIDR[®]-PG) or at STAI (P=0.3; 12.6 \pm 0.4mm, 14d CIDR[®]-PG; 13.2 \pm 0.4mm, 18d CIDR[®]-PG),

nor were any differences observed between treatments in concentrations of E₂ at PG (P=0.8; 1.1±0.19 pg/ml, 14d CIDR[®]-PG; 1.1±0.19 pg/ml, 18d CIDR[®]-PG) or STAI (P=0.6; 3.8±0.19 pg/ml, 14d CIDR[®]-PG; 3.6±0.19 pg/ml, 18d CIDR[®]-PG). Results presented in Chapter 3 suggest that similarities in response observed among heifers based on length of CIDR[®] treatment provide flexibility in scheduling without compromising reproductive outcomes.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Managing beef herds on a reproductive basis has profound impacts on efficiency, production, and profitability; however, the majority of annual losses to beef herds in the United States are the result of reproductive failure (USDA-APHIS, 2008). Estrus synchronization and fixed-time artificial insemination represent two of the more significant ways in which to manage herds reproductively. These can increase the proportion of females that exhibit estrus, the number of AI pregnancies, and the proportion of females that conceive early in a breeding season. Early conception increases the proportion of females that calve early in the calving season, and managing heifers to conceive early in the first breeding season results in greater lifetime production (Lesmeister et al., 1972; Cushman et al., 2013).

Heifers are the future of the cow herd and are, therefore, an indicator of an operation's management and production goals. Ensuring reproductive success of replacement heifers is imperative to a successful breeding program. Estrus synchronization systems provide ways in which to increase the proportion of heifers that attain puberty early and hence conceive earlier during the breeding season. Furthermore, implementing a focused breeding program affords producers multiple opportunities to make selection and culling decisions. Success of the program, however, is still limited by

the effectiveness of the estrus synchronization protocol and the type of artificial insemination system that is used.

Research continues to elucidate improved methods of estrus synchronization and artificial insemination. The outcome of these systems is dependent upon a number of factors including development status, stage of the estrous cycle, and stage of production. A system that maximizes estrous response and pregnancy rates regardless of individual factors provides the greatest overall return.

Recently, a new approach to timed artificial insemination was developed and referred to as split-time AI (STAI), which works on the concept that females that express estrus achieve greater breeding success (Perry et al., 2005; Thomas et al., 2014a; Richardson et al., 2016). Using a STAI approach, non-estrous females (as assigned at AI) are provided an additional opportunity to express estrus and insemination is delayed 20-24h after the predetermined fixed time. In the case of the 14d CIDR[®]-PG estrus synchronization protocol used with beef heifers, STAI significantly improved pregnancy rates to AI compared to FTAI (Thomas et al., 2014b; Bishop et al., 2016). Split-time AI demonstrates potential to be used as an alternative approach to AI and conventional heat-detection systems.

The literature review provided in this chapter focuses on factors related to puberty onset in beef heifers, the estrous cycle in cattle, development of estrus synchronization protocols for beef cattle, and split-time artificial insemination. In support of this thesis, particular emphasis will be placed on reproductive management of beef heifers.

PUBERTY IN HEIFERS

Puberty is a complex developmental process where animals reach sexual maturity and are able to successfully reproduce. In the heifer, this process results from a changing communication in the reproductive endocrine (hypothalamic-pituitary-ovarian) axis and is highly dependent upon the secretion of GnRH at the frequency and amount needed to stimulate the anterior pituitary gland to secrete gonadotropins (McLeod et al., 1984; Schillo et al., 1992). Gonadotropins (LH and FSH) are then responsible for the secondary sexual characteristics and behaviors associated with maturity, in addition to gametogenesis and steroidogenesis (Fortune and Hansel, 1985; Fortune and Quirk, 1988). The definition of puberty in females varies but includes one or several of the following components: time of first estrus, time of first ovulation, or time when the individual can successfully support pregnancy (Moran et al., 1989; Senger, 2005). Age at puberty often refers to the first ovulatory estrus in a heifer (Moran et al., 1989). However, the neuroendocrine and hormonal systems that drive this first estrus are already fully functional by the time of estrus (MacKinnon et al., 1978; McLeod et al., 1984).

Establishing mature, cyclic patterns of gonadotropin release occurs gradually leading up to puberty (Gonzalez-Padilla et al., 1975a). During maturation of the hypothalamus, GnRH release becomes sensitive to stimulation by the neuronal protein, kisspeptin (Han et al., 2005; Mondal et al., 2015). Prior to the onset of puberty, the hypothalamus releases low-frequency pulses of GnRH, which increase with stimulation by kisspeptin as a heifer approaches puberty (Han et al., 2005). During the prepubertal period, LH release from the anterior pituitary is sensitive to negative feedback from E₂

(Schillo et al., 1982; Day et al., 1984). As a heifer approaches the onset of puberty, LH pulsatility increases despite no noticeable decline in E_2 ; this is explained by a decrease in the sensitivity to negative feedback from E_2 (Figure 1.1; Gonzalez-Padilla et al., 1975a; Schillo et al., 1982; Day et al., 1987). This relationship was originally described in rats as the “gonadostat” theory and results from a decline in the receptors for E_2 at the level of the hypothalamus and pituitary gland (Ramirez and McCann, 1963; Day et al., 1984; Day et al., 1987). Increased production of LH then causes an increase in production of E_2 by follicles, resulting in increased growth and maturation of the uterus (Day et al., 1987).

Progesterone is required to induce the pattern of LH release characteristic of pubertal heifers (Gonzalez-Padilla et al., 1975a), and was shown to originate from luteal tissue prior to puberty onset (Berardinelli et al., 1979). When comparing P_4 concentrations in samples collected following estrus in heifers bred on the pubertal estrus compared to a subsequent estrus, concentrations of P_4 were significantly higher at the pubertal estrus, however the first ovulation was usually followed by the formation of a CL with a shortened life span (Byerley et al., 1987a). Furthermore, pregnancy rates were decreased for heifers bred on a pubertal estrus compared to a second or third estrus (Byerley et al., 1987b; Roberts et al., 2018). Lower pregnancy rates in the study by Byerley et al. (1987b) may have resulted from the occurrence of non-pubertal estrus: an anovulatory first estrus preceding the first ovulatory (pubertal) estrus (Rutter and Randel, 1986). For these reasons, breeding heifers of mixed or unknown status regarding estrous cyclicity requires special consideration of a heifer’s pubertal status and potential fertility differences in fertility that may result.

THE BOVINE ESTROUS CYCLE

Introduction

After heifers attain puberty, a regular pattern of reproductive cyclicality begins. During this time, females experience repeated hormonal and physiological changes, collectively referred to as estrous cycles (Figure 1.2). Cattle are considered to be polyestrous in which case females may experience estrous cycles that occur uniformly throughout the year. Polyestrous females have multiple opportunities to conceive throughout the year, during any season. Average length of the estrous cycle is 21 d but can range from 17-24 d (Hammond, 1927; Wishart, 1972). The estrous cycle can be broken down into phases based on presence of ovarian structures and the associated hormonal events/changes that occur (Table 1.1). Approximately 20% of the estrous cycle is made up of the follicular phase and consists of proestrus and estrus (Senger, 2005). The majority and remaining 80% of the cycle is made up of the luteal phase and consists of metestrus and diestrus. During proestrus, formation of ovulatory follicles occurs, along with the subsequent secretion of E_2 . The secretion of E_2 peaks during estrus as the female becomes sexually receptive to the male (Allrich, 1994). After the dominant follicle ovulates, a CL forms from the follicular wall and P_4 begins to be secreted; this marks the beginning of the luteal phase and metestrus (Donaldson and William, 1965; Fritz and Fitz, 1991). Diestrus occurs during the remaining portion of the luteal phase and is defined by sustained P_4 secretion (Garverick et al., 1971). As P_4 concentrations decline at the end of diestrus and E_2 concentrations begin to again rise, a new estrous cycle ensues.

Proestrus

Declining P₄ concentrations mark the end of diestrus and the beginning of proestrus and are primarily the result of luteolysis (regression of the CL). Although functional luteolysis occurs at approximately the third or fourth day before estrus, morphological luteolysis occurs more gradually over the duration of proestrus (Garverick et al., 1971). Early evidence from Wiltbank and Casida (1956) supported the fact that a luteolytic compound may be of uterine origin, as most hysterectomized ewes or cows did not experience CL regression during the study period (100 or 154 d for ewes or cows; Wiltbank and Casida, 1956). The primary luteolysin in sheep, PG, was identified and confirmed to be of uterine, endometrial origin (McCracken et al., 1972; Hixon and Hansel, 1974). This compound is now recognized as the primary luteolytic substance in cattle (Hixon and Hansel, 1974; Lauderdale et al., 1974; McCracken et al., 1999).

A close relationship between the ovary, uterus, and brain exists that controls the events surrounding upregulation of PG synthesis (McCracken, 1980; McCracken et al., 1996). A critical concentration of oxytocin and the presence of oxytocin receptors on endometrial cells are required for PG synthesis (McCracken et al., 1996; McCracken et al., 1999). During the end of diestrus, response to P₄ begins to decline (due to autonomous downregulation of P₄ receptors), which releases a break in E₂ receptor formation. By releasing this break, circulating E₂ from the developing dominant follicle binds to receptors on certain neurons, increasing the firing frequency of the hypothalamic oxytocin pulse generator (McCracken et al., 1996). Pulses of oxytocin released by large luteal cells directly correspond to PG release. Prostaglandin F_{2α} moves by a

countercurrent diffusion system connecting the utero-ovarian vein to the ovarian artery and CL (McCracken et al., 1996; Lee et al., 2010). Although still controversial, a certain number of these pulses may be required to induce complete luteolysis (McCracken et al., 1996; Lee et al., 2010). In addition to increasing the pulses necessary for luteolysis, E₂ was noted to have an influence on anterior pituitary gland function by priming cells to secrete gonadotropins (Kesner et al., 1981; Padmanabhan et al., 1982).

Luteolysis and the associated decline in P₄ removes negative inhibition on GnRH release, subsequently allowing release of LH and FSH (Schallenberger et al., 1984; Bergfeld et al., 1996). Neurons in the hypothalamus release GnRH, which binds to gonadotrophs in the adenohypophysis, stimulating the release of LH and FSH (Schally et al., 1971; Roche, 1996; Foradori et al., 2002). Release of LH is episodic and changes from low to high frequency with positive estradiol feedback (Rahe et al., 1980; Schallenberger et al., 1984). Increasing concentrations of FSH and LH promote follicular growth (Schallenberger et al., 1984); luteinizing hormone stimulates theca interna cells of preovulatory follicles to produce androgens, and FSH stimulates conversion of androgens to E₂ by aromatase in granulosa cells. This concept is known as the “two-cell, two-gonadotropin concept” (Fortune and Quirk, 1988; Allen et al., 2016). During follicle growth, more E₂ is produced and additional LH receptors are acquired, increasing responsiveness to LH (Fortune and Quirk, 1988; Allen et al., 2016). This marks the beginning of estrus.

Estrus

On average, estrus lasts 12-18h and ovulation occurs 24-32h after the onset of estrus (Hammond, 1927; Christenson et al., 1975; Allrich, 1994). Rising concentrations of E₂ act upon the hypothalamus to induce behavioral estrus after reaching a threshold point (Allrich, 1994). Signs of behavioral estrus include increased vocalization, increased movement, and standing to be mounted (Orihuela, 2000; Gaude et al., 2017). In the time leading up to ovulation, E₂ prepares the reproductive tract for breeding and arrival of sperm (Hawk, 1983). The capacity of a follicle to ovulate increases with an increase in LH receptors in the granulosa cells with most follicles reaching ovulatory capacity at 10mm in diameter (Sartori et al., 2001). The preovulatory surge in LH needed for ovulation occurs as a result of an increase in frequency and amplitude of LH pulses which induces a series of physiological events that lead to ovulation (Walters and Schallenberger, 1984). The LH surge causes an increase in blood flow to the ovary and dominant follicle, increasing follicular pressure, and weakening the follicle wall (Epsey, 1994). Physical ovulation and rupture of the follicle wall is also associated with contraction of ovarian smooth muscle and release of lysosomal enzymes and collagenases (Curry et al., 1985; Epsey, 1994). The process of ovulation terminates with rupture of the follicle wall and production of a haploid oocyte that is capable of being fertilized.

Metestrus

Metestrus lasts approximately 5d and marks the beginning of the luteal phase of the estrous cycle where the ruptured follicle forms a corpus luteum. John Hammond

originally noted these changes in the cow in 1927, where hemorrhage and ovulation of the dominant follicle resulted in formation of a structure that would later be named the corpus hemorrhagicum. This is an early form of the corpus luteum.. Following the gonadotropin surge, LH induces luteinization, which continues following ovulation during development of the CL (Donaldson et al., 1965; Keyes, 1969; Smith et al., 1994). Although the concentration of LH is reduced during the early luteal phase, LH is still the major luteotropic hormone in the cow and is responsible for growth and development of the corpus luteum (Donaldson et al., 1965; Wiltbank, 1994). Theca cells of the follicle luteinize to become small luteal cells and granulosa cells luteinize to become large luteal cells, which both secrete P₄ (Donaldson and William, 1965; Fritz and Fitz, 1991; Smith et al., 1994; Romereim et al., 2016). Proper development of the CL is dependent upon rapid angiogenesis, or formation of blood vessels (Smith et al., 1994; Reynolds et al., 2000). Production of P₄ from the CL is highly correlated with ovarian blood flow, a testament to the importance of angiogenesis (Reynolds et al., 2000). Increasing production of P₄ is a function of the switch from E₂ production that results from a decrease in the activity of cytochrome P450 aromatase and P450 17 α -hydroxylase, enzymes involved in production of steroid precursors to E₂ (Voss and Fortune, 1993). Secretion of P₄ continues to increase over the duration of metestrus until reaching near-maximum concentrations.

Diestrus

Diestrus is characterized as having sustained, high concentrations of P₄ released from the corpus luteum 5d following the end of estrus (Garverick et al., 1971; Wiltbank, 1994). During diestrus P₄ functions to inhibit estrus by suppressing GnRH pulsatility

from the hypothalamus and ovulation of a dominant follicle; P₄ additionally promotes endometrial secretions and maintenance of pregnancy (Schallenberger et al., 1984; Adams et al., 1992; Bergfeld et al., 1996). Compared to concentrations of P₄ during estrus, P₄ during diestrus is approximately two-fold greater (Garverick et al., 1971). Luteal phase production of P₄ is sensitive to LH stimulation early in diestrus, however sensitivity to LH decreases by mid-luteal phase, particularly in large luteal cells (Wiltbank, 1994). Steroidogenic activity at this time instead, has greater dependence on lipoproteins supplied by the extensive vascular network within the CL (Wiltbank, 1994).

FOLLICULAR DYNAMICS

Folliculogenesis

Folliculogenesis is the sequence of events in the ovary leading to formation of a dominant (Graafian) follicle from a pool of primordial follicles (Erickson, 1966a; Fortune, 1994). Ruminants develop a pool of primordial follicles during fetal life from which all follicles grow after puberty (Fortune, 1994). The number of primordial follicles decreases with age from approximately 133,000 at birth to 3,000 in the mature aged cow (Erickson, 1966b; Spicer and Echternkamp, 1986). Development of a dominant follicle from the primordial pool occurs through a wave-like process of recruitment, selection, and dominance (Fortune, 1994; Ginther et al., 1996; Webb et al., 1999; Ginther et al., 2001; Jaiswal et al., 2009). The majority of cows and heifers will have two or three

follicular waves within an estrous cycle (Jaiswal et al., 2009), each of which begin with follicles at the primordial or primary stage.

Primordial follicles consist of a single oocyte surrounded by a layer of squamous granulosa cells. Primordial follicles develop into primary follicles after gaining a single layer of cuboidal cells (Pederson and Peters, 1968; Braw-Tal and Yossefi, 1997). Secondary (small to large preantral) follicles develop from primary follicles and have several more layers of follicular cells, including a zona pellucida, which surrounds the oocyte directly (Pederson and Peters, 1968; Braw-Tal and Yossefi, 1997). With age, the follicle acquires a fluid filled cavity known as the antrum and develops an extensive vasculature (Jiang et al., 2003). When an antrum has developed, the follicle is classified as a tertiary follicle (Pederson and Peters, 1968; Braw-Tal and Yossefi, 1997). In order to be classified as a Graafian follicle, antral follicles must be dominant over other follicles (Pederson and Peters, 1968; Braw-Tal and Yossefi, 1997).

Recruitment

Recruitment occurs at the beginning of a follicular wave and refers to the assembly of a group or cohort of follicles (Bao et al., 1997). In cattle, initiation of a follicular wave occurs at several time points throughout the estrous cycle. In beef heifers with three follicular waves, this typically occurs on days 2, 9, and 16 (during metestrus and diestrus), or in the case of heifers with two follicular waves, this occurs on days 2 and 11 (Sirois and Fortune, 1988; Fortune, 1994). Follicles that are recruited in the

second or third wave in two or three wave heifers respectively, will continue to grow, however follicles in earlier recruited waves become atretic (Sirois and Fortune, 1988).

Early recruitment of follicles is associated and dependent upon FSH (Adams et al., 1992; Ginther et al., 1996). As FSH concentrations rise, the cohort of recruited follicles (> 4 mm diameter) begins to grow and granulosa cell layers increase (Richards and Midgley, 1976; Ginther et al., 1996; Webb et al., 1999). At the time of recruitment, P450_{scc} (cholesterol side-chain cleavage enzyme) and P450_{arom} (aromatase) mRNA is detectable in the granulosa layers (Bao et al., 1997). Genomic studies identified more genes and signaling pathways that may play a role in cohort recruitment and follicular growth, although these are not yet fully understood (Li et al., 2016). A recruited cohort will continue to grow together until one follicle surpasses the others in growth and size (Ginther et al., 2001).

Selection

Selection, or follicular deviation, occurs as the largest follicle in a recruited cohort exerts dominance over the second largest follicle approximately 36-48 hours after a cohort is recruited (Ginther et al., 1996; Bao et al., 1997; Ginther et al., 2001). As the growing follicle acquires additional granulosa cells, more E₂ and inhibin are produced, suppressing FSH production and depriving the subordinate follicles of FSH needed for growth (Ginther et al., 1996; Ginther et al., 2001; Knight and Glister, 2001). At deviation, LH stimulates insulin-like growth factor and steroid systems, allowing further growth of the selected follicle (Ginther et al., 2001). Increased responsiveness to LH occurs after

the switch from FSH to LH dependency in the selected follicle (Ginther et al., 1996). This theory is supported by data collected by Bao et al. (1977) showing increased expression of mRNA for the LH receptor in granulosa cells at the time of selection.

Dominance

After selection, a dominant follicle continues to grow to an advanced stage whether the follicle is destined for ovulation or atresia (when a CL is present; Webb and Armstrong, 1998). For several days following selection, both ovulatory and anovulatory follicles experience a period of accelerated growth (Pierson and Ginther, 1984). The dominant ovulatory follicle continues to produce E₂, which aids in priming of the pituitary cells to release LH at the time of ovulation (Kesner et al., 1981; Padmanabhan et al., 1982). Ovulatory capacity is largely dependent on the number of LH receptors present on the follicle; these increase in number during dominant follicle growth (Hawk, 1983). On average, a follicle of 10mm has enough LH receptors to respond to the LH surge and ovulate (Hawk, 1983).

ESTRUS SYNCHRONIZATION

Introduction

Early conception and calving in heifers leads to greater lifetime production and can be accomplished through estrus synchronization (Cushman et al., 2013). Estrus

synchronization involves manipulation of the estrous cycle to control the approximate time of estrus and ovulation. This can then be used to influence earlier conception in both natural service and artificial insemination systems (Patterson et al., 2017). Early research of the estrous cycle led to the development of compounds that affect ovarian dynamics and provided the foundation for the study of estrus synchronization. These studies and those that followed provided the basis for development of estrus synchronization protocols that are used in the cattle industry today. Manipulation of the estrous cycle is largely accomplished through the use of three major categories of compounds: progestins and progesterone to suppress estrus and ovulation, PG to induce luteolysis, and GnRH to manipulate follicular waves and/or induce ovulation.

Progestins and progesterone

Progesterone was used for management of menstrual cycles in women, and in addition was used as a therapeutic agent in women experiencing various menstrual problems (Bishop, 1949). Progesterone was first used in sheep and pigs for ovarian cycle suppression prior to its application in bovine research (Dutt and Casida, 1948; Bishop, 1949). Ulberg et al. (1951) demonstrated that subcutaneous administration of progesterone in corn oil at a dose above 12.5 or 25 mg/d inhibited estrus and ovulation in dairy heifers. Trimberger and Hansel (1955) later showed that administration of progesterone at 50, 75, or 100mg/d successfully inhibited the expression of estrus, however conception rates were higher at the second estrus following progesterone removal compared to the first. These early studies stimulated a continued interest in manipulation of the estrous cycle for use in reproductive management.

Progestins are synthetic analogs of progesterone and are capable of binding to and activating the progesterone receptor (Figure 1.3). Early investigation of progestins for estrus synchronization in cattle began with medroxyprogesterone acetate (MAP; Figure 1.3) and chlormadinone acetate (CAP). In one of the earliest studies involving progestins, 6-methyl-17-acetoxy progesterone (MAP), an anti-ovulatory compound first studied in sheep, was fed in soybean meal and found to inhibit estrus when fed for a period of 20 d (Hansel et al., 1961; Hulet, 1966). In this trial, half of the cows were administered estradiol at AI, however, the inclusion of estradiol at the time of artificial insemination (3-5d following treatment) did not improve conception rate (Hansel et al., 1961). Another orally active progestin, 6-chloro- Δ^6 -dehydro-17-acetoxyprogesterone (CAP) achieved similar estrus synchronization rates, although fertility was reduced when compared to that of cows fed MAP for 18 d (Hansel et al., 1966). This comparison and subsequent trials that determined the minimal effective dose of MAP that is required to inhibit estrus led to approval of Repromix[®], the first commercially available estrus synchronization product (Zimbelman, 1963; Hansel et al., 1966; Lauderdale, 2010). The Upjohn Company produced Repromix[®] (MAP) which was fed for a period of 18 d at 180mg/head/d (Lauderdale, 2010). The sale of Repromix[®] in the United States continued from 1965-1967 but was later terminated due to high expense (Lauderdale, 2010).

Research continued for cheaper and more effective progestin compounds. Wiltbank et al. (1967) used dihydroxyprogesterone acetophenide (DHPA) to synchronize estrus in cattle by feeding 500mg/d for 20 d or 400mg/d for 9 d, with 5mg estradiol valerate administered on the second day of the feeding period (Wiltbank et al., 1967). The 20d feeding resulted in 96% of the heifers exhibiting estrus within 48 h after

progestin removal, whereas the 9 d treatment resulted in 84% of the heifers exhibiting estrus within 96h (Wiltbank et al., 1967). Miksch et al. (1978) investigated a 9d progestin treatment of norgestomet as a subcutaneous implant that included an injection of estrogen, progestin, or a combination of both steroids at the time of implant. In this study, administration of norgestomet and estradiol valerate achieved the greatest success in synchronizing estrus (Miksch et al., 1978). Spitzer et al. (1978) tested a 9d implant with 5mg estradiol valerate and 4mg norgestomet administered as a single injection at the time norgestomet implants were inserted, and reported synchronization rates in heifers ranging from 85-100%. This led to the development of the Syncro-Mate-B[®] protocol (Spitzer et al., 1978). Despite apparent success in synchronizing estrus, conception rates to the synchronized estrus were often lower for prepubertal heifers or anestrus cows compared to estrous cycling females (McGuire et al., 1990). McGuire et al. (1990) demonstrated that in addition to intact cycling females, ovariectomized or anestrus females in some cases also exhibited estrus. This study suggested that females in some cases were able to exhibit estrus in the absence of ovaries, and suggested too, that estradiol valerate was in all likelihood acting at the level of the brain to elicit signs of behavioral estrus that occurred independent of the reproductive tract. Therefore, estrus expression following SMB was not always a reliable indicator of ovarian function (McGuire et al., 1990). Despite some success with SMB (Spitzer et al., 1981), the work by McGuire et al. (1990) contributed to its discontinuation and replacement with more reliable and practical methods of estrus synchronization.

Two progestins are currently available in the United States for use in cattle to synchronize estrus: melengestrol acetate (MGA[®]; Figure 1.3) for heifers and controlled

internal drug release (CIDR[®]) for heifers and cows. Zimbelman and Smith (1966a,b) reported that melengestrol acetate (MGA[®]) suppressed estrus and inhibited ovulation when fed at a dosage of 0.4mg/hd/d for feeding periods of 18 to 32 d. Additionally, MGA feeding was associated with an increase in follicular activity. Melengestrol acetate was shown to improve weight gain and feed efficiency which led to label approval of MGA[®] as a growth promotant for use in feedlot heifers (Bloss et al., 1966). Melengestrol acetate was later approved for use to synchronize estrus in replacement heifers when fed at a rate of 0.5/mg/hd/d (Federal Register, 1997). Although earlier research suggests that MGA[®] is effective in heifers and cows, the legally approved label for MGA[®] only applies to heifers (Beal and Good, 1986; Patterson et al., 1995; Kesler et al., 1996; Federal Register, 1997; Patterson et al., 2016). The CIDR[®], however, is approved for use in both heifers and cows.

The concept of administering progesterone using an intravaginal device to synchronize estrus in cattle produced inconsistent results compared to a progestin administered in the feed (Carrick and Shelton, 1967). Intravaginal pessaries (sponge-like devices) impregnated with progesterone were first studied in sheep but when adapted for cattle, retention rates were reported to be a problem (Sreenan, 1975). Short-term treatment (10d) with progestin pessaries improved retention rates compared to long-term treatment (20d) and demonstrated satisfactory estrus suppression and subsequent fertility (Sreenan, 1975). In a series of trials, Roche (1976a) improved retention rates by designing a ring device covered with silastic rubber and impregnated with progesterone. Progesterone-releasing intravaginal devices (PRIDs) were examined in heifers and shown to be capable of maintaining luteal-concentrations of progesterone when inserted for 7 d

or less (Roche and Ireland, 1981). These were the precursors to the more reliable, T-shaped progesterone releasing device known today as the controlled-internal drug release (CIDR[®]). The CIDR[®] (Zoetis) is a t-shaped device consisting of a nylon spine and silicone cover impregnated with 1.38g of progesterone. Although originally designed and tested in New Zealand, the CIDR[®] was not approved by the FDA until 2001 to be used in combination with PG (Lucy et al., 2001; Lauderdale, 2010). Lucy et al. (2001) found that when PG was administered on day 6 of a 7d CIDR[®] treatment synchrony of estrus was improved in beef heifers and cows. Although not highlighted in this section, research later evaluated other methods of combining progestins or progesterone with gonadotropins and PG for use in estrus synchronization.

Prostaglandin F_{2α}

The luteolytic action of PG in ruminants was reported in sheep and later studied as a method of luteal control in cattle (McCracken et al., 1972; Lauderdale, 1972). Early studies showed that PG was effective in inducing luteal regression in cattle, particularly during diestrus when the CL was 5 d or older (Louis et al., 1973; Lauderdale et al., 1974). Rowson et al. (1972) demonstrated that a PG analog (cloprostenol) was also capable of inducing luteal regression. Currently, two PG analogs (cloprostenol sodium) are available for estrus synchronization in cattle: Estrumate[®] and estoPLAN[®] (generic; Lauderdale, 2015). Other PG products available for use in estrus synchronization include Lutalyse[®], ProstaMate[®] (generic) and In Synch[®] (generic), all of which are dinoprost tromethamine (Lauderdale, 2015). When used at the recommended dose, no differences in efficacy were observed among products, although the dosage for the PG analogs is

reduced (Lauderdale, 2005). Interval to estrus following injection of PG or a PG analog among diestrus females varies (2-5d) due to “two periods of growth and atresia of large antral follicles”, or in other words, stage of a follicular wave (MacMillan and Henderson, 1984). MacMillan and Henderson (1984) suggested that estrus synchrony could be improved with greater control of follicular activity, which led to further research into the inclusion of GnRH in estrus synchronization protocols.

Gonadotropin-releasing hormone

The first GnRH product (Cystorelin[®], gonadorelin diacetate tetrahydrate) was approved in 1978 for the treatment of follicular cysts in dairy cattle (Federal Register, 1978). Today, the following synthetic forms of GnRH (gonadorelins), are used in the United States for the purpose of estrus synchronization: Cystorelin[®], Factrel[®], Fertagyl[®], GONAbreed[®] and OvaCyst[®] (Lauderdale, 2015). These GnRH products are labeled for the treatment of ovarian follicular cysts in dairy cattle and Factrel[®] is additionally labeled for the treatment of follicular cysts in all beef cattle. GONAbreed[®], Factrel[®] and Fertagyl[®] have label approval for use in estrus synchronization in dairy cattle in combination with a specific PG product. However, GONAbreed[®] and, recently, Cystorelin[®] are the only gonadorelins with label approval for estrus synchronization in beef cows (Federal Register, 2013).

Gonadotropin-releasing hormone causes the LH surge that is largely responsible for ovulation (Schally et al., 1971; Walters and Schallenberger, 1984). Ovulatory capacity is typically reached when a dominant follicle is selected and attains a size of at

least 10mm in diameter (Hawk, 1983; Roche et al., 1999; Sartori et al., 2001). In most females with a dominant follicle, an LH surge is induced 2-4h following administration of GnRH, with ovulation occurring 24-36h later (Christenson et al., 1975; Gumen and Seguin, 2003). Response to GnRH and initiation of a subsequent follicular wave is a function of the stage of follicular growth (Hawk, 1983; Roche et al., 1999; Sartori et al., 2001). Gonadotropin-releasing hormone induces formation of an accessory CL that is responsive to PG 6-7d later (Pursley et al., 1995). The administration of GnRH at AI was a critical step in the development of FTAI systems (Pursley et al., 1995; Geary and Whittier, 1998; Kesler, 2005). There is significant variability, however, between cows and heifers in response to GnRH, with only 66% of cows responding to GnRH when administered on a random day of the estrous cycle compared to 50% of heifers (Pursley et al., 1995). This requires special consideration when selecting an estrus synchronization protocol. In heifers particularly, there is greater variation in number of follicular waves, and therefore emergence of the dominant, ovulatory follicle (Thatcher et al., 1993; Pursley et al., 1995; Twagiramungu et al., 1995). This variability, among other inconsistencies, ultimately led to the establishment of estrus synchronization protocols that include a combination of estrus synchronization products.

MGA[®]-PG protocol

Melengestrol acetate (MGA[®]) was first shown to suppress estrus and ovulation in beef heifers when fed at a rate of 0.4mg/hd/d or for feeding periods of 18 to 32 days (Zimbelman and Smith, 1966a,b; Zimbelman et al., 1970). When comparing heifers that were fed MGA[®] for 18d to heifers fed for 23-26 d, a greater proportion of heifers in the

18d group exhibited estrus within 6d after withdrawal of MGA than those that were fed longer (Smith and Zimbelman, 1968). The first estrus and associated ovulation following treatment, however, was one of apparent reduced fertility. This association was noted in other studies with progestins as well (Zimbelman and Smith, 1966b; Smith and Zimbelman, 1968; Patterson et al., 1989b). Shortening progestin or progesterone treatment in heifers that are estrous cycling results in greater fertility, however, a decrease in estrous response was noted (Roche, 1974; Patterson et al., 1989b). Improvements in estrous response to short-term progesterone treatment (7d) was noted when combined with administration of a PG analog after removal of progesterone (Roche, 1976b).

In a study utilizing a 7d MGA[®] treatment followed by administration of PG on day 7, Patterson et al. (1989a) noted that conception rate was dependent upon stage of the estrous cycle when the protocol was initiated. In other words, heifers that were at a later day in the estrous cycle when treatment with MGA was initiated and had regressed CL during the treatment period, were found to have had decreased conception rates at the synchronized estrus. These heifers exhibited estrus in response to withdrawal of MGA from the feed. On the other hand, fertility was greater among heifers that were at an early point in the estrous cycle when treatment was initiated. These heifers had CL at the time feeding of MGA ended and instead exhibited estrus in response to PG. Reduced fertility among heifers that were late in the estrous cycle at the time feeding of MGA began and that regressed CL during treatment was attributed to delayed ovulation of an aged oocyte from a dominant follicle (Patterson et al., 1986; Kinder et al., 1996). Brown et al. (1988) developed the basis of the MGA[®]-PG protocol used today in an attempt to improve

synchrony of estrus and resulting conception and pregnancy rates during the synchronized period

Brown et al. (1988) fed MGA[®] for 14-16d and administered 25mg PG 16 or 17d after MGA[®] withdrawal. This resulted in improvements in both synchrony of estrus and conception rates. Further improvements in synchrony and conception rates were made when PG was administered 19d versus 17d following a 14d feeding period of MGA[®] (Nix et al., 1998; Lamb et al., 2000; Deutscher, 2000). This may be due to a greater proportion of large, pre-ovulatory follicles that are present at the time PG is administered (Nix et al., 1998; Lamb et al., 2000; Deutscher, 2000).

Currently, the MGA[®]-PG protocol consists of a 14d feeding period of MGA[®] with PG administered 19d after MGA[®] withdrawal. Estrus detection is performed for approximately 6d following administration of PG when inseminating heifers on the basis of observed estrus. When the protocol is used in conjunction with timed artificial insemination, AI is performed concurrent with GnRH administration 72h following PG (Figure 1.4). Larson et al. (1996) compared pregnancy rates in heifers after timed AI at 72h to heifers that were inseminated on the basis of detected estrus. Higher pregnancy rates were achieved when inseminations were performed on the basis of detected estrus, however TAI at 72h resulted in pregnancy rates that were considered to be acceptable.

7d CO-Synch + CIDR[®] protocol.

A series of studies conducted by Lucy et al. (2001) led to approval by the FDA for use of the CIDR[®] (InterAg, Hamilton, NZ) in the U.S. In these early studies, a

CIDR[®] was inserted for 7d with PG administered on day 6 of CIDR[®] treatment (Lucy et al., 2001). This led to improvements in both synchrony of estrus and conception rate to AI over a 31d breeding period compared to control or prostaglandin-only treatments (Lucy et al., 2001). Patterson et al. (2017) referenced unpublished data by Dejarnette noting that in order to reduce the number of times that heifers were handled, PG was administered on day 7 compared to day 6 of the 7d CIDR[®] protocol. No differences were noted in synchrony of estrus or conception rates for groups administered PG on day 6 or 7, although estrus occurred later when PG was administered on day 7 (Patterson et al., 2017).

Lamb et al. (2006) evaluated the CIDR[®]-PG protocol (PG on day 7) with or without GnRH at CIDR[®] insertion in a study involving a combination of estrus detection and AI followed by a clean-up AI 84 h after PG, or TAI at 60h. Lamb et al. (2006) reported seeing no differences in pregnancy rates among treatments and concluded that a combination of detecting estrus and AI before clean-up AI enhanced pregnancy rates over FTAI. Leitman et al. (2008) found that synchrony of estrus and ovulation following PG was decreased in heifers that received GnRH at CIDR[®] insertion, with no difference in total estrous response compared to heifers that did not receive GnRH (Leitman et al., 2008). These studies raised questions concerning the need to administer GnRH at the initiation of a 7-day CIDR[®]-PG protocol in heifers.

The 7d CO-Synch + CIDR[®] protocol involves the administration of GnRH coincident with CIDR[®] insertion at the beginning of a 7 d CIDR[®] treatment. At CIDR[®] removal, PG is administered. Approximately 54 hours after CIDR[®] removal and PG, AI may be performed concurrent with the administration of GnRH (Figure 1.5).

14d CIDR[®]-PG protocol.

Studies were initiated to substitute the CIDR[®] in place of MGA[®] for a 14 d treatment period following the approval of the CIDR[®] for use in estrus synchronization in cattle. Kojima et al. (2004) compared the MGA[®]-Select protocol (Wood et al., 2001; Figure 1.6) to a CIDR[®]-Select protocol (Figure 1.7), in which case GnRH was administered on day 23 for CIDR[®] treated heifers or day 26 for MGA[®] treated heifers. Prostaglandin F_{2α} was administered 7d following GnRH administration, and inseminations were performed 12h after the onset of estrus (Kojima et al., 2004). There was no difference in estrous response between treatments, however pregnancy rate to AI was greater among heifers in the CIDR[®] treated group (Kojima et al., 2004). Busch et al. (2007) compared the 7-day CO-Synch + CIDR[®] and CIDR[®]-Select protocols in heifers. Heifers assigned to the CIDR[®]-Select protocol exhibited a more highly synchronized estrous response with significantly higher pregnancy rates to TAI (Busch et al., 2007). Estrous response rates following treatment with the 14d CIDR[®] were similar when comparing heifers that were estrous cycling or pre- or peripubertal prior to treatment initiation (Leitman et al., 2008).

These studies (Leitman et al., 2009a) began to raise questions concerning the need to administer GnRH following a 14 day CIDR[®] treatment, 7 days prior to PG. Leitman et al. (2009b) compared a 14 day CIDR[®]-PG protocol with or without the administration of GnRH on day 23. Despite the fact there was no difference in estrous response between the two treatments, the variance for interval to estrus was reduced among heifers assigned to the CIDR[®]-PG treatment (Leitman et al., 2009b), indicating that synchrony of estrus

was improved among heifers that did not receive GnRH as part of the treatment schedule. In addition, heifers assigned to the CIDR[®]-PG protocol had greater conception and pregnancy rates to AI compared to CIDR[®]-Select treated heifers (Leitman et al., 2009b). Leitman et al. (2009b) suggested that the mean interval to estrus among CIDR[®]-Select treated heifers was extended as a result of delayed recruitment of the follicular wave that emerged following administration of GnRH on day 23 of the treatment schedule. In total, these data clearly showed that GnRH failed to provide any improvement in results following administration of the 14 day CIDR[®]-PG protocol.

Currently, the 14d CIDR[®]-PG protocol involves insertion of a CIDR[®] for 14d, followed 16d later (day 30) with the administration of PG. In an estrus-detection and TAI system, heifers are observed for signs of behavioral estrus after PG and inseminated based on estrus expression; however, any remaining heifers that fail to exhibit estrus three days following PG are administered GnRH to induce ovulation and AI is performed. In contrast, and regardless of estrus status, heifers are administered GnRH concurrent with AI 66h following PG in a FTAI system (Figure 1.8).

HEIFER MANAGEMENT

Introduction.

Early management of replacement heifers influences overall productivity of the cowherd (Patterson et al., 1992). Heifers that calve earlier in a calving season remain in the herd longer than late-calving heifers and achieve greater overall lifetime production (Lesmeister et al., 1972). Furthermore, early-born calves exhibit an advantage in

productivity over later-born calves with an increased likelihood of early born heifer calves to conceive early themselves (Lesmeister et al., 1972; Funston et al., 2012; Whittier, 2013). Early calving is directly correlated with earlier conception in the breeding season. In many ways, early conception is a function of pubertal status and early expression of estrus (Short and Bellows, 1971; Patterson et al., 1992; Perry, 2016). Programs designed to develop replacement beef heifers should reflect management decisions that will advance age at puberty and then determine success of the selection and development program through use of pre-breeding examinations (Patterson et al., 1992; Poock and Payne, 2013).

Age at puberty.

Age at which puberty occurs is highly variable and can be influenced by a variety of factors. Factors that are commonly considered to influence age at puberty include genetics, nutrition, season, management, and treatment with a progestin (Gonzalez-Padilla et al., 1975b; Martin et al., 1992; Patterson et al., 1992; Schillo et al., 1992; Perry, 2016). All of these factors contribute to endocrine maturation either directly or indirectly, and advance age at puberty (Perry, 2016).

Age at puberty is considered to be a more highly heritable trait ($h^2 = 0.4$) compared to other reproductive traits, indicating the potential to select heifers for earlier age at puberty (Martin et al., 1992). When considering bulls, scrotal circumference can be used as an indicator trait for puberty in heifers (Martin et al., 1992). According to Martin et al. (1992), producers can indirectly select for a younger pubertal age by selecting for

traits that promote expression of fertility (such as milk production or body size). Furthermore, improvements can be made by selecting breeds of cattle that reach puberty at younger ages and by taking advantage of heterosis through the use of crossbreeding (Dow et al., 1982; Martin et al., 1992; Perry, 2016). Indicine breeds or crosses of cattle, however, are slower to mature than *Bos taurus* cattle, highlighting the need for different management practices between species (Dow et al., 1982; Sartori et al., 2010).

Age at puberty is affected by nutrition and final weight. These factors should be considered when developing a feeding program for heifers (Patterson et al., 1992). When females are fed a low-plane of energy, the switch to positive estradiol feedback is delayed, whereas an increasing plane of energy promotes positive over negative feedback from estradiol (Day et al., 1986; Schillo et al., 1992). In other words, higher energy diets are associated with decreased age at puberty by positively impacting LH pulsatility. Although breed dependent, choosing and feeding to a target weight aids in early attainment of puberty and feeding to 65% of mature body weight was effective in advancing puberty and increasing 45d pregnancy rates (Patterson et al., 1989a).

Gonzalez-Padilla et al. (1975b), advanced age at puberty by administering a progestogen in prepubertal heifers near the time puberty would be expected to occur. This was based on the concept that progesterone is required for heifers to reach puberty (Gonzalez-Padilla et al., 1975a; Gonzalez-Padilla et al., 1975b). Use of commercially available progestins (CIDRs[®] and MGA[®]) can be used to promote earlier attainment of puberty in prepubertal heifers.

Pre-breeding examinations

When managing and selecting replacement heifers, producers make decisions that affect profitability of an entire cowherd (Patterson et al., 2017). Reproductive performance of the herd can be improved by selecting females that exhibit earlier reproductive success and fewer reproductive losses (Patterson et al., 1992; Patterson et al., 2013; Roberts et al., 2015). When considering replacement females, pelvic measurements and evaluation of the reproductive tract via ultrasonography or rectal palpation paint a picture of the heifer's reproductive potential prior to breeding. Culling decisions based on pelvic size are a common aspect of a heifer's pre-breeding evaluation and reduce the risk of dystocia when heifers are culled for small pelvic area (Holm et al., 2014). Assessment of the reproductive tract includes examination of the ovaries and uterus relative to pubertal status. The RTS system developed by Anderson et al. (1991) has been used in research and heifer management programs and has been noted for its use as an indicator of heifer fertility (Holm et al., 2009).

Reproductive tract scoring

The RTS system consists of rectal palpation of the ovaries and uterus of a heifer to determine pubertal status as a yearling, prior to estrus synchronization and breeding (Anderson et al., 1991; Figure 1.2). Ovaries are palpated for relative size and structures present at the time the exam is performed (follicles, corpora lutea, or lack thereof). The uterus is palpated for relative size, completeness, and tone (Anderson et al., 1991). Upon palpation, the heifer is assigned a RTS ranging from one through five based on combined

assessment of the ovaries and uterus (Anderson et al., 1991; Figure 1.2). A RTS of one indicates that the reproductive tract is infantile with no palpable follicles on the ovaries and lack of tone in the uterine horns (Anderson et al., 1991). The uterine horns measure less than 20 mm in diameter. Heifers assigned a RTS of two have palpable follicles (8mm in diameter) along with increased uterine diameter (20-25mm), although no distinguishable uterine tone. As a heifer acquires uterine tone and the follicles increase in size (8-10mm), a RTS of three may be assigned. Uterine tone continues to increase as a heifer approaches puberty, enough to cause the uterine horns to coil tightly. Heifers whose reproductive tracts exhibit an increase in tone and size of the uterine horns (30mm) with larger follicle size (>10mm) are assigned a score of four and are assumed to be in the follicular stage of the estrous cycle. A score of five indicates the heifer is in the luteal phase of the estrous cycle, with uterine horns greater than 30mm in diameter and a palpable CL present at the time of the exam. A score of one refers to a “prepubertal” heifer that is ≥ 30 d from puberty onset, whereas “peripubertal” heifers are assigned scores of two or three and likely are ≤ 30 d from reaching puberty. Heifers assigned scores of four or five are considered to be estrous cycling or “pubertal.” (Anderson et al., 1991)

Rosenkrans and Hardin (2003) determined that the RTS system developed by Anderson et al. (1991), was an effective and consistent method to determine pubertal status in heifers. The RTS system was repeatable between veterinarians and no advantage was found for ultrasonography over rectal palpation (Rosenkrans and Hardin, 2003). Furthermore, the RTS system was effective in predicting initial reproductive success of heifers, with females assigned a RTS of one or two frequently outperformed from a reproductive standpoint by heifers assigned higher reproductive tract scores (Hall, 2005;

Gutierrez et al., 2014). Application of the RTS system is a useful tool that producers may use to improve initial reproductive success and overall productivity of the cowherd.

ARTIFICIAL INSEMINATION

The development of artificial insemination in the beef cattle industry in the United States has a long and interesting history. Although artificial insemination was first performed in dogs by Lazzaro Spallanzani in 1784, significant efforts were not made until the late 1800's and early 1900's to widen the use of AI in livestock production (Foote, 2002). Many of these efforts to use AI in livestock came from the Russian scientist, Ivanovich Ivanoff (Foote, 2002). Subsequent research at the Royal Veterinary College in Copenhagen, Denmark involved with AI in dairy cattle provided the necessary impetus to increase adoption of AI in the United States (Foote, 2002).

Extensive research with artificial insemination in dairy cattle in the early 1930's and 1940's led to the establishment of AI cooperatives between producers and Cornell University (Foote, 2002). Confinement systems used by the industry, the desire to improve genetics, and the risk associated with housing dairy bulls on-farm provided the necessary rationale for adoption of AI by dairy producers over time. In contrast, use of AI by beef producers required better methods to synchronize estrus and ovulation before widespread adoption of AI would be realized (Patterson et al., 2017).

Protocols that were first developed to facilitate use of AI required estrus detection, which was more time intensive and problematic for larger beef operations. However, development of protocols that facilitated fixed-time artificial insemination

made the use of AI more feasible. One critical step toward the development of FTAI was the inclusion of GnRH at the time of insemination to induce ovulation (Pursley et al., 1995; Geary and Whittier, 1998; Perry et al., 2002; Kesler, 2005). This allowed for a pre-determined scheduling of insemination wherein females could be inseminated on the same day at approximately the same time. Perry and Smith (2018) noted however, that the time of insemination in a FTAI protocol is a compromise between maximizing estrus expression prior to AI, and not reducing fertility by waiting too long for females that expressed estrus earliest (Perry and Smith, 2018).

SPLIT-TIME ARTIFICIAL INSEMINATION

Background

As noted previously, the time of insemination in a fixed-time AI protocol is a compromise between maximizing the number of females that express estrus and not waiting too long to perform insemination so as to decrease fertility of females that express estrus earliest (Perry and Smith, 2018). This is based on research showing that females that express estrus prior to AI achieve higher pregnancy rates compared to those that do not (Busch et al., 2008; Nash et al., 2012; Smith et al., 2012; Thomas et al., 2014b). In a study by Perry et al. (2005) relating follicle size to pregnancy success, pregnancy rates were lower and late embryonic loss was higher for females that had follicles less than 11mm at AI that were induced to ovulate with exogenous GnRH. Furthermore, follicles that ovulated spontaneously showed no decrease in pregnancy rate

to AI, regardless of follicle size (Perry et al., 2005). Reduced pregnancy rates were associated with a delayed rise in progesterone and lower estradiol concentrations at AI that culminated in a reproductive tract that was ill-prepared to transport sperm and maintain pregnancy, in addition to an oocyte that was physiologically immature and therefore sub-fertile (Perry et al., 2005). These findings stimulated a continued interest in timing of insemination in relation to the time when GnRH is administered, the overall use of GnRH, and the relationship of estrus expression and pregnancy rates to timed-AI.

Development of split-time artificial insemination

Split-time artificial insemination is based on the concept that females that express estrus tend to have higher pregnancy rates to artificial insemination compared to females that do not express estrus. Thomas et al. (2014a) classified females as early- (estrous) or late- (non-estrous) ovulating relative to FTAI, as it was proposed that in many cases inseminations during FTAI are performed too early among later ovulating females. This led to the consideration of delayed insemination of non-estrous females as a way to optimize success to TAI (Thomas et al., 2014a; Thomas et al., 2014b).

Thomas et al. (2014a, b) developed the concept of delayed or “split-time” AI by comparing a 20h delayed insemination from GnRH versus standard FTAI. Following the 14d CIDR[®]-PG protocol in beef heifers, delayed insemination of non-estrous heifers (as measured 66h after PG) resulted in higher pregnancy rates to AI compared to the standard FTAI of non-estrous heifers (49 vs. 34%, respectively; Thomas et al., 2014b). Improvements in AI pregnancy rates following delayed insemination likely depend on the

proportion of heifers that express estrus during the delay period (Thomas et al., 2014b). These findings support the work by Perry et al. (2005), relating follicle maturity to reproductive success.

Research related to split-time AI raised further questions regarding the appropriate timing and use of GnRH when using STAI. Thomas et al. (2014b) noted that females that express estrus by the standard time of AI ovulate in response to an endogenous LH surge that occurs prior to the time insemination is performed (Thomas et al., 2014b). This suggests that administration of GnRH should not be necessary among females that have already expressed estrus (Thomas et al., 2014b). This idea was supported from earlier research (Lucy and Stevenson, 1986) in which GnRH-induced LH surges were lower in magnitude in dairy cows and heifers compared to an endogenous LH surge. Furthermore, a GnRH induced LH surge and resulting ovulation is associated with reduced estradiol production via downregulation of aromatase enzymatic activity (Voss and Fortune, 1993; Thomas et al., 2014b).

A series of experiments were designed by Bishop et al. (2016, 2017a, b) to evaluate timing of GnRH administration in conjunction with STAI. Bishop et al. (2016) evaluated use of GnRH following treatment with the 14d CIDR[®]-PG protocol in beef heifers and found that GnRH administration at 66h concurrent with AI did not affect pregnancy rate resulting from AI (Bishop et al., 2016). Bishop et al. (2016) therefore concluded that it is not necessary to administer GnRH to heifers that exhibit estrus by 66h after PG following administration of the 14d CIDR[®]-PG protocol. Bishop et al. (2016) recommended that GnRH only be administered coincident with AI to heifers that fail to exhibit estrus during the delayed time period, 90 h after the administration of PG.

Administration of GnRH to females that express estrus by the standard time of AI is unnecessary, due to the animal's own endogenous LH surge (Bishop et al., 2016; Bishop et al., 2017a; Bishop et al., 2017b; Hill et al., 2016). Results from these studies indicated that STAI can be used to decrease use of GnRH in a timed AI program.

Split- time artificial insemination

Split-time AI was shown to improve estrous response and pregnancy rates in beef heifers and cows and produced acceptable pregnancy rates using sex-sorted semen in beef heifers following administration of the 14d CIDR[®]-PG protocol (Thomas et al., 2014a,b; Bishop et al., 2016, 2017a,b; Hill et al., 2016; Thomas et al., 2017). Delayed insemination of non-estrous females allows for increased estrus expression and the potential for better alignment of ovulation with insemination to optimize fertility (Thomas et al., 2014b; Thomas et al., 2014a; Bishop et al., 2016; Bishop et al., 2017a; Bishop et al., 2017b). Furthermore, STAI may also offer an advantage for certain bulls that may be classified as low-fertility based on reduced lifespan of sperm in the female reproductive tract (Macmillan and Watson, 1975; Thomas et al., 2014b; Thomas et al., 2017).

SUMMARY

The National Animal Health Monitoring System reported that the majority of annual losses in beef cattle in the U.S. result from reproductive failure (USDA-APHIS, 2008). Improvements to existing or development of new reproductive technologies will limit these losses and improve beef production. The significance in use of estrus synchronization and artificial insemination in the beef cattle industry is undeniable. These practices increase production efficiency and overall value of a calf crop. Recent research with split-time AI in beef heifers following treatment with a long-term CIDR[®]-based protocol provides the opportunity to more effectively optimize pregnancy rates when utilizing estrus synchronization and artificial insemination. To date, few studies are reported in beef heifers using STAI. The research presented in this thesis was intended to expand our current understanding of ways in which split-time AI may be used in beef heifers to improve pregnancy rates following a single insemination. Estrus synchronization protocols that are discussed for use with STAI include long-term, short-term, and extended progestin-based protocols.

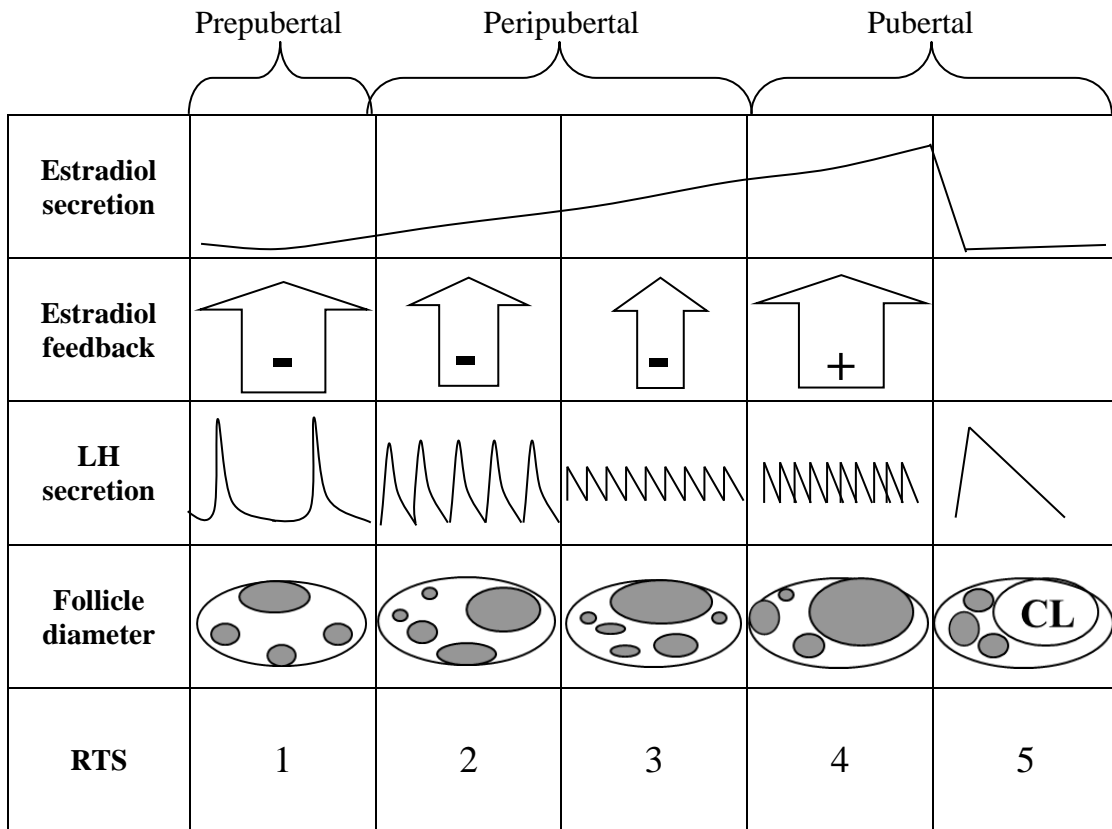


Figure 1.1. Endocrine events of puberty onset. Figure includes related reproductive tract scores (RTS; Adapted from Anderson et al., 1991; Day and Anderson, 1998; Patterson et al., 1999). LH = luteinizing hormone, CL = corpus luteum

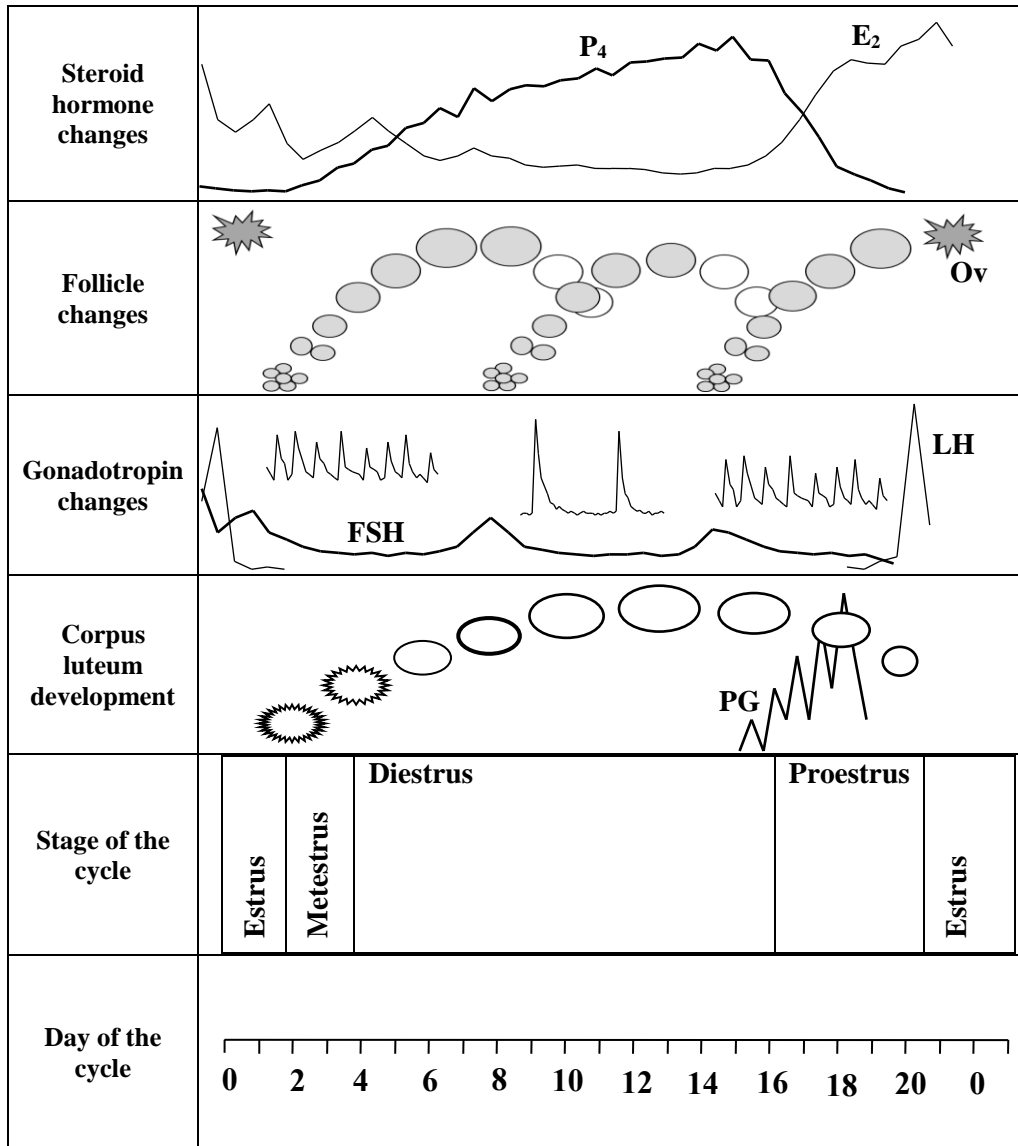


Figure 1.2. The bovine estrous cycle. Figure is characteristic of a 21 d estrous cycle with three follicular waves. Adapted from Kojima and Patterson (2003). P₄ = progesterone, E₂ = estradiol-17β, Ov = ovulation (24-32 h after onset of estrus; Christenson et al., 1975; Allrich, 1994), FSH = follicle stimulating hormone, LH = luteinizing hormone, PG = Prostaglandin F_{2α}

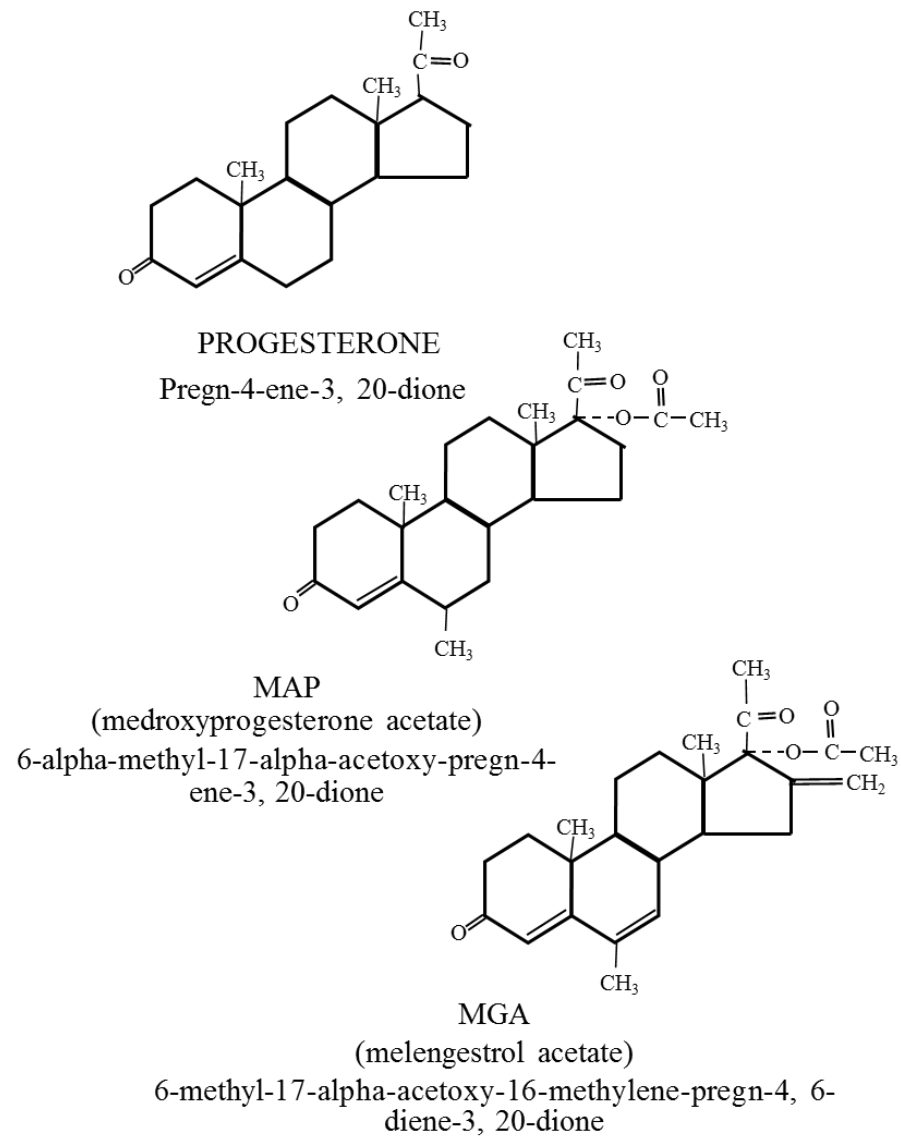


Figure 1.3. Chemical structures of progesterone (P₄), medroxyprogesterone acetate (MAP), and melengestrol acetate (MGA[®]).

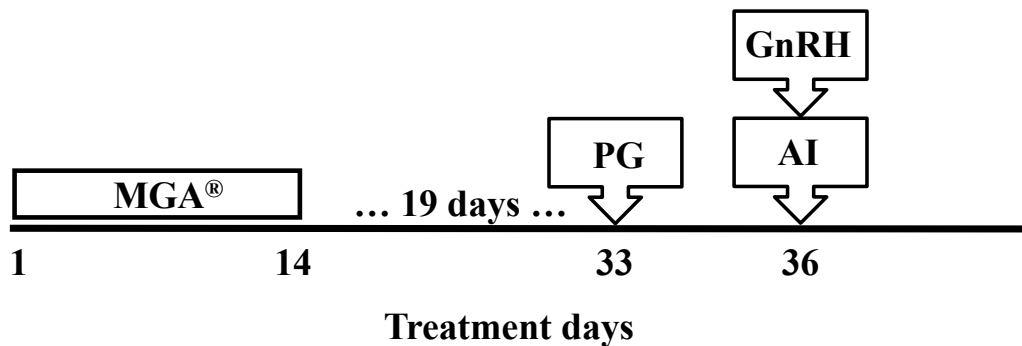


Figure 1.4. The MGA[®] - PG estrus synchronization protocol for fixed-time artificial insemination (FTAI). Adapted from Lamb et al. (2000). MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day, prostaglandin F_{2α} (PG) administration on day 19, and FTAI at 72 h following PG concurrent with gonadotropin-releasing hormone (GnRH) administration.

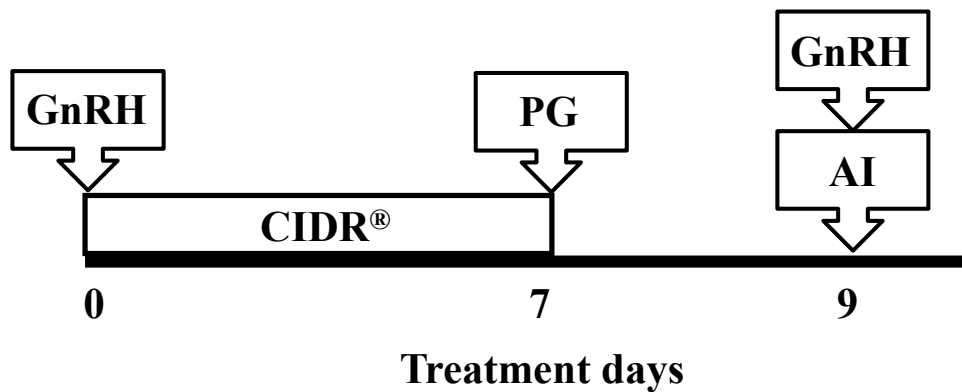


Figure 1.5. The 7d CO-Synch + CIDR[®] estrus synchronization protocol for fixed-time artificial insemination (FTAI). Adapted from the 7d CO-Synch + CIDR[®] protocol (Lamb et al., 2001; Lamb et al., 2006). This protocol consists of a 7 d CIDR[®], gonadotropin-releasing hormone (GnRH) administration at CIDR[®] insertion, and prostaglandin F_{2α} (PG) administration at CIDR[®] removal on day 7. For FTAI, heifers are inseminated at 54 h following PG concurrent with GnRH administration.

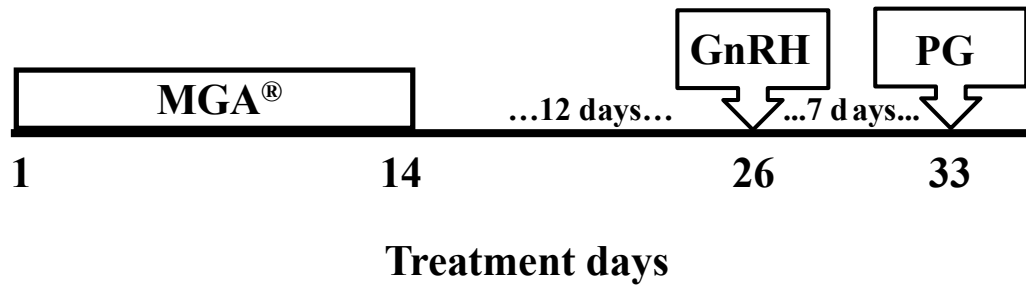


Figure 1.6. The MGA[®] Select estrus synchronization protocol. Adapted from Wood et al. (2001). MGA[®] Select consists of MGA[®] feeding for 14 d at a rate of 0.5 mg/head/day, gonadotropin-releasing hormone (GnRH) administration 12 d after progestin removal and prostaglandin F_{2α} (PG) administration 7 d following GnRH.

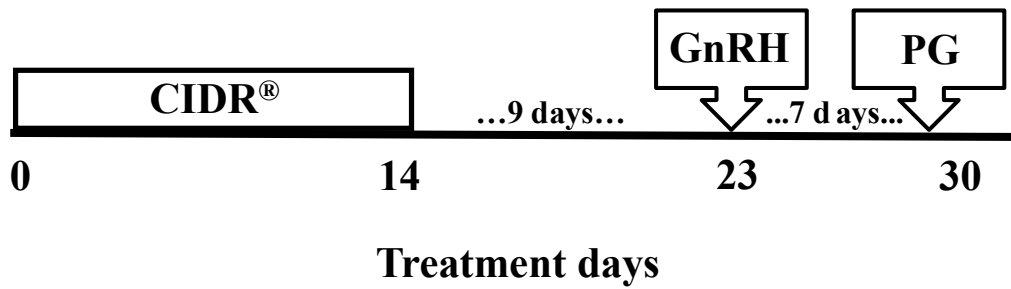


Figure 1.7. The CIDR[®] Select estrus synchronization protocol. Adapted from Kojima et al. (2004). CIDR[®] Select consists of a 14 d CIDR[®], gonadotropin-releasing hormone (GnRH) administration 9 d after progestin removal and prostaglandin F_{2α} (PG) administration 7 d following GnRH.

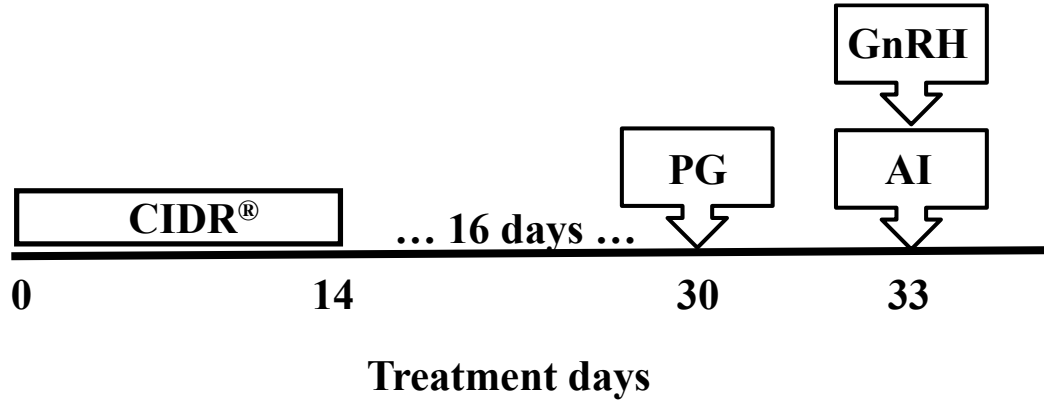


Figure 1.8. The 14d CIDR[®] - PG estrus synchronization protocol for fixed-time artificial insemination (FTAI). Adapted from the 14d CIDR[®] - PG and Show-Me-Synch protocols (Leitman et al., 2009a, b; Mallory et al., 2010, 2011). The 14d CIDR[®] -PG protocol consists of a 14 d CIDR[®] and prostaglandin F_{2α} (PG) administration 16 d after CIDR[®] removal. For FTAI, heifers are inseminated at 66 h following PG concurrent with gonadotropin-releasing hormone (GnRH) administration.

Table 1.1. Characteristics of the 21 d^a estrous cycle

Period	Day of the estrous cycle	Characteristics
Estrus ^b	0	Behavioral estrus (heat)
Metestrus ^c	1 – 4	Ovulation, CL formation
Diestrus ^d	5 – 16	CL growth and maintenance, progesterone secretion
Proestrus ^e	17 - 21	Luteolysis, follicular growth

^a Wishart, 1972

^b Allrich, 1994

^c Donaldson and William, 1965; Fritz and Fitz, 1991

^d Garverick et al., 1971; Wiltbank, 1994

Table 1.2. Reproductive tract scoring^a (RTS)

RTS	Cyclicity Status	Uterine Horns	Ovaries
1	Prepubertal (>30d to puberty)	<20mm diameter Infantile, no tone	No palpable structures
2	Peripubertal (<30d to puberty)	20-25mm diameter No tone	8 mm follicles
3	Peripubertal (<30d to puberty)	25-30mm diameter Slight tone	8-10 mm follicles
4	Pubertal (follicular phase)	30mm diameter Coiled	>10 mm follicles
5	Pubertal (luteal phase)	>30mm Distended	CL present

^a Adapted from Anderson et al., 1991

CHAPTER 2

EVALUATION OF SPLIT-TIME ARTIFICIAL INSEMINATION FOLLOWING ADMINISTRATION OF A LONG- OR SHORT-TERM PROGESTIN-BASED ESTRUS SYNCHRONIZATION PROTOCOL IN BEEF HEIFERS

ABSTRACT

Two experiments were designed to evaluate estrous response and pregnancy rates resulting from fixed-time (FTAI) or split-time (STAI) artificial insemination in beef heifers. Fixed-time and split-time AI were compared following administration of the melengestrol acetate (MGA[®]) prostaglandin F_{2α} protocol (Experiment 1), and the 7d CO-Synch + controlled internal drug release (CIDR[®]) protocol (Experiment 2). Heifers (n = 524) in Experiment 1 were managed in 10 pens and assigned within pen to balanced treatments based on weight and reproductive tract score (RTS; Scale 1-5). Heifers were fed MGA[®] (0.5 mg·animal⁻¹·d⁻¹) in a 1.8 kg grain carrier for 14 d. Prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) was administered 19 d after MGA[®] withdrawal. Estrus detection aids (Estroject[®]) were applied at PG, and estrous status was recorded at 72 h after PG for heifers assigned to FTAI, and 72 and 96 h after PG for heifers assigned to STAI. Estrus was defined as removal of ≥ 50% of the coating from the Estroject[®] patch. Heifers assigned to FTAI were inseminated 72 h after PG and GnRH (100 µg im gonadorelin acetate) was administered at AI. At 72 h after PG, heifers in the STAI

treatment that exhibited estrus were inseminated; however, AI was postponed until 96 h for heifers that were non-estrous at 72 h. Only heifers that failed to exhibit estrus by 96 h received GnRH at AI. Estrous response 72 h after PG did not differ between treatments; however, total estrous response was increased ($P < 0.001$) among heifers assigned to STAI (88%, STAI; 72%, FTAI). Pregnancy rates resulting from AI were greater ($P < 0.04$) for heifers assigned to STAI compared to FTAI (55% vs 46%, respectively), and were enhanced ($P < 0.05$) among heifers that exhibited estrus. Heifers ($n=456$) in Experiment 2 were managed at one location in three pens and assigned within pen to one of two balanced treatments based on weight and reproductive tract score (RTS; Scale 1-5). All heifers were subject to the 7d CO-Synch + controlled internal drug release (CIDR[®]) estrus synchronization protocol and managed in two separate groups. Gonadotropin-releasing hormone (100 μ g gonadorelin acetate) was administered coincident with CIDR[®] [1.38g progesterone (P_4); Zoetis, Madison, NJ] insertion in heifers at the initiation of the protocol, and CIDR[®] inserts were removed after 7 d. Prostaglandin $F_{2\alpha}$ (PG; 250 μ g im cloprostenol sodium) was administered and estrus detection aids (Estroject[®]) were applied coincident with CIDR[®] removal. Estrous status was recorded at 54 h after PG for heifers assigned to FTAI, and 54 and 78 h after PG for heifers assigned to STAI. Estrous was defined as removal of $\geq 50\%$ of the grey coating from the Estroject[®] patch. Heifers assigned to FTAI were inseminated 54 h after PG and GnRH (100 μ g im gonadorelin acetate) was administered at AI. Heifers in the STAI treatment were inseminated 54 h after PG when considered in estus; however, AI was postponed until 78 h for heifers that were non-estrous at 54 h. Only heifers that failed to exhibit estrus by 78 h received GnRH at AI. Estrous response 54 h after PG did not differ

between treatments; however, total estrous response was increased ($P < 0.001$) among heifers assigned to STAI (74%, STAI; 47%, FTAI). Pregnancy rates resulting from AI were not different for heifers assigned to ST- compared to FTAI (48% and 46%, respectively; $P=0.6$), and were enhanced ($P < 0.05$) among heifers in the STAI treatment that exhibited estrus. In summary, STAI improved estrous response and pregnancy rate to AI following treatment with the MGA[®]-PG protocol. When compared to FTAI, STAI enhanced estrous response following the 7d CO-Synch + CIDR[®] protocol, however, this did not result in an increase in pregnancy rate to AI.

Key Words: beef heifer, estrus synchronization, split-time artificial insemination

INTRODUCTION

Estrus synchronization and fixed-time artificial insemination represent two of the more significant ways in which to manage beef herds reproductively, however, adoption of these technologies is limited (USDA-APHIS, 2008). In many cases, producers resist adopting new technologies, based on the perception that there is no benefit to use of the technology or the potential risk associated with their use (Elliot et al., 2013). Replacement heifers represent a unique class of females within a beef herd and are, in many respects, the simplest class of females in which reproductive technologies can be applied. Early conception increases the proportion of females that calve early in the calving season (Lesmeister et al., 1972; Cushman et al., 2013), and managing cows to calve early results in greater lifetime production (Lesmeister et al., 1972). Similarly,

managing heifers to conceive early in the first breeding season results in greater lifetime production (Cushman et al., 2013).

Recent research demonstrated a benefit to using split-time artificial insemination in beef heifers following treatment with the 14d CIDR[®]-PG estrus synchronization protocol (Thomas et al., 2014a; Thomas et al., 2014b). This approach to FTAI is based on the concept that females that express estrus prior to insemination experience greater reproductive success as a result of increased estrous response and pregnancy rates that result from AI (Perry et al., 2005; Thomas et al., 2014b; Bishop et al., 2016; Richardson et al., 2016; Bishop et al., 2017a; Bishop et al., 2017b). When STAI is utilized, females that are non-estrous at the standard time of FTAI are given an additional opportunity to express estrus prior to insemination 20 to 24 h later (Thomas et al., 2014b; Bishop et al., 2016; Bishop et al., 2017a; Bishop et al., 2017b). In addition, STAI reduces the use of GnRH to induce ovulation, as only those females that are non-estrous at the delayed time point receive GnRH (Bishop et al., 2016; Bishop et al., 2017a; Bishop et al., 2017b). Although benefits that result from use of split-time AI were reported following administration of the 14d CIDR[®]-PG protocol, research regarding use of STAI has been limited in terms of the various other protocols currently recommended to synchronize estrus in beef heifers.

The following experiments were therefore designed to compare split-time and fixed-time AI following administration of the MGA[®]-PG (Experiment 1) and 7d CO-Synch + CIDR[®] (Experiment 2) protocols in beef heifers. Comparisons were made on the basis of estrous response and pregnancy rate to AI following administration of each of the two protocols and on the basis of estrous cyclicity status of heifers prior to the

initiation of treatment. We hypothesized that split-time AI would result in an increase in estrous response and pregnancy rates to AI following administration of each of these two protocols.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee.

Experiment 1

Experimental design

Yearling, Angus, beef heifers (n=524) at a single location were managed in 10 pens and assigned within pen to one of two balanced treatments based on weight and reproductive tract score (RTS; scale 1-5). Heifers that were assigned a RTS of one were not retained for the trial (n=7). All heifers were placed on the melengestrol acetate (MGA[®]) prostaglandin F_{2α} protocol to synchronize estrus and assigned to one of two treatments. Heifers were fed MGA[®] (0.5 mg·animal⁻¹·d⁻¹) in a 1.8 kg grain carrier for 14 d. Prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) was administered 19 d after MGA[®] withdrawal, and estrus detection aids (EstroTECT[®]) were applied at the time PG was administered. Estrous status was recorded at 72 h after PG for heifers assigned to

FTAI, and 72 and 96 h after PG for heifers assigned to STAI. Estrous was defined as removal of $\geq 50\%$ of the grey coating from the Estroject[®] patch. Heifers assigned to FTAI were inseminated 72 h after PG and GnRH (100 μg im gonadorelin acetate) was administered at AI. Heifers in the STAI treatment were inseminated 72 h after PG based on expressed estrus; however, AI was postponed until 96 h for heifers that were non-estrous at 72 h. Only heifers that failed to exhibit estrus 96 h after PG received GnRH at AI. Inseminations were performed by one of two experienced technicians that were assigned on the basis of the heifer's weight, reproductive tract score, and treatment. Inseminations were performed using conventional semen from a single sire. Heifers were exposed for breeding for a period of 60 days beginning two weeks after FT- or STAI was performed.

Pregnancy diagnosis

Pregnancy rate resulting from FT- or STAI and final pregnancy rate were determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell WA). Pregnancy diagnoses were performed 90d following insemination. All ultrasounds were performed by or under the supervision of a licensed veterinarian. Pregnancies resulting from AI service were determined on the basis of fetal size (Stroud, 2006).

Statistical Analyses

Treatment differences for RTS and weight were analyzed using the TTEST procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in weight based on cyclicity status were analyzed using the TTEST procedure of SAS (SAS Inst. Inc., Cary, NC). Chi-square contingency tables (PROC FREQ; SAS Inst. Inc., Cary, NC) were used to determine differences between treatments in estrous response 72 h after PG. Differences between treatments relative to estrous response at 72 h, overall estrous response, pregnancy rate to AI, and pregnancy rate at the end of the 60 d breeding season were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment, estrous cyclicity status (estrous cycling, RTS=4 or 5; non-cycling, RTS = 2 or 3), and the treatment x estrous cyclicity status interaction included in the model. Differences in AI pregnancy rates by technician were determined using the GLIMMIX procedure of SAS with treatment, technician, and the treatment x technician interaction included in the model (PROC GLIMMIX; SAS Inst. Inc., Cary, NC).

Experiment 2

Experimental design

Yearling Angus and Angus crossbred beef heifers (n=456) at one location were managed in three pens and assigned within pen to one of two treatments that were balanced based on weight and reproductive tract score (RTS; Scale 1-5). One pen did not have access to a scale and balanced treatments were assigned based on RTS. Heifers that were assigned a RTS of one or two were not retained for the trial (n=4). All heifers were

subject to the 7d CO-Synch + CIDR[®] protocol to synchronize estrus and assigned to one of two treatments. Gonadotropin-releasing hormone (100 µg gonadorelin acetate) was administered at CIDR[®] insertion [1.38g progesterone (P₄); Zoetis, Madison, NJ], and CIDR[®] inserts were removed 7 d later. Prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) was administered and estrus detection aids (Estroject[®]) were applied coincident with CIDR[®] removal on day 7. Estrous status was recorded 54 h after PG for heifers assigned to FTAI, and 54 and 78 h after PG for heifers assigned to STAI. Estrous was defined as removal of ≥ 50% of the grey coating from the Estroject[®] patch. Heifers assigned to the FTAI treatment were inseminated 54 h after PG, and GnRH (100 µg im gonadorelin acetate) was administered at AI. Heifers in the STAI treatment were inseminated 54 h after PG based on having expressed estrus; however, AI was postponed until 78 h for heifers that were non-estrous at 54 h. Only heifers that failed to exhibit estrus by 78 h received GnRH at AI. Heifers were inseminated by one of three experienced technicians. Technicians were preassigned to perform inseminations based on RTS, weight, and treatment. Conventional semen from a single sire was used. Heifers were exposed to natural service 10 d following AI for a 60 day breeding season.

Pregnancy diagnosis

Pregnancy rate resulting from FT- or STAI and final pregnancy status were determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell WA). Pregnancy diagnoses were performed approximately 90d after insemination. All ultrasounds were performed by or under the supervision of a licensed veterinarian. Pregnancies resulting from artificial

insemination or natural service were distinguished from one another on the basis of fetal size (Stroud, 2006).

Statistical Analyses

Treatment differences for RTS and weight were analyzed using the TTEST procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in weight based on cyclicity status were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Differences between treatments relative to estrous response at 54 h, overall estrous response, pregnancy rate to AI, and pregnancy rate at the end of the 60 d breeding season were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment, estrous cyclicity status (estrous cycling, RTS=4 or 5; non-cycling, RTS = 3), and the treatment x estrous cyclicity status interaction included in the model. Differences in AI pregnancy rates by technician were determined using the GLIMMIX procedure of SAS with treatment, technician, and the treatment x technician interaction included in the model (PROC GLIMMIX; SAS Inst. Inc., Cary, NC).

RESULTS

Experiment 1

Treatments and Animals

An overall treatment summary is presented in Table 2.1; treatments were balanced within each of the 10 pens on the basis of RTS and weight. The average reproductive tract score for heifers assigned to each of the two treatments was 3.9 ± 0.06 (mean \pm SE). Average weights of heifers did not differ between treatments; average weights were 333 ± 2.4 kg and 331 ± 2.4 kg for heifers assigned to FT- or STAI treatments, respectively (mean \pm SE). Heifers that were determined to be estrous-cycling prior to MGA[®] feeding (RTS = 4 or 5; 341 ± 2.0 kg) weighed more ($P < 0.0001$) compared to peripubertal heifers (RTS = 2 or 3; 313 ± 2.6 kg).

Estrous response

A summary of estrous response rates for heifers assigned to FT- and STAI treatments is presented in Table 2.2. Estrous response at 72 h did not differ ($P = 0.8$) between treatments (74%, STAI; 72%, FTAI); however, total estrous response was increased ($P < 0.001$) among heifers assigned to STAI (88%, STAI; 72%, FTAI).

Furthermore, no difference ($P=0.2$) was noted between overall estrous response based on estrous cyclicity status. Heifers that were determined to be estrous cycling or non-cycling in the FTAI treatment prior to MGA[®] feeding achieved estrous response rates of 73% and 72%, respectively; whereas, heifers that were estrous cycling or non-cycling prior to MGA[®] feeding in the STAI treatment achieved overall estrous response rates of 89% and 85%, respectively.

Pregnancy rates

Pregnancy rates resulting from artificial insemination were greater ($P < 0.04$) for heifers assigned to ST- compared to FTAI (55% vs 46%, respectively), and were enhanced ($P < 0.05$) among heifers that exhibited estrus (Table 2.3). Within treatment there were no differences in pregnancy rate to AI based on pre-treatment estrous cyclicity status, and there were no differences ($P = 0.4$) between technicians in pregnancy rates resulting from AI. Pregnancy rates at the end of the breeding season were similar ($P=0.1$) between treatments (82%, STAI; 81%, FTAI; Table 2.4).

Experiment 2

Treatments and Animals

An overall treatment summary is presented in Table 2.5; treatments were balanced within pen on the basis of RTS and weight. The average reproductive tract score for heifers assigned to each of the two treatments was 4.3 ± 0.07 (mean \pm SE). Average weights of heifers did not differ between treatments; average weights were 395 ± 2.5 kg and 395 ± 2.7 kg for heifers assigned to FT- or STAI treatments, respectively (mean \pm SE). Heifers that were determined to be estrous-cycling prior to CIDR[®] insertion (RTS = 4 or 5; 399 ± 2.0 kg) weighed more ($P < 0.01$) than peripubertal heifers (RTS = 3; 377 ± 3.7 kg).

Estrous response

A summary of estrous response rates for heifers assigned to FT- and STAI treatments is presented in Table 2.6. Estrous response at 54 h did not differ ($P = 0.3$) between treatments (51%, STAI; 47%, FTAI); however, total estrous response was increased ($P < 0.001$) among heifers assigned to STAI (74%, STAI; 47%, FTAI). In addition, estrous response rates were higher among heifers that were assigned to STAI that were estrous cycling prior to progestin treatment ($P < 0.03$; 79%, estrous cycling; 57%, non-cycling).

Pregnancy rates

Pregnancy rates resulting from fixed-time and split-time artificial insemination are shown in Table 2.7. Pregnancy rate to AI did not differ ($P = 0.4$) between treatments (46%, FTAI; 48%, STAI). In addition, there were no statistical differences in pregnancy rates resulting from AI within treatment based on estrous cyclicity status (assigned prior to CIDR[®] insertion) of heifers. Pregnancy rates resulting from AI did not differ between heifers that exhibited estrus or that failed to exhibit estrus at the time FTAI was performed ($P > 0.05$; 51%, estrous; 41%, non-estrous); however, pregnancy rates to AI were higher among heifers in the STAI treatment that exhibited estrus prior to AI ($P < 0.05$; 53%, estrous; 31%, non-estrous). There were no differences ($P = 0.7$) among technicians in pregnancy rates resulting from AI, and pregnancy rates at the end of the breeding season (Table 2.8) were similar between treatments ($P = 0.6$; FTAI, 79%; STAI, 81%).

DISCUSSION

Split-time artificial insemination was developed to improve pregnancy rates to TAI by inseminating females at a more precise time based on estrus expression (Thomas et al. 2014a, b). This approach to TAI was shown to improve estrous response and pregnancy rates in beef heifers and was used successfully with sex-sorted semen (Thomas et al., 2014a, b; Bishop et al., 2016; Bishop et al., 2017a; Thomas et al., 2017). Pregnancy rates were higher among heifers assigned to ST- versus FTAI treatments following administration of the 14d CIDR[®]-PG protocol, with the greatest increase seen among heifers that were non-estrous at 66 hour after PG. Here, the increase in pregnancy rate was seen among heifers that were non-estrous at 66 hours, in which case insemination was postponed by 24 hours (STAI, 49%; FTAI, 34%; Thomas et al., 2014b). Improvement in pregnancy rates resulting from STAI occurred as a result of an increase in estrous response that occurs during the delay period (Thomas et al., 2014b; Bishop et al., 2016).

In this study, estrous response rates 72 h after PG were similar for heifers assigned to FT- or STAI treatments following administration of the MGA[®]-PG protocol (74%, STAI; 72%, FTAI). However, by postponing insemination 24 h for heifers in the STAI treatment that failed to exhibit estrus by 72, overall estrous response increased 16% compared to heifers in the FTAI treatment (72%, FTAI; 88%, STAI).

Figure 2.3 illustrates estrus distribution patterns obtained using HeatWatch[®] (Wood et al., unpublished data; Mallory et al., 2010) following synchronization of estrus with the MGA[®]-PG protocol. Overall estrous response rates from these combined studies is illustrated by the respective time intervals at which STAI was performed in the current

study (72 and 96 h after PG). The figure clearly illustrates the similarity in estrous response rates recorded using HeatWatch[®] over a six day period to estrous response rates recorded in Experiment 1 using Estroject[®] patches to determine estrous status by 72 or 96 h. Based on the data presented in Figure 2.3, approximately 70% of heifers exhibited estrus by 72 h after PG with an additional 12% of the heifers exhibiting estrus by 96 h. Similarly, in the STAI treatment in Experiment 1, 74% of the heifers exhibited estrus by 72 h with an additional 14% of the heifers exhibiting estrus by 96 h.

Pregnancy rates resulting from AI differed between treatments in Experiment 1 and were higher for heifers in split-time compared to fixed-time AI treatments (STAI, 55%; FTAI, 46%). Furthermore, pregnancy rates to AI were enhanced among heifers that exhibited estrus. In the FTAI treatment, 52% of the estrous heifers and 30% of the non-estrous heifers conceived to AI; whereas 60% of the estrous heifers and 22% of the non-estrous heifers conceived to AI in the STAI treatment. These results support previous studies which showed an improvement in pregnancy rates after STAI that occurred as a result of the reduced number of females that are classified as non-estrous (STAI, 12%; FTAI, 28%; Thomas et al., 2014b; Bishop et al., 2016). Pregnancy rates to AI are consistent with other reports following the MGA[®]-PG estrus synchronization protocol (Bonacker et al., 2018), although a recent study (Vraspir et al., 2014) reported higher pregnancy rates compared to those reported by Bonacker et al. (2018). Pregnancy rates reported among heifers assigned to the STAI treatment (55%) in this study are similar to those reported by Mallory et al. (2010) following inseminations performed on the basis of detected estrus. Consideration of these data supports the point that comparable pregnancy rates can be achieved by inseminating heifers at one of two fixed time points using a

split-time AI approach, compared to detecting estrus and inseminating heifers over a six day period.

Results from Experiment 1 showed no difference in estrous response or pregnancy rates resulting from AI between heifers that were classified as estrous cycling or pre- or peri-pubertal prior to MGA[®] feeding. These similarities may be attributed to successful induction of puberty that occurred in pre- or peri-pubertal heifers after treatment with MGA[®] (Gonzalez-Padilla et al., 1975b; Patterson et al., 1990; Mallory et al., 2010). Because no differences were noted between treatments based on pre-treatment estrous cyclicity status of heifers in Experiment 1, higher pregnancy rates for heifers in the STAI treatment can be largely attributed to the increased estrous response that occurred during the delay period.

Experiment 2 was designed to compare fixed-time to split-time AI approaches following administration of the short-term, 7d CO-Synch + CIDR[®] protocol in heifers. Estrous response rates that were observed 54 hours after CIDR[®] removal and PG in this study are similar to those reported previously (Lamb et al., 2006; Busch et al., 2007; Leitman et al., 2008). Estrous response was increased by 23% among heifers in the STAI treatment by delaying insemination 24 hours for heifers that were non-estrous 54 hours after CIDR[®] removal and PG. However, in contrast to what occurred in Experiment 1, the increase in estrous response did not result in an increase in pregnancy rates resulting from AI.

Pregnancy rates observed in Experiment 2 that resulted from split-time (48%) or fixed-time AI (46%) were consistent with previous studies following use of the 7d CO-Synch + CIDR[®] protocol in heifers (Busch et al., 2007; Bonacker et al., 2018). However,

these results contrast those reported in Experiment 1, and raise important questions regarding the absence of difference in pregnancy rates despite increased estrous response using split-time versus fixed-time AI approaches (Thomas et al., 2014b; Bishop et al., 2016; Richardson et al., 2016). Additionally, there appears to be a trend toward higher pregnancy rates resulting from AI among heifers that were estrous cycling prior to CIDR[®] insertion; this, again, contrasts results from Experiment 1.

Differences in results between Experiments 1 and 2 may have occurred as a result of differences that occur following treatment with long- versus short-term progestin-based protocols. Although short-term treatment with a progestin was reported to effectively induce estrous cyclicity in pre- or peri-pubertal heifers (Gonzalez-Padilla et al., 1975b; Short et al., 1976; Sheffield and Elliott, 1982; Patterson et al., 1990; Hall et al., 1997), there may be an advantage to long-term treatments when considering resulting pregnancy outcomes (Busch et al., 2007; Kasimanickam et al., 2015; Bonacker et al., 2018). This point is worth considering based on the observation that fertility associated with the first pubertal ovulation is typically reduced compared to subsequent ovulations (Byerley et al., 1987a; Perry, 2016). Based on these differences, it is reasonable to assume that long-term progestin-based protocols may offer an advantage over short-term protocols, since inseminations are not performed on the pubertal estrus and ovulation. Another point worth considering relates to when insemination is performed with respect to when treatment with a progestin ends. Because inseminations are performed on the first estrus after CIDR[®] removal (7-day CO-Synch + CIDR[®]) versus the second estrus following MGA[®] withdrawal (MGA[®]-PG), reduced fertility following short- versus long-

term progestin treatment may be related to the presence and resulting ovulation of a persistent follicle that forms during progestin treatment.

Persistent follicles develop during short- or long-term progestin treatment and form in relation to stage of the estrous cycle when treatment begins (Beal et al., 1988; Patterson et al., 1989a). As a result, a portion of the heifers in Experiment 2 may have been inseminated after ovulation of a persistent follicle. This possibility is worth considering given the variability in response to GnRH in heifers compared to cows (Thatcher et al., 1993; Pursley et al., 1995; Twagiramungu et al., 1995; Atkins et al., 2008), and may have contributed to similarity in pregnancy rates between treatments despite differences in estrous response.

Collectively, results from Experiment 1 using split-time AI following treatment with the MGA[®]-PG protocol support results following administration of the 14d CIDR[®]-PG protocol in heifers (Thomas et al. 2014a,b; Bishop et al., 2016); in both cases long-term progestin-based protocols were used. Improvements in pregnancy rates that resulted from a split-time AI approach in both cases resulted from an increase in estrous response. This however, was not the case in Experiment 2, wherein the increase in estrous response using a split-time AI approach did not translate into an associated increase in pregnancy outcome. These results raise interesting questions pertaining to use of long- versus short-term progestin-based protocols to synchronize estrus in replacement beef heifers. These experiments highlight considerations related to fixed- versus split-time AI approaches, relationships to the type of progestin-based protocol that is used (long- versus short), and the pubertal status (estrous cycling versus pre- or peri-pubertal) of heifers at the time

treatment with a progestin is initiated. Clearly, more research is needed to elucidate factors that contribute to these various differences.

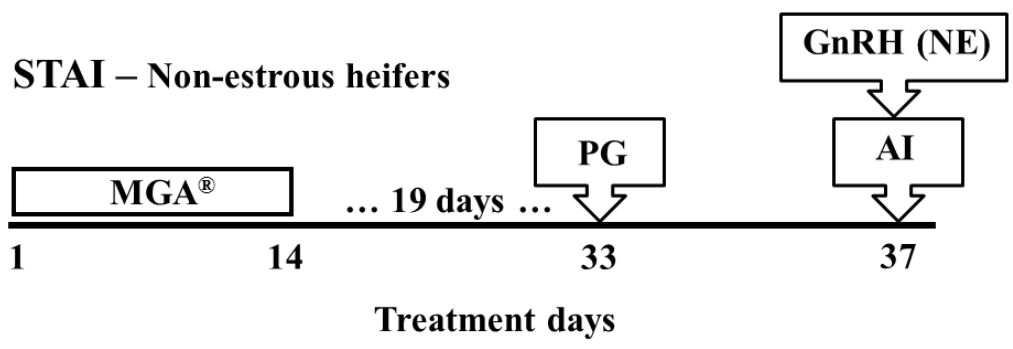
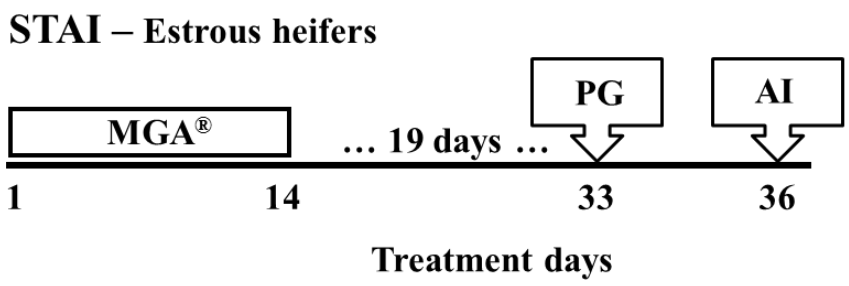
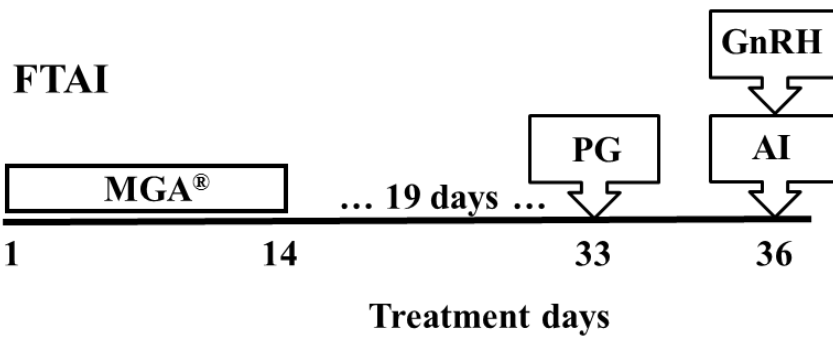
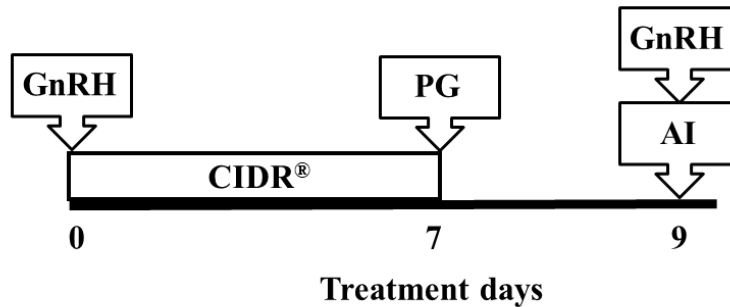
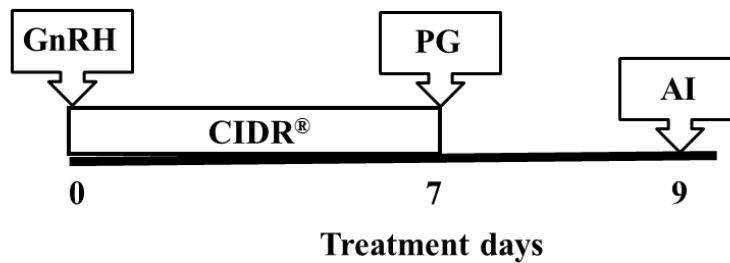


Figure 2.1. Treatment schedules for Experiment 1. Top schedule is the fixed-time artificial insemination (FTAI) treatment and bottom schedule is the split-time artificial insemination (STAI) treatment for the heifers classified as estrous (top) or non-estrous (bottom) at 72 h. Adapted from the MGA[®]-PG protocol for FTAI (Lamb et al., 2000). MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day, prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administration on day 19, and FTAI at 72 h following PG concurrent with gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate) administration. Heifers in the STAI treatment were inseminated at 72 h if an activated EstroTECT[®] was observed. If heifers in the STAI treatment did not have an activated EstroTECT[®], AI was postponed to 96 h. Only heifers in the STAI treatment that were non-estrous at 96 h received GnRH. NE = Non-estrous

FTAI



STAI – Estrous heifers



STAI – Non-estrous heifers

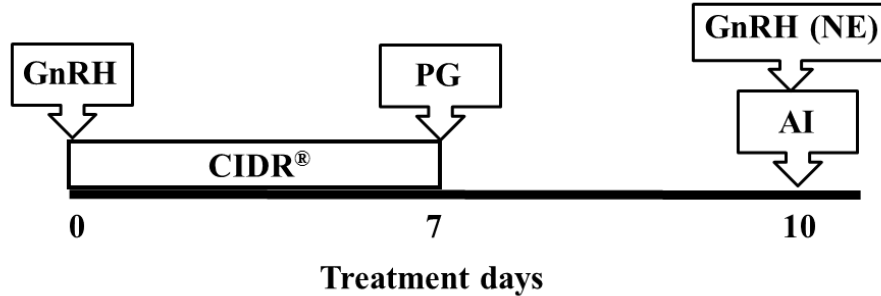


Figure 2.2. Treatment schedules for Experiment 2. Top schedule is the FTAI treatment and bottom schedule is the STAI treatment for heifers classified as estrous (top) or non-estrous (bottom) at 54 h. Adapted from the 7d CO-Synch + CIDR[®] protocol (Lamb et al., 2001; Lamb et al., 2006). This protocol consists of a 7 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone), gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate) administration at CIDR[®] insertion, and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered at CIDR[®] removal on day 7. Heifers in the FTAI treatment were inseminated at 54 h following PG concurrent with GnRH (100 µg im gonadorelin acetate) administration. Heifers in the STAI treatment were inseminated at 54 h if an activated EstroTECT[®] was observed. If heifers in the STAI treatment did not have an activated EstroTECT[®], AI was postponed to 78 h. Only heifers in the STAI treatment that were non-estrous at 78 h received GnRH. NE = Non-estrous

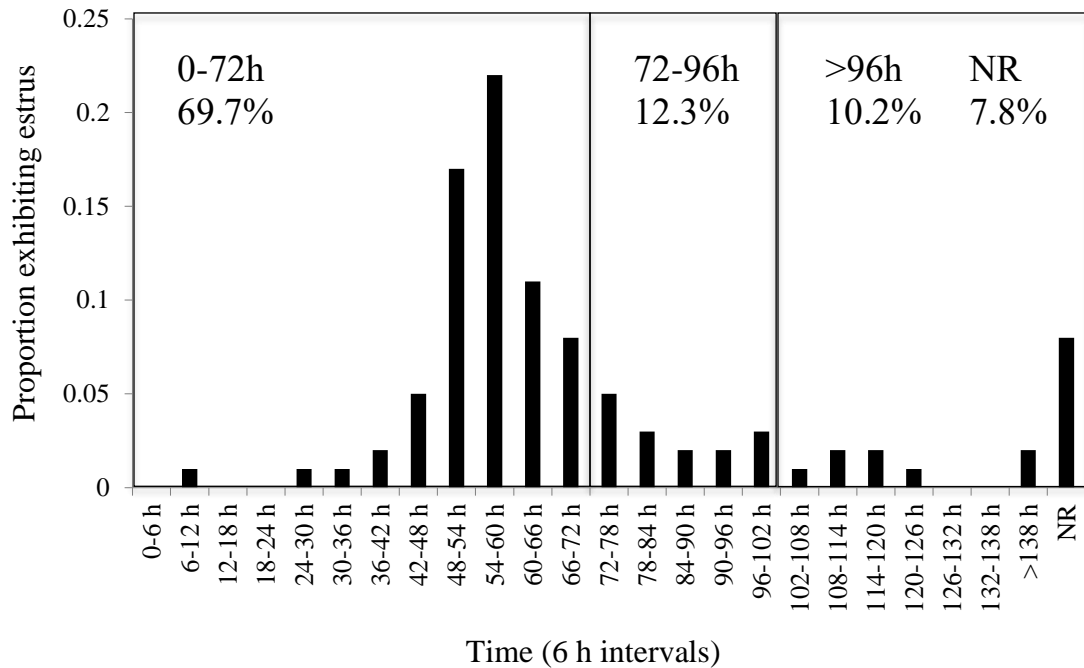


Figure 2.3. Distribution of estrus following the MGA[®]-PG estrus synchronization protocol. Proportion of heifers (N=373) exhibiting estrus by 6 h intervals and subdivided by relative insemination times for split-time artificial insemination (72 or 96 h). MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day and prostaglandin F_{2α} (PG) on day 19. Figure includes Heatwatch[®] data (Wood et al., unpublished; Mallory et al., 2010). NR=Non-responders

Table 2.1. Number of heifers, mean weight (Wt), and mean reproductive tract score (RTS) by treatment^a for Experiment 1^b.

	N	RTS ^c	Wt ^{c,d}
FTAI	265	3.9 ± 0.06	333 ± 2.4
STAI	259	3.9 ± 0.06	331 ± 2.4

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 72 h following PG concurrent with GnRH (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate) administration. Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 72 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 96 h. Only heifers in the STAI treatment that were non-estrous at 96 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of STAI and FTAI following the MGA[®]-PG estrus synchronization protocol. MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) on day 19.

^cData presented as mean values ± SE.

^dWt recorded in kg.

Table 2.2. Estrous response within treatment^a for Experiment 1^b based on estrous cyclicity status of heifers prior to MGA[®].

		Estrous cycling ^c		Non-cycling ^d		Total	
		N	%	N	%	N	%
	Estrous by 72 h	116/159	73	76/106	72	192/265	72
FTAI	Estrous by 96 h ^e	—	—	—	—	—	—
	Overall	116/159	73 ⁱ	76/106	72 ⁱ	192/265	72 ⁱ
	Estrous by 72 h	118/158	75	73/101	72	191/259	74
STAI	Estrous by 96 h	23/40	58	13/28	46	36/68	53
	Overall	141/158	89 ^j	86/101	85 ^j	227/259	88 ^j

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 72 h following PG concurrent with GnRH administration (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate). Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 72 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 96 h. Only heifers in the STAI treatment that were non-estrous at 96 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of STAI and FTAI following the MGA[®]-PG estrus synchronization protocol. MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) on day 19.

^cEstrous cycling heifers defined as having a RTS=4 or 5 prior to MGA[®] feeding.

^dNon-cycling heifers defined as having a RTS=2 or 3 prior to MGA[®] feeding.

^eHeifers inseminated at 72 h and no estrous response data was recorded at 96 h.

^{i,j}Values within column with different superscripts differ (P<0.001).

Table 2.3. Pregnancy rates to AI within treatment^a for Experiment 1^b based on estrous cyclicity status of heifers prior to MGA[®].

		Estrous cycling ^c		Non-cycling ^d		Total	
		N	%	N	%	N	%
	Overall estrous ^e	60/116	52	40/76	53	100/192	52 ⁱ
FTAI	Overall non-estrous ^f	12/43	28	10/30	33	22/73	30 ^j
	Total	72/159	45	50/106	47	122/265	46 ^m
	Estrous by 72 h	70/118	59	44/73	60	114/191	60
	Non-estrous by 72 h	16/40	40	13/28	46	29/68	43
	Estrous by 96 h	14/23	61	8/13	62	22/36	61
STAI	Non-estrous by 96 h	2/17	12	5/15	33	7/32	22
	Overall estrous	84/141	60	52/86	60	136/227	60 ⁱ
	Overall non-estrous	2/17	12	5/15	33	7/32	22 ^j
	Total	86/158	54	57/101	56	143/259	55 ⁿ

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 72 h following PG concurrent with GnRH (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate) administration. Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 72 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 96 h. Only heifers in the STAI treatment that were non-estrous at 96 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of STAI and FTAI following the MGA[®]-PG estrus synchronization protocol. MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) on day 19.

^cEstrous cycling heifers defined as having a RTS=4 or 5 prior to MGA[®] feeding.

^dNon-cycling heifers defined as having a RTS=2 or 3 prior to MGA[®] feeding.

^ePregnancy rate to AI for overall estrous heifers equals pregnancy rate to AI for estrous heifers by 72 h.

^fPregnancy rate to AI for overall non-estrous heifers equals pregnancy rate to AI for non-estrous heifers by 72 h.

^{ij}Values within column with different superscripts differ (P<0.05).

^{m,n}Values within column with different superscripts differ (P<0.04).

Table 2.4. Pregnancy rates resulting from AI and at the end of a 60d breeding season for heifers that were assigned to fixed- or split-time AI treatments^a in Experiment 1^b and based on estrous cyclicity status prior to MGA[®].

Estrous Status	FTAI				STAI			
	AI		Overall		AI		Overall	
	N	%	N	%	N	%	N	%
Estrous cycling ^c	72/159	45	131/159	82	86/158	54	126/158	80
Non-cycling ^d	50/106	47	84/106	79	57/101	56	86/101	85
Total	122/265	46 ⁱ	215/265	81	143/259	55 ^j	212/259	82

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 72 h following PG concurrent with GnRH (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate) administration. Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 72 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 96 h. Only heifers in the STAI treatment that were non-estrous at 96 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of STAI and FTAI following the MGA[®]-PG estrus synchronization protocol. MGA[®]-PG consists of melengestrol acetate (MGA[®]) feeding for 14 d at a rate of 0.5 mg/head/day and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) on day 19.

^cEstrous cycling heifers defined as having a RTS=4 or 5 prior to MGA[®] feeding.

^dNon-cycling heifers defined as having a RTS=2 or 3 prior to MGA[®] feeding.

^{ij}Values within row with different superscripts differ (P<0.03).

Table 2.5. Number of heifers, mean weight (Wt), and mean reproductive tract score (RTS) by treatment^a for Experiment 2^b.

	N	RTS ^c	Wt ^{c,d}
FTAI	229	4.3 ± 0.07	395 ± 2.5
STAI	227	4.3 ± 0.07	395 ± 2.7

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 54 h following PG concurrent with GnRH (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate) administration. Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 54 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 78 h. Only heifers in the STAI treatment that were non-estrous at 78 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of FTAI to STAI following the 7d CO-Synch + CIDR[®] estrus synchronization protocol. The 7d CO-Synch + CIDR[®] protocol consists of a 7 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone), with GnRH administered (100 µg im gonadorelin acetate) at CIDR[®] insertion, and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered at CIDR[®] removal, on day 7.

^cData presented as mean values ± SE.

^dWt recorded in kg.

Table 2.6. Estrous response rates of heifers within treatment^a for Experiment 2^b based on estrous cyclicity status prior to CIDR[®] insertion.

		Estrous cycling ^c		Non-cycling ^d		Total	
		N	%	N	%	N	%
	Estrous by 54 h	94/187	50	14/42	33	108/229	47
FTAI	Estrous by 78 h ^e	—	—	—	—	—	—
	Overall	94/187	50 ^m	14/42	33	108/229	47 ^q
	Estrous by 54 h	94/180	52	21/47	45	115/227	51
STAI	Estrous by 78 h	48/86	56	6/26	23	54/112	48
	Overall	142/180	79 ^{i,n}	27/47	57 ^j	169/227	74 ^r

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 54 h following PG concurrent with GnRH administration (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate). Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 54 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 78 h. Only heifers in the STAI treatment that were non-estrous at 78 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of FTAI to STAI following the 7d CO-Synch + CIDR[®] estrus synchronization protocol. The 7d CO-Synch + CIDR[®] protocol consists of a 7 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone), with GnRH (100 µg im gonadorelin acetate) administered at CIDR[®] insertion, and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered at CIDR[®] removal, on day 7.

^cEstrous cycling heifers defined as having a RTS=4 or 5 prior to CIDR[®] insertion.

^dNon-cycling heifers defined as having a RTS=3 prior to CIDR[®] insertion.

^eHeifers inseminated at 54 h and no estrous response data was recorded at 78 h.

^{i,j}Values within row with different superscripts differ (P<0.03).

^{m,n}Values within column with different superscripts differ (P<0.002).

^{q,r}Values within column with different superscripts differ (P<0.001).

Table 2.7. Pregnancy rates of heifers resulting from AI within treatment^a for Experiment 2^b based on estrous cyclicity status prior to CIDR[®] insertion.

		Estrous cycling ^c		Non-cycling ^d		Total	
		N	%	N	%	N	%
	Overall estrous ^e	50/94	53	5/14	36	55/108	51
FTAI	Overall non-estrous ^f	39/93	42	11/28	39	50/121	41
	Total	89/187	48	16/42	38	105/229	46
	Estrous by 54 h	51/94	54	11/21	52 ^q	62/115	54
	Non-estrous by 54 h	42/86	49 ⁱ	4/26	15 ^{j,r}	46/112	41
	Estrous by 78 h	27/48	56	1/6	17	28/54	52
STAI	Non-estrous by 78 h	15/38	39	3/20	15	18/58	31
	Overall estrous	78/142	55	12/27	44	90/169	53 ^u
	Overall non-estrous	15/38	39	3/20	15	18/58	31 ^v
	Total	93/180	52 ^m	15/47	32 ^m	108/227	48

^aHeifers in the fixed-time artificial insemination (FTAI) treatment were inseminated at 54 h following PG concurrent with GnRH administration (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate). Heifers in the split-time artificial insemination (STAI) treatment were inseminated at 54 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 78 h. Only heifers in the STAI treatment that were non-estrous at 78 h received GnRH (100 µg im gonadorelin acetate).

^bComparison of FTAI to STAI following the 7d CO-Synch + CIDR[®] estrus synchronization protocol. The 7d CO-Synch + CIDR[®] protocol consists of a 7 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone), with GnRH (100 µg im gonadorelin acetate) administered at CIDR[®] insertion, and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered at CIDR[®] removal, on day 7.

^cEstrous cycling heifers defined as having a RTS=4 or 5.

^dNon-cycling heifers defined as having a RTS=3.

^{i,j}Values within row with different superscripts differ (P<0.02).

^{m,m}Values within row with the same superscripts did not differ (P=0.06).

^{q,r}Values within column with different superscripts differ (P<0.03).

^{u,v}Values within column with different superscripts differ (P<0.05).

Table 2.8. Pregnancy rates of heifers resulting from AI and at the end of a 60d breeding season in Experiment 2^a that were assigned to fixed- or split-time AI treatments^b and based on estrous cyclicity status prior to CIDR insertion.

Estrous Status	FTAI				STAI			
	AI		Overall		AI		Overall	
	N	%	N	%	N	%	N	%
Estrous cycling ^c	89/187	48	152/187	81	93/180	52 ⁱ	150/180	83
Non-cycling ^d	16/42	38	29/42	69	15/47	32 ⁱ	34/47	72
Total	105/229	46	181/229	79	108/227	48	184/227	81

^aComparison of fixed-time artificial insemination (FTAI) to split-time artificial insemination (STAI) following the 7d CO-Synch + CIDR[®] estrus synchronization protocol. The 7d CO-Synch + CIDR[®] protocol consists of a 7 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone), with GnRH (gonadotropin-releasing hormone; 100 µg im gonadorelin acetate) administered at CIDR[®] insertion, and prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered at CIDR[®] removal, on day 7.

^bHeifers in the FTAI treatment were inseminated at 54 h following PG concurrent with GnRH administration (100 µg im gonadorelin acetate). Heifers in the STAI treatment were inseminated at 54 h based on an activated Estroject[®]. If heifers in the STAI treatment did not have an activated Estroject[®], AI was postponed to 78 h. Only heifers in the STAI treatment that were non-estrous at 78 h received GnRH (100 µg im gonadorelin acetate).

^cEstrous cycling heifers defined as having a RTS=4 or 5.

^dNon-cycling heifers defined as having a RTS=3.

^{i,i}Values within column with the same superscripts did not differ but approached significance (P=0.06).

CHAPTER 3

ALTERING LENGTH OF THE PRESYNCHRONIZATION PERIOD WITH A PROGESTIN PRIOR TO PROSTAGLANDIN F_{2α} FOLLOWED BY TIMED ARTIFICIAL INSEMINATION IN BEEF HEIFERS

ABSTRACT

An experiment was designed to compare long-term progestin-based estrus synchronization protocols for use with split-time artificial insemination (STAI) in beef heifers. Heifers were assigned to either an 18d (Day 0 to 18) or 14d (Day 4 to Day 18) CIDR[®] treatment (1.38 g progesterone controlled internal drug release insert; Zoetis, Madison, NJ), with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered to heifers in both treatments 16 days after CIDR[®] removal (Day 34). Heifers (n = 193) at location one were evaluated over three consecutive years, and heifers at location two (n=649) were managed as two groups during the same year. Heifers at each location were assigned to treatment based on reproductive tract score (RTS; Scale 1-5) and weight. Heifers that were assigned a RTS of one were not retained for the trial (n=6). At location one, radiotelemetric pressure-sensitive devices (Heatwatch[®]) were applied to determine time of estrus onset following PG administration. In addition, estrus detection aids (EstroTECT[®]) were applied at PG to heifers at both locations. Estrous status was recorded at 66 and 90 h after PG. Estrous was defined as removal of ≥ 50% of the grey coating from the EstroTECT[®] patch, and STAI was performed at either 66 or 90 h after PG based on

timing of estrus onset (Heatwatch[®]; location one) or activation of the Estroject[®] patch. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate), which was administered at the time AI was performed. At location one, blood samples were collected from all heifers in both treatments at PG and AI, and transrectal ovarian ultrasound was performed to detail ovarian structures on a subset of heifers (n=73) at both time points. Estrous response 66 h after PG did not differ between treatments (P = 0.3; 60%, 14d CIDR[®]-PG; 60%, 18d CIDR[®]-PG), nor were any differences observed during the 24 h delay period from 66 to 90 h after PG (P=0.4; 60%, 14d CIDR[®]-PG; 60%, 18d CIDR[®]-PG). Pregnancy rate to STAI did not differ between treatments (P = 0.3; 52%, 14d CIDR[®]-PG; 50%, 18d CIDR[®]-PG), or at the end of the 60 d breeding season (P=0.2; 86%, 14d CIDR[®]-PG; 82%, 18d CIDR[®]-PG). There were no differences between treatments in mean dominant follicle diameters at PG (P=0.6; 10.9±0.4mm, 14d CIDR[®]-PG; 11.0±0.4mm, 18d CIDR[®]-PG) or at STAI (P=0.3; 12.6±0.4mm, 14d CIDR[®]-PG; 13.2±0.4mm, 18d CIDR[®]-PG), nor were any differences observed between treatments in concentrations of E₂ at PG (P=0.8; 1.1±0.19 pg/ml, 14d CIDR[®]-PG; 1.1±0.19 pg/ml, 18d CIDR[®]-PG) or STAI (P=0.6; 3.8±0.19 pg/ml, 14d CIDR[®]-PG; 3.6±0.19 pg/ml, 18d CIDR[®]-PG). These data suggest that similarities in responses observed among heifers based on length of CIDR[®] treatment provides flexibility in scheduling without compromising reproductive outcomes.

Key Words: split-time artificial insemination, beef heifer, estrus synchronization

INTRODUCTION

Long-term, progestin-based protocols have been used effectively to synchronize estrus in replacement beef heifers (Patterson et al., 2017). These protocols involve a period of pre-synchronization using an intravaginal progesterone insert (controlled internal drug release, CIDR[®]) or the orally active progestin, melengestrol acetate (MGA[®]). Recommendations for use of these protocols (14d CIDR[®]-PG and MGA[®]-PG) are based on past research that determined the optimal interval from progestin withdrawal to PG administration (Brown et al., 1988; Nix et al., 1998; Lamb et al., 2000; Deutscher, 2000), effectiveness of gonadotropin-releasing hormone (GnRH) as part of the treatment schedule (Wood et al., 2001; Kojima et al., 2004; Busch et al., 2007, Leitman et al., 2008, 2009a,b; Mallory et al., 2010, 2011), and the optimal time following administration of PG at which to perform fixed-time AI (Larson et al., 1996; Leitman et al., 2009a,b; Mallory et al., 2010, 2011). Furthermore, these protocols (14d CIDR[®]-PG and MGA[®]-PG) were compared and found to perform similarly when used to synchronize estrus in heifers prior to inseminations that were performed on the basis of detected estrus or when used in conjunction with fixed- or split-time AI (Mallory et al., 2010; Vraspir et al., 2014; Knickmeyer et al., 2018).

Aside from successfully synchronizing estrus in beef heifers that are pubertal or estrous cycling, pre-synchronization with a progestin offers the added benefit of effectively inducing estrous cyclicity among pre- or peripubertal heifers (Gonzalez-Padilla et al., 1975b; Short et al., 1976; Sheffield and Elliott, 1982; Patterson et al., 1990; Hall et al., 1997; Mallory et al., 2010). Although short-term treatment with a progestin

was reported to effectively induce estrous cyclicity in heifers that have not yet reached puberty (Gonzalez-Padilla et al., 1975b; Short et al., 1976; Sheffield and Elliott, 1982; Patterson et al., 1990; Hall et al., 1997), there may be an advantage to long-term treatments when considering resulting pregnancy outcomes (Busch et al., 2007; Kasimanickam et al., 2015; Bonacker et al., 2018). The mechanism by which a 14 d progestin treatment with CIDR[®] or MGA[®] results in a highly synchronized expression of estrus in heifers is well characterized among pubertal or estrous cycling heifers. Treatment with a progestin does not prevent luteolysis; therefore, cyclic females in the luteal phase of the estrous cycle undergo luteolysis during progestin treatment, dependent upon the day or stage of cycle at the time progestin treatment began (Sirois and Fortune, 1990). In the absence of endogenous luteal progesterone, treatment with a progestin inhibits atresia of a dominant follicle (Beal et al., 1988; Patterson et al., 1989a; Sirois and Fortune, 1990) which is maintained as a persistent follicle that can be expected to ovulate after progestin withdrawal (Sirois and Fortune, 1990; Kinder et al., 1996). Ovulation of a persistent follicle is generally regarded to result in compromised fertility resulting from ovulation of an aged oocyte (Patterson et al., 1986; Kinder et al., 1996). For this reason, producers are advised to postpone breeding to the estrus that occurs following the administration of PG, subsequent to progestin withdrawal. In estrous cycling females, differences in fertility following short-term progestin-based protocols is largely based on day or stage of the cycle at treatment initiation (Patterson et al., 1986; Sirois and Fortune, 1990).

Protocols designed to synchronize estrus should result in a highly synchronized estrous response that achieves optimal fertility with limited animal handlings. There is

some evidence to suggest that extended treatment with a progestin compared to short-term treatments may be more advantageous when considering pre- or peripubertal females and the potential for puberty induction (Busch et al., 2007; Bonacker et al., 2018). Furthermore, extending the period of treatment with a progestin provides estrous cycling females, depending upon stage of cycle, an appropriate amount of time to regress CL during treatment and develop persistent follicles. Although past research supports a 14d treatment period when using a long-term CIDR[®]-based protocol, there is limited research regarding the implications of extending the treatment period beyond 14 days (Macmillan and Peterson, 1993). This experiment was therefore designed to compare the 14d CIDR[®]-PG protocol to an extended progestin treatment for 18 days (18d CIDR[®]-PG) followed by split-time artificial insemination in replacement beef heifers.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee.

Experimental design

Heifers were assigned to either an 18d (Day 0 to 18) or 14d (Day 4 to Day 18) CIDR[®] treatment (1.38 g progesterone controlled internal drug release insert; Zoetis, Madison, NJ), with prostaglandin F_{2α} (PG; 250 μg im cloprostenol sodium) administered

to heifers in both treatments 16 days after CIDR[®] removal (Day 34). Angus heifers (n = 193) at location one were evaluated over three consecutive years, and Angus and Red Angus heifers at location two (n=649) were managed as two groups during the same year. Heifers at each location were assigned to treatments based on reproductive tract score (RTS; Scale 1-5) and weight. Heifers that were assigned a RTS of one were not retained for the trial (n=6). At location one, radiotelemetric pressure-sensitive devices (Heatwatch[®]) were applied to characterize timing of estrus onset. At both locations, estrus detection aids (Estroject[®]) were applied at PG. Estrous status was recorded at 66 h and 90 h after PG. Estrous was defined as removal of $\geq 50\%$ of the grey coating from the Estroject[®] patch at both locations and split-time artificial insemination (STAI) was performed at either 66 h or 90 h after PG based on timing of estrus onset (Heatwatch[®]; location one) or activation of the Estroject[®]. Only heifers that were non-estrous at 90 h received GnRH (100 μ g gonadorelin acetate) concurrent with AI. At location one, blood samples were collected from all heifers in both treatments at PG and AI and transrectal ovarian ultrasound was performed on a subset of heifers (n=73) at PG and AI. Technicians were preassigned to perform inseminations based on RTS, weight, and treatment. Conventional semen from a single sire was used each year at location one; whereas, semen from eight sires was used at location two and assigned equally to treatments based on heifer weight and RTS. Heifers were exposed for natural service one or two weeks following AI for a 60 day breeding season at locations two or one, respectively.

Pregnancy diagnosis

Pregnancy rate resulting from STAI and final pregnancy status were determined by transrectal ultrasonography. Pregnancy diagnoses were performed 90 d (location one; SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell WA) or 80 d (location two; Aloka 500V with a 5MHz linear-array transducer; Corometrics Inc., Wallingford, CT) after insemination. All ultrasounds were performed by or under the supervision of a licensed veterinarian. Pregnancies resulting from artificial insemination versus natural service were distinguished from one another on the basis of fetal size. At location two, crown to nose or crown to rump measurements were taken on every fetus to more precisely determine age (Stroud, 2006).

Blood sampling and RIA.

At location one, blood samples were collected from all heifers (n=192) at PG administration and at STAI to determine serum estradiol-17 β (E₂) concentrations. Blood samples were allowed to clot and stored at 4 °C for 24 h, with serum collected by centrifugation and stored at -20 °C. Samples were assayed in duplicate for E₂, using a single-antibody charcoal extraction RIA, as described by Kirby et al. (1997). The assays utilized estradiol antibody (#07-138216; MP Biomedicals) and 3-¹²⁵Iodo-Estradiol-17 β (#07138226; MP Biomedicals). Separation of bound and free estradiol was done through charcoal extraction using dextran-coated charcoal solution followed by centrifugation at 2,500 g. The supernatant was poured off and counted for 5 minutes in the gamma counter. Four estradiol assays were performed over two weeks. The intra-assay and inter-assay coefficients of variation were 9% and 5%, respectively. Sensitivity of the assay was 0.5 ng/ml.

Ovarian Ultrasonography.

At location one, transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA) was performed for a subset of heifers (n = 73) to assess ovarian follicle size. Follicles were measured and measurements reported are the largest follicle diameter (LFD). Ultrasonography was performed at PG and STAI.

Statistical Analyses

Treatment differences for RTS and weight were analyzed using the TTEST procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in estrous cyclicity status based on RTS (estrous cycling, RTS=4 or 5; non-cycling, RTS =2 or 3) and weight between treatments were analyzed by ANOVA using the linear statistical model of year, location, treatment, and all relevant interactions using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in estrous response at 66 h, estrous response during the delay period (66 h to 90 h), and overall estrous response were analyzed using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment, estrous cyclicity status, and all relevant interactions included in the model. Differences in AI pregnancy rates were determined using the PROC GLIMMIX procedure of SAS with treatment, estrous cyclicity status, estrous response rates at 66 h, during the delay period, and overall estrous response, and all relevant interactions included in the model. Based on location and where appropriate, differences in AI pregnancy rates were compared among

technicians and AI sires using the PROC GLIMMIX procedure of SAS. Differences in pregnancy rates at the end of the 60 d breeding season were determined using ANOVA with year, location, treatment, estrous cyclicity status, overall estrous response, and all relevant interactions included in the model (PROC GLIMMIX; SAS Inst. Inc., Cary, NC). Serum concentrations of E₂ and differences in mean LFD for heifers at Location 1 at PG and STAI were analyzed using PROC GLIMMIX and the methods described by (Littell et al., 1998).

RESULTS

Treatments

An overall treatment summary is presented in Table 3.1. The average reproductive tract score for heifers assigned to each of the two treatments was 4.1 ± 0.05 (mean \pm SE). Average weights of heifers did not differ between treatments; average weights were 396 ± 1.6 kg and 396 ± 1.7 kg for heifers assigned to the 14d CIDR[®]-PG and 18d CIDR[®]-PG treatments, respectively (mean \pm SE).

Estrous response

A summary of estrous response rates for heifers assigned to the 14d and 18d CIDR[®]-PG treatments is presented in Table 3.2. Estrous response rates observed 66 h

after PG, during the delay period from 66 to 90 h after PG, and total estrous response did not differ ($P > 0.2$) between treatments.

Pregnancy rates

Pregnancy rates resulting from split-time artificial insemination following administration of the 14d and 18d CIDR[®]-PG protocols are shown in Table 3.3. Pregnancy rate to AI did not differ ($P = 0.3$) between treatments (52%, 14d CIDR[®]-PG; 50%, 18d CIDR[®]-PG); however, pregnancy rates resulting from AI were enhanced based on overall estrous response ($P < 0.0001$). Pregnancy rate resulting from STAI was lower at location one for one year compared to all other project groups ($P < 0.02$); however, there was no treatment by project group interaction ($P = 0.9$). Pregnancy rate resulting from AI was not affected by sire or AI technician. Pregnancy rates at the end of the 60 d breeding season were similar for each treatment ($P = 0.2$; 86%, 14d CIDR[®]-PG; 82%, 18d CIDR[®]-PG) and among project groups ($P = 0.8$).

Dominant follicle diameters

Mean dominant follicle diameters at the time PG was administered did not differ between treatments (Table 3.4; $P = 0.6$; 10.9 ± 0.4 mm, 14d CIDR[®]-PG; 11.0 ± 0.4 mm, 18d CIDR[®]-PG; mean \pm SE); nor were any differences observed at the time STAI was performed ($P = 0.3$; 12.6 ± 0.4 mm, 14d CIDR[®]-PG; 13.2 ± 0.4 mm, 18d CIDR[®]-PG; mean \pm SE). Mean dominant follicle diameters were larger ($P < 0.003$) at STAI than PG among heifers in both treatments.

Estradiol-17 β concentrations

Mean serum concentrations of E₂ at PG and STAI are shown in Table 3.4. Serum concentrations of E₂ at PG were similar between treatments (P=0.8; 1.1 \pm 0.19 pg/ml, 14d CIDR[®]-PG; 1.1 \pm 0.19 pg/ml, 18d CIDR[®]-PG; mean \pm SE), and were similar at the time STAI was performed (P=0.6; 3.8 \pm 0.19 pg/ml, 14d CIDR[®]-PG; 3.6 \pm 0.19 pg/ml, 18d CIDR[®]-PG; mean \pm SE). Serum concentrations of E₂ were higher (P<0.0001) at STAI (66 or 90 h) than at PG and were higher among heifers that expressed estrus at 66 h (P<0.01; 4.3 \pm 0.2 pg/ml, estrous; 2.5 \pm 0.3 pg/ml, non-estrous; mean \pm SE). In total, when considering heifers that expressed estrus either at 66 or 90 h after PG, concentrations of E₂ at STAI were higher for heifers that exhibited estrus overall (P< 0.05; 3.8 \pm 0.2 pg/ml, estrous; 2.7 \pm 0.5 pg/ml, non-estrous; mean \pm SE).

DISCUSSION

Early efforts to control the estrous cycle in ruminants (cattle and sheep) utilized exogenous progestins with the intended purpose to manipulate or mimic the luteal phase of the estrous cycle. Exogenous progestins were used in these studies to inhibit behavioral estrus and ovulation (Nellor and Cole, 1956; Hansel et al., 1961; Lamond, 1964; Lamond et al., 1971). Furthermore, progestin treatment was intended to increase the number of females that exhibited estrus in a synchronous pattern after withdrawal of the progestin. Later studies, however, demonstrated that more effective control of the bovine estrous cycle could be achieved through the combined use of a progestin and PG.

When this was done, more precise control of estrous behavior and subsequent luteal function was possible (Heersche et al., 1974; Hansel and Convey, 1983; Higgins et al., 1986; Folman et al., 1990). These early studies laid the necessary foundation for development of the two long-term progestin-based protocols that are currently recommended for use in beef heifers (MGA[®]-PG; 14d CIDR[®]-PG). Leitman et al. (2009a,b) reported the first published evidence demonstrating the efficacy of the 14d CIDR[®]-PG protocol for use in beef heifers. This protocol is regarded as one of the more widely recommended protocols used in the beef industry (Bonacker et al., 2018).

Figure 3.2 illustrates what is expected to occur during a 14 day treatment with MGA[®] or CIDR[®] with respect to heifers that are estrous cycling and in consideration of potential differences in stage of the estrous cycle when treatment begins. Furthermore, Figure 3.2 illustrates the rationale in conducting this study in which 14 versus 18 day CIDR[®] treatments were compared. Although a 14 d CIDR[®] treatment was shown to work effectively in pre-synchronizing heifers prior to the administration of PG (Leitman et al., 2009a, b), extending the CIDR[®] treatment by four additional days may further enhance synchrony of estrus prior to AI. Either treatment schedule would effectively support induction of puberty in pre- or peripubertal heifers (Gonzalez-Padilla et al., 1975b; Short et al., 1976; Sheffield and Elliott, 1982; Patterson et al., 1990; Hall et al., 1997; Mallory et al., 2010). However, in considering the various scenarios presented in Figure 3.2, an 18 d CIDR[®] treatment would logically provide all estrous cycling heifers the opportunity to regress CL during treatment, despite differences in stage of the estrous cycle at the time treatment is initiated. Although the hypothesis and rationale for extending CIDR[®] treatment by an additional 4 days was a logical consideration in the

design of this experiment, similarities in estrous response rate, pregnancy outcome, follicle dynamics, and estradiol concentrations between treatments suggest that no tangible benefit was realized by extending the treatment period.

These results are supported by previous studies that varied the length of MGA[®] feeding and culminated in the current recommendation to feed MGA[®] for a period of 14-days with PG administered 19 days after MGA[®] withdrawal (Zimbelman and Smith, 1966a, b; Brown et al., 1988; Deutscher et al., 2001; Lamb et al., 2001). Early studies found that extending MGA[®] feeding beyond 18 days resulted in delayed expression of estrus following MGA[®] withdrawal (Smith and Zimbelman, 1968). This point is worth considering; however, it is important to contrast differences between MGA[®] and CIDR[®] as it relates to physiological clearance of these progestins following treatment (Perry et al., 2004; Mallory et al., 2010). These differences are best illustrated from the study by Mallory et al. (2010), which suggests that extended CIDR[®] treatment may not result in the same response seen following an extended feeding period with MGA[®].

Finally, the similarities demonstrated between treatments in this study suggest flexibility associated with extending the length of CIDR[®] treatment from 14 to 18 days; however, in the event CIDR[®] treatment is extended, a 16-day interval between CIDR[®] removal and PG should be maintained. Although the current 14d CIDR[®]-PG treatment schedule appears to be optimal, extending the treatment schedule to 18 days produced acceptable results and provides flexibility for extenuating circumstances.

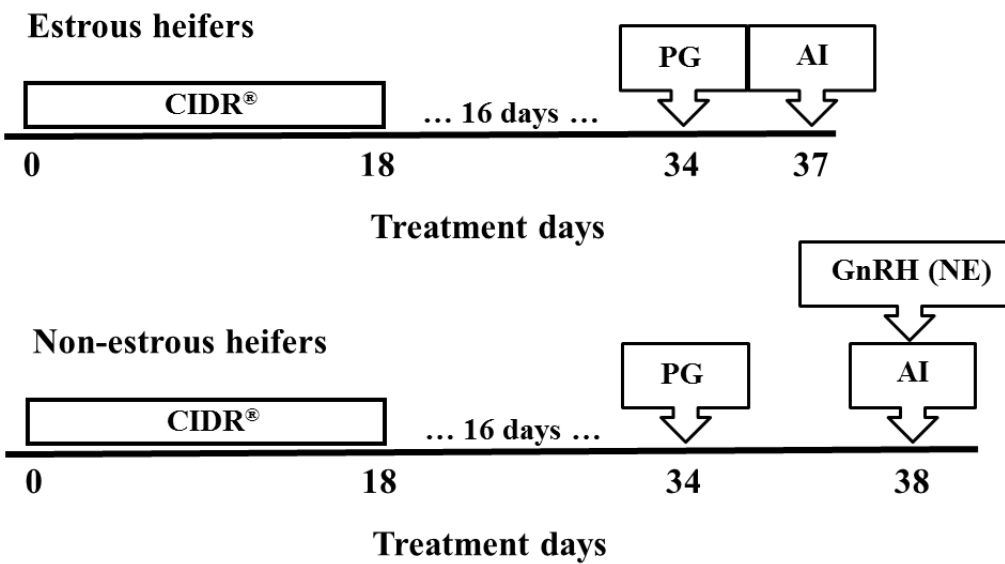
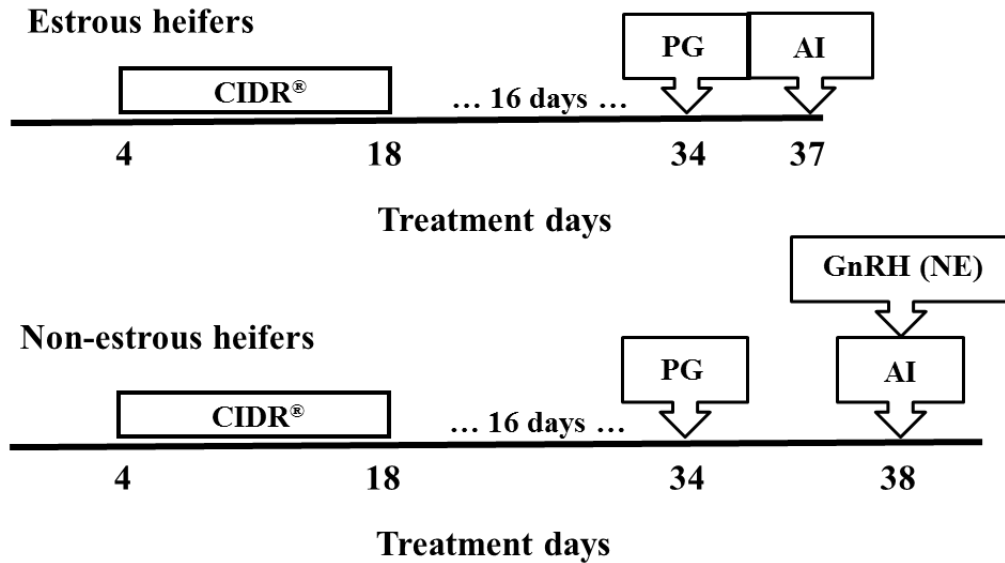


Figure 3.1. Treatment schedules for the 14d CIDR[®]-PG (top) and 18d CIDR[®]-PG (bottom) estrus synchronization protocols with split-time artificial insemination (STAI). The treatments consisted of a 14d or 18d CIDR[®] (controlled internal drug release, intravaginal insert; 1.38g progesterone) with prostaglandin F_{2α} (PG; 250 μg im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination was performed at 66 h when an activated Estrotest[®] was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estrotest[®]. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 μg im gonadorelin acetate). NE = non-estrous

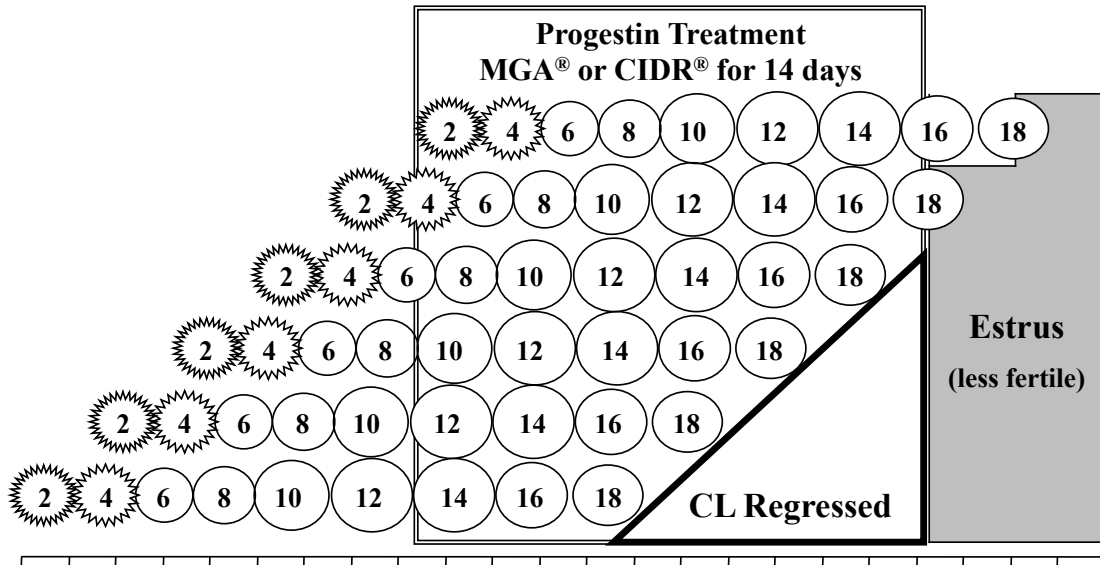


Figure 3.2. Progestin (MGA[®] or CIDR[®]) treatment. Adapted from Kojima and Patterson (2003). Circles represent corpora lutea (CL) and numbers indicate day of the estrous cycle. Females that were subject to progestin treatment starting between days 6 and 12 regressed CL during the 14 d progestin treatment. Estrus is less fertile immediately following progestin treatment due to ovulation of persistent follicles containing aged oocytes (Patterson et al., 1986; Kinder et al., 1996). MGA[®] = melengestrol acetate, CIDR[®] = controlled internal drug release

Table 3.1. Number of heifers, mean weight (Wt), and reproductive tract score (RTS) by treatment.

	N	RTS ^c	Wt. ^d
14d CIDR [®] -PG ^a	427	4.1±0.05	396±1.6
18d CIDR [®] -PG ^b	415	4.1±0.05	396±1.7

^aThe 14d CIDR[®] -PG treatment consisted of a 14 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone) with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination (AI) was performed at 66 h when an activated Estroject[®] was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject[®]. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate).

^bThe 18d CIDR[®] -PG treatment consisted of a 18 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone) with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination (AI) was performed at 66 h when an activated Estroject[®] was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject[®]. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate).

^cRTS presented as mean ± SE.

^dWt presented in kg as mean ± SE.

Table 3.2. Estrous response of heifers following the 14 or 18 d CIDR®-PG estrus synchronization protocol.

Estrous response	14d CIDR®-PG ^a		18d CIDR®-PG ^b	
	N	%	N	%
Estrous by 66 h	257/427	60	248/415	60
Estrous by 90 h	102/170	60	100/167	60
Overall estrous	359/427	84	348/415	84

^aThe 14d CIDR® -PG treatment consisted of a 14 d controlled internal drug release, intravaginal insert (CIDR®; 1.38g progesterone) with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination (AI) was performed at 66 h when an activated Estroject® was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject®. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate).

^bThe 18d CIDR® -PG treatment consisted of a 18 d controlled internal drug release, intravaginal insert (CIDR®; 1.38g progesterone) with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination (AI) was performed at 66 h when an activated Estroject® was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject®. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate).

Table 3.3. Pregnancy rates to STAI^a of heifers following the 14d or 18d CIDR[®]-PG protocol.

Estrous response	14d CIDR [®] -PG ^b		18d CIDR [®] -PG ^c	
	N	%	N	%
Estrous by 66 h	142/257	55	137/248	55
Non-estrous by 66 h	79/170	46	72/167	43
Estrous by 90 h	58/102	57	57/100	57
Non-estrous by 90 h	21/68	31	15/67	18
Overall estrous	200/359	56 ⁱ	194/348	56 ⁱ
Overall non-estrous	21/68	31 ^j	15/67	22 ^j
Total	221/427	52	209/415	50

^aSplit-time artificial insemination (STAI). Artificial insemination (AI) was performed at 66 h when an activated Estroject[®] was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject[®]. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 µg im gonadorelin acetate).

^bThe 14d CIDR[®] -PG treatment consisted of a 14 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone) with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered 16 d following progestin removal.

^cThe 18d CIDR[®] -PG treatment consisted of a 18 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone)with prostaglandin F_{2α} (PG; 250 µg im cloprostenol sodium) administered 16 d following progestin removal.

^{i,j} Values within column with different superscripts differ (P<0.001).

Table 3.4. Large follicle diameter and mean estradiol-17 β (E₂) concentrations at prostaglandin F_{2 α} (PG) and split-time artificial insemination (STAI).

	Treatment			
	14d CIDR [®] -PG ^b		18d CIDR [®] -PG ^c	
	mean \pm SE	N	mean \pm SE	N
LFD ^a at PG	10.9 \pm 0.4 ^e	36	11.0 \pm 0.4 ^e	37
LFD ^a at AI	12.6 \pm 0.4 ^f	36	13.2 \pm 0.4 ^f	37
Mean E ₂ at PG, pg/ml	1.1 \pm 0.19 ⁱ	96	1.1 \pm 0.19 ⁱ	96
Mean E ₂ at AI, pg/ml	3.8 \pm 0.19 ^j	96	3.6 \pm 0.19 ^j	96

^aLFD=large follicle diameter in mm.

^bThe 14d CIDR[®] -PG treatment consisted of a 14 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone) with prostaglandin F_{2 α} (PG; 250 μ g im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination (AI) was performed at 66 h when an activated Estroject[®] was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject[®]. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 μ g im gonadorelin acetate).

^cThe 18d CIDR[®] -PG treatment consisted of a 18 d controlled internal drug release, intravaginal insert (CIDR[®]; 1.38g progesterone) with prostaglandin F_{2 α} (PG; 250 μ g im cloprostenol sodium) administered 16 d following progestin removal. Artificial insemination (AI) was performed at 66 h when an activated Estroject[®] was recorded. Artificial insemination was postponed to 90 h for heifers having failed to exhibit estrus based on a non-activated Estroject[®]. Only heifers that were non-estrous at 90 h received gonadotropin-releasing hormone (GnRH; 100 μ g im gonadorelin acetate).

^{e,f} Values within column with different superscripts differ (P<0.003).

^{ij} Values within column with different superscripts differ (P<0.0001).

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VITA

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