TIMING GNRH ADMINISTRATION WITH SPLIT-TIME ARTIFICIAL INSEMINATION FOLLOWING ADMINISTRATION OF CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND OVUALTION IN BEEF HEIFERS AND COWS

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

TIMING GNRH ADMINISTRATION WITH SPLIT-TIME ARTIFICIAL INSEMINATION FOLLOWING ADMINISTRATION OF CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND OVUALTION IN BEEF HEIFERS AND COWS

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DEDICATION

This thesis is dedicated to my family, for their endless support throughout this journey:

To my parents, Mark and Terry, who have led by example that hard work, dedication, and love can truly take you anywhere in life that you may want to go. They have instilled within me a passion for animal agriculture and have always challenged me to be an active learner. I can only hope to one day be such a great role model to my family as they have been to me.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
BCS	Body condition score
CIDR	Controlled internal drup release insert
CL	Corpus luteum
cm	Centimeter(s)
d	Day(s)
E_2	Estradiol-17β
FSH	Follicle stimulating hormone
FTAI	Fixed-time artificial insemination
g	Gram(s)
GnRH	Gonadotropin-releasing hormone
h	Hour(s)
hd	Head
i.m.	Intramuscular
kg	Kilogram(s)
LH	Luteinizing hormone
mg	Milligram(s)
MGA	Melengestrol acetate
mL	Milliliter(s)

ng	Nanogram(s)
OT	Oxytocin
P_4	Progesterone
PGE_2	Prostaglandin E
$PGF_{2\alpha}$, PG	Prostaglandin $F_{2\alpha}$
SAS	Statistical Analysis System
SE	Standard error
STAI	Split-time artificial insemination
µg	Microgram(s)

ABSTRACT

Split-time artificial insemination (STAI) was developed as a novel breeding strategy that delays insemination by 20 to 24 h for cows and heifers that fail to express estrus prior to a predetermined fixed time. Split-time AI improved pregnancy rates in cows when sex-sorted semen was used in conjunction with the 7-day (d) CO-Synch + CIDR protocol and in heifers inseminated with conventional semen following synchronization of estrus with the 14-d CIDR-PG protocol. It is unclear whether improvements in pregnancy rates after STAI should be attributed to fertility associated effects related to lifespan of sperm in the female reproductive tract when considering the timing of induced ovulations, or to an increase in overall estrous response prior to insemination. These considerations raise questions pertaining to the timing and use of GnRH when STAI is practiced.

Two experiments (Chapter 2) evaluated timing of GnRH administration in beef heifers and cows based on estrous status during STAI following treatment with CIDR-based protocols. In experiment 1, estrus was synchronized for 816 heifers using the 14-d CIDR-PG protocol and in experiment 2, estrus was synchronized for 622 cows using the 7-d CO-Synch + CIDR protocol. For both experiments, estrus detection aids (Estrotect) were applied at $PGF_{2\alpha}$, with estrus recorded at 66 and 90 h after $PGF_{2\alpha}$. Treatments were balanced across locations for heifers using reproductive tract score and weight; whereas for cows, treatments were assigned and balanced to treatment according to age, body condition score, and days postpartum. Timing of AI for heifers and cows was based on estrus expression 66 h after $PGF_{2\alpha}$. Females in each treatment that exhibited estrus by 66 h were inseminated at 66 h, whereas AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for females

failing to exhibit estrus by 66 h. Females in treatment 1 received GnRH 66 h after $PGF_{2\alpha}$ irrespective of estrus expression; however, in treatment 2, GnRH was administered coincident with delayed AI only to females not detected in estrus at 66 h after $PGF_{2\alpha}$. Among heifers, there was no effect of treatment on overall estrous response (P = 0.49) or AI pregnancy rate (P = 0.54). Pregnancy rate for heifers inseminated at 66 h was not influenced by GnRH (P = 0.65) and there were no differences between treatments in estrous response during the 24 h delay period (P = 0.22). More cows in treatment 2 (P = 0.04) exhibited estrus during the 24 h delay period resulting in a greater overall estrous response (P = 0.04), but this did not affect AI pregnancy rate at 90 h (P = 0.51) or total AI pregnancy rate (P = 0.89). Pregnancy rate resulting from AI for cows inseminated at 66 h was not influenced by GnRH (P = 0.50). In summary, when split-time AI is used with the 14-d CIDR-PG protocol in heifers or the 7-d CO-Synch + CIDR protocol in cows, administration of GnRH at AI to females that exhibited estrus by 66 h after $PGF_{2\alpha}$ was not necessary. Furthermore, among heifers for which AI was delayed based on failure to exhibit estrus by 66 h after $PGF_{2\alpha}$, timing of GnRH (66 vs 90 h after $PGF_{2\alpha}$) was more flexible. However, delayed administration of GnRH to 90 h after $PGF_{2\alpha}$, coincident with AI for cows that fail to exhibit estrus by 66 h improved overall estrous response.

A third experiment (Chapter 3) was designed to evaluate STAI in beef heifers following administration of the 14-d CIDR-PG protocol and to compare pregnancy rates among non-estrous heifers based on administration of GnRH at AI. Estrus was synchronized for 1,138 heifers across six locations. Heifers received a CIDR insert (1.38 g progesterone) on Day 0 with removal on Day 14. Estrus detection aids (Estrotect) were applied at $PGF_{2\alpha}$ (25 mg) 16 d after CIDR removal on Day 30. Treatments were balanced

across locations for heifers using reproductive tract score and weight. Split-time AI was performed at 66 and 90 h after $PGF_{2\alpha}$, and estrus was recorded at these times. Heifers in both treatments that exhibited estrus by 66 h were inseminated at that time and did not receive GnRH, whereas AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for heifers that failed to exhibit estrus by 66 h. For heifers in treatment 1 that were inseminated at 90 h, GnRH (100 μg) was administered concurrent with AI at 90 h. Heifers in treatment 2 that were inseminated at 90 h did not receive GnRH. Estrous response did not differ between treatments at 66 h ($P = 0.58$) or 90 h ($P = 0.21$) after $PGF_{2\alpha}$. There was no effect of treatment on total AI pregnancy rate ($P = 0.60$) or on AI pregnancy rate for heifers inseminated at 66 h ($P = 0.86$) or 90 h ($P = 0.50$) after $PGF_{2\alpha}$. Ovulation was confirmed via ultrasonography for a subset of heifers that failed to exhibit estrus prior to 90 h after $PGF_{2\alpha}$. Treatments did not differ in ovulation rate for heifers failing to exhibit estrus by 90 h ($P = 0.64$) and ovulation rate did not affect AI pregnancy rate ($P = 0.97$). In summary, when split-time AI is used in conjunction with the 14-d CIDR-PG protocol in heifers, administration of GnRH is not necessary.

This series of experiments supports previous studies which demonstrate that STAI improves AI pregnancy rates as a result of increased estrus expression during the 20 to 24 h delay period. Delayed administration of GnRH had no effect on AI pregnancy rate in heifers or cows, although it increased the overall estrous response in cows. Furthermore, these results indicate that GnRH is not required when STAI is practiced in conjunction with the 14-d CIDR-PG protocol in heifers.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

The use of artificial insemination (AI) and estrus synchronization have had profound effects on beef production in the United States. Artificial insemination facilitates use of semen from high accuracy, genetically superior sires. Estrus synchronization facilitates expanded use of AI by reducing time and labor required to implement an AI program and increases the number of calves born earlier during the calving period. Additionally, implementation of a successful AI program reduces the number of natural service sires required to breed cows during the breeding season which reduces breeding costs. These tools, when combined, provide greater control of breeding programs and allow beef producers to expand management practices within their herds that lead to new marketing opportunities.

Artificial insemination and estrus synchronization are most widely used by beef operations with 200 cows or more, but in total are implemented by less than 10% of herds across the United States. The most common reasons cited by beef producers for not using reproductive technologies include constraints related to time and labor, cost of implementing the technology, or the perception that implementation of the technology is too complicated to successfully accomplish [1]. In order to overcome these barriers, a number of protocols were developed that facilitate use of fixed time artificial insemination (FTAI) wherein all females are inseminated at a predetermined fixed time to reduce labor associated with estrus detection. Development of new protocols that effectively

synchronize estrus and ovulation led to improvements in pregnancy rate to AI, reduced the cost of AI on a per pregnancy basis, and supported increased use of estrus synchronization in beef cows and heifers.

One disadvantage in using FTAI compared to insemination performed on the basis of detected estrus is that not all females express estrus prior to the time insemination is performed. In a meta-analysis of 26 studies including over 10,000 beef heifers and cows, estrous females at the time of AI achieved a 27% higher pregnancy rate than those females that failed to exhibit estrus prior to AI [2]. Split-time artificial insemination (STAI), a strategy that delays insemination of non-estrous cows and heifers by 20 to 24 h, was developed by Thomas et al. to better manage females based on estrous status at the time of AI [3, 4]. Split-time AI allows more time for females to exhibit estrus before insemination is performed and was shown to increase pregnancy rates in heifers inseminated with conventional semen and in cows inseminated with sex-sorted semen. Although the results from these studies are promising, questions arise regarding the necessity and timing of administration of GnRH to heifers and cows involved in breeding programs that practice STAI. This chapter reviews literature relating to the bovine estrous cycle and estrus synchronization, with an emphasis on the efficacy of administering GnRH to both heifers and cows when STAI is performed.

A REVIEW OF THE BOVINE ESTROUS CYCLE

The estrous cycle. The bovine estrous cycle is characterized by hormonally driven physiological events that allow females multiple opportunities to become pregnant within

a given breeding season. The length of the estrous cycle averages 21 d, but ranges from 17 to 24 d. The estrous cycle can be divided into two phases, the follicular phase and the luteal phase, represented by the dominant structure present during each phase of the cycle. The follicular phase makes up about 20% of the estrous cycle and can be broken down further into proestrus and estrus. During proestrus, luteolysis of the corpus luteum (CL) reduces circulating concentrations of progesterone (P_4) while follicular recruitment, selection, and dominance ensue that result in rising levels of estradiol (E_2). Following proestrus, onset of estrus occurs gradually and is the most recognizable stage of the estrous cycle during which the female is receptive to mounting and displays other unique behaviors, driven by a peak in E_2 secretion. Estrus culminates in ovulation of the dominant follicle. The remainder of the estrous cycle, the luteal phase, can be further broken down into two phases: metestrus and diestrus. During metestrus, the ovulatory follicle luteinizes, forming a new CL and P_4 secretion is initiated. The CL reaches maximal function during diestrus when it secretes high circulating concentrations of P_4 for a prolonged period, rendering the uterus capable of establishing and maintaining pregnancy. If pregnancy is not established, this phase ends in luteolysis, in which the CL is lysed and the cycle resumes [5].

Proestrus. Proestrus begins after luteolysis, at which time circulating P_4 concentrations decline to baseline. Progesterone normally inhibits secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, however during proestrus negative feedback inhibition is removed and GnRH pulse frequency increases [6, 7]. Gonadotropin-releasing hormone acts on the anterior pituitary to increase synthesis and pulsatile release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) [8, 9]. Production of GnRH occurs in two separate areas of the hypothalamus, known as the

tonic and surge centers. The tonic center is responsible for release of GnRH over long periods of time which supports follicular development, whereas the surge center is responsible for the preovulatory surge of GnRH which triggers the surge release of LH and subsequent ovulation. Both LH and FSH contribute to follicular development and estradiol synthesis through the "two-cell, two gonadotropin model" [10]. Membrane receptors for LH located on cells of the theca interna bind LH, activating a cascade of events that convert cholesterol to testosterone within the cell [11]. Steroidogenesis begins with the rate limiting step involving the conversion of cholesterol to pregnenolone by side chain cleavage using the mitochondrial cytochrome P450 enzyme system [12]. Pregnenolone is then enzymatically converted to P_4, which is further cleaved by enzymes to produce testosterone [11, 13]. Testosterone then diffuses out of the theca interna and into the granulosa cells, where FSH binds to its receptor causing testosterone to be converted to E_2 by the enzyme aromatase [14, 15]. Estradiol production increases to threshold levels at which time positive feedback to the hypothalamus stimulates the preovulatory LH surge and the onset of estrus.

Estrus. Estrus is the period of sexual receptivity, which ends in ovulation of a dominant follicle. The dominant follicle produces high levels of E_2 which peak 36 h prior to ovulation. Estradiol elicits a number of effects at estrus including changes in behavior and sexual receptivity, preparation of the reproductive tract for mating and establishment of pregnancy, and stimulation of the surge center in the hypothalamus resulting in the preovulatory GnRH and LH surge [16, 17, 18, 19]. The defining characteristic of estrus is a heifer or cow standing to be mounted, although there are many secondary signs of estrus such as changes in cervical mucus, increased physical activity, and increased vocalization

[20, 21, 22]. Estrus typically lasts from 10 to 18 h, and beef cows that are mounted more during estrus achieve a higher AI pregnancy rate [23, 24].

Adequate numbers of LH receptors must be present on the granulosa cells of the dominant follicle in order for final maturation and ovulation of the follicle to occur [25]. The preovulatory LH surge leads to ovulation through its effects on prostaglandin and P_4 production. Prostaglandin E_2 (PGE_2) is responsible for increasing blood flow to the ovary and dominant follicle, whereas Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is responsible for contractions of smooth muscle within the ovary and release of lysosomal enzymes. Additionally, the dominant follicle begins producing P_4 rather than E_2, which increases collagenase production. Pressure builds in the follicle due to muscular contraction of the ovary and edema from increased local blood flow, and the follicular wall weakens due to the action of lysosomal enzymes and collagenase. These events culminate in ovulation [5].

Metestrus. Ovulation marks the beginning of metestrus, in which the CL forms from the recently ovulated follicle under the influence of LH. The CL is made up of a homogeneous mixture of small and large luteal cells developed from thecal and granulosa cells of the follicle wall [26, 27]. Angiogenesis, the formation of new blood vessels, is aided by multiple factors which peak two to three d after ovulation [28]. Ovarian blood flow is highly correlated with P_4 production, and almost all steroidogenic cells of the mature CL are in contact with one or more capillaries [29, 30]. By the seventh day of the estrous cycle, the CL reaches mature size and is fully functional [15, 31].

Diestrus. During diestrus high levels of P_4 are secreted by the CL, peaking around day 10 of the estrous cycle. Progesterone acts on the hypothalamus to maintain low frequency pulsatile release of GnRH, which inhibits preovulatory follicles from developing

and secreting high levels of E_2 [15]. This in turn inhibits estrus. Progesterone also functions by inhibiting uterine contractions and stimulating endometrial glands to support the conceptus [32].

Diestrus ends in luteolysis, or regression of the CL, caused by oxytocin and P_4 from the CL and $PGF_{2\alpha}$ from the uterus. The first step in luteolysis is downregulation of progesterone receptors by circulating P_4. As a result, the inhibitory effects of P_4 on the hypothalamus are reduced and E_2 levels begin to rise with development of a dominant follicle. As E_2 levels increase, oxytocin (OT) is released from the hypothalamus and upregulates uterine OT receptors [33]. Activation of oxytocin receptors in the uterus cause $PGF_{2\alpha}$ to be secreted, which acts on luteal cells containing large amounts of OT. When luteal OT is released from the CL, $PGF_{2\alpha}$ pulse amplitude and frequency increase, leading to regression of the CL [34]. Prostaglandin $F_{2\alpha}$ reaches the CL through countercurrent exchange between the utero-ovarian vein and ovarian artery and aids in luteolysis through vasoconstriction of arteries supplying the CL. In addition to the effect of reduced blood supply to the CL, capillaries within the CL degrade during luteolysis and deprive luteal cells of nutrients required for survival [5].

Folliculogenesis. Folliculogenesis is the process by which Graafian, or preovulatory, follicles are matured from a resting pool of primordial follicles [35]. As follicles mature they must pass through a number of stages: primordial, primary, secondary, tertiary, and lastly Graafian follicles. A number of factors are responsible for initiating the development of follicles from the primordial to primary stage, and once development is initiated follicles are destined to ovulate or become atretic and degenerate [36, 37]. Heifer

calves are born with 100,000 to 150,000 primordial follicles, although this number can exceed two million during fetal development [38, 39]. The number of primordial follicles at birth dictates the number of preantral and antral follicles that can be found developing at any time throughout the lifespan of the heifer or cow. Although primordial follicles are continuously recruited to become primary follicles throughout the lifespan of an animal, the number recruited at any one time decreases as the pool of primordial follicles is depleted [15, 38, 40].

Follicular wave development. There are three processes that antral follicles pass through before their eventual ovulation: recruitment, selection, and dominance. The discovery of follicular wave dynamics was aided by use of ultrasonography, as the number and size of antral follicles can be recorded on a daily basis, with changes charted over time [41].

Recruitment. Recruitment defines the growth of a cohort (or group) of follicles which begin to produce E_2. Independent of GnRH stimulation, initial recruitment occurs, in which primordial follicles are continually recruited and undergo folliculogenesis. Alternatively, during the follicular phase when P_4 levels are low, GnRH causes cyclic recruitment to occur, in which follicles pass through stages of development leading to ovulation [42]. Surges in FSH precede the emergence of follicular waves at two or three points during the interovulatory interval, beginning with an FSH surge at estrus [43, 44]. The number of follicular waves during each estrous cycle is determined in part by length of the luteal phase [45].

Selection. Soon after cyclic recruitment a subordinate group of follicles will become atretic but the remaining healthy follicles are selected and continue to grow. In cattle, only one follicle is typically selected for continued growth at the time of follicular divergence [46]. This follicle produces moderate levels of E_2 and inhibin. The E_2 produced by this follicle initially provides negative feedback on the surge center of the hypothalamus and inhibits the preovulatory surge of GnRH and LH [7]. Both inhibin and E_2 provide negative feedback on the anterior pituitary in order to decrease FSH release and inhibit growth of new follicles [46, 47, 48].

Dominance. Continued growth of the selected follicle leads to dominance, a LH dependent phase in which recruitment of other follicles is inhibited. Luteinizing hormone is critical for growth of the dominant follicle and initiation of ovulation [49]. If P_4 concentrations remain low, as they are during the follicular phase of the estrous cycle, then E_2 production at threshold levels provides positive feedback on the surge center of the hypothalamus, producing a surge release of GnRH and LH that induce ovulation. When P_4 concentrations are high during the luteal phase of the estrous cycle or pregnancy, the dominant follicle will undergo atresia and a new follicular wave will begin [5].

A REVIEW OF ESTRUS SYNCHRONIZATION PRODUCTS

Progesterone. Progesterone was the first product used to manipulate the estrous cycle in cattle, as exposure to exogenous P_4 mimics the role of the CL and can be used to either extend or establish the luteal phase of the estrous cycle [50]. Progesterone feedback on the hypothalamus inhibits the release of GnRH, hindering final maturation and ovulation

of a dominant follicle [15]. Early studies showed that when heifers were administered progesterone daily, estrus was inhibited although development of a dominant follicle still occurred. When the treatment period ended, heifers exhibited estrus and ovulated. Long term administration of P_4, however, led to turnover of follicles and replacement with a new preovulatory follicle two to three weeks later [51]. The use of ultrasonography and the discovery of follicular waves aided in the development of protocols using P_4, specifically to determine the optimal length of exposure to P_4 when combining its use with other synchronization products.

In addition to suppressing estrus, P_4 exposure can induce cyclicity in prepubertal heifers and postpartum cows by mimicking the natural rise in P_4 that occurs before the onset of puberty in heifers or resumption of estrous cyclicity in cows [52]. In the weeks leading up to puberty there are two rises in plasma concentrations of P_4, presumably due to luteinization of follicles, which are followed by changes in LH pulsatility and overall greater concentrations of LH in blood [53, 54]. Progestins induce estrous cyclicity through an increase in LH pulsatility after withdrawal of P_4 exposure that allows for follicle maturation [55, 56]. Additionally, receptors for E_2 are downregulated in the hypothalamus, removing negative feedback on LH secretion which allows for final maturation and ovulation of a dominant follicle [56, 57]. Similarly, in cows, concentrations of P_4 increase prior to the resumption of estrous cyclicity [58] and exposure to exogenous P_4 can induce cyclicity as early as 21 days postpartum [59].

Melengestrol Acetate. The two progestin products commonly used in the United States are melengestrol acetate (MGA) and the EAZI-BREED^{TM} Controlled Internal Drug Release (CIDR; Zoetis, Madison, NJ). Melengestrol acetate, an orally active progestin that

is approved for use in heifers, was originally developed to improve rate of gain in feedlot heifers by suppressing estrus [60]. Many studies showed that feeding MGA at a rate of 0.4 mg/d per head (hd) suppressed estrus in feedlot heifers and improved rate of gain when compared to ovariectomized heifers, but not when compared to control groups [61, 62]. The current recommendation for feeding MGA for the purpose of estrus synchronization is a rate of 0.5 mg/hd/d to suppress estrus behavior and ovulation and to induce puberty in heifers [55, 63, 64]. A carrier of 1.4 to 2.3 kg/hd/d is often mixed with MGA to ensure that an adequate amount is consumed by each heifer daily, and adequate bunk space of 60 linear cm per hd is required to ensure that all heifers have access to the ration [50]. Melengestrol acetate is frequently used to synchronize estrus in beef heifers because of low cost and reduced labor required for administration compared to the CIDR.

Controlled Internal Drug Release. The CIDR is a vaginal insert that is approved for use in both heifers and cows in the United States. The T-shaped nylon spine of the CIDR is coated with silicon containing 1.38 g of P_4, and the amount of P_4 released from the CIDR is consistent over a 15 d period [65]. Concentrations of P_4 in the blood are maintained above 2.0 ng/mL by the CIDR and are dependent on the stage of the estrous cycle [66]. The greatest advantages of the CIDR over MGA are the consistency of exposure to P_4 and rapid clearance of P_4 from the bloodstream when the CIDR is removed. Trials comparing a 14-d progestin treatment with MGA or CIDR showed that heifers exhibited estrus more rapidly after CIDR removal compared to MGA withdrawal, which improved synchrony of estrus during the synchronized period [67, 68].

Prostaglandin $F_{2\alpha}$. Administration of $PGF_{2\alpha}$ after Day five of the estrous cycle causes luteolysis of the CL and subsequent synchronization of estrus [69, 70]. Within the CL, specific binding of $PGF_{2\alpha}$ to membrane receptors increases from Day three to Day 20, but declines by Day 21-24 due to luteal regression [71]. Although heifers and cows do not respond to administration of $PGF_{2\alpha}$ during luteolysis, they still express estrus within the synchronized period. Although expression of estrus occurs anywhere from one to seven d after $PGF_{2\alpha}$, synchrony of estrus depends on stage of the estrous cycle at the time $PGF_{2\alpha}$ is administered, as heifers and cows with smaller follicles experience longer intervals to estrus compared to those with larger follicles [41, 72, 73]. Prostaglandin $F_{2\alpha}$ does not induce cyclicity in heifers or cows, so protocols that combine use of a progestin and $PGF_{2\alpha}$ are recommended whenever cyclicity is a concern [74].

Prostaglandin $F_{2\alpha}$ products. There are many approved $PGF_{2\alpha}$ products for estrus synchronization including Lutalyse®, ProstaMate®, InSynch®, Estrumate®, and estroPlan®. The products ProstaMate and InSynch are generics of Lutalyse, and estroPlan is a generic of Estrumate [75]. A number of trials have been conducted that compared efficacy of Lutalyse and Estrumate. No differences between products were reported when estrous response or pregnancy rates resulting from AI were compared. [74].

Gonadotropin-releasing hormone. Gonadotropin-releasing hormone induces the LH surge and ovulation of a dominant follicle and initiates development of a new follicular wave. The need for synchronization of follicular waves arose when it was determined that the interval from $PGF_{2\alpha}$ administration to estrus was dependent on size of the dominant follicle [41]. Although P_4 and $PGF_{2\alpha}$ are capable of synchronizing estrus, the addition of

GnRH allows for synchronization of follicular waves and ovulation. Ovulation typically varies over an eight h period between 28 and 32 h following the administration of GnRH to synchronized cows, allowing for higher pregnancy rates to be achieved at a single insemination [76].

Protocols that were designed initially to synchronize estrus that included GnRH (Figure 1.1) improved synchrony of estrus and ovulation when compared to treatment with $PGF_{2\alpha}$ alone [77, 78]. Gonadotropin-releasing hormone causes ovulation and luteinization of a dominant follicle, followed by recruitment of a new follicular wave in two to three d [79]. Estrus is inhibited in most females following administration of GnRH and subsequent ovulation until the time when $PGF_{2\alpha}$ is administered [80]. The newly formed CL can be regressed with $PGF_{2\alpha}$ six or seven d after GnRH is administered resulting in the synchronized expression of estrus within a four d period, peaking on the second and third d after $PGF_{2\alpha}$ administration [80, 81].

The interval to estrus following administration of the GnRH-$PGF_{2\alpha}$ protocol is too variable to perform a single fixed-time insemination (FTAI). This observation led to the development of the GnRH-$PGF_{2\alpha}$-GnRH protocol [50]. The second GnRH, administered two d after $PGF_{2\alpha}$, allows FTAI to be performed as it induces a preovulatory LH surge and ovulation of the dominant follicle. Two variations of this protocol were developed to account for differences in dairy and beef cattle: Ovsynch (Figure 1.2) and CO-Synch (Figure 1.3). Ovsynch is implemented in dairy herds and is the template for most dairy synchronization protocols. Due to daily handling of dairy cows, insemination 16-24 h after a second GnRH injection allows more time for ovulation before FTAI and yields reliable pregnancy rates to a single insemination [76, 82, 83, 84]. This protocol, however, does not

successfully synchronize estrus in dairy heifers and yields significantly lower pregnancy rates compared to synchronization with $PGF_{2\alpha}$ alone [84]. The CO-Synch protocol for beef cattle differs from Ovsynch in that FTAI is performed concurrent with the second GnRH administration in order to reduce handling of cows, although this results in lower pregnancy rates to TAI [85]. CO-Synch and other GnRH-$PGF_{2\alpha}$ protocols are not recommended for use in beef heifers because heifers do not respond consistently to the initial GnRH treatment [76, 86, 87, 88].

Gonadotropin-releasing hormone products. Cystorelin®, Factrel®, Fertagyl®, OvaCyst®, and GONAbreed® are the five GnRH products currently available in the U.S. Fertagyl, OvaCyst, and GONAbreed are generics of Cystorelin. The original product labeling for GnRH was intended for treatment of ovarian follicular cysts in dairy cows. Consequently, a limited number of these products are approved for estrus synchronization, especially in beef cattle. Factrel, Fertagyl, and GONAbreed are approved for estrus synchronization in dairy cattle when used in combination with specific progestins and prostaglandins, however GONAbreed® is the only GnRH product approved for use in beef cattle [75].

Why cows and heifers respond differently to GnRH. Numerous studies have shown that response to GnRH in heifers is inconsistent when compared to cows [76, 86, 87]. Furthermore, the requirement for inclusion of GnRH in estrus synchronization protocols specifically designed for beef heifers has been questioned [89, 90, 91]. A common reason for failure to respond to GnRH among both heifers and cows is the stage of development of the dominant follicle at the time GnRH is administered. Treatment with GnRH at random

stages of the estrous cycle is estimated to induce ovulation in 66% of cows but only 50% of heifers [76, 85].

In heifers, GnRH was initially shown to be 100% effective at causing disappearance of the first wave dominant follicle during the growth phase, decreasing to 33% during the plateau phase, and finally decreasing to 0% effective during the regression phase [92]. Further experiments in both beef and dairy heifers classified treatments by five d of the cycle that can be assigned to these three phases. Ovulation did not occur in any of the treated heifers on Day two of the estrous cycle due to lack of a dominant follicle, but increased during growth phases on Day five and 15 of the estrous cycle. Day ten was considered the plateau phase, and the response to GnRH was low. The only discrepancy between the two studies was on Day 18, when few beef heifers but all dairy heifers responded to GnRH, possibly due to differences in cycle length of heifers with two or three follicular waves. These studies further assessed GnRH-$PGF_{2\alpha}$-GnRH protocols by confirming ovulation after treatment with the second GnRH and found that the initial day on which GnRH is administered may influence response to the second or ovulatory GnRH, thereby affecting resulting AI pregnancy rates [87, 93]. These results highlight the need to pre-synchronize follicular waves to more effectively manage control of estrus and ovulation.

Ovulatory response to GnRH is dependent on LH receptors in the follicle, which increase in number during growth of the dominant follicle. Once the dominant follicle undergoes atresia, the number of LH receptors decrease and ovulation subsequently fails to occur in response to the administration of GnRH [94, 95, 96]. The number of follicular waves per estrous cycle is inconsistent in heifers and may impact a heifer's response to

GnRH even on a known day of the estrous cycle. There are conflicting data for whether two or three waves are more common in heifers, but there are no known genetic or environmental reasons for the emergence of two or three waves during each cycle [41, 45]. Most importantly, the interval from follicular growth to atresia for each follicular wave is dependent on the number of follicular waves per estrous cycle, which makes it difficult to determine an effective time at which to administer GnRH to heifers in order to perform FTAI [45].

A REVIEW OF SPLIT-TIME ARTIFICIAL INSEMINATION

Split-time artificial insemination allows females to be managed based on estrous response at the time of insemination in order to increase pregnancy rates compared to FTAI. With STAI, insemination is delayed for non-estrous females by 20 to 24 h, whereas with FTAI all heifers are inseminated at a single predetermined time. Experiments designed to evaluate STAI were conducted in heifers following synchronization of estrus with the 14-d CIDR- $PGF_{2\alpha}$ protocol and in cows following the 7-d CO-Synch + CIDR protocol [3, 4]. This section will review the development of these protocols and the original experiments performed using STAI.

Protocols used in conjunction with split-time artificial insemination. The 7-d CO-Synch + CIDR protocol (Figure 1.4) is a modification of the GnRH-$PGF_{2\alpha}$-GnRH protocol, otherwise known as CO-Synch. When estrus is synchronized for cows using the CO-Synch protocol, a proportion of cows fail to respond to the initial GnRH treatment resulting in 8-10% of cows subsequently exhibiting estrus prior to administration of $PGF_{2\alpha}$ [85].

Incorporation of a progestin for seven d from the initial GnRH treatment to $PGF_{2\alpha}$ administration, however, successfully suppressed estrus in those cows that failed to respond to the first GnRH [97]. Additionally, progestin exposure improved estrous response and pregnancy rates for anestrous cows by 20%, and overall pregnancy rates by 10% or more [98, 99]. Pregnancy rates resulting from FTAI for cows following synchronization of estrus using the 7-d CO-Synch + CIDR protocol are consistent, ranging from 61 to 67% [100, 101, 102, 103]. Timing of insemination is recommended to be performed 60 to 66 h following the administration of $PGF_{2\alpha}$, however the highest pregnancy rates were reported when AI was performed at 66 h after $PGF_{2\alpha}$ [100, 103].

The 7-d CO-Synch + CIDR protocol has also been used in heifers, but with lower pregnancy rates resulting from AI. This protocol is less effective for use in synchronizing estrus in heifers because a proportion of heifers fail to respond to the initial administration of GnRH and fail to ovulate a dominant follicle or initiate recruitment of a new follicular wave [104]. Follicular waves, however, can be successfully synchronized in heifers without the use of GnRH with long-term administration of P_4, which led to development of the 14-d CIDR-PG protocol [50, 90, 91, 105].

To synchronize estrus using the 14-d CIDR-PG protocol (Figure 1.5), an Eazi-Breed CIDR insert is applied for 14 d, with 70% of heifers expressing estrus two to three d following CIDR removal [68]. Prostaglandin $F_{2\alpha}$ is administered 16 d after CIDR removal on Day 30 and all heifers are administered GnRH concurrent with AI at 66 h following $PGF_{2\alpha}$. It is not recommended to inseminate heifers at the initial estrus after P_4 removal due to reduced fertility after long-term progestin exposure [106]. Fertility of the dominant follicle decreases as the period of dominance is extended from four to eight d, and is even

further reduced after dominance is maintained for 10 d [107]. The second estrus, however, is fertile and highly synchronized, with 88% of heifers expressing estrus within two to three d following $PGF_{2\alpha}$ administration [90, 91, 108]. Pregnancy rates to FTAI following estrus synchronization with the 14-d CIDR-PG protocol averaged 49% over five years in the Missouri Show-Me-Select Replacement Heifer Program [109].

The efficacy of GnRH has been questionable even following pre-treatment with a progestin. The long-term progestin protocol, CIDR Select, included the administration of GnRH on day 23, 7 d before $PGF_{2\alpha}$, to synchronize recruitment of a new follicular wave that would produce a dominant follicle by Day 30. A field trial comparing the 14-d CIDR Select and CIDR-PG protocols reported no difference in estrous response between the two protocols, however synchrony of estrus and pregnancy rate resulting from AI were improved among heifers assigned to the CIDR-PG protocol. The addition of GnRH on Day 23 lengthened the interval to estrus following $PGF_{2\alpha}$, which raised questions regarding the potential negative effect of GnRH on a newly recruited follicular wave [91].

Split-time artificial insemination. Split-time AI was developed to manage females based on expression of estrus prior to FTAI. All STAI experiments were conducted following administration of the 14-d CIDR-PG protocol in heifers (Figure 1.7) and the 7-d CO-Synch + CIDR protocol in cows (Figure 1.8) [3, 4]. Females that express estrus prior to AI ovulate on average 28 to 32 h after the onset of estrus and in response to an endogenous surge of GnRH and LH around the onset of estrus. Females that fail to express estrus prior to AI are administered GnRH concurrent with the time at which insemination is performed, and are expected to ovulate within 28 to 32 h later [76]. This results in a tendency for estrous females to ovulate earlier in a FTAI protocol [110].

The initial hypothesis for STAI was that delayed insemination of non-estrous females to 20 h after GnRH was administered would yield higher pregnancy rates by better aligning the lifespan of viable sperm with the timing of ovulation [3, 4]. The first trial involving STAI was performed with sex-sorted semen. Thomas et al. reported that STAI improved pregnancy rates compared to FTAI in beef cows when inseminations were performed using sex-sorted semen, prompting a similar investigation in both beef heifers and cows using conventional, non-sex-sorted semen [3, 4].

In the trial involving sex-sorted semen, pregnancy rates for cows that failed to exhibit estrus improved from 3% when cows were inseminated at the time GnRH was administered to 36% when cows were inseminated 20 h following the administration of GnRH. In a third treatment, FTAI was performed with conventional semen from the same sire, with non-estrous cows achieving a similar 37% pregnancy rate. The resulting improvement in pregnancy rates using STAI compared to FTAI among estrous and non-estrous cows inseminated with sex-sorted semen was 13% [3]. Thomas et al. then conducted a similar trial using conventional semen in both heifers and cows. Although conventional semen is not damaged by the flow cytometry process, longevity of non-sex-sorted sperm could be negatively affected by the freeze-thaw process due to precapacitation, an early, induced capacitation of sperm that can limit the number of viable sperm remaining in the female reproductive tract at the time of ovulation [111]. Delayed insemination with conventional semen increased total estrous response prior to insemination for the STAI treatments, although more heifers expressed estrus during the 20 h delay period than cows. Higher pregnancy rates were achieved during the delay period for both heifers (66 vs 29%) and cows (67 vs 40%) that expressed estrus compared to those

that did not. Overall, pregnancy rates resulting from STAI were higher for heifers (46% FTAI vs 54% STAI), but not for cows (59% for both FTAI and STAI treatments). Questions were raised regarding differences between heifers and cows when using STAI. It was suggested that high estrous response rates prior to 66 h may have minimized the effects of STAI for cows in this particular experiment. Additionally, the high rate of estrus expression among heifers during the 20 h delay period was unexpected and suggests that GnRH administration at 66 h after $PGF_{2\alpha}$ may not be as effective in heifers when compared to cows [4].

Heifers and cows that exhibit estrus prior to TAI consistently yield higher pregnancy results compared to those that do not, suggesting that timing of ovulation may not be the only factor contributing to differences between these females [2]. The expression of estrus in cattle follows a rise in serum concentrations of estradiol, which in turn controls critical processes involved with the establishment of pregnancy, including effects on follicular cells, the oocyte, gamete transport, and preparation of the uterus for pregnancy [112]. For this reason, improvements in pregnancy rate following STAI in beef heifers using conventional semen were attributed to an increase in overall estrous response prior to insemination rather than the advanced administration of GnRH. [3, 4].

A similar series of experiments was conducted following estrus synchronization using the 7-d CO-Synch + CIDR protocol in cows and the 14-d CIDR-PG protocol in heifers. In these trials however, insemination was performed at 58 or 76 h after $PGF_{2\alpha}$ rather than 66 and 86 h for cows and heifers, respectively. Delayed insemination of non-estrous cows failed to improve pregnancy rates in cows, although there was a tendency for increased pregnancy rates in heifers following delayed insemination [113]. Based on estrus

distribution data collected using HeatWatch, insemination at 54 h coincides with the peak onset of estrus in heifers and precedes the peak of estrous activity in cows [68, 90, 91, 102]. Insemination at 56 h rather than the recommended time for TAI (60-66 h for cows and 66 ± 2 h for heifers), likely decreased pregnancy rates at the initial insemination [100]. Additionally, early insemination resulted in increased estrous activity at the time Estrotect patches were evaluated, which may have confounded the results of this experiment [113].

Delayed insemination was also evaluated in beef heifers after estrous was synchronized using the MGA-PG protocol (Figure 1.6). Heifers that expressed estrus by 72 h were inseminated 12 h after detected estrus and achieved a 70% pregnancy rate. Heifers that failed to exhibit estrus by 72 h were administered GnRH at 72 h with one group receiving AI at that time, and the other receiving delayed insemination 16 h after GnRH was administered. Non-estrous heifers that were inseminated at the time GnRH was administered achieved similar pregnancy rates to those that received delayed insemination (56 vs 47%) [114]. Markwood et al. then performed a series of experiments using the MGA-PG protocol in beef heifers focusing on non-estrous heifers at 72 h. In this trial insemination was delayed by 9, 12, or 18 h. No differences in pregnancy rates were reported [113]. These experiments may have failed to demonstrate improvements in pregnancy rate after delayed insemination because of differences in estrous response between long-term CIDR- and MGA-based protocols. The overall estrous response in heifers is greater following administration of the 14-d CIDR-PG protocol and the interval to estrus for these heifers is also reduced, allowing a larger proportion of non-estrous heifers to exhibit estrus in the period prior to delayed insemination. After a peak in estrus expression from 48 to 72 h following $PGF_{2\alpha}$, using the MGA-PG protocol, synchrony of estrus expression is

reduced, with 20% of heifers exhibiting estrus over the next 48 h. Between heifers that exhibit estrus late and those that do not exhibit estrus at all, over 30% of heifers typically fail to exhibit estrus prior to a second insemination, resulting in potentially lower pregnancy rates [90, 91, 108]. Results from these experiments would have been explained more thoroughly if estrous activity had been recorded when delayed insemination was performed.

Experiments were also conducted to evaluate the optimal timing of GnRH administration for non-estrous beef cows when a STAI approach is used. Hill et al. conducted an experiment in which estrus was synchronized for cows using the 7-d CO-Synch + CIDR protocol with AI at 60 and 75 h. Cows that expressed estrus by 60 h were inseminated concurrent with GnRH administration at 60 h and achieved a 66% pregnancy rate. Cows that failed to exhibit estrus prior to 60 h were divided into three treatments. The first treatment involved FTAI, where cows were administered GnRH and inseminated at 60 h. The next two treatments received delayed AI, but one treatment received GnRH at 60 h and the other received GnRH at 75 h concurrent with AI. Although no difference in pregnancy rate was found between the two treatments for which insemination was delayed (55% if GnRH was administered at 60 h vs 53% if GnRH was delayed to 75 h), pregnancy rates were significantly higher than the FTAI treatment in which AI was performed at 60 h (44%) [115].

It is possible that pregnancy rates were lower among cows assigned to the FTAI treatment because inseminations were performed at 60 h instead of 66 h. Only 40% of cows are expected to express estrus prior to insemination at 60 h, whereas 60-70% are expected to express estrus prior to 75 h [102]. Earlier insemination of cows would likely decrease

pregnancy rates after FTAI due to lower estrous response rates at 60 h, which would theoretically increase the chance of realizing an improvement in pregnancy rate when delayed insemination was performed. If this is in fact the reason that a significant advantage was observed among cows that received delayed insemination that were involved in this experiment, it may still, however, be more economical to perform a single insemination at 66 h rather than two inseminations at 60 and 75 h.

SUMMARY

There are a number of protocols available for beef producers that can be used effectively to synchronize estrus, facilitate use of artificial insemination, and enhance reproductive performance within a herd. These protocols range in time and labor requirements, costs, and expected pregnancy rates, allowing producers to select the protocol that best fits their program. Split-time AI requires additional time and labor, but affords the potential to improve pregnancy rates resulting from AI. Although STAI was shown to improve pregnancy rates in heifers, results in cows have been inconsistent.

Published studies involving STAI raised questions concerning the potential effect(s) of GnRH on estrus expression and the significance of these effects on subsequent pregnancy rates in both heifers and cows. Additionally, there are no published studies that evaluate the effects of GnRH on beef heifers and cows that exhibit estrus prior to the time AI is performed. These considerations form the basis for the experiments presented in this thesis.

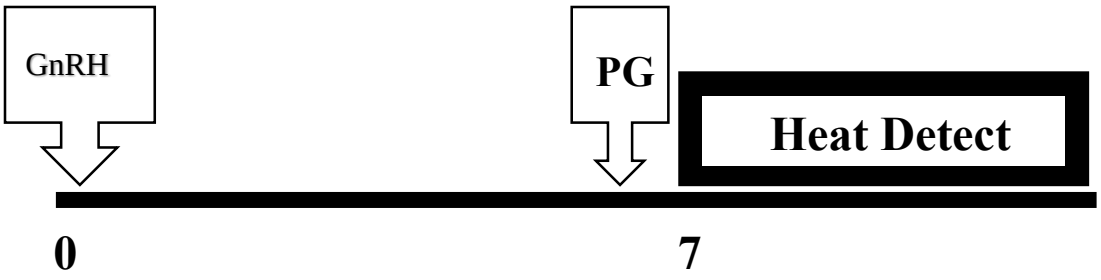

Figure 1.1. Treatment schedule for the GnRH-$PGF_{2\alpha}$ protocol [132].

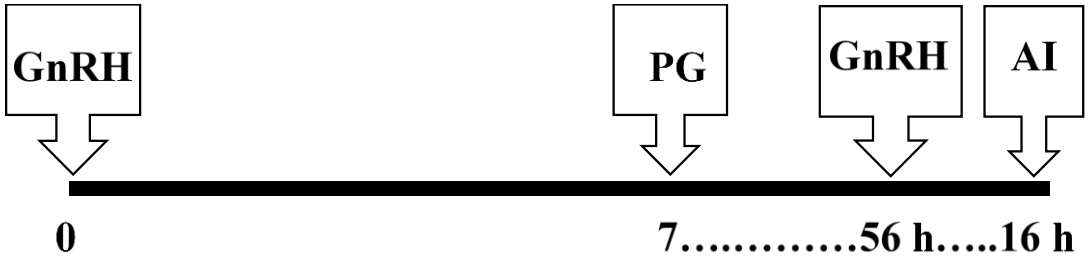

Figure 1.2. Treatment schedule for the Ovsynch protocol [84].

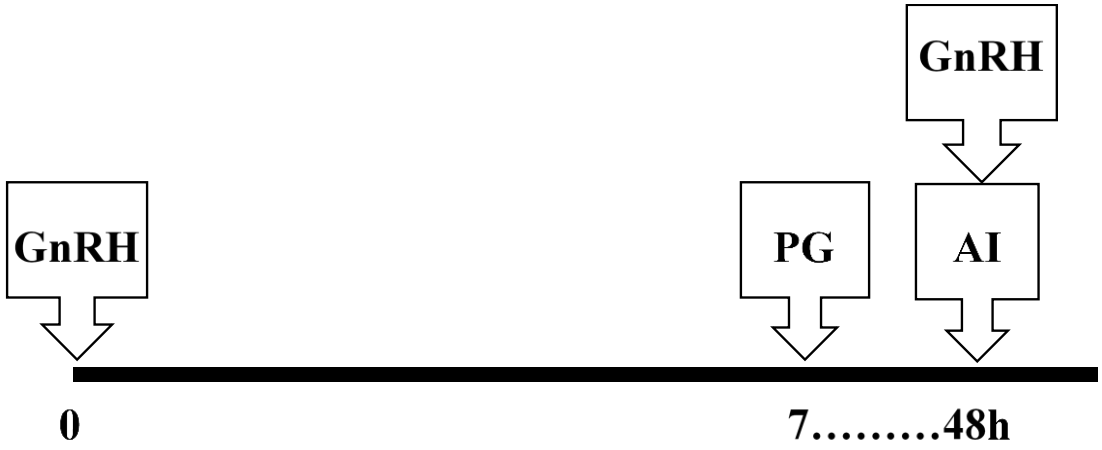

Figure 1.3. Treatment schedule for the CO-Synch protocol [85].

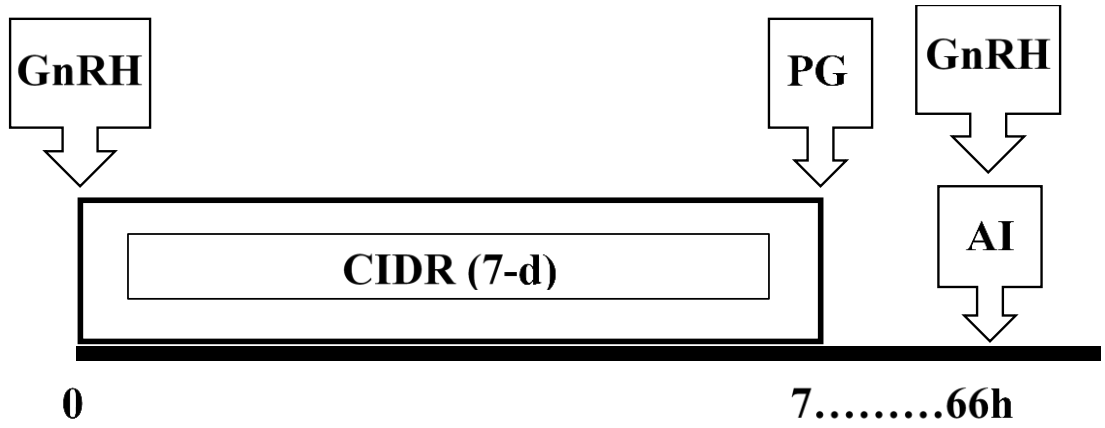

Figure 1.4. Treatment schedule for the 7-d CO-Synch + CIDR protocol [91, 101].

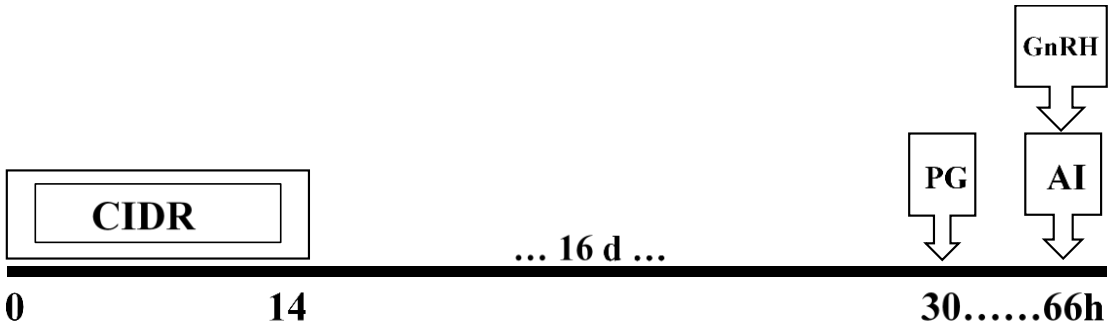

Figure 1.5. Treatment schedule for the 14-d CIDR-PG protocol [91, 105].

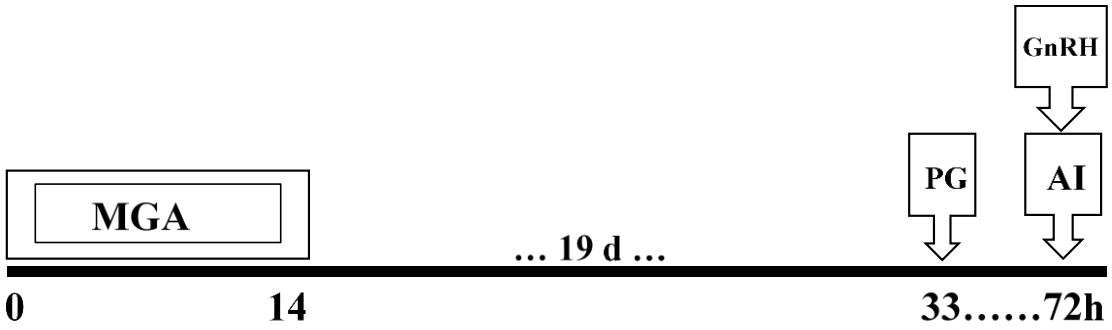

Figure 1.6. Treatment schedule for the MGA-PG protocol [99, 133].

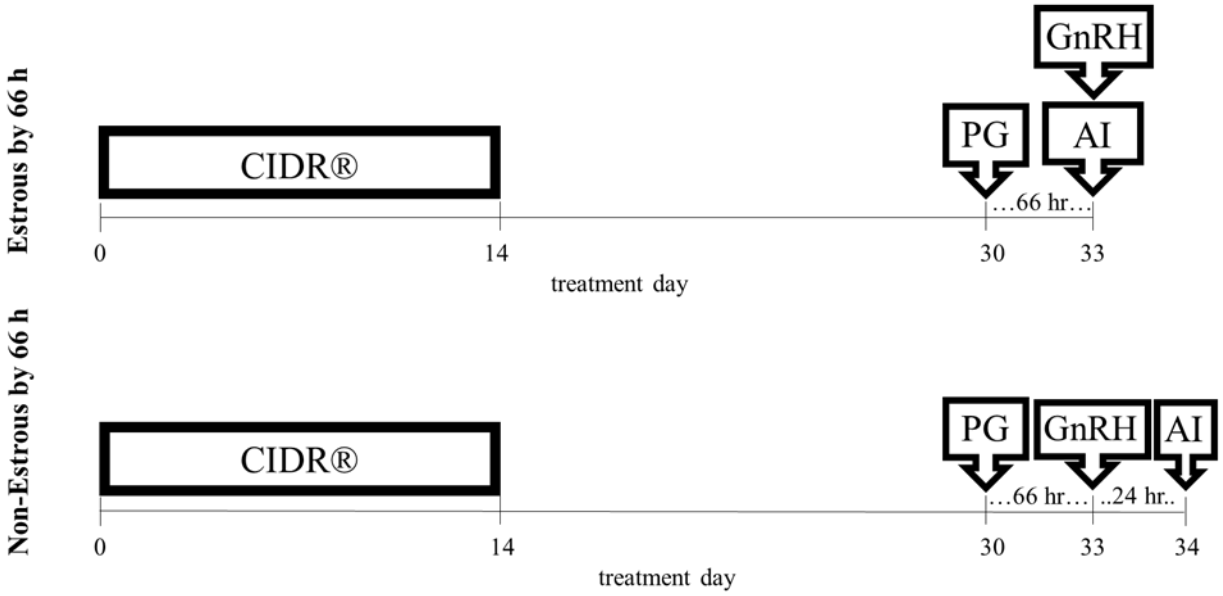

Figure 1.7. Treatment schedule for the 14-d CIDR-PG protocol with STAI [3, 4].

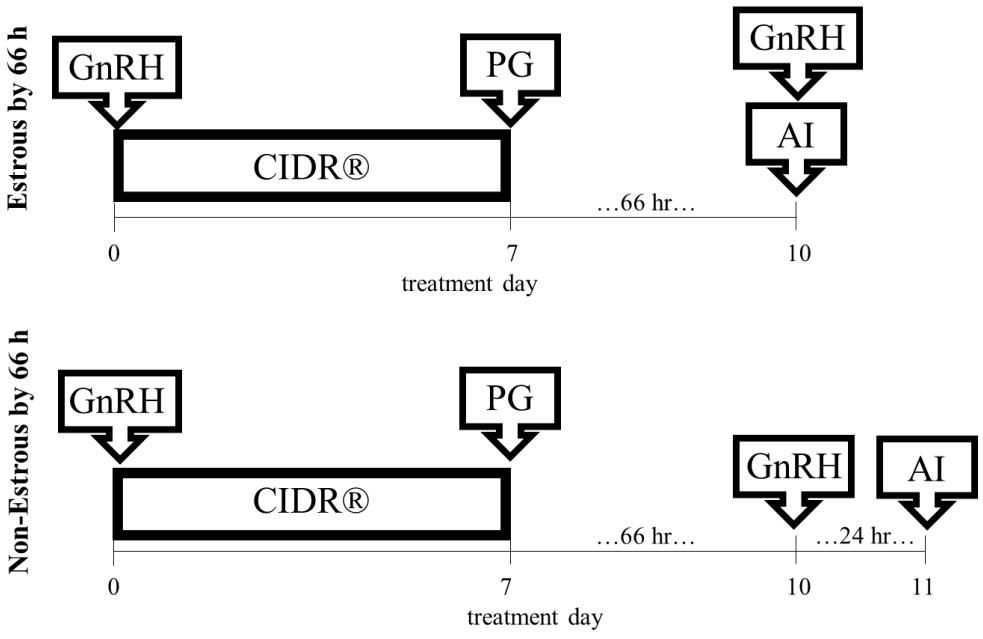

Figure 1.8. Treatment schedule for the 7-d CO-Synch + CIDR protocol with STAI [3, 4].

CHAPTER 2

SPLIT-TIME ARTIFICIAL INSEMINATION IN BEEF CATTLE: I. USING ESTROUS RESPONSE TO DETERMINE THE OPTIMAL TIME(S) AT WHICH TO ADMINISTER GNRH IN BEEF HEIFERS AND POSTPARTUM COWS

ABSTRACT

Two experiments evaluated timing of GnRH administration in beef heifers and cows based on estrous status during split-time artificial insemination (STAI) following controlled internal drug release (CIDR) based protocols. In experiment 1, estrus was synchronized for 816 heifers using the 14-day (d) CIDR-$PGF_{2\alpha}$ (PG) protocol and in experiment 2, estrus was synchronized for 622 cows using the 7-d CO-Synch + CIDR protocol. For both experiments, estrus detection aids (Estrotect) were applied at PG, with estrus recorded at 66 and 90 h after PG. Treatments were balanced across locations for heifers using reproductive tract score and weight; whereas for cows, treatments were assigned and balanced to treatment according to age, body condition score, and days postpartum. Timing of AI for heifers and cows was based on estrus expression 66 h after PG. Females in each treatment that exhibited estrus by 66 h were inseminated at 66 h, whereas AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for females failing to exhibit estrus by 66 h. Females in treatment 1 received GnRH 66 h after $PGF_{2\alpha}$ irrespective of estrus expression; however, in treatment 2, GnRH was administered coincident with delayed AI only to females not detected in estrus at 66 h after PG. Among heifers, there was no effect

of treatment on overall estrous response (P = 0.49) or AI pregnancy rate (P = 0.54). Pregnancy rate for heifers inseminated at 66 h was not influenced by GnRH (P = 0.65) and there were no differences between treatments in estrous response during the 24 h delay period (P = 0.22). Cows in treatment 2 had a greater (P = 0.04) estrous response during the 24 h delay period resulting in a greater overall estrous response (P = 0.04), but this did not affect AI pregnancy rate at 90 h (P = 0.51) or total AI pregnancy rate (P = 0.89). Pregnancy rate resulting from AI for cows inseminated at 66 h was not influenced by GnRH (P = 0.50). In summary, when split-time AI is used with the 14-d CIDR-PG protocol in heifers or the 7-d CO-Synch + CIDR protocol in cows, administration of GnRH at AI to females that exhibited estrus by 66 h after $PGF_{2\alpha}$ was not necessary. Furthermore, among heifers for which AI was delayed based on failure to exhibit estrus by 66 h after PG, timing of GnRH (66 vs 90 h after PG) was more flexible. However, delayed administration of GnRH to 90 h after $PGF_{2\alpha}$ coincident with AI for cows that fail to exhibit estrus by 66 h improved overall estrous response.

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

INTRODUCTION

The development of protocols that effectively facilitate synchronization of estrus and ovulation has enabled producers to increase the use of fixed time artificial insemination in beef heifers and cows. One of the greatest challenges in using FTAI in heifers and cows

is that females that fail to express or exhibit estrus by the time of insemination achieve lower pregnancy rates to FTAI [2, 3, 4, 100, 101, 102, 105, 108, 116].

Split-time artificial insemination involves a single insemination performed at one of two time points, and allows beef heifers and cows to be managed based on estrous status following the administration of an estrus synchronization protocol. This is in contrast to FTAI, in which all females are inseminated at a single predetermined time concurrent with the administration of GnRH, irrespective of estrous status. Split-time AI was shown to improve pregnancy rates among non-estrous cows when using sex-sorted semen in a timed AI setting. Delayed insemination (20 h) for cows that did not exhibit estrus prior to the time of appointment breeding allowed cows that received delayed insemination with sex sorted semen to achieve similar pregnancy rates to non-estrous cows inseminated with conventional semen after FTAI. Split-time AI has also been used with conventional semen in cows synchronized using the 7-d CO-Synch + CIDR protocol and heifers synchronized using the 14-d CIDR-PG protocol. To date, STAI has been shown to significantly improve AI pregnancy rates in heifers but not in cows [3, 4].

In the original studies that compared STAI to FTAI, GnRH was administered to all females (estrous and non-estrous) at 66 h following PG. Insemination of estrous females was performed concurrent with administration of GnRH, whereas, insemination of non-estrous females was delayed by 20 h (from 66 to 86 h following PG) [3, 4]. These studies raised questions pertaining to the appropriate time at which to administer GnRH when STAI is performed. The present work, performed among heifers following the 14-d CIDR-PG protocol (experiment 1) and cows following the 7-d CO-Synch + CIDR protocol (experiment 2), evaluates the need for administration of GnRH to estrous females at 66 h

after $PGF_{2\alpha}$ and the effect of delaying the administration of GnRH to 90 h on overall estrous response and STAI pregnancy rate.

MATERIALS AND METHODS

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

Experimental design: Experiment 1

Estrus was synchronized in 816 Angus and Angus-cross heifers across four locations using the 14-d CIDR-PG protocol (Figure 2.1). Heifers received an Eazi-Breed CIDR insert (1.38 g progesterone; Zoetis, Madison, NJ) on Day 0 with removal on Day 14; $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse, Zoetis, Madison, NJ) was administered 16 d after CIDR removal on Day 30; and GnRH (100 μg, i.m.; Cystorelin, Merial, Athens, GA) on Day 33 or 34 depending on treatment. Estrus detection aids (Estrotect, Rockway Inc, Spring Valley, WI) were applied at $PGF_{2\alpha}$ on Day 30, with estrus recorded at 66 and 90 h after $PGF_{2\alpha}$ on Days 33 and 34, respectively. Timing of insemination was based on expression of estrus 66 h after PG, with estrus being defined as having at least 50% of the coating rubbed off of the Estrotect patch. Heifers were preassigned to treatments across locations using reproductive tract score (RTS) and weight (BW) recorded at CIDR insertion [117, 118, 119]. Heifers with a RTS of one were removed from the experiment. Technicians that performed AI and AI sires were preassigned to treatments based on RTS and BW to ensure that treatments were not biased. Heifers in each treatment that exhibited estrus by 66 h were

inseminated, whereas AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for heifers failing to exhibit estrus by 66 h. Heifers in treatment 1 were administered GnRH 66 h after $PGF_{2\alpha}$ irrespective of estrus expression; however, in treatment 2, heifers were administered GnRH coincident with delayed insemination at 90 h only if not detected in estrus at 66 h after PG. For each heifer, times were recorded at which $PGF_{2\alpha}$ and GnRH were administered and AI was performed. Heifers were exposed to fertile bulls beginning 14 d after AI.

Experimental design: Experiment 2

Estrus was synchronized in 622 Angus and Angus-cross cows across six locations using the 7-d CO-Synch + CIDR protocol (Figure 2.2). Cows received GnRH (100 μg) and an Eazi-Breed CIDR insert (1.38 g progesterone) on Day 0; $PGF_{2\alpha}$ (25 mg) at CIDR removal on Day 7; and GnRH (100 μg) was administered on Day 10 or 11 depending on treatment. Estrus detection aids were applied at $PGF_{2\alpha}$ on Day 7, with estrus recorded at 66 and 90 h after $PGF_{2\alpha}$ on Days 10 and 11, respectively. Timing of insemination was based on expression of estrus 66 h after PG, with estrus being defined as having at least 50% of the coating rubbed off of the Estrotect patch. Cows were preassigned to treatments across locations by age, body condition score (BCS), and day postpartum (DPP). Technicians that performed AI and AI sires were preassigned to treatment based on age, BCS, and DPP. Cows in each treatment that exhibited estrus by 66 h were inseminated; however, AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for cows that failed to exhibit estrus by 66 h. Cows in treatment 1 were administered GnRH 66 h after $PGF_{2\alpha}$ irrespective of estrus expression, whereas in treatment 2, cows were administered GnRH coincident with delayed insemination at 90 h only if not detected in estrus at 66 h after PG. For each cow, times

were recorded at which $PGF_{2\alpha}$ and GnRH were administered and AI was performed. Cows were exposed to fertile bulls beginning 14 d after AI.

Pregnancy diagnosis.

Pregnancy rate to AI was determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA) 60 to 90 d after AI.

Statistical analysis.

Estrous response and AI pregnancy rate in both heifers and cows were evaluated to assess the effectiveness of GnRH administered at 66 or 90 h following PG. Differences between treatments for overall estrous response and AI pregnancy rate, as well as estrous response during the 24 h delay period were analyzed by using a contingency X^2 analysis (PROC FREQ; SAS Inst. Inc., Cary, NC). Variables of age, BCS, and DPP for cows and BW and RTS for heifers did not differ based on treatments by the TTEST procedure of SAS. An ANOVA analysis was performed to confirm the PROC FREQ analysis using the statistical model with treatment, estrus expression at 66 and 90 h, and the treatment by estrus expression interaction (PROC GLIMMIX; SAS Inst. Inc., Cary, NC).

RESULTS

Experiment 1

Treatment summary

The number of heifers, mean weight, and RTS for each location and treatment are shown in Table 2.1. For heifers in treatment 1 that were administered GnRH at the standard time, the interval from $PGF_{2\alpha}$ to GnRH (mean ± SEM) was 66.0 ± 0.1 h. For heifers in treatment 1 that failed to exhibit estrus by 66 h and in which case insemination was delayed, the interval from GnRH to insemination was 21.6 ± 0.1 h, or 87.2 ± 0.2 h from PG. For heifers in treatment 2 that expressed estrus by 66 h, the interval from $PGF_{2\alpha}$ to insemination (without administration of GnRH) was 65.9 ± 0.1 h. For heifers in this treatment that failed to exhibit estrus by 66 h and received delayed insemination, the interval from $PGF_{2\alpha}$ to GnRH administration, concurrent with insemination, was 87.6 ± 0.2 h.

Estrous response

Estrous response for heifers within each location and treatment is shown in Table 2.2. Estrous response did not differ at 66 h (1 = 70%; 2 = 69%). For all non-estrous heifers at 66 h that received delayed insemination at 90 h, heifers in treatment 1 that received GnRH at 66 h achieved a similar estrous response (50%) to heifers in treatment 2 that received GnRH at 90 h (58%, $P = 0.22$). Overall estrous response did not differ ($P = 0.49$) based on the time at which GnRH was administered (treatment 1, GnRH at 66 h = 85%; treatment 2, GnRH at 90 h = 87%).

Pregnancy rate to AI

Pregnancy rates of heifers to AI based on location, estrous response, and treatment are shown in Table 2.3. Heifers that expressed estrus by 66 h but did not receive GnRH concurrent with insemination achieved similar pregnancy rates ($P = 0.65$) to heifers that

received GnRH (64% for treatment 2 versus 62% for treatment 1). There were no differences between treatments in pregnancy rate resulting from AI for heifers that expressed estrus during the 24 h delay period (treatment 1, GnRH at 66 h = 59%; treatment 2, GnRH at 90 h = 61%). There was no significant effect of treatment on overall AI pregnancy rate (1 = 55%; 2 = 58%; P = 0.54). Finally, there were no differences in AI pregnancy rate based on sire or AI technician.

Experiment 2

Treatment summary

The number of cows, mean age, body condition score (BCS), and days postpartum (DPP) are shown in Table 2.4. For cows in treatment 1 that were administered GnRH at the standard time, the interval from $PGF_{2\alpha}$ to GnRH (mean ± SEM) was 66.6 ± 0.1 h. For cows in treatment 1 that failed to exhibit estrus by 66 h and received delayed insemination, the interval from GnRH to insemination was 22.9 ± 0.1 h, or 89.4 ± 0.1 h from PG. For cows in treatment 2 that expressed estrus by 66 h and did receive GnRH, the interval from $PGF_{2\alpha}$ to insemination was 66.6 ± 0.1 h. For cows in this treatment that failed to exhibit estrus by 66 h and received delayed insemination, the interval from $PGF_{2\alpha}$ to GnRH administration, concurrent with insemination, was 89.5 ± 0.1 h.

Estrous response

Estrous response for cows within each location and treatment is shown in Table 2.5. Estrous response at 66 h did not differ between treatments (1 = 73%; 2 = 75%). Delayed administration of GnRH to 90 h after $PGF_{2\alpha}$ resulted in a higher (P = 0.04) estrous

response during the 24 h delay period for cows in treatment 2 (61%) compared to treatment 1 (45%). This translated to a higher ($P = 0.04$) overall estrous response in treatment 2 (90%) compared to treatment 1 (85%).

Pregnancy rate to AI

Pregnancy rate to AI based on location, estrous response, and treatment is shown in Table 2.6. Cows that expressed estrus by 66 h that did not receive GnRH concurrent with insemination achieved similar pregnancy rates ($P = 0.89$) to cows that received GnRH (57% for treatment 2 versus 58% for treatment 1). There were no differences between treatments in pregnancy rate resulting from AI for cows that expressed estrus during the 24 h delay period (treatment 1, GnRH at 66 h = 44%; treatment 2, GnRH at 90 h = 49%; $P = 0.51$). There was no significant effect of treatment on overall AI pregnancy rate (1 = 58%; 2 = 57%; $P = 0.89$). Finally, there were no differences in AI pregnancy rate based on sire or AI technician.

DISCUSSION

Split-time artificial insemination delays insemination of non-estrous cows and heifers, allowing females to be managed based on estrous status at the time of AI. Split-time AI improved pregnancy rates compared to FTAI in beef cows when inseminations were performed using sex-sorted semen, although not with conventional semen. Differences in pregnancy rate were hypothesized to result from fertility associated effects related to lifespan of sperm in the female reproductive tract when considering the timing

of induced ovulations. In contrast, improvements in pregnancy rate following STAI in beef heifers using conventional semen were attributed to an increase in overall estrous response prior to insemination [3, 4]. The experiments involving STAI reported here were conducted to evaluate the effect of timing and administration of GnRH based on estrous status following treatment with the 14-d CIDR-PG or the 7-d CO-Synch + CIDR protocols in beef heifers and cows respectively.

Thomas et al. designed a series of experiments with STAI to determine whether delayed insemination of non-estrous females would optimize fertility by better aligning the timing of ovulation with insemination [3, 4]. Gonadotropin-releasing hormone is used routinely to synchronize ovulation in a TAI protocol to reduce the variation in timing of ovulation from PG. Among synchronized cows and heifers, ovulation occurred within an eight h time period between 24 and 32 h after GnRH was administered compared to a 36 h time period from 84 to 120 h after $PGF_{2\alpha}$ when GnRH was not administered [76]. Furthermore, GnRH-induced LH surges occur later than those that occur spontaneously [110]. Therefore, when managing females following the administration of protocols that facilitate TAI, non-estrous females that respond to exogenous GnRH are expected to ovulate later than females that exhibit estrus and experience their own endogenous LH surge [110]. By separating females on the basis of those that have expressed estrus versus those that have not in order to accommodate STAI, we are therefore able to determine more precisely the efficacy of GnRH administered at different times based on estrous status of individual females.

Pregnancy rates that result from TAI are expected to be higher in females that express estrus prior to insemination compared to those that do not [2, 3, 4, 100, 101, 102,

105, 108, 116]. The expression of estrus in cattle follows a rise in serum concentrations of estradiol, which in turn controls critical processes involved with the establishment of pregnancy, including effects on follicular cells, the oocyte, gamete transport, and preparation of the uterus for pregnancy [112]. Estradiol exerts its effect on oocyte maturation within the follicle both directly on the oocyte and indirectly on surrounding cumulus cells, and estradiol was shown to increase the likelihood of development to the blastocyst stage from studies conducted *in vitro*. Increases in follicular diameter are also positively correlated with increases in estradiol production and oocyte fertility [120, 121, 122]. Additionally, sperm transport is optimal at estrus, and the mechanism of estradiol action on sperm motility can be explained by changes in uterine pH following exposure to estradiol [123]. Cows that expressed estrus had increased concentrations of estradiol in serum and decreased uterine pH [124, 125]. These effects are known to decrease sperm motility, but also increase TAI pregnancy rates [126, 127]. Perry and Perry proposed that the expression of estrus in cattle increases pregnancy rates resulting from TAI through a pH-mediated decrease in sperm motility that increases sperm longevity. Increases in estradiol also prepare the uterus for pregnancy by regulating protein expression of numerous uterine secretions and receptors [124, 125, 128].

Aids used to detect estrus facilitated the development of new strategies that base timing of insemination on estrus expression, but previous experiments have not elicited answers to questions related to the necessity of GnRH administration in females that exhibit estrus prior to AI. In studies reported to date involving STAI when used in conjunction with the 14-d CIDR-PG and 7-d CO-Synch + CIDR protocols in beef heifers and cows, GnRH was administered to all females that exhibited estrus by 66 h after $PGF_{2\alpha}$ [3, 4].

However, these initial studies that described use of STAI were not designed to compare pregnancy rates that resulted on the basis of whether GnRH was administered to females expressing estrus by the time AI was performed. The results reported here clearly demonstrate that GnRH is not required for cows or heifers that exhibit estrus prior the time(s) at which STAI is performed. Furthermore, these results indicate that among females that failed to exhibit estrus by 66 h and that were inseminated 24 h later, pregnancy rates were affected more by expression of estrus during the 24 h delay period than by altering the timing of GnRH after PG. In each treatment, females that exhibited estrus during the 24 h delay period attained pregnancy rates that were up to 30% higher than those that failed to express estrus prior to AI. If in fact administering GnRH earlier was beneficial to heifers or cows that received delayed insemination, higher pregnancy rates would have been expected among females that were administered GnRH at 66 h and inseminated at 90 h, specifically among those females that failed to exhibit estrus during the 24 h delay period. This outcome was not observed.

We hypothesized that delayed administration of GnRH would maximize estrous response during the 24 h delay period in heifers and cows that were non-estrous at 66 h and therefore increase pregnancy rates that resulted from STAI. When GnRH is administered there is an expected decrease in estrus expression because an LH surge is induced, luteinization begins, and the endocrine function of granulosa cells shifts from secretion of androgen or estrogen to progesterone [129]. Lucy et al. reported that dairy cows and heifers that experienced GnRH-induced LH surges failed to exhibit estrus and achieved lower pregnancy rates. These females, in addition, had lower estradiol profiles compared to heifers and cows that did not receive GnRH, and those females that had LH surges before

or near the time GnRH was administered [110]. A higher proportion of cows that received delayed insemination in this study exhibited estrus during the 24 h delay period when the administration of GnRH was postponed to 90 h (61% vs 45%). Because pregnancy rates resulting from AI are generally higher when insemination is performed on the basis of estrus, we expected that delayed administration (90 h after PG) of GnRH would result in higher pregnancy rates than among cows that were administered GnRH earlier (66 h after PG). This increase was expected due to the anticipated increase in estrous response prior to AI. There was, however, no resulting increase in pregnancy rate, despite the increased number of cows that expressed estrus. The failure to detect differences in resulting pregnancy rates, despite the significant difference between treatments in the proportion of cows that exhibited estrus may be explained by high initial estrous response rates and the resulting low number of non-estrous cows per treatment. Differences in estrous response rates resulting from delayed administration of GnRH were not significant in heifers, and no differences in pregnancy rates between treatments were observed.

Although STAI allows more heifers and cows to express estrus by the time AI is performed, there remains a proportion of females that fail to exhibit estrus during the 24 h delay period. Pregnancy rates among this group of females are expected to be low. Failure to respond to GnRH in these cases may result from inadequate secretion of estradiol by Graafian follicles coincident with the time at which GnRH is administered. The likelihood of GnRH being able to induce an LH surge may be compromised when GnRH is administered prematurely because maturation of the Graafian follicle is most likely incomplete [110]. This theory is supported by reports that lower serum concentrations of estradiol coincident with the time at which GnRH is administered were directly

proportional to decreased magnitude and duration of LH release. These results provide evidence to explain the reduction in fertility that occurs when GnRH-induced ovulations were not accompanied by an estrous response [110].

It also remains possible that GnRH fails to induce ovulation in situations where females did not respond to an estrus synchronization protocol as expected. Numerous studies have shown that response to GnRH in heifers is inconsistent when compared to cows [76, 86, 87]. The results from this study are of interest when considering the use of GnRH in protocols designed to synchronize estrus and ovulation in beef heifers, as the requirement for inclusion of GnRH in estrus synchronization protocols specifically designed for beef heifers has been questioned [89, 90, 91]. In beef heifers, inclusion of GnRH at the beginning of a short-term CIDR-based protocol failed to increase pregnancy rates; however, the standard deviation of pregnancy rates was increased when GnRH was not included. In addition, Leitman et al. reported that long-term progestin-based protocols (14-d CIDR-PG) have been shown to be successful in synchronizing estrus in both prepubertal and estrous-cycling beef heifers by facilitating pre-synchronization of follicular waves without the use of GnRH prior to $PGF_{2\alpha}$ [90, 91].

Split-time AI in heifers following synchronization of estrus with the 14-d CIDR-PG protocol reduces the need for GnRH; first, by using a long-term progestin treatment to synchronize follicular development, and second, by delaying insemination of non-estrous heifers at 66 h to allow heifers more time to exhibit estrus before insemination is performed 24 h later. The reduced variance for interval to estrus following administration of this protocol and the resulting synchrony of estrus that results explain the consistency in pregnancy rates that can be achieved when using the protocol to facilitate FT- or STAI [90,

91]. Figure 2.3 illustrates estrus distribution patterns obtained using HeatWatch for 511 heifers over three years at a single location, following synchronization of estrus with the 14-d CIDR-PG protocol. Overall estrous response from these combined studies [90, 91, 108] is illustrated by the respective time intervals at which STAI was performed (66 and 90 h after PG) in the current study. The figure clearly illustrates the similarity in estrous response rates recorded over a six day period using HeatWatch to estrous response rates recorded in the present experiment using Estrotect patches to determine estrous status based on STAI. Consideration of these results makes the point that pregnancy rates resulting from AI were similar for heifers that were inseminated based on detected estrus over a six day period compared to pregnancy rates that resulted from STAI, where all heifers were inseminated at one of two time points within a 24 h period [90, 91, 108].

In summary, these data indicate that GnRH is not required for estrous females at 66 h following $PGF_{2\alpha}$ when the 14-d CIDR-PG or the 7-d CO-Synch + CIDR protocols are used prior to STAI in beef heifers and cows, respectively. In addition, these data indicate that among non-estrous females, the timing of administration of GnRH (66 or 90 h after PG) did not affect pregnancy rate resulting from AI. In cows, however, it is worthwhile to note that overall estrous response was increased when the administration of GnRH was delayed to 90 h, coincident with AI, for cows that were non-estrous at 66 h. These experiments addressed questions pertaining to the timing and use of GnRH when using split-time AI in beef heifers and cows. Further studies are needed to carefully evaluate the use and efficacy of GnRH in heifers that fail to exhibit estrus when using STAI, and the potential for improving pregnancy rates with ST- over FTAI in cows when administration of GnRH is delayed to 90 h.

Table 2.1. Heifer weight (BW) and reproductive tract score (RTS) based on location and treatment[a].

Location	Treatment	*n*	BW, kg	RTS
1	Treatment 1	39	354.1 ± 4.5	3.8 ± 0.16
	Treatment 2	40	355.0 ± 4.1	3.7 ± 0.15
2	Treatment 1	66	402.3 ± 3.2	4.4 ± 0.04
	Treatment 2	69	401.7 ± 3.2	4.3 ± 0.04
3	Treatment 1	78	370.8 ± 3.3	4.3 ± 0.08
	Treatment 2	70	364.7 ± 3.6	4.3 ± 0.10
4	Treatment 1	222	389.9 ± 1.7	4.3 ± 0.04
	Treatment 2	232	392.7 ± 1.6	4.4 ± 0.03
Total	Treatment 1	405	384.9 ± 1.7	4.3 ± 0.04
	Treatment 2	411	385.8 ± 1.7	4.3 ± 0.04

Data presented as mean values (±SEM)

Abbreviations: BW, weight; RTS, reproductive tract score

[a]Treatment 1: GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all heifers, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h; Treatment 2: TAI without GnRH administration for heifers having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination with GnRH administered concurrently at 90 h for heifers failing to express estrus prior to 66 h (Figure 2.1).

Table 2.2. Estrous response in heifers based on location and treatment[a].

		Treatment 1		Treatment 2	
Location	Estrous Status	Proportion	%	Proportion	%
1	Overall estrous response	38/39	97	35/40	88
	Estrous by 66 h	36/39	92	33/40	83
	Estrous 66-90 h	2/3	67	2/7	29
2	Overall estrous response	53/66	80	60/69	87
	Estrous by 66 h	44/66	67	48/69	70
	Estrous 66-90 h	9/22	41	12/21	57
3	Overall estrous response	67/78	86	60/70	86
	Estrous by 66 h	56/78	72	42/70	60
	Estrous 66-90 h	11/22	50	18/28	64
4	Overall estrous response	187/222	84	202/232	87
	Estrous by 66 h	149/222	67	160/232	69
	Estrous 66-90 h	38/73	52	42/72	58
Combined	Overall estrous response	345/405	85	357/411	87
	Estrous by 66 h	284/405	70	283/411	69
	Estrous 66-90 h	61/120	50	74/128	58

[a]Treatment 1: GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all heifers, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h; Treatment 2: TAI without GnRH administration for heifers having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination with GnRH administered concurrently at 90 h for heifers failing to express estrus prior to 66 h (Figure 2.1).

Table 2.3. Pregnancy rates[a] in heifers resulting from split-time artificial insemination based on location, estrous response, and treatment[b].

		STAI pregnancy rate			
		Treatment 1		Treatment 2	
Location	Estrous response	Proportion	%	Proportion	%
Location 1	Estrous by 66 h	25/36	69	20/33	61
	Estrous by 90 h	0/2	0	0/2	0
	Non-estrous 90 h	0/1	0	1/5	20
	Total	25/39	64	21/40	53
Location 2	Estrous by 66 h	22/43	51	27/48	56
	Estrous by 90 h	6/10	60	9/12	75
	Non-estrous 90 h	4/13	31	2/9	22
	Total	32/66	48	38/69	55
Location 3	Estrous by 66 h	32/56	57	27/42	64
	Estrous by 90 h	6/11	55	10/18	56
	Non-estrous 90 h	2/11	18	2/10	20
	Total	40/78	51	39/70	56
Location 4	Estrous by 66 h	96/149	64	106/160	66
	Estrous by 90 h	24/38	63	26/42	62
	Non-estrous 90 h	7/35	20	6/30	20
	Total	127/222	57	138/232	59
Total	Estrous by 66 h	175/284	62	180/283	64
	Estrous by 90 h	36/61	59	45/74	61
	Non-estrous 90 h	13/60	22	11/54	20
	Total	224/405	55	236/411	57

Abbreviations: STAI, Split-time AI

[a]Pregnancy rate to timed insemination at 66 h and 90 h after $PGF_{2\alpha}$, determined by ultrasound 60 to 90 d after AI.

[b]Treatment 1: GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all heifers, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h; Treatment 2: TAI without GnRH administration for heifers having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination with GnRH administered concurrently at 90 h for heifers failing to express estrus prior to 66 h (figure 2.1).

Table 2.4. Cow age, body condition score[b], and days postpartum[c] based on location and treatment[a].

Location	Treatment	*n*	Age	BCS	DPP
1	Treatment 1	37	5.4 ± 0.4	6.5 ± .08	68.1 ± 2.3
	Treatment 2	37	5.4 ± 0.4	6.4 ± .07	68.5 ± 2.0
2	Treatment 1	39	5.0 ± 0.4	6.1 ± 0.1	81.3 ± 1.9
	Treatment 2	40	5.0 ± 0.4	5.9 ± .09	84.0 ± 2.1
3	Treatment 1	39	8.0 ± 0.4	5.5 ± .08	67.0 ± 2.8
	Treatment 2	41	8.0 ± 0.4	5.7 ± .08	68.4 ± 2.5
4	Treatment 1	65	3.0 ± 0.1	5.8 ± .07	85.7 ± 2.6
	Treatment 2	62	3.1 ± 0.1	5.8 ± .07	85.8 ± 2.7
5	Treatment 1	67	5.4 ± 0.2	5.6 ± .05	82.3 ± 1.4
	Treatment 2	63	5.2 ± 0.2	5.7 ± .06	83.0 ± 1.4
6	Treatment 1	66	5.9 ± 0.2	5.8 ± .08	82.2 ± 1.0
	Treatment 2	66	6.0 ± 0.2	5.7 ± .07	82.7 ± 1.1
Total	Treatment 1	313	5.3 ± 0.1	5.8 ± .03	79.2 ± .91
	Treatment 2	309	5.3 ± 0.1	5.8 ± .03	80.0 ± .89

Data presented as mean values (±SEM)

Abbreviations: BCS, body condition score; DPP, days postpartum

[a]Cows in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg, i.m.) on d 0. The CIDR insert was removed on d 7 and $PGF_{2\alpha}$ (25 mg, i.m.) was administered. Cows were assigned to 1 of 2 treatments: 1) GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all cows, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h or 2) TAI without GnRH administration for cows having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination with GnRH administered concurrently at 90 h for cows failing to express estrus prior to 66 h (Figure 2.2).

[b]BCS of cows at the time of $PGF_{2\alpha}$ (25 mg, i.m.) injection (1 to 9 scale, where 1 = emaciated and 9 = obese).

[c]DPP calculated from calving date to breeding date.

Table 2.5. Estrous response in cows based on location and treatment[a].

		Treatment 1		Treatment 2	
Location	Estrous Status	Proportion	%	Proportion	%
1	Overall estrous response	28/37	76	33/37	89
	Estrous by 66 h	25/37	68	26/37	70
	Estrous 66-90 h	3/12	25	7/11	64
2	Overall estrous response	35/39	90	36/40	90
	Estrous by 66 h	31/39	79	35/40	88
	Estrous 66-90 h	4/8	50	1/5	20
3	Overall estrous response	28/39	72	36/41	88
	Estrous by 66 h	21/39	54	28/41	68
	Estrous 66-90 h	7/18	39	8/13	62
4	Overall estrous response	57/65	88	54/62	87
	Estrous by 66 h	51/65	78	44/62	71
	Estrous 66-90 h	6/14	43	10/18	56
5	Overall estrous response	63/67	94	58/63	92
	Estrous by 66 h	53/67	79	48/63	76
	Estrous 66-90 h	10/14	71	10/15	67
6	Overall estrous response	55/66	83	62/66	94
	Estrous by 66 h	47/66	71	52/66	79
	Estrous 66-90 h	8/19	42	10/14	71
Combined	Overall estrous response	266/313	85[b]	279/309	90[b]
	Estrous by 66 h	228/313	73	233/309	75
	Estrous 66-90 h	38/85	45[c]	46/76	61[c]

[a]Cows in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg, i.m.) on d 0. The CIDR insert was removed on d 7 and $PGF_{2\alpha}$ (25 mg, i.m.) was administered. Cows were assigned to 1 of 2 treatments: 1) GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all cows, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h or 2) TAI without GnRH administration for cows having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination with GnRH administered concurrently at 90 h for cows failing to express estrus prior to 66 h (Figure 2.2).

[b]Overall estrous responses differ between treatments ($P = 0.04$)

[c]Estrous responses from 66 to 90 h differ between treatments ($P = 0.04$)

Table 2.6. Pregnancy rates[a] in cows resulting from STAI based on location, estrous response, and treatment[b]

		STAI pregnancy rate			
		Treatment 1		Treatment 2	
Location	Estrous response	Proportion	%	Proportion	%
Location 1	Estrous by 66 h	14/25	56	13/26	50
	Estrous 66 h - 90 h	2/3	67	1/7	14
	Non-estrous 90 h	2/9	22	1/4	25
	Total	18/37	49	15/37	41
Location 2	Estrous by 66 h	21/31	68	25/35	71
	Estrous 66 h - 90 h	3/4	75	0/1	0
	Non-estrous 90 h	3/4	75	0/4	0
	Total	27/39	69	25/40	63
Location 3	Estrous by 66 h	13/21	62	17/28	61
	Estrous 66 h - 90 h	1/7	14	6/8	75
	Non-estrous 90 h	1/11	9	0/5	0
	Total	15/39	38	23/41	56
Location 4	Estrous by 66 h	34/51	67	29/44	64
	Estrous 66 h - 90 h	6/6	100	8/10	80
	Non-estrous 90 h	3/8	38	5/8	63
	Total	43/65	66	42/62	68
Location 5	Estrous by 66 h	33/53	62	27/48	56
	Estrous 66 h - 90 h	6/10	60	4/10	40
	Non-estrous 90 h	2/4	50	1/5	20
	Total	41/67	61	32/63	51
Location 6	Estrous by 66 h	28/47	60	28/52	54
	Estrous 66 h - 90 h	5/8	63	9/10	90
	Non-estrous 90 h	3/11	27	2/4	50
	Total	36/66	55	39/66	59
Total	Estrous by 66 h	143/228	63	139/223	60
	Estrous by 90 h	23/38	61	28/46	61
	Non-estrous 90 h	14/47	30	9/30	30
	Total	180/313	58	176/309	57

[a]Pregnancy rate to timed insemination at 66 h and 90 h after $PGF_{2\alpha}$, determined by ultrasound 60 to 90 d after AI.

[b]Cows in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg, i.m.) on d 0. The CIDR insert was removed on d 7 and $PGF_{2\alpha}$ (25 mg, i.m.) was administered. Cows were assigned to 1 of 2 treatments: 1) GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all cows, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h or 2) TAI without GnRH administration for cows having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination with GnRH administered concurrently at 90 h for cows failing to express estrus by 66 h (Figure 2.2)

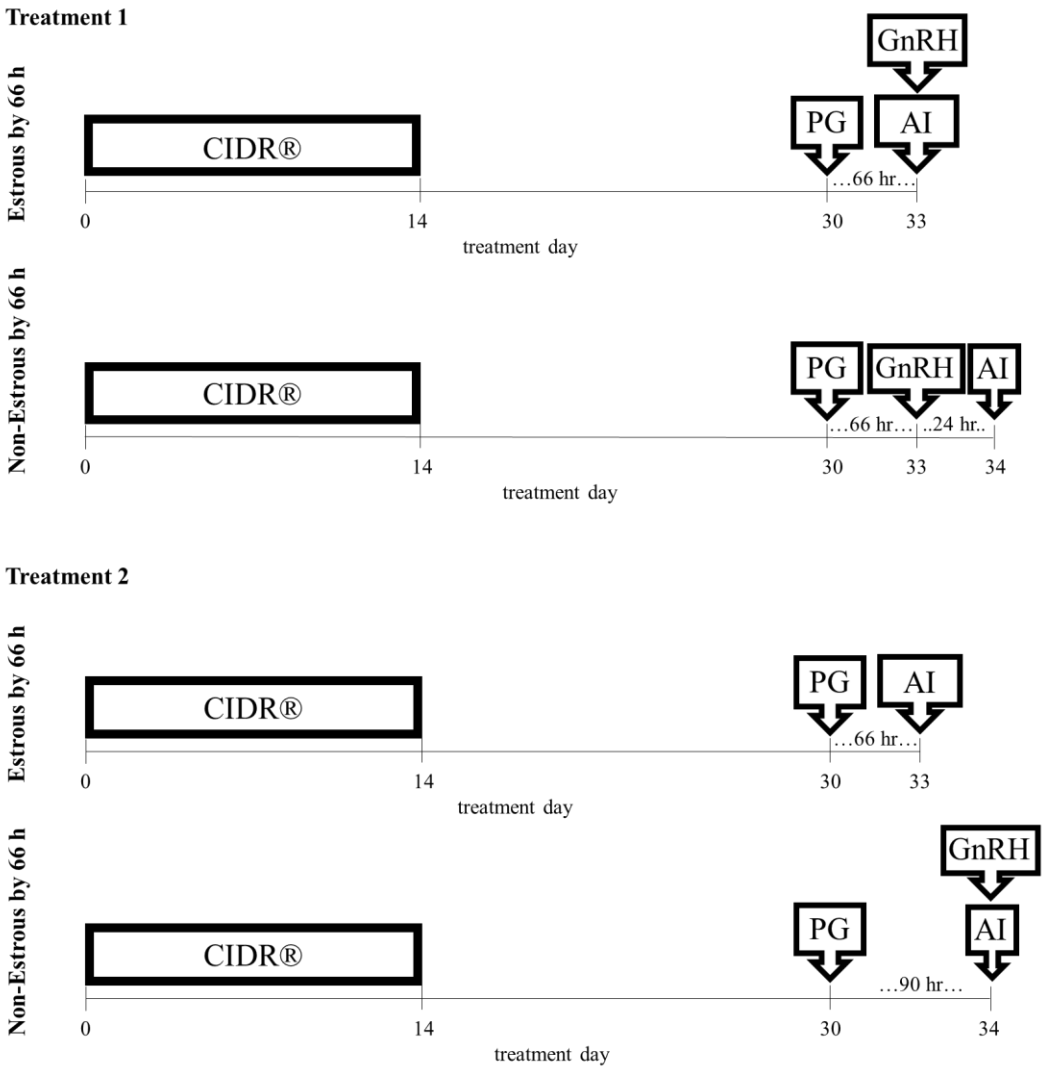

Figure 2.1. Treatment diagrams for Experiment 1; STAI in heifers following the 14-d CIDR-PG protocol. Treatment 1: GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all heifers, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h; Treatment 2: TAI without GnRH administration for heifers having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination concurrent with GnRH administration at 90 h for heifers failing to express estrus prior to 66 h.

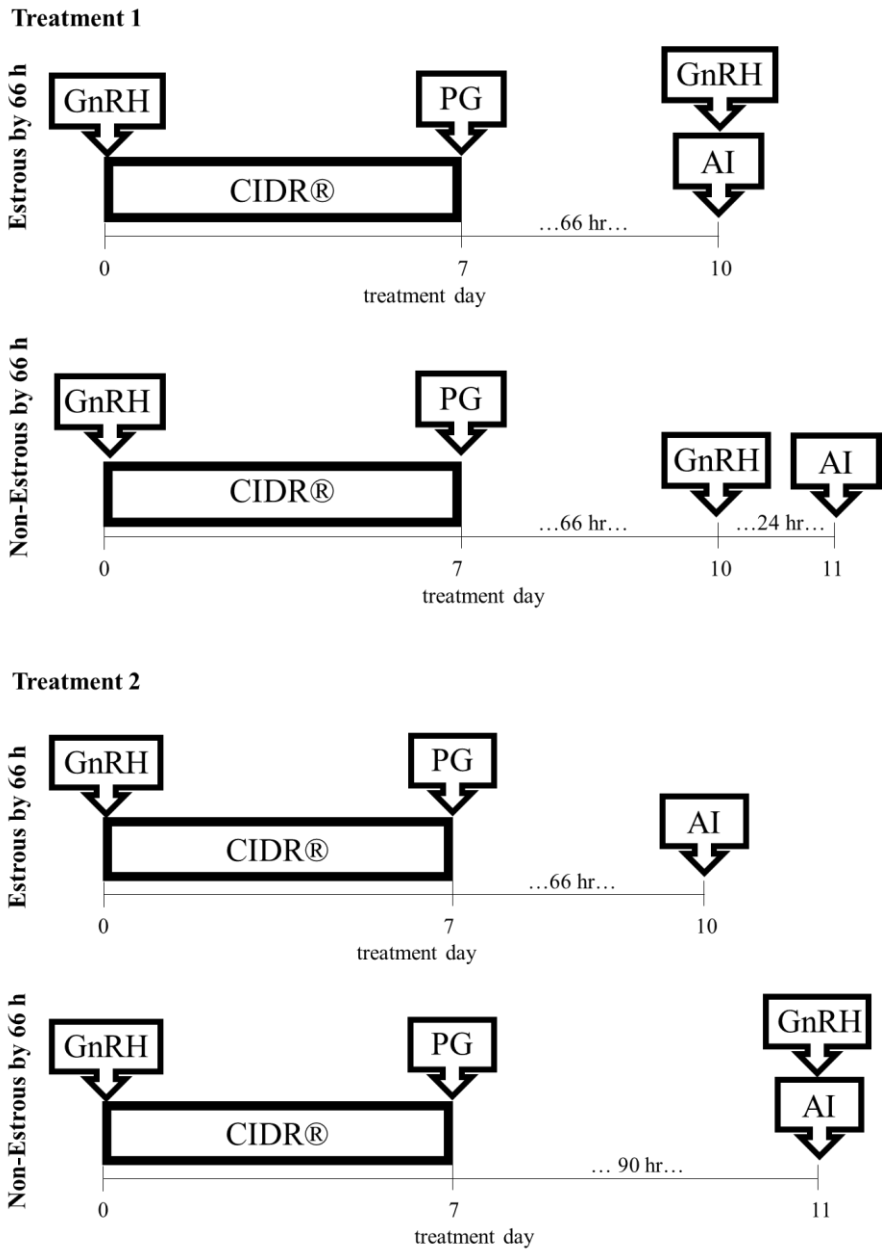

Figure 2.2. Treatment diagrams for Experiment 2; STAI in cows following the 7-d CO-Synch + CIDR protocol. Cows in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg, i.m.) on d 0. The CIDR insert was removed on d 7 and $PGF_{2\alpha}$ (25 mg, i.m.) was administered. Cows were assigned to 1 of 2 treatments: 1) GnRH (100 μg, i.m.) at 66 h after $PGF_{2\alpha}$ for all cows, with concurrent TAI for those having expressed estrus prior to 66 h and delayed insemination at 90 h for those failing to express estrus prior to 66 h or 2) TAI without GnRH administration for cows having expressed estrus prior to 66 h after $PGF_{2\alpha}$ and delayed insemination concurrent with GnRH administration at 90 h for cows failing to express estrus prior to 66 h.

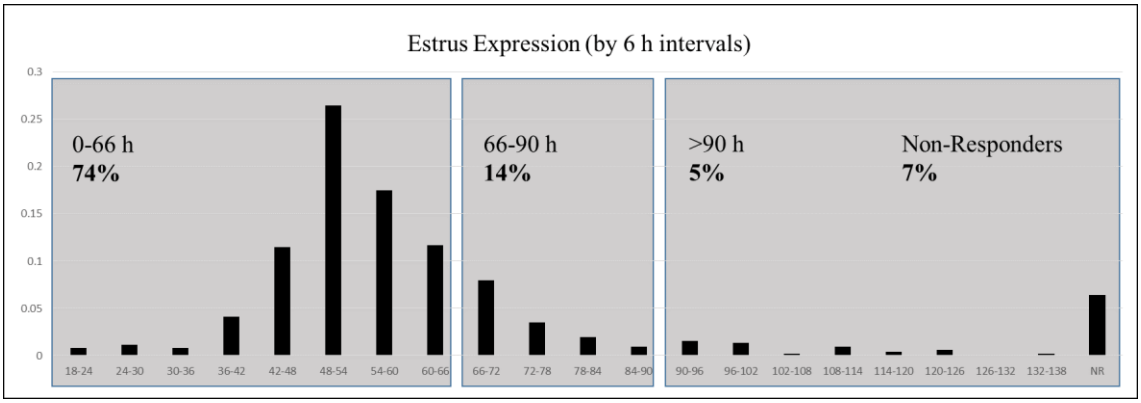

Figure 2.3. Estrus distribution obtained using HeatWatch for 511 heifers over three years at a single location, following synchronization of estrus with the 14-d CIDR-PG protocol. Overall estrous response is broken down into the respective time intervals at which STAI is performed (66 and 90 h after PG) [90, 91, 108].

CHAPTER 3

SPLIT-TIME ARTIFICIAL INSEMINATION IN BEEF CATTLE: II. COMPARING PREGNANCY RATES AMONG NON-ESTROUS HEIFERS BASED ON ADMINISTRATION OF GNRH AT AI

ABSTRACT

This experiment was designed to evaluate split-time artificial insemination (STAI) in beef heifers following administration of the 14-day (d) controlled internal drug release (CIDR)-$PGF_{2\alpha}$ (PG) protocol and to compare pregnancy rates among non-estrous heifers based on administration of GnRH at AI. Estrus was synchronized for 1138 heifers across six locations. Heifers received a CIDR insert (1.38 g progesterone) on d 0 with removal on d 14, then estrus detection aids (Estrotect) were applied at $PGF_{2\alpha}$ (PG, 25 mg) 16 d after CIDR removal on d 30. Treatments were balanced across locations for heifers using reproductive tract score and weight. Split-time AI was performed at 66 and 90 h after PG, and estrus was recorded at these times. Heifers in both treatments that exhibited estrus by 66 h were inseminated at 66 h without the administration of GnRH, whereas AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for heifers failing to exhibit estrus by 66 h. For heifers in treatment 1 that were inseminated at 90 h, GnRH (100 μg) was administered concurrent with AI at 90 h. Heifers in treatment 2 that were inseminated at 90 h were not administered

GnRH. Ovulation was confirmed via ultrasonography for a subset of heifers that failed to exhibit estrus prior to 90 h after PG. There was no effect of treatment on total AI pregnancy rate (P = 0.60) or on AI pregnancy rate for heifers inseminated at 66 h (P = 0.86) or 90 h (P = 0.50) after PG. Estrous response did not differ between treatments at 66 (P = 0.58) or 90 h (P = 0.21) after PG. Treatments did not differ in ovulation rate for heifers that failed to exhibit estrus by 90 h (P = 0.64) and ovulation rate did not affect AI pregnancy rate (P = 0.97). In summary, administration of GnRH is not necessary when split-time AI is used in conjunction with the 14-d CIDR-PG protocol in heifers.

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

INTRODUCTION

Gonadotropin-releasing hormone is used in timed AI (TAI) protocols for beef heifers to ovulate a dominant follicle at the time of insemination, but response to GnRH in heifers is inconsistent and can result in poor pregnancy rates [84, 86]. Furthermore, pregnancy rates to AI are lower in heifers that fail to express estrus prior to insemination, especially when smaller follicles are ovulated in response to administration of GnRH [2, 3, 4, 100, 101, 102, 105, 108, 116].

Split-time artificial insemination following the 14-d CIDR-PG protocol in heifers allows females to be managed based on estrous response at the time of insemination in order to increase pregnancy rates compared to FTAI. In STAI, insemination is delayed for non-estrous females by 20 to 24 h, whereas with FTAI all heifers are inseminated at a single

time. In the original field trials that compared STAI and FTAI all heifers were administered GnRH at 66 h irrespective of estrous status. The working hypothesis in that study was that delayed insemination of non-estrous heifers would allow more time for heifers to respond to GnRH and better align the timing of insemination with ovulation [3, 4].

Later experiments compared the timing of GnRH administration during STAI. Results from these studies showed that administration of GnRH was not required for heifers that exhibit estrus prior to AI and that administration of GnRH for heifers that fail to exhibit estrus by 66 h could be delayed to 90 h, concurrent with insemination. This work confirmed the fact that higher pregnancy rates resulting from STAI compared with FTAI are due to higher estrous response rates prior to insemination, in contrast to the theory that earlier administration of GnRH allows more time for ovulation to occur before insemination is performed [130, 131]. Despite the high overall estrous response that is observed in heifers when split-time AI is practiced, a percentage of heifers fail to exhibit estrus prior to 90 h after PG. Pregnancy rates resulting from AI are generally reduced among these heifers compared with those that exhibit estrus, despite the fact GnRH is routinely administered concurrent with AI. The present experiment was designed to further evaluate STAI in heifers following synchronization of estrus with the 14-d CIDR-PG protocol and to determine the need for administering GnRH to heifers that fail to exhibit estrus by 90 h after PG.

MATERIALS AND METHODS

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

Experimental Design.

Estrus was synchronized in 1138 Angus and Angus-cross heifers across six locations using the 14-d CIDR-PG protocol (Figure 3.1). Heifers received an Eazi-Breed CIDR insert (1.38 g progesterone; Zoetis, Madison, NJ) on Day 0 with removal on Day 14; $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse, Zoetis, Madison, NJ) was administered 16 d after CIDR removal on Day 30; and GnRH was administered (100 μg, i.m.; Cystorelin, Merial, Athens, GA) on Day 34 depending on treatment. Estrus detection aids (Estrotect, Rockway Inc, Spring Valley, WI) were applied at $PGF_{2\alpha}$ on Day 30, with estrus recorded at 66 and 90 h after $PGF_{2\alpha}$ on Days 33 and 34, respectively. Timing of insemination was based on expression of estrus 66 h after $PGF_{2\alpha}$, with estrus being defined as having at least 50% of the coating rubbed off of the Estrotect patch. Heifers were preassigned to treatments across locations using reproductive tract score (RTS) and weight (BW) recorded at CIDR insertion [117, 118, 119]. Heifers with a RTS of one were removed from the experiment. Technicians that performed AI and AI sires were preassigned to treatments based on RTS and BW to ensure that treatments were not biased. Heifers in each treatment that exhibited estrus by 66 h were inseminated, whereas AI was delayed 24 h until 90 h after $PGF_{2\alpha}$ for heifers failing to exhibit estrus by 66 h. Heifers in treatment 1 were administered GnRH 90 h after $PGF_{2\alpha}$ irrespective of estrus expression, whereas, in treatment 2, GnRH was not

administered. For each heifer, times were recorded at which $PGF_{2\alpha}$ and GnRH were administered and AI was performed. For heifers that failed to exhibit estrus by 90 h after PG, ovulation was confirmed via transrectal ultrasonography. Heifers were exposed to fertile bulls beginning 14 d after AI.

Determining Ovulation Status

Ovulatory status was determined for a subset of heifers that failed to express estrous prior to 90 h after $PGF_{2\alpha}$ via transrectal ultrasonography of ovaries (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA). Heifers that failed to express estrus by 90 h after PG, were inseminated at 90 h with or without the administration of GnRH at AI. Ovaries of these heifers were then re-examined 48 h after AI to determine disappearance of the dominant follicle.

Pregnancy Diagnosis

Pregnancy rate to AI was determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA) 60 to 90 d after AI.

Statistical Analysis

Total AI pregnancy rate and ovulatory status for heifers that failed to express estrous by 90 h after $PGF_{2\alpha}$ were evaluated to assess the effectiveness of GnRH administered 90 h following PG. Differences between treatments for overall AI pregnancy rate, estrous response at 66 and 90 h, and ovulation rate were analyzed by using a

contingency X2 analysis (PROC FREQ; SAS Inst. Inc., Cary, NC). Pregnancy rate to AI for heifers that failed to exhibit estrus by 90 h after $PGF_{2\alpha}$ was analyzed using logistic regression (PROC GLIMMIX; SAS Inst. Inc., Cary, NC) with treatment, ovulation rate, and their interaction in the model. Pregnancy rate to AI for heifers inseminated at 66 and 90 h was also analyzed using PROC GLIMMIX with treatment, estrous response at 66 and 90 h, and their interaction in the model. Variables of BW and RTS for heifers did not differ based on treatments by the TTEST procedure of SAS.

RESULTS

Estrous response

Estrous response for heifers within each location and treatment is shown in Table 3.2. Estrous response did not differ at 66 h (1 = 70%; 2 = 71%; $P = 0.58$) or 90 h (1 = 59%; 2 = 52%; $P = 0.21$). Overall estrous response therefore was similar between treatments (1 = 88%; 2 = 86%; $P = 0.50$).

Ovulation Rate

Within the subset of heifers for which ultrasound was performed to determine ovulatory status, a similar number of heifers ovulated after insemination at 90 h independent of GnRH administration (Treatment 1, GnRH at 90 h = 52%; Treatment 2, no GnRH = 50%; $P = 0.42$). A dominant follicle was present in 93% of heifers in Treatment 1 and 84% of heifers in Treatment 2. Diameter of the largest follicle (LFD) did not differ

between treatments (1 = 11.5; 2 = 12.3; P = 0.29) and heifers that ovulated in each treatment had similar LFDs (1 = 11.7; 2 = 13.4; *P* = 0.11).

Pregnancy rate to AI

Pregnancy rates of heifers to AI based on location, estrous response, and treatment are shown in Table 3.3. Heifers in both treatments that expressed estrus by 66 h achieved similar pregnancy rates (1 = 58%; 2 = 62%; *P* = 0.86). During the 24 h delay period there were no differences between treatments in pregnancy rate resulting from AI for heifers that expressed estrus (treatment 1, GnRH at 90 h = 59%; treatment 2, no GnRH = 59%; *P* = 0.95) or for heifers that failed to express estrus (treatment 1, GnRH at 90 h = 23%; treatment 2, no GnRH = 16%; *P* = 0.35). There was no significant effect of treatment on overall AI pregnancy rate (1 = 54%; 2 = 56%; *P* = 0.60). Finally, there were no differences in AI pregnancy rate based on sire or AI technician.

Within the subset of heifers for which ultrasound was performed to determine ovulatory status, pregnancy rate did not differ between treatments (1 = 24%; 2 = 15%; P = 0.97). For heifers in which ovulation was confirmed, pregnancy rates were similar between treatments (1 = 15%; 2 = 31%; *P* = 0.36). In Treatment 2, four heifers became pregnant to AI that were not confirmed to have ovulated a dominant follicle within 48 h, whereas none of the heifers failing to ovulate in Treatment 1 became pregnant, however, these differences were not significant (*P* = 0.97).

DISCUSSION

Split-time artificial insemination was developed as a novel breeding strategy that improves AI pregnancy rates in heifers following estrus synchronization using the 14-d CIDR- $PGF_{2\alpha}$ protocol. Although initial trials with STAI recommended administering GnRH to non-estrous heifers at 66 h with delayed insemination 20 h later, pregnancy rates were similar for non-estrous heifers that received delayed insemination concurrent with administration of GnRH at 90 h [3, 4, 130, 131]. Split-time AI has been used successfully in beef heifers because of increased estrous expression during the 20 to 24 h delay period, which supports results from previous studies that showed that as estrous response prior to AI increased pregnancy rates were improved by as much as 27% [2].

Bishop et al. compared early and delayed administration of GnRH, which raised questions regarding the use of GnRH in heifers following STAI. First, estrous response recorded during the delay period was similar for heifers that received GnRH at 66 or 90 h after $PGF_{2\alpha}$ (50% and 58%, respectively; $P = 0.22$). These data were in contrast to results in cows where estrous response was decreased by 16% ($P = 0.04$) when GnRH was administered at 66 compared to 90 h after $PGF_{2\alpha}$ [130, 131]. Administration of GnRH is expected to reduce estrus expression as luteinization is initiated in response to GnRH, which then decreases the production of follicular estradiol [129]. The marked difference in estrous expression between heifers and cows following administration of GnRH suggests that GnRH perhaps fails to induce luteinization in a proportion of heifers when administered at 66 h after $PGF_{2\alpha}$. The second important observation from that experiment is that heifers that failed to exhibit estrus prior to 90 h after $PGF_{2\alpha}$ achieved very low

pregnancy rates of 20-22% [130, 131]. This may indicate that GnRH fails to induce ovulation in a proportion of heifers or that GnRH induced ovulations are less fertile in heifers than in cows.

There were no significant differences in pregnancy rate resulting from AI in this study when GnRH was omitted from the STAI protocol. This observation supports the theory that GnRH may be ineffective at inducing ovulation among non-estrous heifers at 90 h when STAI is practiced. Ovulatory status was determined for a subset heifers in both treatments to assess differences among heifers based on whether or not GnRH was administered coincident with AI at 90 h. There was no difference in the number of heifers that ovulated a dominant follicle based on whether GnRH was or was not administered to heifers that failed to exhibit estrus by 90 h after $PGF_{2\alpha}$ (Treatment 1, GnRH at 66 h = 52%; Treatment 2, no GnRH = 50%; $P = 0.42$). Additionally, there were no differences in AI pregnancy rate for heifers that ovulated a dominant follicle (Treatment 1 = 15%; Treatment 2 = 31%; $P = 0.36$).

These results are not the first to demonstrate a failure of GnRH to induce ovulation of a dominant follicle in heifers. Treatment with GnRH at random stages of the estrous cycle is estimated to induce ovulation in 66% of cows but only 50% of heifers, with stage of development of the dominant follicle being the determining factor for whether or not heifers respond [76, 85]. For this reason, long-term progestin-based protocols are more successful at pre-synchronizing follicular waves in heifers compared to shorter protocols such as the 7-d CO-Synch + CIDR protocol that require GnRH administration to reset follicular development at the time a CIDR is inserted [90, 91, 104, 105]. Even when

follicular waves were pre-synchronized using a progestin, GnRH administration seven d prior to AI failed to increase synchrony of estrus at AI [90, 91].

A possible explanation for failure of GnRH to induce ovulation when STAI is used in conjunction with the 14-d CIDR- $PGF_{2\alpha}$ protocol may be related to stage of the estrous cycle at which time GnRH is administered. When experiments were performed assessing ovulation rate on different d of the estrous cycle, only 20% of beef heifers responded to GnRH as late as Day 18 [93]. Ovulatory response to GnRH is dependent on LH receptors in the dominant follicle, which increase in number during its growth, but decrease once the dominant follicle undergoes atresia [94, 95, 96]. The number of follicular waves per estrous cycle is inconsistent in heifers and affects the interval from follicular growth to atresia during each follicular wave. This narrows the window of time at which a dominant follicle is responsive to GnRH [41, 45]. With STAI following administration of the 14-d CIDR- $PGF_{2\alpha}$ protocol, CIDRs are removed on Day 14 and estrus subsequently occurs between Days 16 and 18 of the protocol. When GnRH is then administered 90 h following PG, this corresponds to Day 34 of the protocol, or Days 16 to 18 after estrus.

This experiment demonstrates that GnRH was ineffective at inducing ovulation on Day 34 of the 14-d CIDR- $PGF_{2\alpha}$ protocol, and eliminates the need to administer GnRH when STAI is performed following its administration. It remains unclear, however why in many instances heifers fail to ovulate a dominant follicle in response to GnRH and raises questions regarding the general efficacy of GnRH in heifers. These data support previously published results that point to the potential to improve pregnancy rates in beef heifers when STAI is used in conjunction with the 14-d CIDR- $PGF_{2\alpha}$ protocol.

Table 3.1. Heifer weight (BW) and reproductive tract score (RTS) based on location and treatment[a].

Location	Treatment	*n*	BW, kg	RTS
1	1	93	374.7 ± 4.2	4.2 ± 0.1
	2	89	372.4 ± 3.9	4.1 ± 0.1
2	1	115	321.2 ± 3.5	4.0 ± 0.1
	2	115	322.7 ± 3.8	4.1 ± 0.1
3	1	38	364.2 ± 2.9	4.0 ± 0.1
	2	40	363.8 ± 3.0	4.0 ± 0.1
4	1	226	380.5 ± 3.9	4.3 ± 0.1
	2	230	379.9 ± 3.7	4.3 ± 0.1
5	1	40	375.0 ± 3.7	4.2 ± 0.1
	2	40	375.9 ± 3.8	4.3 ± 0.1
6	1	56	373.9 ± 4.0	3.9 ± 0.1
	2	56	374.0 ± 3.9	4.0 ± 0.1
Total	1	568	365.4 ± 1.6	4.2 ± 0.0
	2	570	365.2 ± 1.6	4.2 ± 0.0

Data presented as mean values (±SEM)

Abbreviations: BW, weight; RTS, reproductive tract score

[a]Heifers in both treatments were inseminated utilizing split-time AI. Heifers in each treatment that exhibited estrus by 66 h after $PGF_{2\alpha}$ were inseminated, however GnRH was not administered to heifers in either treatment at the time AI was performed. Heifers in both treatments that failed to exhibit estrus by 66 h were inseminated at 90 h. When AI was performed at 90 h, heifers in Treatment 1 were administered GnRH at AI, however GnRH was not administered to heifers in Treatment 2 (Figure 3.1).

Table 3.2. Estrous response in heifers based on location and treatment[a].

		Treatment 1		Treatment 2	
Location	Estrous Status	Proportion	%	Proportion	%
1	Overall estrous response	85/93	91	76/89	85
	Estrous by 66 h	74/93	80	69/89	78
	Estrous 66-90 h	11/19	58	7/20	35
2	Overall estrous response	99/115	86	97/115	84
	Estrous by 66 h	84/115	73	84/115	73
	Estrous 66-90 h	15/31	48	13/31	42
3	Overall estrous response	35/38	92	35/40	88
	Estrous by 66 h	28/38	74	31/40	78
	Estrous 66-90 h	7/10	70	4/9	44
4	Overall estrous response	193/226	85	207/230	90
	Estrous by 66 h	144/226	64	160/230	70
	Estrous 66-90 h	49/82	60	47/70	67
5	Overall estrous response	33/40	83	31/40	78
	Estrous by 66 h	27/40	68	22/40	55
	Estrous 66-90 h	6/13	46	9/18	50
6	Overall estrous response	52/56	93	45/56	80
	Estrous by 66 h	38/56	68	39/56	70
	Estrous 66-90 h	14/18	78	6/17	35
Total	Overall estrous response	497/568	88	491/570	86
	Estrous by 66 h	395/568	70	405/570	71
	Estrous 66-90 h	102/173	59	86/166	52

[a]Heifers in both treatments were inseminated utilizing split-time AI. Heifers in each treatment that exhibited estrus by 66 h after $PGF_{2\alpha}$ were inseminated, however GnRH was not administered to heifers in either treatment at the time AI was performed. Heifers in both treatments that failed to exhibit estrus by 66 h were inseminated at 90 h. When AI was performed at 90 h, heifers in Treatment 1 were administered GnRH at AI, however GnRH was not administered to heifers in Treatment 2 (Figure 3.1).

Table 3.3. Pregnancy rate[a] in heifers resulting from split-time artificial insemination based on location, estrous response, and treatment[b].

		STAI pregnancy rate			
		Treatment 1		Treatment 2	
Location	Estrous response	Proportion	%	Proportion	%
1	Estrous by 66 h	51/74	69	46/69	67
	Estrous by 90 h	8/11	73	6/7	86
	Non-estrous 90 h	1/8	13	3/13	23
	Total	60/93	65	55/89	62
2	Estrous by 66 h	51/84	61	53/84	63
	Estrous by 90 h	10/15	67	10/13	77
	Non-estrous 90 h	2/16	13	1/18	6
	Total	63/115	55	64/115	56
3	Estrous by 66 h	14/28	50	22/31	71
	Estrous by 90 h	3/7	43	3/4	75
	Non-estrous 90 h	0/3	0	1/5	20
	Total	17/38	45	26/40	65
4	Estrous by 66 h	78/144	54	87/160	54
	Estrous by 90 h	29/49	59	21/47	45
	Non-estrous 90 h	7/33	21	7/23	30
	Total	114/226	50	115/230	50
5	Estrous by 66 h	16/27	59	13/22	59
	Estrous by 90 h	5/6	83	6/9	67
	Non-estrous 90 h	6/7	86	1/9	11
	Total	27/40	68	20/40	50
6	Estrous by 66 h	21/38	55	32/39	82
	Estrous by 90 h	5/14	36	5/6	83
	Non-estrous 90 h	0/4	0	0/11	0
	Total	26/56	46	37/56	66
Total	Estrous by 66 h	231/395	58	253/405	62
	Estrous by 90 h	60/102	59	51/86	59
	Non-estrous 90 h	16/71	23	13/80	16
	Total	307/568	54	317/570	56

Abbreviations: STAI, Split-time AI

[a]Pregnancy rate to timed insemination at 66 h and 90 h after PG, determined by ultrasound 60 to 90 d after AI.

[b]Heifers in both treatments were inseminated utilizing split-time AI. Heifers in each treatment that exhibited estrus by 66 h after $PGF_{2\alpha}$ were inseminated, however GnRH was not administered to heifers in either treatment at the time AI was performed. Heifers in both treatments that failed to exhibit estrus by 66 h were inseminated at 90 h. When AI was performed at 90 h, heifers in Treatment 1 were administered GnRH at AI, however GnRH was not administered to heifers in Treatment 2 (Figure 3.1).

Table 3.4. Pregnancy rate[a] based on ovulatory status[b] of heifers that failed to express estrus prior to 90 h after PG.

	Treatment[c]	*n*	Mean LFD at 90 h	Success of Ovulation		Mean LFD at 90 h	AI Pregnancy Rate	Overall Pregnancy Rate
Follicles > 7mm[d]	1	25	11.5 ± 0.4	Yes	13/25 = 52%	11.7 ± 0.4	2/13 = 15%	6/25 = 24%
				No	12/25 = 48%	11.1 ± 0.6	4/12 = 33%	
	2	26	12.3 ± 0.8	Yes	13/26 = 50%	13.4 ± 1.4	4/13 = 31%	4/26 = 15%
				No	13/26 = 50%	11.2 ± 0.4	0/13 = 0%	

Abbreviations: LFD, largest follicle diameter

[a]Pregnancy rate to timed insemination at 90 h after PG, determined by ultrasound 60 to 90 d after AI.

[b]Ovulation rate determined by ultrasonography at 90 h after PG, at which time AI was performed with or without GnRH administration. Ovaries were re-examined 48 h after AI to determine disappearance of the dominant follicle.

[c]Heifers in both treatments were inseminated utilizing split-time AI. Heifers in each treatment that exhibited estrus by 66 h after PG were inseminated, however GnRH was not administered to heifers in either treatment at the time AI was performed. Heifers in both treatments that failed to exhibit estrus by 66 h were inseminated at 90 h. When AI was performed at 90 h, heifers in Treatment 1 were administered GnRH at AI, however GnRH was not administered to heifers in Treatment 2.

[d]Table only includes heifers with dominant follicles greater than 7mm (Treatment 1, 25 of 27; Treatment 2, 26 of 31).

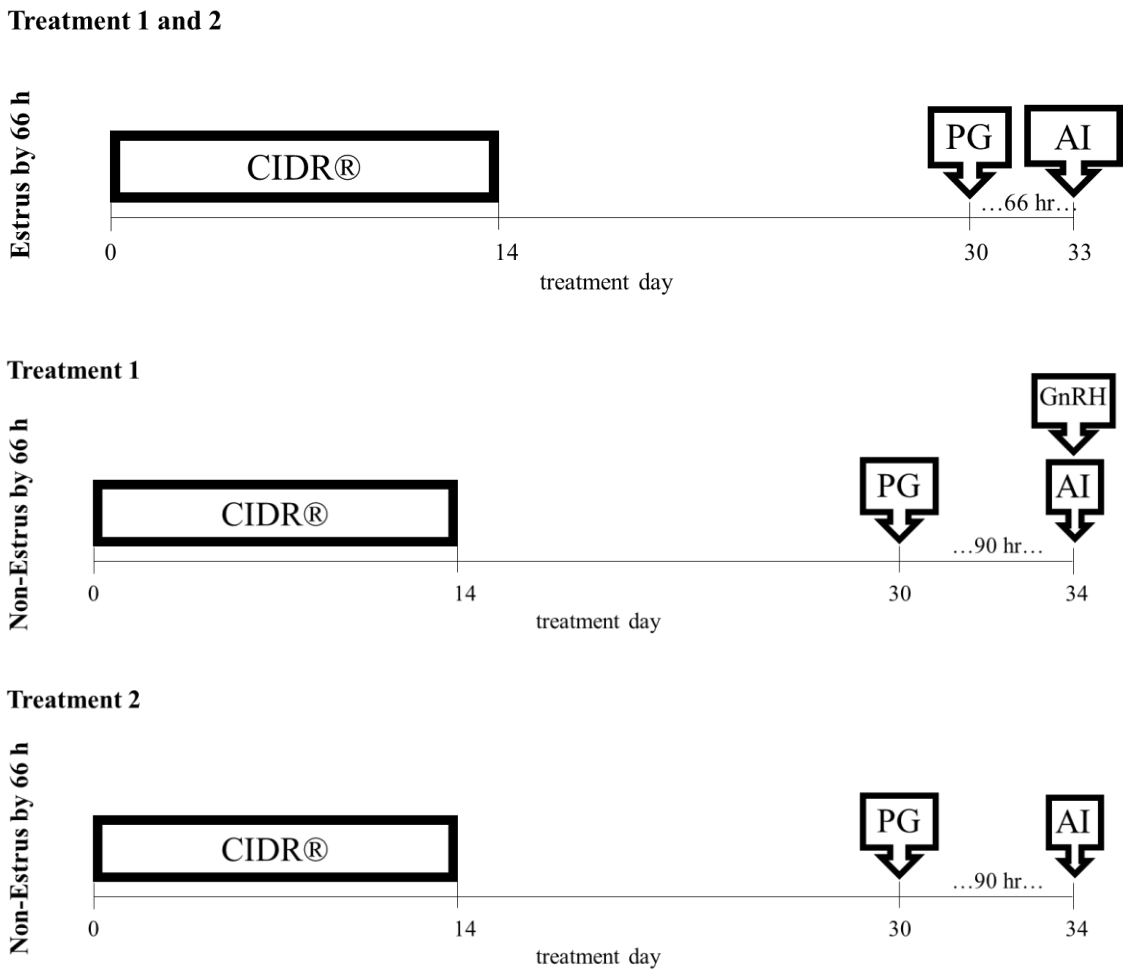

Figure 3.1. Split-time AI treatment diagrams for heifers. Heifers in both treatments were inseminated utilizing split-time AI. Heifers in each treatment that exhibited estrus by 66 h after $PGF_{2\alpha}$ were inseminated, however GnRH was not administered to heifers in either treatment at the time AI was performed. Heifers in both treatments that failed to exhibit estrus by 66 h were inseminated at 90 h. When AI was performed at 90 h, heifers in Treatment 1 were administered GnRH at AI, however GnRH was not administered to heifers in Treatment 2.

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VITA

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