Effect of delaying insemination in beef heifers not expressing estrus by 48 hours after a 7-d CO-Synch plus controlled internal drug release timed artificial insemination protocol

by

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Abstract

Synchronizing estrus before AI is an effective way to shorten the breeding season, and increasing the number of pregnancies per AI may lead to greater use and acceptance of synchronization protocols among beef producers. Our objective was to determine if pregnancy rates to fixed-time AI (FTAI) would be improved by delaying insemination in heifers not expressing estrus before FTAI in a 7-d CO-Synch + controlled internal drug release (CIDR) estrus-synchronization protocol. In Experiment 1, yearling beef heifers (n = 465) at three locations of commercial and purebred herds were treated with GnRH (Cystorelin 100 μg im) and a CIDR insert (1.38 g of progesterone) on Day 0. On Day 7 CIDR inserts were removed and all heifers received $PGF_{2\alpha}$ (Lutalyse 25 mg im) and were fitted with an estrus-detection patch (Estrotect; Rockway, Inc.). Heifers were assigned to three treatments based on estrus-detection patch color at 48 h after $PGF_{2\alpha}$: (1). Estrus-Red 48 h (Red 48; n = 180), heifers that expressed estrus and were inseminated at 48 h; (2). Non-Estrus-Gray 48 h (Gray 48; n = 137) heifers that did not express estrus and were inseminated at 48 h; and (3). Non-Estrus Delayed- 56 h (Gray 56; n = 148), heifers that did not express estrus at 48 h, and were not inseminated until 56 h after $PGF_{2\alpha}$. Pregnancy rate to AI was greatest (P < 0.0001) for Red 48 heifers (67.8%) compared with heifers in the Gray 48 (39.4%) and Gray 56 (42.6%) treatments. Heifers assigned to Gray 48 and Gray 56 achieved similar (P = 0.83) pregnancy rates. In Experiment 2, yearling beef heifers (n = 257) at two different locations were treated with the same 7-d CO-Synch protocol, but heifers were assigned to three different treatments based on estrus-detection patch color at 48 h after $PGF_{2\alpha}$: (1). Estrus-Red 48 h (Red 48; n =95), heifers that expressed estrus and were inseminated at 48 h; (2). Non-Estrus-Gray 48 h (Gray 48; n = 84), heifers that did not express estrus but were inseminated at 48 h; and (3). Non-Estrus Delayed- 72 h (Gray 72; n = 78), heifers that did not

express estrus at 48 h, and were not inseminated until 72 h after $PGF_{2\alpha}$. Pregnancy rate to AI was greatest (P = 0.004) for Red 48 heifers (62.1%) compared with heifers in Gray 48 (40.5%), and Gray 72 (46.2%). No difference in pregnancy rates (P = 0.75) was detected between heifers assigned to treatments Gray 48 and Gray 72. Delaying insemination in heifers not expressing estrus by 48 h after $PGF_{2\alpha}$ did not improve pregnancy rates to AI.

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Chapter 1 - Literature Review

Introduction

Reproductive performance in cattle is considered to be the most economically important trait and is essential for the success of an operation [1]. The technologies of estrous synchronization and artificial insemination (AI) have become widely available to producers [2], and have had positive effects on beef production in the United States. These technologies have made it possible for producers to use semen from multiple sires with superior genetics without purchasing larger numbers of herd bulls.

During the last decade, use of AI only, without bull exposure, has increased in popularity in the beef cattle industry [3,4]. Unfortunately, even with this increase, the National Animal Health Monitoring System [4] reported that only 7.6% of beef cattle producers use AI and only 16.3% of beef heifers were inseminated artificially. Time and labor have been reported as the main reasons for not utilizing AI [4]. To promote the use of AI, time, labor, and animal handlings must be minimal, and the percentage of females that conceive to AI needs to be increased. Methods of estrous synchronization that result in a fertile estrus and ovulation will help to facilitate use of AI and fixed-timed artificial insemination (FTAI) [5]. Estrus-detection aids have been available for several years [6,7], but were not used as much initially because of their poor efficiency in detecting estrus. Foote [6] reported the KaMar detector was expensive, easily lost, and gave false readings. Estrus-detection aids have increased in popularity in recent years as they have become more efficient and reduce labor costs [8]. Estrus-detection aids, such as estrus-detection patches, help validate occurrence of estrus, and could be used to reduce labor and costs, and increase the number of pregnancies to AI.

Fixed-time AI was initially created to decrease time and labor issues producers faced with AI. Unfortunately, with a one-time insemination, a portion of females do not express estrus before timed AI (TAI) [9]. Females that express estrus before AI have greater pregnancy rates than females that do not express estrus before AI [10,11,12]. New protocols that better link estrous expression with time of insemination are being developed. Split-time AI, or delayed insemination, is a protocol that delays insemination of females not expressing estrus by TAI. This delay allows extra time for females to express estrus before being inseminated. Studies involving split-time AI in heifers have only compared the 14-d CIDR $PGF_{2\alpha}$ protocol and melengestrol acetate (MGA)- $PGF_{2\alpha}$ with a delay of 20 or 24 h [13,14]. These studies produced inconsistent pregnancy rates in heifers after delayed insemination. Use of a 14-d CIDR $PGF_{2\alpha}$ protocol also increases the total amount of time needed for estrous synchronization in heifers compared with a 7-d CO-Synch + CIDR protocol. Therefore, research is needed to evaluate pregnancy rates in heifers exposed to split-time AI in a 7-d CO-Synch + CIDR protocol.

Review of the Bovine Estrous Cycle

A basic understanding of the estrous cycle is necessary to improve current reproductive technologies and protocols. The estrous cycle is divided into a follicular phase and luteal phase [15]. The follicular phase is approximately 8 d and is divided into 2 stages: proestrus and estrus. The luteal phase spans ovulation to luteolysis of the corpus luteum (CL), which is approximately 13 d and is divided into 2 stages: metestrus and diestrus.

Proestrus

Proestrus is the first stage of the follicular phase and is characterized by the presence of a maturing dominant follicle. During this stage, there is an abrupt decline in progesterone caused by luteolysis. Because progesterone concentration is decreasing, this negative feedback of

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progesterone on the hypothalamic-pituitary axis is removed and GnRH is released at a greater amplitude and frequency than when negative feedback was present. A decrease in progesterone allows an increase in circulating estradiol, resulting from the maturation of the preovulatory follicle and secretion of more estradiol, which induces a greater secretion of GnRH. An increase in GnRH secretion also increases the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The hypothalamus plays a role in regulating the estrous cycle by controlling the release of GnRH, and therefore the gonadotropins LH and FSH [15].

In order for increased production of estradiol, a series of events must occur. Estradiol production occurs in the granulosa cells of the follicle. The theca cells of the follicle have LH receptors, and the granulosa cells have FSH receptors. As the follicle becomes dominant, granulosa cells acquire receptors for LH. The binding of LH to theca cell LH-receptors of a growing dominant follicle drives the conversion of cholesterol to pregnenolone. The theca cells are capable of converting the pregnenolone to androstenedione by use of the delta-5 pathway [16]. Androstenedione diffuses through the cell membrane of the granulosa cells and is converted into testosterone by 17β- hydroxysteroid dehydrogenase. Testosterone is then converted to estradiol-17β by P450 aromatase in response to FSH. Progesterone secreted by the CL has a negative effect on hypothalamic GnRH neurons, and subsequently on LH, FSH, and estradiol. When luteolysis occurs and progesterone concentrations are reduced, the negative feedback is removed and a positive feedback loop is established. The positive feedback loop allows for an increase in circulating estradiol, which causes an increase in the release of GnRH from the hypothalamus. This in turn causes an increase in the release of LH and FSH. The growing follicle continues to secrete increasing concentrations of estradiol that results in sexual behavior, estrus, and the induction of an LH surge.

Estrus

The willingness of a cow or heifer to accept a male is known as standing estrus. When estradiol becomes the dominant hormone during the estrous cycle, a female will display physical signs of sexual receptivity and mating. A cow or heifer is in estrus for approximately 15 h [15]. As estradiol concentrations continue to increase, they will eventually reach a threshold, and initiate a surge of GnRH from the hypothalamus. This surge of GnRH results in a preovulatory surge of LH from the anterior pituitary gland, which occurs near the onset of estrus. Ovulation of the preovulatory follicle will occur approximately 28 h after the LH surge [15].

Metestrus

The luteal phase begins when a female no longer displays sexual interest or standing heat, otherwise known as metestrus. Heifers and cows ovulate during this stage. Ovulation occurs because the LH surge activates a series of enzymes that thin the ovarian wall and cause the follicle to rupture. A CL forms by massive remodeling and differentiation of the ovulated follicle. The CL is made up of luteal cells that differentiated from the theca and granulosa cells of the follicle. Progesterone production increases as the CL matures, and by the seventh day of the estrous cycle, the CL is mature and fully functional [17].

Diestrus

Progesterone is secreted at greatest concentrations from the CL during diestrus. These levels of progesterone inhibit estrus and prevent uterine contractions to help support the conceptus growth. If no conceptus is detected, diestrus ends with luteolysis of the CL. Luteolysis begins with a decrease in the number of progesterone receptors on the GnRH producing neurons of the hypothalamus, which allows for greater LH pulse secretion to promote growth of a dominant follicle and more estradiol secretion. An increase in estradiol increases the frequency

of oxytocin release from the posterior pituitary. When the oxytocin receptors of the uterus are activated, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is secreted from the endometrium and causes a release of luteal oxytocin. This increases the release of $PGF_{2\alpha}$, initiates luteolysis, and the CL begins to regress.

Expression of Estrus

Increasing the effectiveness of all AI protocols requires controlling the expression of estrus and time of ovulation. Heifers and cows that express estrus before FTAI have greater pregnancy rates than females that do not express estrus before FTAI [9,18,19,20, 21]. Estrus is initiated in cattle by a rise in estradiol concentration [22]. This rise in estradiol contributes to several physiological factors that influence pregnancy. A few of these factors include gamete transportation and preparation of the uterine environment, as well as having an effect on the oocyte and CL development [23,24,25]. Sperm can be transported more efficiently in females that are in estrus [26]. Inadequate concentrations of estradiol could decrease pregnancy rates by potentially decreasing the number of sperm that are transported efficiently through the female reproductive tract.

A decrease in estradiol also could have an influence on the development of a CL, and therefore, a developing embryo. Treatment with GnRH has been shown to induce ovulation in FTAI protocols regardless of estrous expression [27], however, ovulation without expression of estrus may cause decreased conception rates [18,28,29,30] resulting from inadequate estradiol concentrations to properly regulate the uterine environment for gamete interaction [31,10]. Perry et al. [10] found that GnRH-induced ovulation of smaller follicles was related to decreased pregnancy rates and increased embryonic and fetal mortality in beef cows because some of the follicles may not have attained functional maturity. A study by Murdoch and Van Kirk [32]

found that luteal insufficiency occurred in ewes when ovulation was induced, and may be associated with immature follicles that did not produce sufficient concentrations of estradiol, and therefore resulted in decreased concentrations of progesterone. Larimore et al. [33] discovered that embryos recovered from heifers that expressed estrus before insemination were of better quality and more advanced in stage of development. Improved embryo quality resulted from the increased estradiol concentrations to which oocytes were exposed [34].

It has also been hypothesized that increased estradiol regulates uterine pH around the onset of estrus [35]. A decrease in the intracellular pH of bull sperm decreases sperm motility, but increases the sperm longevity [36]. Perry and Perry [35] discovered that uterine pH decreased in estrual cows compared with non-estrual cows. This indicates that estradiol concentrations may also influence fertilization by altering the uterine pH.

Research by Busch et al. [9,19] found that in a FTAI protocol, 50% of cows and 45 to 55% of heifers fail to express estrus by TAI. Richardson et al. [12] conducted a meta-analysis comparing five of the most popular FTAI protocols in beef females. These protocols consisted of CO- Synch, 7-d CO-Synch + CIDR, 5-d CO-Synch + CIDR, PG 6-d CIDR, and 14-d CIDR. The results from this meta-analysis showed beef cows that expressed estrus before FTAI had a 27% overall improvement in pregnancies per AI. These studies indicate that estrous expression before insemination is important to increase fertility.

To assist producers with the accuracy and efficiency of estrus-detection, estrus-detection aids have been developed and marketed during the last few decades. A few of these aids include: tail head markings using chalk or paint, electronic mount detectors that transmit standing estrus occurrence to a computer, and estrus-detection patches that adhere to the tail head. The patches that adhere to the tail head have a surface layer that is scratched off as the female is mounted. It

takes approximately 6 to 7 mounts to remove the top surface layer of the patches [37]. Research has shown a 91% positive predictive value that a heifer is in estrus if a patch has 50% or more of the surface removed. The probability of no change in patch color and heifers not being in estrus was 76.3% [38]. These probabilities indicate that there is still potential to miss females in estrus and are not beneficial for heifers having synchronized ovulation without estrous expression.

Factors That Affect Expression of Estrus

Puberty

In order for estrous expression to occur in heifers, the heifer must first achieve puberty. One of the most important steps in beef heifer development is the ability to achieve puberty in a timely manner. When a heifer does not achieve puberty by her first breeding season, the probability she will become pregnant is reduced.

During the prepubertal period, GnRH neurons are extremely sensitive to stimulation from neighboring kisspeptin neurons, which are activated by estradiol. As the heifer approaches the peripubertal period, there is a decrease in sensitivity of GnRH neurons to estradiol negative feedback allowing an increase in GnRH secretion, and a subsequent increase in LH release from the anterior pituitary. Increased LH causes dominant follicles to increase in size and duration of dominance, and also to secrete more estradiol [39,40]. The decline in estradiol negative feedback, and increase in LH secretion eventually reach adequate concentrations to induce the pubertal surge of LH [41,42]. Achievement of puberty is indicated by ovulation following the surge of LH.

Age of puberty in beef heifers has been shown to decrease when exposed to an exogenous source of progesterone. Progesterone reduces estradiol negative feedback by decreasing the number of estradiol receptors on the kisspeptin neurons of the hypothalamus.

Kisspeptin neurons are responsible for stimulating GnRH secreting neurons in the hypothalamus. This allows LH pulse frequency to increase and induce an LH surge, which induces ovulation for the first time [43]. This stimulatory effect of endogenous progesterone is why many estrus-synchronization protocols use a progestin source to induce puberty in heifers and improve conception rates.

Nutrition

Nutritional management can have a large effect on estrous expression and age of puberty. Short and Adams [44] created a ranking determining the order of priority for use of available energy. In this ranking, reproduction is listed as a low priority in terms of energy utilization, and is one of the first priorities to be eliminated when dietary energy is limiting. When nutrition is limited and energy intake is insufficient, puberty can be delayed in heifers. Day et al. [41,45] reported that when nutrition and energy intake are limited, LH secretion remains more sensitive to the negative feedback of estradiol, and the prepubertal surge of LH that induces ovulation is delayed. Imakawa et al. [46] found that heifers that are anestrous because of a lack of energy intake also do not respond to a progestin treatment to induce estrus. Body condition plays an important role in determining puberty and estrous cyclicity in beef heifers. It is recommended that heifers reach 60% of their mature weight before breeding because age and inadequate body weight can influence the onset of puberty [47] and decrease the chance of conception [48].

After parturition, cows are anestrous, and when fed a restricted energy diet, duration of anestrus may be prolonged. One way to determine nutritional status is by using body condition scoring (BCS). Body condition scoring evaluates females based on a scale of 1 to 9, with 1 being malnourished, and 9 being obese. Beef cows should have a score of 5 or 6 before breeding and calving [49]. Richards et al. [50] found that nutritionally induced anestrous was associated with a

decrease in LH pulse frequency. In contrast, when nutrition was not limiting and females reached a BCS of 4.6, estrous cycles were reinitiated. Imakawa et al. [51] suggested that this decrease in LH pulse frequency from nutritional deprivation could be caused by hypothalamic inactivity. Partitioning of energy and nutrients is of great importance to establish estrous expression and normal estrous cycles to ensure pregnancy establishment.

Timed Artificial Insemination Protocols

As artificial insemination protocols and estrus-synchronization were developed, some confusion occurred for beef producers. In order to better inform beef producers about these new technologies, the Beef Reproductive Task Force was created. The Beef Reproductive Task Force is comprised of industry specialists from different universities in the United States who work to educate producers and promote the benefits of using reproductive technologies. Different insemination protocols from the Beef Reproductive Task Force for beef heifers are in Figure 1.1 [52].

Seven-day CO-Synch + CIDR

Because of a large variability in the interval to estrus and ovulation following methods of artificially manipulating the estrous cycle, researchers began combining different elements of synchronization strategies in beef cattle [53]. A significant element in this variability is failure to manage follicular waves [5]. When using a CO-Synch protocol, a percentage of heifers fail to ovulate in response to the initial GnRH, and express estrus before the subsequent injection of $PGF_{2\alpha}$ [53]. Premature estrus and ovulation occurred before the expected period of AI, resulting in missed insemination opportunities.
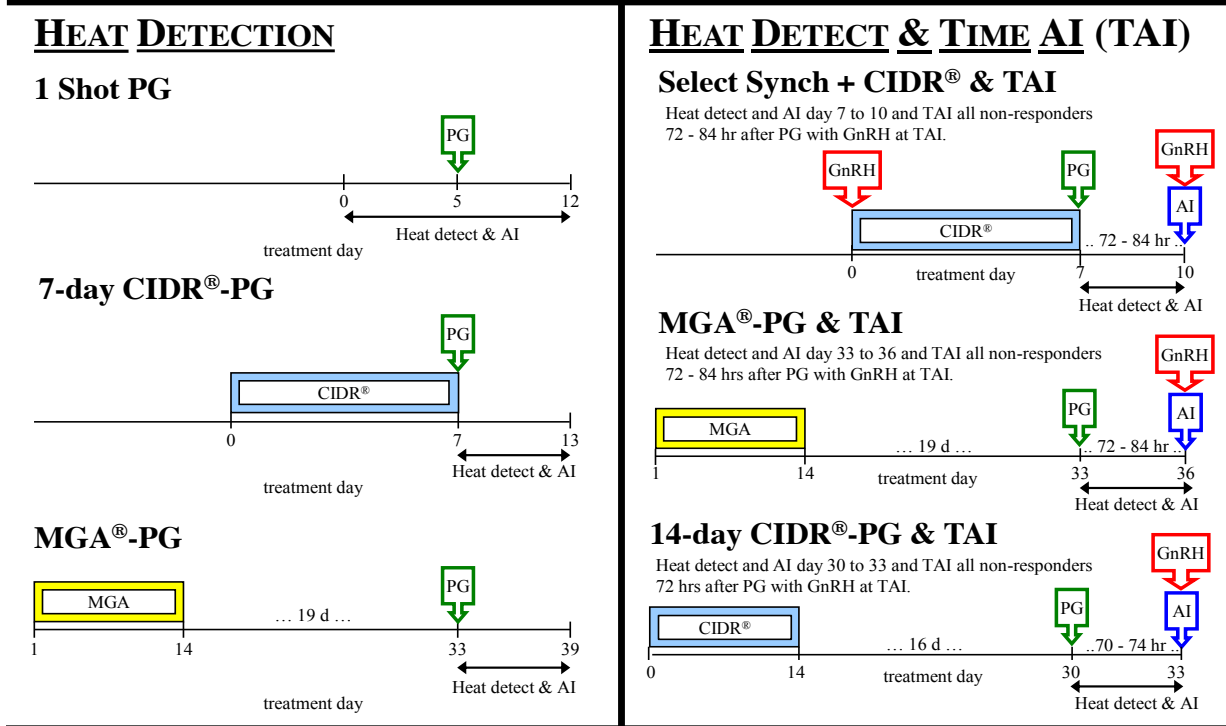
Researchers developed the 7-d CO-Synch + CIDR as a modification for the CO-Synch protocol [54]. In the CO-Synch protocol, the lack of a progestin allowed some heifers to express

estrus before $PGF_{2\alpha}$. Addition of the progestin insert during the week before $PGF_{2\alpha}$ injection suppressed estrus, preventing those females with a regressing CL to come into estrus. Use of progestin inserts has been shown to increase pregnancy rates in cycling cows by 36%, and by 20% in anestrous cows [55] compared with females not receiving a progestin insert. This increase in pregnancy rate occurs because the addition of progesterone suppresses estrus in cycling females whose CL regresses during progesterone treatment. Pregnancy rates in cows have been greater than 60% following a 7-d CO-Synch + CIDR protocol [9,20].

Progestin inserts have been reported to induce puberty in heifers [56]. Progestin administration induces puberty by accelerating the peripubertal decline of estradiol negative feedback on LH secretion [57]. Pregnancy rates in heifers following a 7-d CO-Synch + CIDR protocol have been considerably lesser than in cows, ranging from 47 to 55% [19, 58]. Timed insemination for beef heifers is recommended to occur 54 h after CIDR removal [52].

Use of a 14-d $CIDR-PGF_{2\alpha}$ protocol in heifers increased estrous response and pregnancy rates when compared with a 7-d CO-Synch + CIDR protocol [19,59]. Inconsistent estrous expression and pregnancy rates when using the 7-d CO-Synch + CIDR protocol results from the inability to synchronize follicular waves with GnRH [59,60]. Improved synchronization of estrus and ovulation with the 14-d $CIDR-PGF_{2\alpha}$ is associated with a greater response to GnRH and better control of follicular waves [18,19]. The 14-d $CIDR-PGF_{2\alpha}$ protocol, however, requires more animal handlings, and the duration of time needed for estrous synchronization compared with the 7-d CO-Synch + CIDR makes it less practical for producers.

BEEF HEIFER PROTOCOLS - 2016

HEAT DETECTION

1 Shot PG

7-day CIDR®-PG

MGA®-PG

HEAT DETECT & TIME AI (TAI)

Select Synch + CIDR® & TAI

Heat detect and AI day 7 to 10 and TAI all non-responders
72 - 84 hr after PG with GnRH at TAI.

MGA®-PG & TAI

Heat detect and AI day 33 to 36 and TAI all non-responders
72 - 84 hrs after PG with GnRH at TAI.

14-day CIDR®-PG & TAI

Heat detect and AI day 30 to 33 and TAI all non-responders
72 hrs after PG with GnRH at TAI.

FIXED-TIME AI (TAI)*

Short-term Protocols

7-day CO-Synch + CIDR®

Perform TAI at 54 ± 2 hr after PG with GnRH at TAI.

5-day CO-Synch + CIDR®

Perform TAI at 60 ± 4 hr after CIDR removal with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.

Long-term Protocols

14-day CIDR®-PG

Perform TAI at 66 ± 2 hr after PG with GnRH at TAI.

MGA®-PG

Perform TAI at 72 ± 2 hr after PG with GnRH at TAI.

* The times listed for "Fixed-time AI" should be considered as
the approximate average time of insemination. This should be
based on the number of heifers to inseminate, labor, and facilities.

GnRH — Cystorelin®, Factrel®, Fertagyl®, OvaCyst®, GONABreed®

PG — estroPLAN®, Estrumate®, In-Synch®, Lutalyse®, ProstaMate®

Approved 8-16-2015

Beef Reproduction Task Force

Figure 1.1

Artificial insemination synchronization protocols used for beef heifers [52].

Fourteen-day CIDR-PGF$_{2\alpha}$

The 14-d CIDR-PGF$_{2\alpha}$ protocol (Figure 1.1) was created to decrease the variability associated with the synchronization of follicular waves in heifers [60]. By inserting a CIDR before PGF$_{2\alpha}$, follicle growth can be manipulated, and this leads to an improvement in the synchronization of new follicular wave emergence [59,61]. In a 14-d CIDR-PGF$_{2\alpha}$ protocol, a CIDR insert is in place for 14 d. When the CIDR is removed, it is recommended that insemination not occur at the initial estrus because records show that a follicle that has maintained dominance for more than 10 d becomes a persistent follicle and a significant reduction in fertility and pregnancy rate is observed [62]. An injection of PGF$_{2\alpha}$ is given 16 d after CIDR removal and insemination occurs approximately 66 h after PGF$_{2\alpha}$. The 14-d CIDR-PGF$_{2\alpha}$ protocol has the ability to increase pregnancy rates, but lasts about 33 d and may not be practical for a producer who might be on a tight breeding schedule. The 7-d CO-Synch + CIDR protocol only takes about 9 d to complete.

Melengestrol Acetate

Melengestrol acetate (MGA) is an orally active progestin, and is administered in feed. Melengestrol acetate was developed to suppress estrus and estrous behavior in heifers on feed for better performance on gain [63]. It has more recently been used to synchronize estrus in replacement heifers. When short feedings of MGA- PGF$_{2a}$ were used, fertility was reduced at the initial synchronized estrus [64]. This decrease in fertility at the initial estrus seemed to take place in heifers that began treatment with MGA after Day 12 of the estrous cycle [64]. Longer feeding periods also were developed at the same time as the short feeding treatment, however, fertility remained poorer in this longer feeding period as well [65, 66]. Anderson and Day [67] found that feeding 0.5 mg of MGA/d for 11 to 14 d resulted in the development of persistent follicles in the

absence of a CL. A persistent follicle is a dominant follicle with a prolonged life span because it was exposed to an exogenous, low concentration source of progestin. This exogenous source of progestin maintains progesterone concentrations for a longer amount of time than what a CL would. These follicles ovulate once the progestin is removed. Other factors associated with a persistent follicle were altered ovulation, abnormal cervical mucus, reduced size and weight of the CL after treatment, and possible interference with sperm transport [68]. These factors were associated with the first synchronized estrus following withdrawal of the exogenous progestin. Researchers began administering $PGF_{2\alpha}$ 17 d after ending treatment of MGA in order for estrus to be synchronized with no reduction in fertility [69]. The protocol used with MGA today uses a wait period of 17 to 19 d before treatment with PGF_{2a}. Unfortunately, the duration of this treatment requires an increased amount of time and management compared with the use of the 7-d CO-Synch + CIDR protocol. Heifers that received a 14-d CIDR-$PGF_{2\alpha}$ protocol had a more synchronized estrus than heifers that received a 14-d MGA-$PGF_{2\alpha}$ protocol because the use of the CIDR offers a more consistent concentration of progesterone [70].

Five-Day CO-Synch + CIDR

The 5-d CO-Synch + CIDR protocol was developed to improve pregnancy rates when using TAI by optimizing oocyte quality at ovulation, and maximizing preovulatory estradiol concentrations [71] (Figure 1.1). Perry et al. [10] found that follicle size in beef cows was important for maximum fertility when the follicle was induced to ovulate. Follicles that spontaneously ovulated, however, attained maturity and produced adequate amounts of estradiol regardless of size. It has also been reported [72] that growing dominant follicles from Day 4 of the follicular wave have increased estradiol concentrations compared with Day 6 or 8 of the follicular wave. The 5-d CO-Synch + CIDR protocol offers a shortened synchronization time

frame because a CIDR is only inserted for 5 d compared with the 7-d CO-Synch + CIDR protocol where the CIDR is inserted for 7 d. The 5-d CO-Synch + CIDR protocol also allows for follicles to be approximately 4 d from ovulation compared with 6 d with the use of a 7-d CO-Synch + CIDR protocol [71].

Variable results as have been reported comparing pregnancy rates after the 5-d CO-Synch + CIDR or 7-d CO-Synch + CIDR protocol [20,71]. The 5-d CO-Synch + CIDR protocol does require two treatments with PGF_{2a}, and an extra animal handling, in order to achieve incomplete luteolysis. Incomplete luteolysis may occur because the CL may be too young to regress after ovulation in response to initial treatment of GnRH. This protocol primarily relies on GnRH to reset follicular waves. Heifers, however, are not as likely to respond to GnRH as cows, resulting in the inability to synchronize follicular waves with GnRH [60]. The 7-d CO-Synch + CIDR protocol offers fewer animal handlings, and does not rely as heavily on the success of the initial GnRH treatment.

Split-Time Artificial Insemination

Split-time artificial insemination is a TAI protocol that delays insemination of heifers not expressing estrus by the predetermined insemination time. Split-time AI or delayed AI allows for more females to achieve estrus by the time of insemination.

Split-time AI was developed to better manage expression of estrous for beef heifers and cows in a FTAI protocol. If a proportion of females were not expressing estrus before FTAI, then insemination would be delayed for those females. In the delay period, extra time is allowed for more females to express estrus before insemination. Females that express estrus at or before FTAI will ovulate approximately 28 to 32 hours after the onset of estrus. In contrast, females that do not express estrus by AI could potentially not ovulate within the time frame of optimum

sperm viability. The optimal time to AI for an oocyte to develop into an embryo of good quality is between 12 and 24 h before ovulation [73]. By better aligning the lifespan of viable sperm and ovulation, greater pregnancy rates can be achieved.

Research conducted by Thomas et al. [13] compared pregnancy rates of heifers to split-time AI following administration of a 14-d CIDR-PGF$_{2\alpha}$ protocol, and also compared pregnancy rates of cows to split-time AI following a 7-d CO-Synch + CIDR protocol. The hypothesis of their study was that greater pregnancy rates could be achieved by delaying insemination after GnRH by 20 h in non-estrous heifers and cows. There was no difference observed in the overall pregnancy rates between the two treatments for cows (59 vs. 59%). However, cows that expressed estrus in the 20 h delay period had a 27 percentage point increase in pregnancy rates (67 vs. 40%, respectively) compared with cows that did not express estrus in the 20 h delay period. Heifers not expressing estrus by 66 h and inseminated 86 h after PGF$_{2\alpha}$ had greater pregnancy rates (54 vs. 46%) than all heifers inseminated at 66 h after PGF$_{2\alpha}$ regardless of estrous expression. Heifers that expressed estrus during the 20 h delay period also achieved greater pregnancy rates than heifers that did not express estrus (66 vs. 29%). It was concluded in this study that delaying insemination for heifers not expressing estrus could improve pregnancy rates in heifers synchronized with a 14-d CIDR-PGF$_{2\alpha}$ split-time AI protocol because more heifers expressed estrus during the delay period.

In contrast, the results from Hill et al. [74] found that delaying insemination to 75 h in cows not expressing estrus by 60 h improved pregnancy rates when synchronized with a 7-d CO-Synch + CIDR protocol. Their hypothesis was that pregnancy rates would increase by dividing cows into two insemination times based on estrous expression. Cows expressing estrus at 60 h were inseminated, and remaining females not expressing estrus were divided into 3 groups.

Cows not expressing estrus were either treated with GnRH and inseminated at 60 h, treated with GnRH at 60 h and inseminated at 75 h, or treated with GnRH and inseminated at 75 h. This study [74] had a greater pregnancy outcome for the delayed insemination compared with the study by Thomas et al. [13], possibly because the delayed insemination occurred at a more optimal time relative to ovulation (75 vs. 86 h).

Markwood et al. [14] compared the pregnancy rates of heifers synchronized with MGA-$PGF_{2\alpha}$ vs. 7-d CO-Synch + CIDR that were inseminated based on Estrotect patch status. Heifers synchronized with MGA that did not express estrus were assigned to insemination at 72 h with no delay, a 12 h delay with insemination at 84 h, or an 18 h delay with insemination at 90 h. Heifers synchronized with a 7-d CO-Synch + CIDR protocol were inseminated at either 58 or 76 h after CIDR removal regardless of estrous expression, and patch status was recorded at 58 h. Regardless of treatment with MGA- $PGF_{2\alpha}$ or 7-d CO-Synch + CIDR protocol, delaying insemination did not result in an increase in pregnancy rates compared with heifers in the non-delayed treatment.

This study [14] also evaluated a 7-d CO-Synch + CIDR protocol in cows. Regardless of estrous expression, cows were either inseminated at 58 h, or insemination was delayed to 76 h. No difference was observed in overall pregnancy rate between the two treatments, and no difference was observed in pregnancy rate in cows not expressing estrus at 58 h or 76 h. This study also conducted a partial budget model comparing the 7-d CO-Synch + CIDR FTAI protocol to the 7-d CO-Synch + CIDR split-time AI protocol, as well as the 7-d CO-Synch + CIDR split-time AI protocol to natural service. It was suggested from this model that delaying insemination with split-time AI in cows not yet expressing estrus could increase the profit per cow when compared with FTAI or natural service.

Summary

Multiple estrus-synchronization protocols are available for producers to use today. The protocols that induce a large proportion of heifers to have synchronized estrus and ovulation reduce the time and labor required for AI. This, in turn, makes AI more acceptable and practical to producers. Split-time AI requires one additional animal handling, but this could be offset by improved pregnancy rates to AI.

Previous studies have not evaluated the effects of a split-time AI in heifers using a 7-d CO-Synch + CIDR protocol. These studies have also only evaluated delayed insemination in heifers not expressing estrus by approximately 20 to 24 h following a 14-d CIDR $PGF_{2\alpha}$ protocol. The hypothesis of the present study proposed that delaying insemination by either 8 or 24 h in heifers not expressing estrus at FTAI would increase pregnancy rates.

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Chapter 2 - Effect of delayed insemination of non-estrual beef heifers after a 7-d CO-Synch plus controlled internal drug release timed artificial insemination protocol

Abstract

Synchronizing estrus before AI is an effective way to shorten the breeding season, and increasing the number of pregnancies per AI may lead to greater use and acceptance of synchronization protocols among beef producers. Our objective was to determine if pregnancy rates to fixed-time AI (FTAI) would be improved by delaying insemination in heifers not expressing estrus before FTAI in a 7-d CO-Synch + controlled internal drug release (CIDR) estrus-synchronization protocol. In Experiment 1, yearling beef heifers (n = 465) across three locations of commercial and purebred herds were treated with 100 µg GnRH im and a CIDR insert (1.38 g of progesterone) on Day 0. On Day 7 CIDR inserts were removed and all heifers received 25 mg $PGF_{2\alpha}$ im and were fitted with an estrus-detection patch (Estrotect; Rockway, Inc.). Heifers were assigned to three treatments based on estrus-detection patch color at 48 h after $PGF_{2\alpha}$: (1). Estrus-Red 48 h (Red 48; n = 180), heifers that expressed estrus and were inseminated at 48 h; (2). Non-Estrus-Gray 48 h (Gray 48; n = 137) heifers that did not express estrus and were inseminated at 48 h; and (3). Non-Estrus Delayed- 56 h (Gray 56; n = 148), heifers that did not express estrus at 48 h, and were not inseminated until 56 h after $PGF_{2\alpha}$. Pregnancy rate to AI was greatest (P < 0.0001) for Red 48 heifers (67.8%) compared with heifers in the Gray 48 (39.4%) and Gray 56 (42.6%) treatments. Heifers assigned to Gray 48 and Gray 56 achieved similar (P = 0.83) pregnancy rates. In Experiment 2, yearling beef heifers (n = 257) at two different locations were treated with the same 7-d CO-Synch protocol, but heifers were assigned to three different treatments based on estrus-detection patch color at 48 h after $PGF_{2\alpha}$:

(1). Estrus-Red 48 h (Red 48; n =95), heifers that expressed estrus and were inseminated at 48 h;

(2). Non-Estrus-Gray 48 h (Gray 48; n = 84), heifers that did not express estrus but were

inseminated at 48 h; and (3). Non-Estrus Delayed- 72 h (Gray 72; n = 78), heifers that did not

express estrus at 48 h, and were not inseminated until 72 h after $PGF_{2\alpha}$. Pregnancy rate to AI was

greatest (P = 0.004) for Red 48 heifers (62.1%) compared with heifers in Gray 48 (40.5%), and

Gray 72 (46.2%). No difference in pregnancy rates (P = 0.75) was detected between heifers

assigned to treatments Gray 48 and Gray 72. Delaying insemination in heifers not expressing

estrus by 48 h after $PGF_{2\alpha}$ did not improve pregnancy rates to AI.

Introduction

Reproductive performance in cattle is considered to be the most economically important

trait and is essential for the success of a cow-calf operation [1]. During the last decade, use of

artificial insemination (AI) only, without bull exposure, has increased in popularity in the beef

cattle industry [2,3]. Unfortunately, even with this increase, the National Animal Health

Monitoring System [3] reported that only 7.6% of beef cattle producers use AI and only 16.3%

of beef heifers were artificially inseminated. The most common reasons for not using this

reproductive technology is its requirement of extra time and labor [3]. In order to increase the

use of estrous synchronization and AI, protocols should have minimal numbers of animal

handlings and increase the percentage of pregnancies to AI in a herd.

Fixed-time artificial insemination (FTAI) reduces time and labor required for estrous

detection. Unfortunately, producers still encounter heifers not expressing estrus at time of AI.

Pregnancy rate to FTAI varies with estrous expression, with females expressing estrus at or

before FTAI having greater pregnancy rates than females not expressing estrus before FTAI

[4,5,6]. When females are inseminated between 54 and 66 h after controlled internal drug release

(CIDR) removal in a 7-d CO-Synch + CIDR protocol as recommended by Applied Reproductive Strategies in Beef Cattle [7], estrous expression varies. A previous study by Busch et al. [8] found that approximately 50% of cows did not express estrus before FTAI at 54 h after CIDR removal in a 7-d CO-Synch + CIDR protocol, and a similar result was observed in beef heifers in a 14-d CIDR $PGF_{2\alpha}$ protocol [9]. A 14-d CIDR $PGF_{2\alpha}$ protocol uses a CIDR insert for 14-d, $PGF_{2\alpha}$ is administered 16 d after CIDR removal, and GnRH administration and AI occur 66 h after $PGF_{2\alpha}$ [7]. New protocols that better manage estrous expression before time of insemination are being developed.

Split-time AI, or delayed insemination, is a protocol that delays insemination in females not expressing estrus by the appointed hour of AI. This delay allows extra time for females to express estrus before being inseminated. Studies involving split-time AI in heifers have only compared the 14-d CIDR $PGF_{2\alpha}$ protocol and melengestrol acetate (MGA)- $PGF_{2\alpha}$ with a delay of 20 or 24 h [13,14]. Melengestrol acetate is an orally active progestin that is fed for 14 d to synchronize estrus in beef heifers [7]. These studies have produced conflicting results to justify whether delaying insemination increases pregnancy rates in heifers. Use of a 14-d CIDR $PGF_{2\alpha}$ protocol also increases the total amount of time needed for protocol implementation in heifers compared with a 7-d CO- Synch + CIDR protocol. Therefore, research is needed to evaluate pregnancy rates that can be obtained in heifers implementing split-time AI in a 7-d CO-Synch + CIDR protocol. We hypothesized that pregnancy rates could be improved by delaying insemination in heifers not expressing estrus by FTAI. The objective of this study was to determine if pregnancy rates would be improved in beef heifers not expressing estrus before FTAI by delaying insemination in a 7-d CO-Synch + CIDR protocol.

Materials and Methods

The experimental procedures were approved by Kansas State University Animal Care and Use Committee.

Experiment 1

Experimental Procedure

Estrus was synchronized using the 7-d CO-Synch + CIDR FTAI protocol (Figure 2.1) across three locations of commercial and purebred beef heifers (n = 465). Heifers were administered 100 μg GnRH im (Cystorelin, Merial, Athens, GA) and an Eazi- Breed CIDR insert (1.38 g of progesterone, Zoetis, Florham Park, NJ) on Day 0. The CIDR inserts were removed 7 d later and heifers received a 25 mg injection of $PGF_{2\alpha}$ im (Lutalyse, Zoetis) and estrus-detection patches (Estrotect; Rockway, Inc., Spring Valley, WI) were applied. All heifers received an injection of GnRH on Day 9 at 48 h after $PGF_{2\alpha}$ regardless of estrous expression (change in patch color). Estrus was defined to have occurred when > 50% of the gray rub-off coating on the Estrotect patch was removed, exposing underlying red color. Heifers were assigned to three treatments based on estrous detection patch color at 48 h after $PGF_{2\alpha}$: (1). Estrus-Red 48 h (Red 48; n = 180) – heifers displayed estrus by 48 h as indicated by red estrous detection patch and inseminated at 48 h after $PGF_{2\alpha}$; (2). Non-Estrus-Gray 48 h (Gray 48; n = 137) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$ as indicated by a gray estrous detection patch, and inseminated at 48 h after $PGF_{2\alpha}$; and (3). Non-Estrus Delayed- 56 h (Gray 56; n = 148) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$, and inseminated at 56 h after $PGF_{2\alpha}$. Estrotect patches remained on all heifers that did not display estrus at 48 h after $PGF_{2\alpha}$, and patch color of these heifers was recorded again at 56 h after $PGF_{2\alpha}$. The actual interval from $PGF_{2\alpha}$ to FTAI for the delayed AI was approximately 8 to 11 h. We chose a delay of 8 h to provide a practical

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implementation of the protocol for producer use. At location 1, semen from two different sires was used, and one AI technician inseminated the heifers, at location 2, heifers were inseminated with semen from one sire were inseminated by two AI technicians, and at location 3, semen from two sires was used and one AI technician inseminated the heifers.

Cycling Status

Ovaries and reproductive tracts were palpated at approximately Day −30 to Day −7 to determine if heifers had reached puberty. Heifer reproductive tracts were scored on a five-point scale. A score of 1 = an immature tract, with no uterine tone and no presence of ovarian structures, and a score of 5 = a mature, large tract with uterine tone and a palpable corpus luteum [15]. Heifers with a tract score of 1, 2, or 3 generally are considered to be prepubertal and heifers with a tract score of 4 or 5 are considered to be pubertal.

Pregnancy Diagnosis

At 30 to 50 d post AI, transrectal ultrasonography (5MHz transrectal transducer, Aloka 500V, Wallingford, CT) was used to confirm pregnancy. Pregnancy to AI was determined by the presence of uterine fluid and an embryo.

Experiment 2

Experimental Procedure

Heifers at two different locations (n = 257) were enrolled in this study. Estrus was synchronized using the 7-d CO-Synch + CIDR FTAI protocol (Figure 2.2). Heifers were administered 100 μg of GnRH im (Cystorelin, Merial) and an Eazi- Breed CIDR insert (1.38 g of progesterone, Zoetis) on Day 0. The CIDR inserts were removed 7 d later and the heifers received 25 mg of $PGF_{2\alpha}$ im (Lutalyse, Zoetis) and estrus-detection patches (Estrotect; Rockway, Inc.) were applied. Estrus was defined as described previously in Experiment 1. At 48 h after

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$PGF_{2\alpha}$, estrous detection patch color was determined and heifers were assigned to treatments

based on the color of the patch: (1). Estrus-Red 48 h (Red 48; n = 95) - heifers displayed estrus

as indicated by red estrous detection patch and inseminated at 48 h after $PGF_{2\alpha}$; (2). Non-Estrus-

Gray 48 h (Gray 48; n = 84) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and inseminated

at 48 h after $PGF_{2\alpha}$; and (3). Non-Estrus Delayed- 72 h (Gray 72; n = 78) - heifers did not display

estrus by 48 h after $PGF_{2\alpha}$ and inseminated at 72 h after $PGF_{2\alpha}$. Heifers assigned to Red 48 and

Gray 48 were administered GnRH on Day 9 at 48 h after $PGF_{2\alpha}$ regardless of estrous expression

(change in patch color), and heifers assigned to Gray 72 were administered GnRH 72 h after

$PGF_{2\alpha}$. The actual interval from $PGF_{2\alpha}$ to FTAI for the delayed AI was approximately 24 to 26 h.

We chose a delay of 24 h to provide a practical application of the protocol for producer use.

Heifers at location 1 were inseminated with semen from 10 different sires and one AI technician,

and heifers from location 2 were inseminated with semen from one sire and were inseminated by

two AI technicians.

Pregnancy Diagnosis

At 30 days post AI, pregnancy was diagnosed as described in Experiment 1.

Statistical Analyses

Experiment 1

Binomial data were analyzed as a completely random design using the FREQ and the

GLIMMIX procedures of SAS (SAS Enterprise Guide 4.3; SAS Inst. Inc., Cary, NC). Heifer

served as the experimental unit, treatment was a fixed effect, and location was a random variable

to determine the effect of treatment on pregnancy rate. Occurrence of estrous also was analyzed

using the FREQ and the GLIMMIX procedures of SAS to determine the effect of estrous

expression on pregnancy rate during the delay period. Heifer served as the experimental unit, treatment was the fixed effect, and location was a random variable. Reproductive tract scores were collected for heifers. Pregnancy outcome as affected by reproductive tract score was analyzed in a separate GLIMMIX model with tract score, treatment, and tract score × treatment as fixed effects, and location as a random variable. When no significant interaction of tract score and treatment was detected, the interaction, and effect of treatment was eliminated from the model. Linear and quadratic contrasts were also used to further evaluate pregnancy rates as affected by reproductive tract scores. Probability values ≤ 0.05 were considered to be significant, and P-values > 0.05 to 0.10 were considered to show a tendency.

Experiment 2

Binomial data were analyzed using the FREQ and the GLIMMIX procedures of SAS. Pregnancy rate as affected by treatment was analyzed using the same model described above for Experiment 1. Occurrence of estrous to determine the effect of estrous expression on pregnancy rate during the delay period was analyzed using the same model as described in Experiment 1. Reproductive tract scores were not analyzed for Experiment 2.

Results

Experiment 1

Pregnancy rates to FTAI based on estrous response and treatment are shown in Table 2.1. Treatment affected pregnancy rate to AI with heifers expressing estrus before FTAI (Red 48; 67.8%) having a greater ($P < 0.001$) pregnancy rate than heifers that had not expressed estrus before FTAI (Gray 48; 39.4%). No difference ($P = 0.83$) in pregnancy rate of heifers not expressing estrus was detected between Gray 48 and Gray 56 (Table 2.1).

Patches remained on all heifers that did not express at 48 h. Heifers that were assigned to Gray 48 (n = 137) had a gray patch and were inseminated at 48 h after $PGF_{2\alpha}$ (Table 2.2). Colors of patches were observed again at 56 h after $PGF_{2\alpha}$, and 66.4% still had a gray patch and did not express estrus, whereas 33.6% had expressed estrus (red patch). A difference in pregnancy rates was not observed between heifers that expressed estrus by 56 h and heifers that did not express estrus by 56 h.

Heifers assigned to Gray 56 (n = 148) had gray patches at 48 h after $PGF_{2\alpha}$, but were not inseminated until 56 h after $PGF_{2\alpha}$. Patch color was evaluated again when the heifers were inseminated at 56 h, with 62.2% of the heifers with a gray patch (no estrus), whereas 37.8% of the heifers had expressed estrus. No difference in pregnancy rates was observed between heifers that expressed estrus by 56 h and heifers that did not express estrus by 56 h. At location 1, heifers assigned to treatment Gray 48 did not have a difference (P = 0.77) in pregnancies compared with heifers assigned to treatment Gray 56.

Overall, in heifers with a reproductive tract score of 1 pregnancy rate was 27.3%. Heifers with a reproductive tract score of 4 had a pregnancy rate of 56.8% (Table 2.1). At location 3, the average reproductive tract score was a 2, and indicated small and immature reproductive tracts. Heifers at this location had no reproductive tract scores of 5, and only one heifer having a score of 4. There was a linear (P = 0.001) effect of reproductive tract score on resulting AI pregnancy rate, indicating that pregnancy rates increased as reproductive tract score increased (Figure 2.3).

Experiment 2

Pregnancy rates to FTAI based on estrous response and treatment are shown in Table 2.3. When contrasting Red 48 to Gray 48 and Gray 72, pregnancy rate was greatest for heifers that expressed estrus by 48 h after $PGF_{2\alpha}$. In contrast, when treatments were compared individually,

there was only a difference (P = 0.01) between Red 48 (62.1%) and Gray 48 (40.5%). There was a tendency for heifers to achieve greater pregnancy rates (P = 0.09) when inseminated and expressing estrus by 48 h compared to heifers that did not express estrus by 48 h and received the delayed insemination 24 h later (Table 2.3).

Patches remained on all heifers not expressing estrus by 48 h. Heifers assigned to Gray 48 (n = 84) had gray patches and did not express estrus by 48 h after $PGF_{2\alpha}$. When patches were evaluated at 72 h after $PGF_{2\alpha}$ (Table 2.4), 71.4% of heifers still had a gray patch, whereas 28.6% of heifers had expressed estrus (red patch). The heifers that did not express estrus by 72 h had a pregnancy rate of 30.0%, whereas heifers that did express estrus by 72 h had a pregnancy rate of 66.7%. Pregnancy rates were greater (P = 0.02) when estrus was expressed by 72 h compared with heifers that did not express estrus.

Heifers assigned to the delayed AI treatment, Gray 72, (n = 78) had gray patches at 48 h after $PGF_{2\alpha}$ but were not inseminated until 72 h after $PGF_{2\alpha}$. When heifers were inseminated, patch color was observed and re-evaluated, 51.3% of the heifers had still not expressed estrus by 72 h, and 48.7% did express estrus and had a red patch. Pregnancy rate in Gray 72 was greater (P < 0.0001) for heifers that did express estrus by 72 h compared with heifers that did not express estrus by 72 h.

Discussion

Split-time AI, or delayed insemination, is a TAI protocol that delays insemination of heifers not expressing estrus. Spit-time AI accommodates delayed insemination for females not in estrus by the appointed time of AI. This delay allows for more females to achieve estrus before AI. In this study, there were essentially two groups of females: females that expressed estrus before FTAI, and females that did not express estrus before FTAI. The results obtained

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from this study are similar to those reported by Richardson et al. [6]. Richardson et al. [6] compared multiple AI synchronization programs in a meta-analysis and determined that females detected in estrus before FTAI, regardless of the estrous synchronization protocol, had a 27% greater pregnancy success than heifers that did not express estrus before FTAI. This increase is thought to be caused by changes in the reproductive tract that are associated with elevated concentrations of estradiol. We hypothesized that delaying insemination in heifers not expressing estrus at FTAI, would increase pregnancy rate by allowing a greater proportion of heifers to come into estrus and develop an optimal uterine environment for AI because of higher concentrations of circulating estradiol. Females expressing estrus before FTAI may have attained adequate concentrations of estradiol to provide a more optimal uterine and reproductive tract environment for fertilization and pregnancy [11,12]. Ovulation without expression of estrus, however, may cause reduced conception rates [13,14,16] resulting from estradiol concentrations not being adequate to properly regulate the uterine environment for gamete interaction [4,17].

Females that express estrus at or before FTAI will ovulate approximately 28 to 32 h after the onset of estrus. In contrast, females that do not express estrus by AI could potentially not ovulate within the time frame of optimum sperm viability. The optimal AI time for an oocyte to develop into a good embryo is between 12 and 24 h before ovulation [18]. Greater pregnancy rates can be achieved by better aligning the viable lifespan of sperm and oocyte because gametes are less likely to be aged at the time of insemination [19].

A recent study conducted by Thomas et al. [10] found that delaying insemination by 20 h after GnRH for heifers not expressing estrus in a 14-d CIDR $PGF_{2\alpha}$ protocol improved pregnancy rates compared with heifers not expressing estrus that were inseminated when GnRH was administrated, 66 h after $PGF_{2\alpha}$, (49 vs. 34%). During the 20 h delay, 54% of heifers

expressed estrus, and the pregnancy rate increased for these heifers compared with heifers that did not express estrus before FTAI (66 vs. 29%). In the present study, we compared the effects of delaying insemination in heifers in a 7-d CO-Synch + CIDR protocol and no increase was observed in pregnancy rate of heifers not expressing estrus in Gray 48 and Gray 56. The pregnancy rate of heifers that expressed estrus by 48 h after $PGF_{2\alpha}$ was 67.8%, and was greater than heifers that did not express estrus by 48 h. The results from the present study support other findings that females that express estrus before insemination have greater pregnancy rates.

The results from the current study also support the findings by Kasimanickam et al. [20] Their study [20] compared the effects of TAI at 56 and 72 h in a 5-d CO-Synch + CIDR protocol in beef heifers. They found that pregnancy rate was greater for heifers inseminated at 56 h after CIDR removal compared with heifers inseminated at 72 h after CIDR removal (66.2 vs. 55.9%). It was noted, however, that a greater proportion of heifers expressed estrus before insemination at 72 h compared with 56 h (82.4 vs. 73.1%). In Experiment 1 of the current research, the cumulative percentage of heifers not expressing estrus by 48 h after $PGF_{2\alpha}$ was 61.3%. No significant difference was observed in pregnancy rates of heifers that expressed estrus by 56 h compared to heifers that did not express estrus by 56 h in Gray 48 and Gray 56. In Experiment 2, however, a difference was observed in pregnancy rates if heifers expressed estrus by 72 h compared to heifers that did not express estrus by 72 h in Gray 48 and Gray 72. These results suggest that perhaps a longer delay period for heifers not expressing estrus is needed to achieve a difference in pregnancy rate when using a 7-d Co-Synch + CIDR protocol.

Another factor that could have affected estrous expression and pregnancy rate was the administration of GnRH at time of AI. In Experiment 1, regardless of treatment, heifers received GnRH 48 h after CIDR removal. Exogenous GnRH induces a surge of LH and subsequent

ovulation [20]; however, if estradiol has not yet reached a threshold before GnRH is administered, estrus will not occur because LH suppresses estradiol release by inhibiting aromatase activity within the follicle [21,22]. In the present study, in Experiment 1, GnRH may have been administered too early to heifers not expressing estrus by 48 h, and that is why we did not see a significant change in estrous response and pregnancy rates in those heifers. This also applies to heifers in Gray 48 in Experiment 2.

When we did not observe an increase in pregnancy rate with the delayed insemination in Experiment 1, we increased the delay period from 8 h to 24 h in Experiment 2, with the intent to increase the number of females that could express estrus during a longer delay. Unpublished data from Kansas State University has shown pregnancy rates of 45 to 55% in heifers when inseminated 48 h after $PGF_{2\alpha}$. These percentages are similar to pregnancy rates observed when insemination occurs at 54 h after $PGF_{2\alpha}$ in heifers [23,24]. Inseminations occurring at 48 or 72 h after $PGF_{2\alpha}$ are convenient because they allow for AI to occur in the morning, and during daylight hours.

By delaying insemination and administration of GnRH until 72 h after $PGF_{2\alpha}$ in heifers that are not expressing estrus by 48 h, a greater percentage of females became pregnant compared to delaying insemination to 56 h after $PGF_{2\alpha}$. There was still no difference in pregnancy rates, however, when comparing the non-estrous expressing treatments. The pregnancy results of the delayed insemination differ from results discovered by Thomas et al. [10]. In the study by Thomas et al. [10] the delay in AI did increase pregnancy rates in heifers that did not express estrus by the appointed time of AI. It is likely that these differences occur because a different TAI protocol was used, as well as different times relative to insemination.

Synchronization of estrous cycles among beef females has the potential to shorten the subsequent breeding season and increase calf uniformity. Development of FTAI has eliminated time and labor required for estrous detection. Expression of estrus before FTAI has been shown to increase pregnancy rates in females, and use of estrous detection patches help determine estrous activity. We did not see an improvement in pregnancy rates by delaying insemination in heifers that did not express estrus by time of AI in either of these studies. We speculate, however, that it could be more practical for producers to use a protocol for delayed insemination on females that are not expressing estrus at time of AI. The delay period allows for more of those females to achieve estrus by insemination, and thus improve pregnancy rates. More research needs to be conducted to determine the optimum timing of delayed insemination in heifers using a short-term CIDR based protocol.

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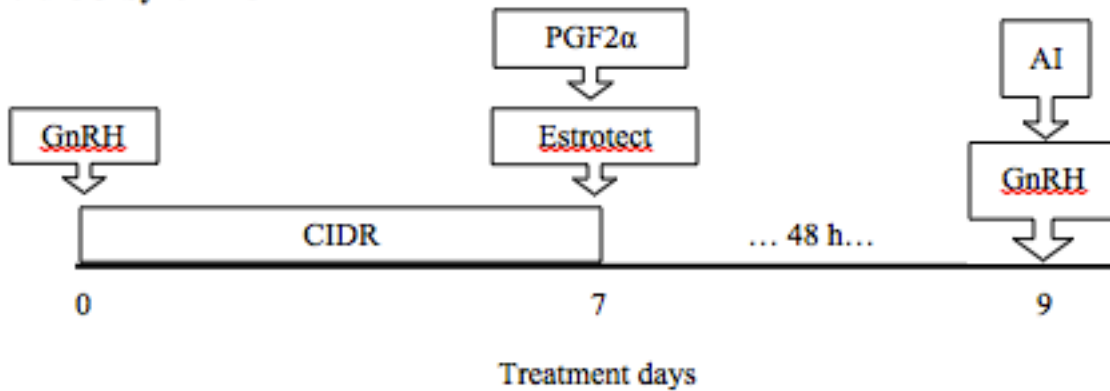
7-d CO-Synch + CIDR

Treatment days
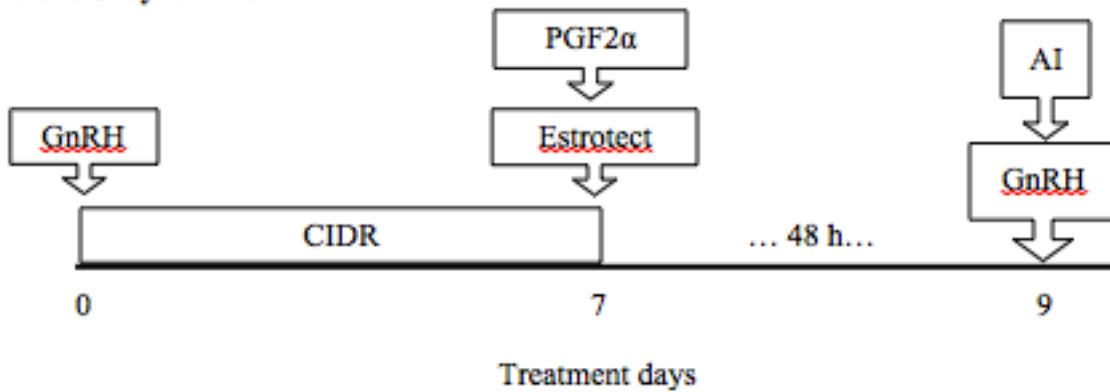
Modified 7-d CO-Synch + CIDR for delayed insemination
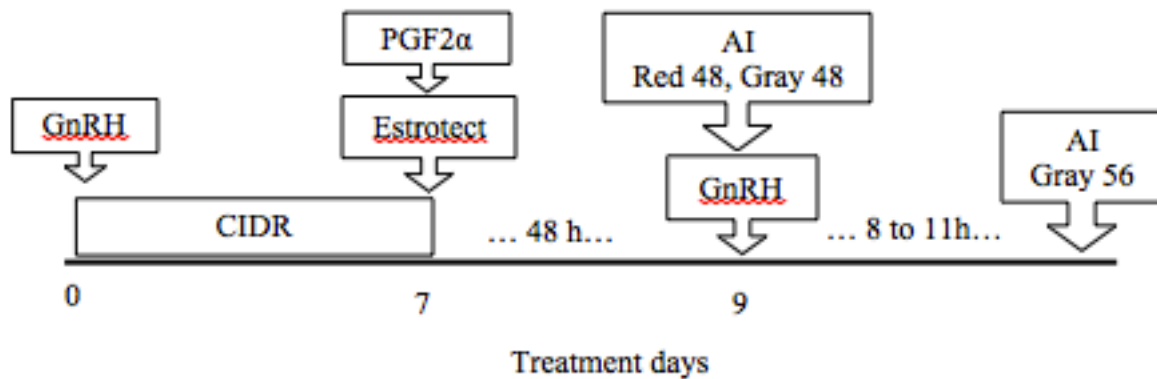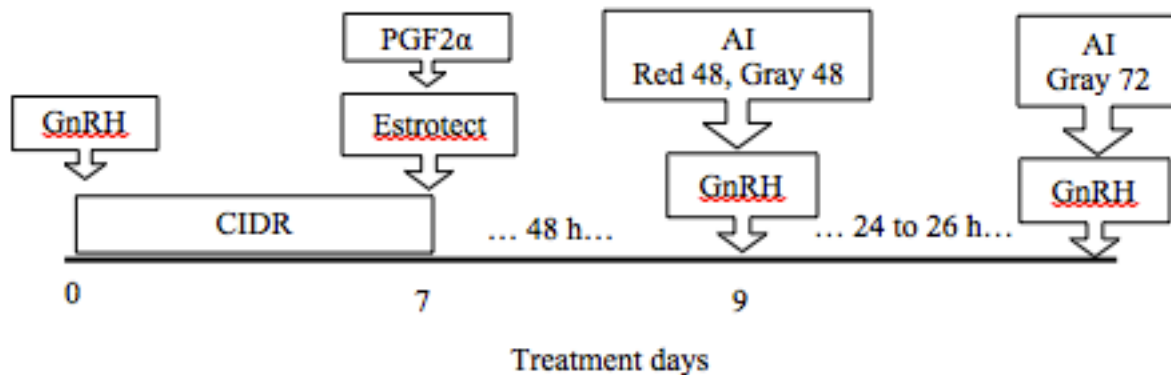

Treatment days

Figure 2.1

Treatment schedule for the 7-d CO-Synch + controlled internal drug-release (CIDR) protocol. Heifers in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 µg im) on Day 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg im) was administered on Day 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers received GnRH (100 µg im) and were allocated to three treatments based on expression of estrus by 48 h. Heifers expressing estrus (Estrus-Red 48 h; Red 48), and a portion of the heifers not expressing estrus (Non-estrus-Gray 48 h; Gray 48) were inseminated at time of GnRH. The remaining portion of heifers not expressing estrus at 48 h (Non- Estrus Delayed- 56 h; Gray 56) was inseminated 56 h after CIDR insert removal and $PGF_{2\alpha}$.

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7-d CO-Synch + CIDR

PGF2α

GnRH → Estrotect

AI → GnRH

CIDR ... 48 h...

| 0 | 7 | 9 |

Treatment days

Modified 7-d CO-Synch + CIDR for delayed insemination

PGF2α

GnRH → Estrotect

AI Red 48, Gray 48 → GnRH

AI Gray 72 → GnRH

CIDR ... 48 h... ... 24 to 26 h...

| 0 | 7 | 9 |

Treatment days

Figure 2.2

Treatment schedule for the 7-d CO-Synch + controlled internal drug-release (CIDR) protocol.

Heifers in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg im) on Day 0. The CIDR insert was removed and PGF$_{2\alpha}$ (25 mg im) was administered on Day 7. At 48 h after CIDR insert removal and PGF$_{2\alpha}$ heifers were allocated to three treatments based on expression of estrus by 48 h. Heifers expressing estrus (Estrus-Red 48 h; Red 48), and a portion of the heifers not expressing estrus (Non-estrus-Gray 48 h; Gray 48) were inseminated and treated with GnRH at 48 h. The remaining portion of heifers not expressing estrus at 48 h (Non- Estrus Delayed- 72 h; Gray 72) were inseminated 72 h after CIDR insert removal and treated with GnRH.

Linear: P = 0.001
Quadratic: P = 0.85
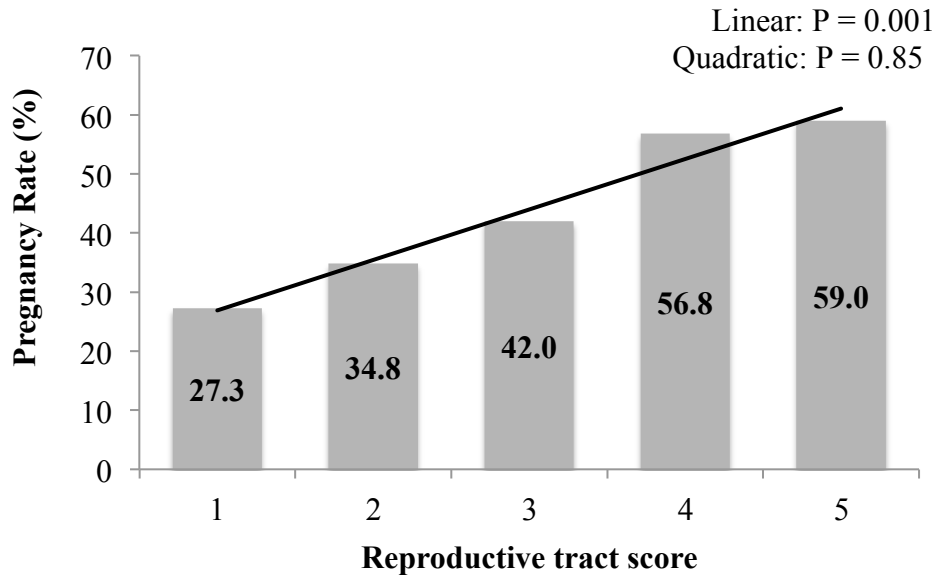
Figure 2.3

Pregnancy rates of heifers with different reproductive tract scores in Experiment 1. There was a

linear effect observed between reproductive tract score on pregnancy rate (P = 0.001).

Reproductive Tract Scores were scored on a scale of 1 to 5 (1 = immature tract, no evidence of

ovarian structures and 5 = mature tract with a palpable CL). Reproductive tract scores were

evaluated Day −7 to Day −30 before CIDR insert (Day 0). Pregnancy rate to AI was determined

by transrectal ultrasonography 30 to 60 d after AI.

Table 2.1. Pregnancy rate of heifers after insemination based on location and treatment for Experiment 1

Location	Treatment[1]	RTS[2]	Pregnancy rate to FTAI[3]	
			Proportion	%
Location 1				
	Red 48	4.3 ± 0.8	21/28	75.0
	Gray 48	3.9 ± 1.0	13/28	46.4
	Gray 56	3.8 ± 1.0	10/26	38.4
Location 2				
	Red 48	4.2 ± 0.7	94/139	67.6
	Gray 48	4.0 ± 0.7	35/78	44.9
	Gray 56	4.2 ± 0.7	41/86	47.7
Location 3				
	Red 48	2.0 ± 0.9	7/13	53.9
	Gray 48	2.1 ± 0.7	6/31	19.4
	Gray 56	1.9 ± 0.8	12/36	33.3
Total				
	Red 48	4.0 ± 0.9	122/180	67.8[a]
	Gray 48	3.5 ± 1.1	54/137	39.4[b]
	Gray 56	3.6 ± 1.2	63/148	42.6[b]
Overall			239/465	51.4

[a,b]Pregnancy rates with different superscripts within columns are different (P < 0.0001).
[1]Heifers received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg im) on Day 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg im) was administered on Day 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers received GnRH (100 μg im) and were assigned to three treatments. Heifers expressing estrus (Estrus-Red 48 h; Red 48), and a portion of the heifers not expressing estrus (Non-estrus-Gray 48 h; Gray 48) were inseminated at 48 h. The remaining portion of heifers not expressing estrus at 48 h (Non- Estrus Delayed- 56 h; Gray 56) was inseminated 56 h after CIDR insert removal and $PGF_{2\alpha}$.
[2]RTS (Reproductive tract score, 1 to 5 scale, 1 = immature tract, no evidence of ovarian structures and 5 = mature tract with a palpable CL) were evaluated Day −7 to Day −30 before CIDR insert (Day 0).
[3]Pregnancy rate to AI was determined by transrectal ultrasonography 30 to 60 days after AI.

Table 2.2. Pregnancy rates after insemination based on estrous expression during the delay period for heifers in treatment Gray 48 and treatment Gray 56 for Experiment 1

Treatment[1]	Estrous response[2]			Pregnancy rate to TAI[3]	
		Proportion	%	Proportion	%
Gray 48	Estrus	91/137	66.4	30/91	33.0
	Non-estrus	46/137	33.6	24/46	52.2
Gray 56	Estrus	92/148	62.2	34/92	37.0
	Non-estrus	56/148	37.8	29/56	51.8

[1]Heifers received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 µg im) on Day 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg im) was administered on Day 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers received GnRH (100 µg im) and were assigned to three treatments. Heifers expressing estrus (Estrus-Red 48 h; Red 48), and a portion of the heifers not expressing estrus (Non-estrus-Gray 48 h; Gray 48) were inseminated at 48 h. The remaining portion of heifers not expressing estrus at 48 h (Non- Estrus Delayed- 56 h; Gray 56) was inseminated 56 h after CIDR insert removal and $PGF_{2\alpha}$.
[2] Estrous response was determined by color of Estrotect patch (Estrotect; Rockway Inc., Spring Valley, WI) during the 8 h period after GnRH administration.
[3]Pregnancy rate to AI was determined by transrectal ultrasonography 30 to 60 days after AI.

Table 2.3. Pregnancy rate of heifers after insemination based on location and treatment for Experiment 2

Location	Treatment[1]	Pregnancy Rate to TAI[2]	
		Proportion	%
Location 1			
	Red 48	17/28	60.7
	Gray 48	16/30	53.3
	Gray 72	15/28	53.6
Location 2			
	Red 48	42/67	62.7
	Gray 48	18/54	33.3
	Gray 72	21/50	42.0
Total			
	Red 48	59/95	62.1[a]
	Gray 48	34/84	40.5[b]
	Gray 72	36/78	46.2
Overall		129/257	50.2

[a,b]Pregnancy rates with different superscripts within columns are different (P < 0.05).
[1]Heifers received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg im) on Day 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg im) was administered on Day 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers were assigned to three treatments. Heifers expressing estrus (Estrus-Red 48 h; Red 48), and a portion of the heifers not expressing estrus (Non-estrus-Gray 48 h; Gray 48) were inseminated at 48 h and treated with GnRH (100 μg im). The remaining portion of heifers not expressing estrus at 48 h (Non- Estrus Delayed- 72 h; Gray 72) were inseminated 72 h after CIDR insert removal and $PGF_{2\alpha}$ and were treated with GnRH at time of insemination.
[2]Pregnancy rate to AI was determined by transrectal ultrasonography 30 to 60 days after AI.

Table 2.4. Pregnancy rates after insemination based on estrous expression during the delay period for heifers in treatment Gray 48 and treatment Gray 72 for Experiment 2

Treatment[1]		Estrous Response[2]		Pregnancy Rate to TAI[3]	
		Proportion	%	Proportion	%
Gray 48	Estrus	24/84	28.6	16/24	66.7[a]
	Non-estrus	60/84	71.4	18/60	30.0[b]
Gray 72	Estrus	38/78	48.7	28/38	73.7[a]
	Non-estrus	40/78	51.2	8/40	20.0[b]

[a,b]Heifers in treatment Gray 48 had different ($P = 0.02$) pregnancy rates based on expression of estrus by 72 h, and heifers in treatment Gray 72 had different ($P < 0.001$) pregnancy rates based on expression of estrus by 72 h.

[1]Heifers received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 μg im) on Day 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg im) was administered on Day 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers were assigned to three treatments. Heifers expressing estrus (Estrus-Red 48 h; Red 48), and a portion of the heifers not expressing estrus (Non-estrus-Gray 48 h; Gray 48) were inseminated at 48 h and treated with GnRH (100 μg im). The remaining portion of heifers not expressing estrus at 48 h (Non- Estrus Delayed- 72 h; Gray 72) were inseminated 72 h after CIDR insert removal and $PGF_{2\alpha}$ and were treated with GnRH at time of insemination.

[2] Estrous response was determined by color of Estrotect patch (Estrotect; Rockway Inc., Spring Valley, WI) during the 8 h period after GnRH administration.

[3]Pregnancy rate to AI was determined by transrectal ultrasonography 30 to 60 days after AI.