

THESIS

EFFECT OF DELAYING TIME OF AI BASED ON ESTROTECT PATCH STATUS ON
PREGNANCY RATES OF BEEF HEIFERS AND NURSING BEEF COWS

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ABSTRACT

EFFECT OF DELAYING TIME OF ARTIFICIAL INSEMINATION BASED ON ESTROTECT PATCH STATUS ON PREGNANCY RATES OF BEEF HEIFERS AND NURSING BEEF COWS

Four studies were conducted to evaluate the efficacy of a delayed timed AI (**TAI**) protocol. The objective was to evaluate the use of Estroprotect patch status to determine optimum TAI implementation.

Experiment 1 was conducted with, 997 cows across 6 locations were administered a 7-d CO-Synch + controlled internal drug release (**CIDR**) estrous synchronization protocol. The experimental design was a 2×2 factorial; factors were patch status at 58 h post PGF_{2 α} injection (activated or inactivated) and time interval from PGF_{2 α} to TAI (58 or 76 h). Patch status was characterized at 58 h post PGF_{2 α} into 4 scores: 1 = 0% activated, 2 = 50% activated, 3 = 100% activated, and 4 = missing. Females with a patch status of 3 were considered to be activated and females with patch status of a 1 or 2 were considered to be not activated. Females with missing patches were removed. There was no treatment \times location interaction for pregnancy rate ($P = 0.96$), so data were pooled across locations. There was a tendency ($P = 0.07$) for an interaction between the main effects for pregnancy rate. Pregnancy rate was greater ($P < 0.01$) in cows with activated patches at 58 h post PGF_{2 α} (67.0%) compared to those with inactivated patches (51.1%). There was no difference ($P = 0.99$) for pregnancy rate when comparing the 58 (59.9%) vs. 76 h PGF_{2 α} to TAI interval (58.7%). Pregnancy rates for cows with a patch status of 3 at 58 h post PGF_{2 α} were greater ($P < 0.05$) than cows with a patch status of 1 or 4 and tended ($P = 0.09$)

to be greater than cows with a patch status of 2. Cows with a patch status of 1 tended ($P = 0.06$) to have increased pregnancy rates by delaying TAI to 76 h post PGF_{2α}. Although, it was not different ($P = 0.13$), there was a 7.4 percentage point increase in pregnancy rates for cows with inactivated patches that received TAI at 76 vs. 58 h.

Experiment 2 was conducted to evaluate delayed TAI on 1,682 heifers across 3 locations. The objective was to evaluate the use of Estroject patch status at various recommended and delayed insemination times for a TAI protocol. Experiment 2a was implemented with 1,159 *Bos taurus* heifers synchronized using a 14 d melengestrol acetate (MGA) – PGF_{2α} protocol. Estroject patches were applied at the time of PGF_{2α} injection and evaluated at the time of insemination. Heifers were subsequently assigned to 5 treatments: 1) recommended 72 h post PGF_{2α} TAI with activated patches, 2) recommended 72 h post PGF_{2α} TAI with inactivated patches, 3) 12 h delayed TAI with inactivated patches, 4) 18 h delayed TAI with inactivated patches, and 5) heifers with missing patches. Experiment 2b was conducted with 449 *Bos taurus* heifers synchronized using a 14 d MGA – PGF_{2α} protocol. Heifers were divided into 3 treatments: 1) recommended 72 h post PGF_{2α} TAI with activated patches, 2) recommended 72 h post PGF_{2α} TAI with inactivated patches, and 3) an 8 h delayed TAI with inactivated patches. Experiment 2c utilized 74 heifers synchronized with a 7-d CO-Synch plus CIDR protocol. All heifers had Estroject patches applied at the time of CIDR removal and PGF_{2α} injection. Patch status was evaluated 58 h post PGF_{2α} injection on all heifers. This experiment was analyzed as a 2 x 2 factorial with patch status 58 h post PGF_{2α} injection (activated or inactivated) and at the time of insemination (58 or 76 h). In experiment 2a, differences ($P < 0.05$) in pregnancy rates were different by patch status across treatments. Similarly, experiment 2b demonstrated resulted in differences ($P < 0.05$) in pregnancy rates when comparing the activated patch treatment to

both the inactivated patch treatment and the delayed inactivated treatment. However, no differences ($P > 0.05$) were seen in either experiment 2a or 2b when comparing the recommended inactivated patch treatments to any of the delayed inactivated patch treatments regardless of delay interval. Experiment 2c demonstrated a tendency ($P = 0.07$) for the interaction pregnancy rates to be increased when activated patches at 58 h were inseminated at 58 h post PGF_{2α} injection and when inactivated patches at 58 h were inseminated at 76 h post PGF_{2α}. Pregnancy rates for the main effects of patch status ($P > 0.05$) and interval inseminated ($P > 0.05$) did not differ. Results show a definitive increase in pregnancy rates when comparing heifers with activated patches to heifers with inactivated patches. However, delaying insemination time of heifers with inactivated patches did not increase pregnancy rates.

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TABLE OF CONTENTS

Abstract.....	ii
Acknowledgments.....	v
Table of Contents.....	vii
List of Tables.....	viii
List of Figures.....	ix
Keywords.....	x
Chapter 1	
1.1 Development of AI and Estrous Synchronization	1
1.2 Progestin use in a Protocol.....	2
1.3 GnRH use in a protocol	3
1.4 GnRH usage in Beef heifers	5
1.5 Timed AI Protocols	5
1.6 Follicle Size	7
1.5 Progesterone.....	8
1.6 Rationale for Current Experiment	8
1.7 Cow Variability.....	10
1.8 Literature Cited	12
Chapter 2	
2.1 Introduction	21
2.2 Materials and Methods.....	22
2.3 Results and Discussion.....	23
2.4 Implications.....	29
2.5 Literature Cited.....	31
2.6 Tables.....	34
Chapter 3	
3.1 Introduction.....	38
3.2 Materials and Methods.....	41
3.3 Results.....	45
3.4 Discussion.....	47
3.5 Implications.....	52
3.6 Literature Cited.....	54
3.7 Tables.....	56
Appendix I	
SAS Code for Chapter II.....	63
SAS Code for Chapter III.....	63

LIST OF TABLES

TABLE 2.1 Average BCS and post-partum intervals by locations and overall.....	40
TABLE 2.2 Pregnancy rates to timed AI and percentage within each patch status by location and overall	40
TABLE 2.3 Distribution of Estroject patch status at 58 h post prostaglandin injection and pregnancy rate among patch status at each interval and overall.....	41
TABLE 2.4 Main effects of time interval (58 vs. 76 h) from PGF _{2α} to timed AI and Estroject patch status at 58 h post PGF _{2α} on pregnancy rate by location.....	42
TABLE 2.5 List of assumptions and sources.....	43
TABLE 2.6 Partial budget for Delayed 7 – d CO – Synch plus CIDR vs. Recommended 7 – d CO – Synch plus CIDR.....	44
TABLE 2.7 Partial budget for Delayed 7 – d CO – Synch plus CIDR vs. Natural Service.....	45
TABLE 3.1 Average BCS among treatments and overall.....	66
TABLE 3.2 Distribution of Estroject patch status at 72 h post prostaglandin injection and pregnancy rate among patch status.....	66
TABLE 3.3 Pregnancy rates to timed AI and percentages within each treatment by bull.....	67
TABLE 3.4 Distribution of pregnancy rate among treatments.....	68
TABLE 3.5 Distribution of Estroject patch status at 72 h post prostaglandin injection and pregnancy rate among patch status.....	69
TABLE 3.6 Distribution of Estroject patch status at 72 h post prostaglandin injection and pregnancy rate among patch status.....	70
TABLE 3.7 Average BCS among each patch status and time interval combination.....	71
TABLE 3.8 Distribution of Estroject patch status at 58 h post prostaglandin injection and pregnancy rate among patch status.....	72
TABLE 3.9 Effect of time interval (58 vs. 76 h) from PGF _{2α} to timed AI and Estroject patch status at 58 h post PGF _{2α} on pregnancy rate by location.....	72

LIST OF FIGURES

FIGURE 2.1 Estrous synchronization protocols administered to nursing beef cows.....	39
FIGURE 3.1 Estrous synchronization protocols for beef heifers in Exp. 1	63
FIGURE 3.2 Estrous synchronization protocols for beef heifers in Exp. 2	64
FIGURE 3.3 Estrous synchronization protocols for beef heifers in Exp. 3	65

KEYWORDS

beef cows, beef heifers, delayed insemination, estrus detection aid, economics, timed AI

CHAPTER I

REVIEW OF LITERATURE

Development of AI and Estrous Synchronization

Estrus synchronization and AI are some of the most important and widely applicable reproductive technologies that are available for beef cattle producers (Seidel, 1995). These technologies allow producers to use elite genetics that would otherwise be unobtainable without purchasing a bull of similar genetic merit. Estrus Synchronization has been around for 60 years. Recently, timed artificial insemination (**TAI**) has become used more readily because of the increased research conducted and advances in ultrasound imaging that allows for real time imaging that can lead to increased pregnancy rates. Protocols are now being developed to effectively synchronize ovulation and improve TAI pregnancy rates in beef and dairy cattle (Twagiramungu et al., 1995).

To promote implementation of AI by beef producers, time and labor inputs must be minimized (Lamb et al., 2006). Protocols are being designed with two key factors in mind to encourage implementation by producers, 1) minimize the number of times cattle are handled through a working facility; and 2) eliminate the detection of estrus by employing TAI (Lamb et al., 2006). Difficulty associated with detection of estrus is one of the primary reasons that many cattle producers do not use AI in their herds (NAHMS, 1994). Estrus detection aids have been around since the 1950's, but within the last 5 years application of these aids has increased. These estrus detection aids could be used as a tool to determine cyclic behavior and help minimize the problem of detecting estrus for producers. Estrus detection patches could be used to reduce labor costs and in turn increase the overall number of cows AI. Compared to the swine

and dairy industry, the beef industry has a much lower utilization of AI. The use of AI and estrus synchronization in 2008 was 7.6% and 7.9% respectively (USDA 2009a) compared to the 76.3% of dairy producers who used AI on cows for first service (USDA 2009b) and 76.1% of the swine producers who use AI in 2006 (USDA 2007).

By using an estrous synchronization protocol that facilitates TAI, more calves can be born earlier in the calving season (Rogers et al., 2012). This is shown by the decrease in the average number of days that calves were born after the start of the calving season for TAI (26.8 d) compared to cows in the natural service group (31.3 d) (Rogers et al., 2012). It has been shown that cows that had estrous synchronized and TAI, had greater weaning weights per cow exposed (193.4 kg) compared to cows that were bred by natural service (175.9 kg) (Rogers et al., 2012). In the study performed by Rogers et al. (2012) bulls that were used for TAI and those used for natural service were of similar genetic merit. Rogers et al. (2012) reported the increased weaning weights were greater for the TAI group most likely as a result of the increased days of age of the calf rather than the increased genetic potential from the bull. If bulls of superior genetics would have been used the potential for greater weaning weights is increased and added profit could be seen. An overall advantage of \$49.14 was seen when comparing TAI to those that were bred via natural service (Rogers et al., 2012). Data reported by Thomas et al. (2013) stated that heifers meeting the minimum qualifications for the Show-Me-Select Replacement heifer Program and were bred via AI had \$192 increase in profits compared to heifers meeting the same requirements and bred via natural service. Similarly heifers that were sired by bulls with high accuracy EPD's and AI had increase profits of \$330 as well as heifers that were sired by bulls with high accuracy EPD's and bred via natural service had increase profits of \$216 over heifers that were sired by a low accuracy bulls and bred via natural service (Thomas et al., 2013).

Progestin use in a Protocol

The development of the Controlled Internal Drug Releasing (**CIDR**) insert has impacted the ability to synchronize estrus in cows and heifers (Day and Geary, 2005). A CIDR releases progesterone at a constant rate, by irritating the vaginal wall, capillaries are opened and progesterone enters the bloodstream. Using a progestin for 7 d works because the CIDR prevents animals in the luteal phase from ovulating and showing estrus. At the same time cows in the follicular phase of the cycle are unresponsive to regression of the CL by PGF_{2α} (Day and Geary, 2005). The CIDR will help to ensure ensures heifers that began treatment early in the estrous cycle will have a CL that will be responsive to PGF_{2α}. A CIDR can also be used to overcome the 5 to 15% of cows that will come into estrus before the PGF_{2α} injection because not all cows are at the same stage of their estrous cycle when synchronization is initiated (Kojima et al., 2000; Lamb et al., 2001). Melengestrol acetate (**MGA**) is another commonly used progestin that is administered through feed inclusion. Today MGA is only FDA labeled for use in heifers. MGA is used in the feedlot industry to suppress estrous in heifers.

Using a CIDR can influence cyclicity of anestrus cows and maximize pregnancy rates, which is an advantage of using a progestin based synchronization protocol. The use of progestin exposure, via a CIDR or MGA, is one of the most powerful tools for cattle producers to induce anestrus cows and pre-pubertal beef heifers to resume estrous cycles or induce puberty (Day and Geary, 2005). This induction to estrous will normally result in a short cycle that is necessary for cows the start their normal 21 d estrous cycle (Day and Geary, 2005). This short cycle of elevated progesterone levels is needed before the synchronized estrus at which insemination will occur to ensure that the next estrous cycle is of normal length (Day and Geary, 2005). Using a progestin based protocol, 66.9% of the anestrus cows and 66.8% of the estrous-cycling cows

conceived as a result of TAI (Wilson et al., 2010). Using a progestin based protocol with PGF_{2α} the estrus synchronization and pregnancy rate was increased compared to control cows (no CIDR or PGF_{2α}) (Lucy et al., 2001). When comparing a CO-Synch protocol to a CO-Synch + CIDR protocol, the CIDR based protocol had higher pregnancy rates to AI than the recommended CO-Synch protocol (Larson et al., 2006).

GnRH use in a protocol

The hypothalamic/pituitary axis is the central axis that controls the female reproductive cycle. Estrogen has a positive feedback loop on the hypothalamus and increased estrogen levels will cause the hypothalamus to produce GnRH which acts positively on the anterior pituitary to release LH and FSH. Luteinizing hormone and FSH will then act on the structures of the ovary. However, progesterone will act negatively on the hypothalamus and prevent the release of GnRH. This information, as well as information discovered about follicular waves via transrectal ultrasonography has aided in the ability to synchronize estrous. Follicular waves can be manipulated through the use of exogenous GnRH. By giving exogenous GnRH, the hypothalamus will cause a surge of LH to be secreted from the anterior pituitary and cause the most dominant follicle to ovulate and a new follicular wave to start development (Twagiramungu et al., 1995). This injection of GnRH will also cause the secretion of FSH from the anterior pituitary and cause recruitment, selection, and dominance of a new follicular wave if a dominant follicle is not present (Twagiramungu et al., 1995). With this discovery, the Select Synch protocol was developed giving GnRH on d 0 and PGF_{2α} on d 7 and heat checking d 7 to 13. This increased pregnancy rates because it allowed for the females to start a new follicular wave and have a dominate follicle by d 7. The interval between GnRH and PGF_{2α} administration allows for the time that is necessary for a new follicular wave to be recruited, along with selection and

maturation of the dominant follicle to a point at which successful ovulation of a healthy oocyte may be achieved (Thatcher et al., 1989; Pursley et al., 1995). A new follicular wave at the beginning of a TAI protocol will yield an increase in the quality of embryos when compared to females who do not ovulate and begin a new follicular wave in response to GnRH at the beginning of an estrous synchronization protocol (Cerri et al., 2009; Perry et al., 2007). In some cows and heifers estrus may not be seen after giving exogenous GnRH. This GnRH induced ovulation is associated with decreased estradiol concentrations in the peripheral circulation and spontaneous estrus is inhibited (Twagiramungu et al., 1995). This is because the LH surge that was triggered by the GnRH will suppress estrogen levels of the follicle and the female will not exhibit estrus. This LH surge will also cause ovulation of the dominant follicle (Twagiramungu et al., 1995). However, ovulation does not always occur. Ovulation is dependent on the stage of development of the follicle at the time of GnRH treatment (Twagiramungu et al., 1995). Ovulation will occur in those females in which the follicle is still growing. The LH receptors that are on the ovary are decreasing in numbers as the follicle is slowing in growth and starting to become atretic (Twagiramungu et al., 1995). Those follicles that have already regressed and committed to atresia will not be rescued from atresia by giving exogenous GnRH (Twagiramungu et al., 1995). While using a CIDR can help to induce estrus in post-partum cows, GnRH can also induce estrus in post-partum cows. Giving an exogenous dose of GnRH can either cause ovulation of a dominant follicle or atresia and reemergence of a new follicular wave that can lead to ovulation. This ovulation will lead to formation of a CL and allow for the positive short term effect that progesterone has on the estrous cycle (Twagiramungu et al., 1995).

GnRH Usage in Beef Heifers

After a GnRH injection at random stages of the estrous cycle 64 - 75% of postpartum beef and dairy cows ovulated a follicle (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004), whereas only 48-60% of heifers ovulated a follicle in response to a GnRH injection (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000). In heifers, pregnancy rates did not differ between heifers receiving GnRH at CIDR insertion of a 7- d protocol and those that did not receive GnRH at CIDR insertion (Lamb et al., 2006). The primary reason is the inability to synchronize follicular waves with a GnRH injection in heifers. Lucy and Stevenson (1986) reported a reduced magnitude of GnRH-induced LH released in heifers compared with cows. However, Schafer et al. (2006) reported that 86% of heifers responded to the GnRH injection after a presynchronization with a 14 - d CIDR. This may be explained by the greater degree of synchrony that resulted from presynchronization using the 14 - d CIDR (Leitman et al., 2008).

Timed AI Protocols

Timed AI protocols have been researched thoroughly over the last 20 years and have aided in the ability to AI cows and heifers without the use of estrus detection. Most TAI protocols require cows to come through the chute 3 or 4 times depending on the protocol, and AI at a given time interval from CIDR removal or PGF_{2α} injection. The Select Synch protocol has shown to effectively synchronize estrous in postpartum beef cows (Kojima et al., 2000; Dejarnette et al., 2001). By adding a CIDR to the Select Synch protocol pregnancy rates were increased (Lamb et al., 2001; Larson et al., 2006). In addition, AI pregnancy rates were 66% and 67% when using the 7 d CO-Synch + CIDR protocol (Schafer et al., 2007; Busch et al., 2008).

When comparing the Select Synch and TAI to the Select Synch + CIDR and TAI a tendency for a greater percentage of cows to be detected in estrus was seen for the Select Synch plus CIDR and TAI (Larson et al., 2006). Furthermore, the time from PGF_{2α} injection to cows exhibiting estrus was similar among the Select Synch + CIDR and TAI (53.4) with the Select Synch and TAI (51.5) (Larson et al., 2006). The 5-d Select Synch + CIDR and the 7-d Select Synch + CIDR showed no differences in pregnancy rates (Wilson et al., 2010). These data yield similar results but the fact that cows had to be run through the chute an additional time and an extra shot of PGF_{2α} was needed for the 5-d Select Synch + CIDR protocol should be factored into the decision of which protocol to use.

Timing of AI using a TAI protocol is critically important. Busch et al. (2008) confirmed that TAI pregnancy rates were greater when AI was performed at 66 vs. 54 h after PGF_{2α} administration of the 7-d CO-Synch + CIDR protocol. On the basis of the odds ratio, cows inseminated 66 h following PGF_{2α} administration are 1.32 times more likely to conceive to the TAI than cows inseminated 54 h following PGF_{2α} administration (Busch et al., 2008). This is supported by the data that Wilson et al. (2010) had shown the average interval from PGF_{2α} to estrus to be 64.8 h. This 60-66 h interval after PGF_{2α} injection is what is recommended by the Beef Reproductive Task Force to AI using a 7 - d CO-Synch + CIDR protocol.

In a particular study done by Busch et al. (2008) estrus response was examined and cows that were inseminated at the 54 h interval had a smaller proportion of cows show estrus (26%) compared to those AI at the 66 h interval (50%). Cows that exhibited estrus before AI had higher pregnancy rates than those who did not show estrus (Busch et al., 2008). This also holds true for those cows inseminated with sexed semen (Thomas et al., 2014). Cows that exhibited estrus between the time of CIDR removal and insemination had higher pregnancy rates

than cows that had not displayed estrus after CIDR removal regardless if given sexed semen or non-sexed semen. (Sa Filho et al., 2012; Thomas et al., 2014). Heifers that were synchronized, had estrus detected, and received AI, had higher pregnancy rates than heifers receiving TAI without estrus detection (Lamb et al., 2006). Heifers that were not detected in estrus at 84 h post CIDR removal had lower pregnancy rates than those heifers detected in estrus (Lamb et al., 2006).

It has been documented that presynchronization has the ability to increase pregnancy rates and benefiting effects to estrus synchronization. The CIDR Select method includes a 14 - d CIDR prior to the start of the CO-Synch protocol on d 23 and is a presynchronization method (Busch et al., 2007). Heifers that were presynchronized using the CIDR Select protocol were 1.86 times more likely to conceive to TAI than heifers synchronized using the 7 - d CO-Synch + CIDR protocol (Busch et al., 2007). Using a 14 - d CIDR presynchronization is similar and has the same effects as feeding MGA to heifers for 14 d. The 14 - d MGA based protocols have been used more commonly by producers because of the cheaper cost of MGA than a CIDR. However, prior planning in advance is required to ensure proper timing of this protocol because of its length. Estrus response was higher for the CIDR Select heifers than the 7 - d CO-Synch + CIDR heifers but the average time from PGF_{2α} to estrus was shorter for the 7 - d CO-Synch + CIDR compared to the CIDR Select heifers (Busch et al., 2007). A peak estrus response was seen 48 to 60 h post PGF_{2α} injection (Busch et al., 2007).

Follicle Size

Cows that had an ovulatory follicle greater than 12 mm at the time of GnRH injection had greater pregnancy rates compared to cows induced to ovulate with ovulatory follicles less than

12 mm (Lamb et al., 2001). In a study performed by Perry et al. (2005), GnRH induced ovulation of follicles less than 11 mm resulted in decreased pregnancy rates and increased the incidence of early embryonic/fetal mortality. However, spontaneous ovulation of follicles less than 11 mm had no effect on pregnancy rate or embryonic/fetal mortality (Perry et al., 2005). Cows that exhibited estrus had a larger diameter follicle than those cows that did not exhibit estrus (Sa Filho et al., 2012). Inducing ovulation of immature follicles can reduce the pregnancy rates of beef cows because the ovulation of an immature follicle can produce an immature oocyte (Busch et al., 2008).

Progesterone

Higher progesterone levels can attribute to higher pregnancy rates. When comparing the progesterone serum concentration levels between induced follicles greater than 12mm and those that ovulated spontaneously there was no difference (Busch et al., 2008). Ovulation of a physically immature follicle induced by GnRH resulted in formation of a CL in which production of progesterone was reduced (Busch et al., 2008). Similar results were seen by Vasconcelos et al. (2001) who reported that induced ovulation of small follicles (11.5) resulted in development of a smaller CL that secreted less progesterone compared to larger follicles (14.2) induced to ovulate in dairy cows. This has led to believe that ovulation of small or immature follicles has a negative impact on pregnancy rates as well as increased early embryonic death which is thought to be from ovulation of an incompetent oocyte, inadequate uterine environment, or both (Busch et al., 2008).

Rational for current Experiment

In any TAI protocol there are 2 groups of cows, those that show estrus before TAI and those that do not (Thomas et al., 2014). Development of estrus detection patches have come about and have aided in determining estrus in cattle. By using these patches producers are able to categorize these cows prior to TAI, and breed according to patch. Thus, turning a TAI into estrus detection and TAI. It was shown that heifers that had estrus detected, had higher pregnancy rates than heifers receiving a TAI (Lamb et al., 2006). Similarly a heat detect and TAI protocol (Select Synch + CIDR and TAI) had similar pregnancy rates to a TAI protocol (CO-Synch + CIDR) (Larson et al., 2006).

In non-lactating dairy cows ovulation occurred 27.6 ± 5.4 h after the first sign of estrus (Walker et al., 1996). Those cows that did not show estrus between the PGF_{2α} injection and TAI will be induced to ovulate from the exogenous GnRH that was given and ovulation will occur 24-32 h after the second GnRH injection of a TAI protocol (Pursley et al., 1995). With the viability of frozen-thawed semen having a lifespan of approximately 24 h, the semen is subject to not being able to survive until ovulation (Trimberger et al., 1984). Sperm transport to the site of fertilization in the oviduct requires a minimum of 4-6 h following insemination in the cow (Hunter and Wilmut, 1984). Therefore, the optimal time for AI is 6-16 h after the onset of estrus (Dransfield et al., 1998) and at 16 after the second GnRH injection of an Ovsynch protocol (Pursley et al., 1998). But inseminating too late could cause problems as well. Pursley et al. (1998) noted that if AI was performed at 32 h after the second GnRH injection of an Ovsynch protocol an increase in pregnancy losses and lower pregnancy rates occurred. They believed this is because they inseminated 0 - 8 h after the time of ovulation, and it takes sperm approximately 4 - 6 h following insemination to reach the site of fertilization, which would put the sperm at the

oocyte 8 - 16 h after ovulation (Pursley et al, 1998). The more time that passed between the onset of estrus and the insemination time, the greater the fertilization rate was seen but a decrease of excellent/good embryos were seen by waiting to inseminate (Dalton et al., 2001). High embryo quality (grades of excellent or good) and lower fertilization rates were seen when insemination early after the onset of estrus but greater fertilization rates and lower embryo quality (grades of fair or poor) is seen when inseminating late after the onset of estrus (Dalton et al., 2001), explaining that there is a compromise in the optimal time to AI cows.

This is where the delaying of insemination of about 18-20 h occurs. Categorization of cows via estrus detection patches as either having displayed estrus or not can be performed at the time of the second GnRH injection of a 7 - d CO-Synch + CIDR protocol. Cows with activated patches at this time are assumed to have expressed estrus and already ovulated a follicle. Those cows that have not exhibited estrus before AI have not yet ovulated a follicle, so the likelihood of the sperm fertilizing an oocyte is reduced. By waiting an additional 18-20 h to AI, semen is deposited closer to the time of ovulation and the viability of the semen is not compromised. Geary and Whittier (1998) examined the different AI times comparing the CO-Synch versus the Ovsynch protocol. The Ovsynch protocol had a 24 h delay from the time of the second GnRH injection to AI, whereas the CO-Synch protocol had AI and GnRH given simultaneously at the same 48 h interval from PGF_{2α}. They saw an increase in pregnancy rates for those cows in the Ovsynch protocol (57%) versus the CO-Synch protocol (49%) (Geary and Whittier, 1998). A similar project was done in dairy cows examining the optimal time to AI after the second GnRH injection (Pursley et al., 1998). Intervals of 0, 8, 16, 24, and 32 - h after the second GnRH injection were examined. Pregnancy rates were lower for the 32 - h group compared to all the others, and were numerically the highest at the 16 - h interval (Pursley et al., 1998). Pregnancy

losses were less for those cows inseminated at the 0 h interval compared the 8, 16, 24, and 32 - h interval, but the 32 - h interval had the highest pregnancy losses numerically of all the intervals (Pursley et al., 1998). These losses suggest that age of oocyte at fertilization appears to affect pregnancy rate and pregnancy loss (Pursley et al., 1998) similar to the data seen by Dalton et al. (2001). Using this protocol they determined that the time of ovulation is between 24 to 32 h after the second injection of GnRH and that ovulation was synchronized 87-100% of the time during this time interval. (Pursley et al., 1995).

Cow Variability

Estrus synchronization and AI is not the only method to ensure cows get pregnant. Fundamental management techniques should always be kept in mind. Going into the breeding season, cows must be in adequate body condition and have an adequate diet or supplementation to meet nutritional needs. Cows will also have a post-partum interval that is needed to be addressed and can be anywhere from 25 - 80 d post-partum at the time of AI. It is suggested that cows need to be at least 40 to 45 days post-partum to allow for full uterine involution after calving (Day and Geary, 2005). It has been recommended that heifers should be at least 60 - 65% of their mature body weight prior to the breeding season (Martin et al., 2008). However previous research performed by Martin et al. (2008) showing that there is no difference in pregnancy rates when comparing heifers developed to 50 or 55% of their mature bodyweight. In any synchronization protocol, there is always the ability to have variation from cow to cow. Whether this is from improper injection technique, improper timing, or improper management, variation seems to occur and not all cows are going to respond to the protocol every time.

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CHAPTER II

INTRODUCTION

Estrous synchronization and AI are some of the most important and widely applicable reproductive technologies available for beef cattle producers (Seidel, 1995). Researchers are developing estrous synchronization protocols using 2 key factors to help encourage implementation: 1) minimize frequency of handling, and 2) eliminate detection of estrus by employing timed AI (**TAI**); (Lamb et al., 2006). Difficulty associated with implementation of an estrous synchronization protocol is one of the primary reasons that many cattle producers do not use AI. Development of estrous detection patches could help eliminate this obstacle and increase implementation (NAHMS, 2008). The activated patches would allow cows to be classified at TAI as having exhibited estrus activity. Those females with an activated patch could receive TAI when the patch was seen as activated while the remaining cows would be induced to ovulate via an injection of exogenous GnRH and subsequent TAI at a later time (16-20 h; Pursley et al., 1995). The exogenous dose of GnRH will induce ovulation of an oocyte 24 to 32 h after injection (Pursley et al., 1995). Semen in the female reproductive tract has a lifespan of 24 h (Trimberger, 1984), which can result in not having viable spermatozoa to reach the oocyte for cows that did not exhibit estrus. Sperm transport to the site of fertilization in the oviduct requires a minimum of 4 to 6 h following insemination in the cow (Hunter and Wilmut, 1983). Therefore, the optimal time for AI is 6 to 16 h after the onset of estrus (Dransfield et al., 1998) and 16 h after the second GnRH injection in an Ovsynch protocol (Pursley et al., 1998). Therefore, we hypothesized that cows with activated patches will have greater pregnancy rates if

inseminated at 58 vs. 76 h post PGF_{2α}, and cows with inactivated patches 58 h post PGF_{2α} will have greater pregnancy rates if TAI is delayed to 76 h post PGF_{2α}.

MATERIALS AND METHODS

All cows in this study were cared for and managed under the approval of the Colorado State University Institutional Animal Care and Use Committee guidelines. Postpartum beef cows (n = 997) in 6 herds and across 3 states were enrolled in this study. All cows had a controlled internal drug release (**CIDR**) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis, Florham Park, NJ) i.m. on d 0. On d 7, all cows had CIDR removed and were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and aerosol adhesive was sprayed onto the tailhead where Estroject patches (Estroject, Spring Valley, WI) were applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, and approximately half of the cows (n = 510 cows) were inseminated, while remaining cows (n = 487) received delayed insemination at 76 h post PGF_{2α} injection. At 58 h post PGF_{2α}, all cows had Estroject patches characterized into 4 scores: 1 = 0% activated, 2 = 50% activated, 3 = 100% activated, and 4 = missing. Cows with a patch status of 1 or 2 were considered to be inactivated, and a patch status of 3 was considered to be activated. Cows with a patch status of 4 (missing) were removed from the data analysis. Cows were randomly designated to the 58 or 76 h TAI group by ear tag number at locations 1 and 2, and randomly selected by chute order at locations 3, 4, 5, and 6. At 58 h, all calves were removed from their dams and held separately until after cows were inseminated. Cows inseminated at 76 h were left separated from their calves beginning at 58 h through insemination at 76 h. The 58 and 76 h intervals were used for this study because of the time constraints and

the ability to facilitate working larger groups of cattle during daylight hours. Pregnancy determination was performed at 35 to 55 d post TAI using an Ibex pro ultrasound machine with a 5.0-MHz linear-array transducer (E.I. Medical Imaging, Loveland, CO) or an Aloka 500V equipped with a 5.0-MHz linear-array transducer (Aloka, Wallingford, CT) to determine pregnancies to TAI by a qualified technician.

Statistical Analyses

Data were analyzed as a 2×2 factorial using the GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC) to produce a general linear mixed model including the fixed variables of BCS, post-partum interval, patch status, PGF_{2 α} to TAI interval, and patch status \times PGF_{2 α} to TAI interval. Location was set as a random variable in the model. There was no treatment \times location interaction ($P > 0.05$), so data were pooled across locations. A contrast statement was used in the model to examine differences between groups of means within the factorial. Means were compared and separated using the LS Means option in SAS.

RESULTS AND DISCUSSION

Body Condition Score and Post-Partum Interval

The number of cows, d postpartum, and mean BCS at the time of CIDR insertion on d 0 are presented in Table 2.1. Body condition score was not taken at location 6. Cows from locations 2 and 3 had greater ($P < 0.05$) BCS than cows at other locations. Post-partum intervals

were not different ($P > 0.05$) when comparing locations 2 and 5. However, all other locations differed from each other for post-partum interval ($P < 0.05$).

Patch Status

The TAI pregnancy rate for cows with a patch status of 3 (67.3%) was greater ($P < 0.05$) than cows with a patch status of 1 or 4 (50.0 and 52.5%), respectively (table 2.2). When the time intervals were combined there was a tendency ($P = 0.09$) for cows with a patch status of 3 to have higher pregnancy rates (67.3%) than cows with a patch status of 2 (56.6%). Similarly, at 58 h post PGF_{2α} pregnancy rate for cows with a patch status of 2 or 3 (65.9 or 69.2%) was greater ($P < 0.05$) than cows with a patch status of 1 (45.7%). Pregnancy rates did not differ ($P > 0.05$) from patch status of 3 vs. 2. However, at 76 h post PGF_{2α} pregnancy rates for patch status of 1 (54.3%) were not different ($P > 0.05$) from patch status 2 cows (45.7%), and cows with a patch status of 3 (65.5%) had greater ($P < 0.05$) pregnancy rates than cows with a patch status of 1 (54.3%). There was a trend ($P = 0.06$) for cows with a patch status of 1 to have greater pregnancy rates at the 76 h interval (54.3%) compared to the 58 h interval (45.7%). Cows with a patch status of 2 tended ($P = 0.08$) to have lower pregnancy rates at the 76 h interval (45.7%) compared to the 58 h interval (65.9%).

These results are similar to data reported by Bridges et al. (2012), in which cows assumed to have shown estrus activity, via rubbing off tail paint before or at TAI, had greater pregnancy rates than those that were not rubbed. The tendency in the current experiment to have an increase in pregnancy rates for cows with patch scores of 1 at 58 h post PGF_{2α} and inseminated at 76 h post PGF_{2α} was similar to the results seen by Thomas et al. (2014). With development of the CO-Synch + CIDR protocol and control of ovulation, it is possible for some cows that have

not shown estrus to conceive. Thomas et al. (2014) reported that 45% of cows that did not show estrus conceived a pregnancy. That is similar to this experiment where 45.7% of cows with a patch status 1 were inseminated at 58 h post PGF_{2α} and were confirmed pregnant. Stevenson et al. (2000) noted that a second injection of GnRH reduced estrus expression from 79.5 to 13.0%. This is because the LH surge triggered by GnRH will suppress estrogen levels of the follicle and the female will not exhibit estrus, leading to ovulation of the dominant follicle (Twagiramungu et al., 1995). At the 58 h post PGF_{2α} when patch status was being evaluated, there was a portion of cows that were still coming into estrus as they were being worked through the chute. If this evaluation was done at a later 60 to 66 h interval, there is a chance that the proportion of cows in each patch status would be different than what was seen. The time intervals of 58 and 76 h post PGF_{2α} were chosen because an 18 h delay was used for the designated delay time. To ensure that the 18 h delay was met and synchronization procedures were performed in daylight hours a time of 58 and 76 h were established.

Delayed Fixed-Time AI Pregnancy Rates

Pregnancy rates for location 4 were lower locations 1, 2, 3, 5, and 6 ($P < 0.05$). While locations 1, 2, 3, 5, and 6 were similar ($P > 0.05$). There was no location x treatment interaction ($P = 0.96$) for pregnancy rates or patch status ($P = 1.00$). Therefore, all data were pooled for final analysis. There tended ($P = 0.07$) to be an interaction between patch status and PGF_{2α} to TAI interval. Since the interaction was a tendency, main effects were evaluated individually. Cows with activated patches had greater ($P < 0.01$) pregnancy rates (67.0%) compared to those whose patch was inactivated (51.1%). This is similar to results reported by Busch et al. (2008) where cows that were observed in estrus had higher pregnancy rates than cows that did not show

estrus prior to TAI. In the current study, there was no effect ($P > 0.05$) on pregnancy rates between 58 (59.9%) and 76 h (58.7%) PGF_{2α} to TAI interval. Previous research by Geary and Whittier (1998) compared CO-Synch to Ovsynch and by using the Ovsynch protocol and waiting an additional 24 h, pregnancy rates were increased by 8 percentage points (Geary and Whittier, 1998). However, this research was done prior to inclusion of CIDR inserts. Data by Geary and Whittier (1998) differs from these data which suggests that 58 or 76 h interval has no effect on pregnancy rates. When comparing cows with inactivated patches and inseminated at 58 h to cows with inactivated patches and inseminated at 76 h, there was only a numerical advantage, 48.5 vs. 53.7% ($P = 0.13$). There was no difference when comparing cows with activated patches and inseminated at 58 h to cows with activated patches and inseminated at 76 h, (70.2 vs 63.5%; $P = 0.26$). An improved strategy was comprised of evaluating patch status at 58 h and insemination of cows with activated patches at 58 h and inseminating cows with inactivated patches at 76 h. Using a contrast statement, this strategy was compared to the overall mean pregnancy rate of this experiment. When evaluating this strategy, pregnancy rates tended to be higher ($P = 0.07$) than the overall pregnancy rate for the experiment (64.2 vs. 59.0). When comparing this strategy to the 58 h PGF_{2α} to TAI main effect, there was no difference in pregnancy rate for the improved strategy (64.2 vs. 59.9%; $P = 0.13$). Similarly, there was no difference when the improved strategy was compared to the 76 h PGF_{2α} to TAI interval (64.2 vs 58.7%; $P = 0.26$).

The Beef Reproductive Task Force recommends that the recommended 7-d CO-Synch + CIDR TAI protocol have a 60 to 66 h interval from PGF_{2α} to TAI for cows. This is supported by data from Wilson et al. (2010) in which the average interval from PGF_{2α} to estrus was 64.8 h when using the 7-d Select Synch plus CIDR protocol. For the current study, an 18 h delay was

used; therefore, CIDR's were pulled early on d 7 so TAI could occur on the afternoon of d 9 and morning of d 10. Slight variation from the recommended 60 to 66 h timing interval was established to ensure that breeding was done in daylight hours and sufficient time was allowed to process all cows.

Partial Budget Analysis

To evaluate the economics of implementing an estrous synchronization protocol a partial budget was used to compare the delayed 7-d CO-Synch + CIDR protocol to the recommended 7-d CO-Synch + CIDR protocol (Table 2.6). A second partial budget analysis was completed to compare cows bred via natural service to the delayed 7-d CO-Synch + CIDR (Table 2.7). Partial budget's are used to compare the incremental cost differences of a new protocol (delayed 7-d CO-Synch + CIDR) to an existing or control protocol (recommended 7-d CO-Synch + CIDR). Partial budgets are unique in the fact that they only include the production costs that are associated with the differences in protocols to capture the effects of the change in the production system. These costs are defined as increased revenue and costs for the new protocol and decreased revenue and costs for the existing protocol. The final result of a partial budget is net profit (or loss) which is calculated:

(1) net profit or loss = (additional revenue + reduced costs) – (additional costs + reduced revenue).

The decision maker will adopt the new protocol if the net profit is positive. If the net profit is negative the decision maker will continue using the existing protocol.

Numerous calculations are needed to calculate the changing revenues and costs in equation (1). Assumptions were made for these calculations from existing literature and results

from this study (Table 2.5). All prices used were calculated on a per head basis. Pregnancy rates and cow numbers used in the partial budget calculations were those reported from the data of this project. The following sections describe the four major components in equation 1 with discussion of the assumptions used simultaneously.

Delayed 7-d vs. Recommended 7-d

Additional revenues are items that increase revenue by using the delayed vs. recommended protocol. Using the delayed protocol results in an increased number of calves born, which was determined by the pregnancy rates from this research. By using the ideal mean that was calculated from the current experiment, a comparison between the ideal delayed protocol mean and the 58 h post PGF_{2α} main effect mean were utilized. An increase of 4.3 percentage points was found from using the delayed protocol (64.2%) compared to the recommended protocol (59.9%). April 2014 market prices of were used to calculate revenue for these calves with an average calf selling price of \$1.99/ 0.45 kg to result in a total revenue increase of \$764,248.36. We assumed calves were 6 to 8 months old at the time of weaning. Based on an ADG of 1.36 kg, weaning weights were expected to be 272.4 kg when they were sold. Research published by Rodgers et al. (2012) indicated that calves born from dams that had estrous synchronized and AI were 17.5 kg greater per cow exposed than calves born from dams that were natural service sired. This 17.5 kg weight increase was assumed to be the increased weight that each estrous synchronized and AI sired calf will have greater than natural service sired calves at weaning and what is used in the partial budget model. A final pregnancy rate of 90% was cited from Anderson and Deaton, (2003) where final pregnancy rates were 90% when using AI for the breeding season and utilizing clean up bulls. The percent of cows pregnant to natural service bulls was determined by using the AI pregnancy rates for each protocol

subtracted from 90%. It was assumed that AI sired calves would receive a premium due to superior genetics. This is because AI sired calves will be heavier at the time of weaning and have the genetic potential to perform better in the feedlot and pasture settings for replacement females. It is difficult to quantify the increased value of AI sired calves, but previous work by Johnson and Jones (2008) suggested a \$0 to 50/head increase in profit for an estrous synchronized AI sired calf vs. natural service based solely on genetic potential. For this project, we assumed a \$25.00 increase in profit for an estrous synchronized AI sired calf was used, which is the median of the Johnson and Jones (2008) range. The \$25.00 increase in price was multiplied by the AI pregnancy rate for the delayed protocol to produce a value for profits based on genetic potential. With these assumptions, total additional revenue of \$1,067,670.68 is produced.

Reduced costs are the costs associated with the recommended protocol. In this analysis the costs associated with delayed do not differ from the recommended protocols, therefore the total reduced cost was \$0.

Additional costs are costs for the new production system (delayed protocol), meaning they did not exist or are different from the recommended protocol. Additional costs for the delayed 7-d CO-Synch + CIDR protocol included an increased labor charge per cow for additional time through the working facility to TAI the females on the delayed protocol. The labor charge was based on 4 workers receiving \$15.00/ h. These laborers are only used in the labor force that process cows through the chute, gather, and sort off calves. The extra time needed to process is based on information found from the current study. Using a breeding barn with 2 AI technicians and 1 person thawing semen and preparing AI guns, an average of 65 cows can be inseminated per hour. We estimated that it would take an additional 1 min and a half per

cow to inseminate cows at 76 h post PGF_{2α} and an additional 30 s per cow to pair the cows back up with their calves. This results in an additional cost of \$2.00 per head, however this cost was only associated with cows that were processed through the working facility at 76 h post PGF_{2α} (563 cows). The other additional cost was Estroject patches, which cost \$1.16 per cow. This is considered to be an additional cost because a producer could breed by timed appointment without using these patches in a recommended TAI protocol. With these assumptions, total additional cost's came to be \$2228.52 or \$2.24 per cow.

Reduced revenue is the revenue that would be received by using the recommended 7-d CO-Synch + CIDR protocol. A reduced number of calves would be born to AI with the recommended vs. the delayed protocol. There were a reduced number of calves born to AI because a reduced pregnancy rate to AI was used for the recommended protocol. Reduced revenue was calculated the same way as additional revenue. However, 59.9% was the pregnancy rate used for AI pregnancy rate and the genetic potential pregnancy rate. This is the mean pregnancy rate of inseminating all cows at 58 h post PGF_{2α}. With these assumptions, total reduced revenue was \$1,063,314.34 or \$1,066.51 per cow.

The net change in profit for this partial budget is calculated by adding together the total additional revenue and total reduced costs, and subtracting the total additional costs and reduced revenue, to result in a net change in profit (Table 2.6). Under all the assumption stated above there is a net change in profit of \$2.13. This net change is specific to the assumptions used, but indicates there is a benefit to switching to the delayed protocol.

The partial budget model can be used for a number of different scenarios and can be beneficial for producers to determine differences of profitability between protocols. The delayed vs. recommended model can also be used for future research to provide producers with

cost/return associated with synchronization protocols. Some of the costs associated and variables can have a bigger impact on net profit or loss. The largest contributing factor in this model is pregnancy rate for each synchronization protocol. Although the final pregnancy rate in the delayed vs. recommended model is the same for each estrous synchronization protocol, a slight change in the final pregnancy rate can have a major impact on the profit or loss in this model. In the delayed vs. recommended model, if the delayed protocol had a 1 percentage point increase (91%) in final pregnancy rates compared to the recommended protocol (90%) a profit of \$13.31 per cow can be accrued. However, if the delayed protocol had a 1 percentage point decrease (89%) in final pregnancy rates compared to the recommended protocol (90%) a loss of \$9.04 is reported. Price fluctuations in the beef market are continuously changing and can have an impact on the variation in profit. Another factor that can influence the outcome of this model is the monetary value used for increase genetic potential. Although Johnson and Jones, (2008) have cited a \$0 to \$50 increase in profit for AI sire calves, it is arguable that the value for AI sired calves could exceed \$50 depending on markets and premiums associated with the genetics and performance of the AI sired calves. As the premium for an AI sired calf is increased the profit for the delayed synchronization program can increase dramatically.

Delayed vs natural service

For the economic analysis of the delayed 7-d CO-Synch + CIDR protocol vs. natural service the same partial budget analysis was used. Additional revenues would be from the increased weight of AI sired calves. These calves will be approximately 17.5 kg heavier at weaning compared to natural service sired calves (Rodgers et al., 2012) and weigh 272.15 kg at weaning. With current market prices of \$1.99/0.45 kg and pregnancy rates to AI (64.2%) from this study being used, an increased revenue of 764,248.36 is calculated. Calves born to AI would

have an increased genetic potential, which result in a \$25 increase in profit per calf born to AI (Johnson and Jones, 2008). A final pregnancy rate of 90% was used to calculate the number of calves born to natural service sires (Anderson and Deaton, 2003). By subtracting the AI pregnancy rate from the final pregnancy rate the percentage of calves born to natural service would be 25.8%. By adding all these values, total additional revenue would be \$1,067,670.68.

Reduced costs are costs that would be reduced by using the delayed protocol compared to the natural service mating's. A bull to cow ratio of 1:25 is assumed for natural service but cows exposed to the delayed protocol will have a 1:50 bull to cow ratio under the assumption that half of the cows will be pregnant from TAI. Therefore, a reduced cost of 20 less bulls can be realized. Under the assumption that bulls cost \$4000 per bull, a reduced cost of \$80,000 is included used in this model. Vet expenses of \$300 per bull would be accrued and a value of \$6000 would be established. Bulls tend to cause damage to facilities and a value of \$20 per bull is estimated for damages. The 20 bulls that are used would cost approximately \$1.85 per bull per day to feed resulting in a value of \$13,505. Bull cost would be \$99,905.00 in total reduced costs.

The additional costs that were accrued by using the delayed 7-d CO-Synch + CIDR protocol were labor charges for 434 cows inseminated at 58 h post PGF_{2α} and 563 cows inseminated at 76 h post PGF_{2α}. This is the number of cows that would be inseminated at these times if evaluating patch status prior to TAI and inseminating females with activated patches at 58 h post PGF_{2α} and inseminating females with inactivated patches at 76 h post PGF_{2α}. Based upon the research conducted in this study, it was estimated that the time needed for the 58 h post PGF_{2α} protocol would take 2.25 min per cow to gather and sort cows 3 times, 1 min per cow to insert CIDR's and give an injection of GnRH, half a min per cow to remove CIDR's and give and injection of PGF_{2α}, and 1.5 min per cow to AI and give an injection of GnRH. This comes to

a total of 5.25 min per cow to process females through the working facility. Using a labor fee of \$15 per person and having 4 people employed, a cost of \$5.25 per cow was associated with processing cows through the working facility at 58 h post PGF_{2α}. There is added cost to inseminate cows at 76 h post PGF_{2α} because of the extra time and labor needed to process the cows through the chute at the 76 h time interval. An additional 1.5 min per cow would be added on to the processing time to AI those females at 76 h post PGF_{2α} and half a min to pair the cows back with the calves that were left sorted off. This results in a time of 7.25 min per cow to process cows in the 76 h post PGF_{2α} TAI interval, resulting in a cost of \$7.25 per cow at 76 h post PGF_{2α}. These times and charges are just for employees processing cows through the working facility. A technician cost of \$8 per cow is coupled with the fee for the insemination process and the personnel needed to thaw semen for AI. Estroject patches costing \$1.16 per cow would be placed on all cows. Synchronization drugs used for this estrous synchronization protocol are \$18.96/cow. Semen used to AI females is assumed to be \$17.00 per cow. A cost of having a working facility to process all the cows through for injections and AI is accrued and results in a \$6.00 fee per cow. This results in a total additional cost of \$57,326.89 or \$57.50/cow.

Reduced revenues would come from calves born by natural service and salvage value of the bull. Calves born to natural service are assumed to have a pregnancy rate of 81% (Anderson and Deaton, 2003) and are assumed to be 17.5 kg lighter (Rodgers et al., 2012) than AI sired calves resulting in a weaning weight of 254.7 kg. With current market prices of \$1.99/ 0.454 kg, a revenue of \$902,366.60 was calculated. There would also be reduced revenue from having less salvage value from selling bulls. For this scenario it is expected that a pregnancy rate of 50% would result from the TAI. Therefore, if using a bull to cow ratio of 1:50 there would only need

to be 20 bulls instead of the 40 needed for a natural service scenario, to service the remaining females that were not pregnant. The 20 bulls that are not needed by using the TAI protocol are used in the model to determine reduced revenues. If current market prices of \$1.05/0.454 kg for April 2014 were assumed for bulls weighing an average of 816.47 kg, a reduced revenue variable cost of \$37,800.00 or would be realized.

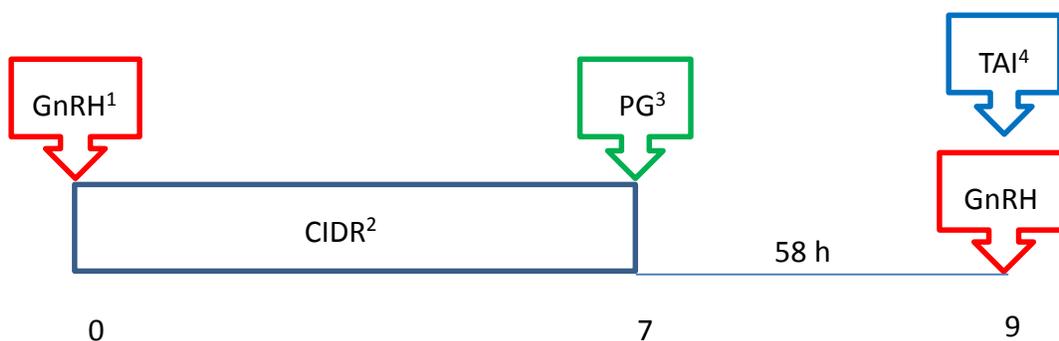
Under the above assumptions a net change in profit of \$170.59 per cow is established for using the delayed estrous synchronization protocol compared to natural service mating. It should be noted that this net change in profit is based on stated assumptions and is specific for this scenario. Final pregnancy rates can cause a variance in the outcome of this model as pregnancy rate is the primary contributor to profitability. If final pregnancy rates are left the same for each model, a net profit of \$70.03 will be produced. Rodgers et al. (2012) reported increased profits of \$49.14 for cows that were exposed to estrous synchronization and TAI compared to cows mated to natural service sires. The bulls used in the experiment performed by Rodgers et al. (2012) were of similar genetic merit and the difference in prices for each calf was more likely related to increased weight due to increased days of age rather than increased genetic potential. However, increased calf weaning weights of cows that have been exposed to AI or estrous synchronization have been attributed to a combination of calves being born earlier in the calving season and improved genetic growth potential (Johnson and Jones, 2008). Johnson and Jones (2008) noted that AI was more economical than natural service when the bull to cow ratio was 1:20 compared to 1:40. The increased genetic potential from using AI could have a significant impact on the beef industry. The outcome of this budget could be altered by fluctuating prices in the market and products used to inseminate cows.

Results suggest the interaction between patch status and PGF_{2α} to TAI interval could be of economic importance for increased overall TAI pregnancy rates. Pregnancy rates tended to differ within cows with a patch that has not been activated by the 58 h interval and pregnancy rates tended to increase by delaying AI to 76 h post PGF_{2α}. Moreover, TAI pregnancy rates increased in cows with activated patches. There was no difference in pregnancy rates when comparing the 58 or 76 h post PGF_{2α} interval. The benefit of delaying TAI on cows with inactivated patches will result in an additional \$2.02 in profit per cow. The delayed protocol vs natural service mating increase profits by \$170.59 per cow.

IMPLICATIONS

The evaluation of patch status at 58 h to determine the time of implementation of TAI tended to yield greater pregnancy rates than the overall mean. This advantage could lead to increased profits for a producer of known AI sired genetics and allow producers to increase profits.

58 h Insemination interval



Delayed 76 h Insemination interval

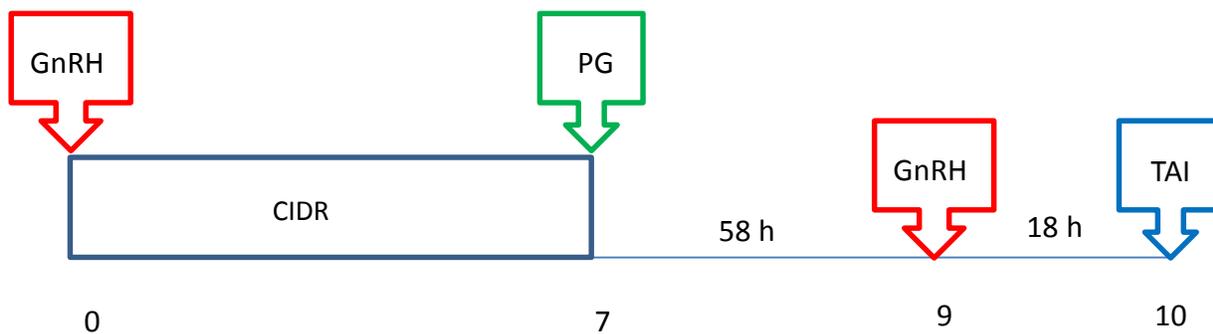


Figure 2.1 Estrous synchronization protocols administered to nursing beef cows.

¹GnRH: 100 µg (Factrel, Gonadorelin, Zoetis)

²Controlled Internal Drug Releasing device, (CIDR; EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ)

³PG: ProstaglandinF_{2α} 25 mg (Lutalyse, Dinoprost tromethamine, Zoetis)

⁴TAI: timed AI

Table 2.1. Average BCS and post-partum intervals among locations and overall

Location	n	BCS ^{1,2}		PPI ³	
		Mean	SE	Mean	SE
1	263	5.1 ^a	0.03	86.2 ^b	0.87
2	93	5.3 ^b	0.07	74.5 ^a	1.43
3	180	5.5 ^c	0.04	116.6 ^c	0.68
4	128	5.1 ^a	0.05	93.3 ^c	1.88
5	155	5.1 ^a	0.03	73.4 ^a	0.72
6	178	.	.	97.3 ^d	1.31
Overall	997	5.2	0.02	91.3	0.79

¹BCS on 1 to 9 scale (Wagner et al., 1988)

²BCS was not taken at location 6.

³PPI = post-partum interval

^{a-e} Within a column means without a common superscripts differ ($P < 0.05$).

Table 2.2. Distribution of Estroject™ patch status and pregnancy rate among patch status at each interval and overall ¹

Patch status ²	58 h Interval	SE	76 h Interval	SE	Overall Pregnancy rate (%)	SE
1	45.7 ^{ax}	3.2	54.3 ^{ay}	3.2	50.0 ^a	2.3
2	65.9 ^{bcy}	7.4	45.7 ^{ax}	8.4	56.6 ^{ab}	5.7
3	69.2 ^c	3.5	65.5 ^b	3.6	67.3 ^b	2.5
4	52.9 ^{ab}	7	51.7 ^{ab}	9.3	52.5 ^a	5.6

¹n = 997

²1 = 0% activated , 2 = 50% activated, 3 = 100% activated, 4 = missing

³ Estroject™, (Spring Valley, WI)

^{a-c} Within a column means without a common superscripts differ ($P < 0.05$).

^{x-y} Within a row means without a common superscript differ ($P < 0.10$)

Table 2.3. ²Distribution of EstroTECT™ patch status at 58 h post prostaglandin injection and pregnancy rate among patch status at each interval and overall ^{1,2}

Location	Pregnancy rate (%)	SE	Patch status at 58 h ³ (%)			
			1	2	3	4
1	59.7 ^b	3.0	58.9 ^e	6.1 ^{ab}	32.7 ^{ab}	2.3 ^a
2	60.2 ^b	5.1	66.7 ^e	2.2 ^a	22.6 ^a	8.6 ^b
3	59.4 ^b	3.7	42.8 ^{bc}	5.0 ^a	51.1 ^c	1.1 ^a
4	41.7 ^a	4.4	55.1 ^{de}	11.8 ^{bc}	32.3 ^{ab}	0.8 ^a
5	63.2 ^b	3.9	29.0 ^a	12.9 ^{bc}	40.0 ^{ac}	18.1 ^c
6	53.9 ^b	3.7	45.1 ^{bcd}	8.0 ^{ac}	26.9 ^b	20.0 ^c
Overall	56.9	1.6	49.1	7.7	35.2	8.1

¹ n = 997

²Cows received a controlled internal drug release (**CIDR**) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) i.m. on d 0. On d 7, cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and EstroTECT patches (EstroTECT, Spring Valley, WI) applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, 510 cows received AI at 58 h post PGF_{2α} while the remaining 487 cows received AI 76 h post PGF_{2α} injection

³1 = 0% activated, 2 = 50% activated, 3 = 100% activated, 4 = missing

^{a-e} Within a column means without a common superscripts differ ($P < 0.05$).

Table 2.4. Main Effects of time interval (58 vs. 76 h) from PGF_{2α} to timed AI and Estroject™ patch status (activated vs. inactivated at 58 h post PGF_{2α} on pregnancy rate by location^{1,2,5}

Location	58 h ³		76 h ³		<i>P</i> values		
	Activated ⁴	Activated ⁴	Inactivated ⁴	Inactivated ⁴	Interaction ⁶	Interval	Patch status
1	76.3	67.5	49.3	54.8	0.26	0.71	<0.01
2	77.7	100.0	50.0	51.7	0.98	0.98	0.97
3	68.4	65.2	41.5	58.5	0.21	0.41	0.03
4	58.4	37.7	35.7	43.8	0.21	0.60	0.46
5	73.5	62.0	64.4	64.6	0.49	0.51	0.69
6	64.1	54.5	56.5	49.7	0.86	0.39	0.51
Overall	71.0 ^c	64.8 ^{bc}	47.0 ^a	54.4 ^b	0.07	0.99	<0.01

¹n = 912

² Cows received a controlled internal drug release (**CIDR**; EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) i.m. on d 0. On d 7 cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and Estroject patches (Estroject, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, 510 cows received AI at 58 h post PGF_{2α} while the remaining 487 cows received AI 76 h post PGF_{2α} injection

³ Prostaglandin to timed AI interval.

⁴ Activated = patch status of a 3 (100% activated); inactivated = patch status of a 1 or 2 (0% activated or 50% activated, respectively) evaluated at 58 h post PGF_{2α}. Patch status of 4 was removed from this analysis.

⁵ Least Squares Means are reported in this table.

⁶There was no treatment x location interaction (*P* = 0.96).

^{a-c} Within a row, means without a common superscripts differ (*P* < 0.05).

Table 2.5 Distribution and source of partial budget input variables

Model input variable	Average	Minimum value	Maximum value	Source
Labor recommended, \$/cow	5.25	4.00	10.00	Estimated from current study
Labor delayed, \$/cow	7.25	5.00	15.00	Estimated from current study
Patches, \$/cow	1.16	1.15	1.19	Current market price, Genex Sales Representative, March 2014
Technician, \$/cow	8.00	6.00	20.00	Genex Sales representative, March 2014
Synch Drugs, \$/cow	18.96	16.98	20.58	Current market price, Valley vet supply March 2014
Semen, \$/cow	17.00	5.00	100.00	Genex Sales representative, March 2014
Facilities, \$/cow	6.00	0.00	15.00.	Estimated from current study
Calf prices, \$/0.454 kg	1.99			Current market price, April 2014
Calves AI, kg at weaning	272.15			Estimated, Rodgers et al., 2012
Calves born via natural service, kg at weaning	254.69			Estimated, Rodgers et al., 2012
AI benefits, \$/calf	25.00	0.00	100.00	Johnson and Jones, 2008
Delayed pregnancy rates, %	64.20			Research from current study 2013
Recommended 7 d CO-Synch + CIDR pregnancy rates, %	59.90	30.00	94.00	Research from current study 2013 , Johnson and Jones, 2008
Natural Service Pregnancy rates, %	81.00	74.00	100.00	Anderson and Deaton, 2003; Johnson and Jones, 2008
Final pregnancy rates, %	90.00	74.00	100.00	Anderson and Deaton, 2003; Johnson and Jones, 2008
Cost of Bull, \$/bull	4000.00	400.00		Estimated, Rodgers et al., 2012; Johnson and Jones, 2008
Salvage value of Bull, \$/0.454 kg	1.05			Current market price, April 2014
Vet Expenses, \$/year	40.00	0.00	500.00	Rodgers et al., 2012
Damage, \$/lifetime	200.00	0.00	1000.00	Estimated
Feed Costs, \$/day	1.85	0.00	5.00	Estimated from current feed prices April 2014

Table 2.6 Partial budget for delayed 7 – d CO – Synch + CIDR vs. recommended 7 – d CO – Synch + CIDR^{1,2,3}

Additional Costs	Budget	Number of cows	price			Additional Revenue	Budget	Number of cows	pregnancy rate	Price ⁵	Kg
Labor	1,072.00	563	2.00			Calves AI	764,248.36	997.00	64.20	1.99	272.15
Patches	1,156.52	997	1.16			AI benefits	16,001.85	997.00	64.20	25.00	
						Calves NS ⁴	287,420.47	997.00	25.80	1.99	254.69
						Final Pregnancy Rate			90.00		
<i>Total Additional cost</i>	2,228.52					<i>Total Additional Revenue</i>	1,067,670.68				
Reduced Revenue	Budget	Number of cows	pregnancy rate	Price ⁵	Kg	Reduced Costs	Budget				
Calves AI	713,060.38	997	59.90	1.99	272.15						
AI Benefits	14,930.08	997	59.90	25.00							
Calves NS ⁴	335,323.89	997	30.10	1.99	254.69						
Final Pregnancy Rate			90.00								
<i>Total Reduced Revenue</i>	1,063,314.34					<i>Total Reduced Costs</i>	0.00				
A. Total Additional Costs & Reduced Revenue	1,065,542.86					B. Total Additional Revenue & Reduced Costs	1,067,670.68				
						Net Change in Profit	2,127.82				
						Net Change per head	2.13				

¹ n = 997

²Cows received a controlled internal drug release (**CIDR**) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) i.m. on d 0. On d 7 cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and Estroject patches (Estroject, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, 510 cows received AI at 58 h post PGF_{2α} while the remaining 487 cows received AI 76 h post PGF_{2α} injection

³ Average pregnancy rates for the cows in the delayed synchronization protocol were the means consisted of inseminating cows with activated patches at 58 h post PGF_{2α} injection and cows with inactivated patches 76 h post PGF_{2α} injection. Pregnancy rates for cows in the recommended protocol were the mean for all the females inseminated at the 58 h PGF_{2α} injection.

⁴Calves born via natural service (**NS**) mating.

⁵Price for Calves AI and Calves NS is based on a dollars/ 0.454 Kg basis.

Table 2.7 Partial budget for delayed 7-d CO-Synch+CIDR protocol vs. Natural Service^{1,2,3}

Additional Costs	budget	cows	Cost	Additional Revenue	budget	cows	price ⁵	pregnancy rate	kg	
Labor recommended	2278.50	434	5.25	Calves AI	764,248.36	997	1.99	64.20	272.51	
Labor delayed	4081.75	563	7.25	AI benefits	16,001.85	997	25.00	64.20		
Patches	1156.52	997	1.16	Calves NS ⁴	287,420.47	997	1.99	25.80	254.69	
Technician	7976.00	997	8.00							
Synch Drugs	18903.12	997	18.96							
Semen	16949.00	997	17.00							
Facilities	5982.00	997	6.00							
<i>Total Additional cost</i>	57,326.89			<i>Total Additional Revenue</i>	1,067,670.68					
Reduced Revenue	budget	cows	price	pregnancy rate	kg	Reduced Costs	budget	bulls	Price	days
	902,366.60	997	1.99	81.00						
Calves NS					254.69	Bull ⁶	80,000.00	20	4000.00	
Salvage value of bull	37,800.00	20	1.05		816.47	Vet Expenses	6,000.00	20	300.00	
						Damage	400.00	20	20.00	
						Feed	13,505.00	20	1.85	365
<i>Total Reduced Revenue</i>	940,166.60					<i>Total Reduced Costs</i>	99,905.00			
A. Total Additional Costs & Reduced Revenue	997,493.49					B. Total Additional Revenue & Reduced Costs	1,167,575.68			
							170,082.19			
						Net Change in Profit	170.59			
						Net Change in Profit per head				

¹ n = 997

²Cows received a controlled internal drug release (CIDR) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) i.m. on d 0. On d 7 cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and Estroject patches (Estroject, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, 510 cows received AI at 58 h post PGF_{2α} while the remaining 487 cows received AI 76 h post PGF_{2α} injection

³ Average pregnancy rates for the cows in the delayed synchronization protocol were the means consisted of inseminating cows with activated patches at 58 h post PGF_{2α} injection and cows with inactivated patches 76 h post PGF_{2α} injection. Pregnancy rates for cows in the recommended protocol were the mean for all the females inseminated at the 58 h PGF_{2α} injection.

⁴ Calves born via natural service (**NS**) mating.

⁵ Price for Calves AI, Calves NS, and salvage value of bull is based on a \$/ 0.454 Kg basis.

⁶ Price for is based on a \$/bull basis

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CHAPTER III

INTRODUCTION

Estrous synchronization and AI techniques are used in less than 10% of producers beef herds (NAHMS, 2008). With the economic value of today's market, AI-sired heifers are worth a premium compared to natural service sired heifers (Thomas et al., 2013). An additional \$192 in profit was seen when pregnant heifers were sold bearing an AI sired calf compared to heifers bearing a natural service sire calf (Thomas et al., 2013). It is critical that heifers conceive to AI and that they conceive early in the breeding season so they are retained longer in the herd and produce greater pounds of weaned calf over their lifetime (French et al., 2013). Progestin allows for the potential to presynchronize heifers and induce heifers that were prepubertal (Day and Geary, 2005). Researchers are developing estrous synchronization protocols using 2 key factors to help encourage implementation: 1) minimize frequency of handling, and 2) eliminate detection of estrus by employing TAI (Lamb et al., 2006). Through these techniques and the adaption of estrus detection patches, producers are now able to focus efforts in a TAI protocol, and evolve it into an estrus detection and TAI protocol, which has shown to have increased pregnancy rates than a TAI without estrus detection (Lamb et al., 2006). When examining patches at the time of insemination heifers could be classified into 2 different categories: those that have exhibited estrus, and those that have not exhibited estrus and will be induced to ovulate an oocyte from the exogenous dose of GnRH that is given (Walker et al., 1996). It is the heifers that have not exhibited estrus prior to TAI that are ultimately lowering the overall AI pregnancy rates of the herd. Delaying the breeding of heifers that did not show any estrus activity will allow for semen to be deposited closer to the time of ovulation, which occurs 24 to 32 h after the

GnRH injection (Pursley et al., 1995). We hypothesize that heifers with inactivated patches that receive delayed insemination 12 or 18 h after a GnRH injection will have greater pregnancy rates than heifers with inactivated patches and inseminated simultaneously with the GnRH injection. We also hypothesized that heifers with activated patches will have greater pregnancy rates at the time of the GnRH injection than heifers that were delayed insemination 12 or 18 h after the GnRH injection.

MATERIALS AND METHODS

All heifers on these studies were cared for under the approval of the Colorado State University Institutional Animal Care and Use Committee guidelines. There were 3 different experiments of heifers at, all of 3 different locations.

Experiment 1

There were 1159 *Bos taurus* heifers located at the Kuner feed yard in Kersey, Colorado. All heifers were housed in feedlot style pens fed a similar growing ration consisting mainly of a high roughage base diet. The protocol consisted of all heifers fed melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis) for 14 d starting on d 0. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had Estrotect (Estrotect, Spring Valley, WI) patches applied to their tailhead. Seventy two h after PGF_{2α} all heifers were sorted by patch status. Heifers with inactivated patches were randomly sorted and approximately one half of the females with inactivated patches were given 100 μg of GnRH (Factrel, Gonadorelin, Zoetis) and subsequently inseminated (n = 271). The other half of the heifers with inactivated patches were randomly sorted into 2 treatments and delayed TAI by 12 (n = 105) or 18 h (n = 130) after the GnRH injection. Heifers with activated or missing patches at 72 h post PGF_{2α} were not given

GnRH and AI 72 h after PGF_{2α} (n = 615). Patches were classified into 3 categories :1 = 100% activated, 2 = < 100% activated, 3 = missing. Heifers with a patch status of 1 were considered to be activated, and a patch status of 2 was considered to be inactivated. Since heifers with a patch status of 3 were missing their patch for various different reasons at the time of reading, they were assigned to their own treatment. Pregnancy determination was performed at 35 d post TAI using an Aloka 500V equipped with a 5.0-MHz linear-array transducer (Aloka, Wallingford, CT) to determine AI pregnancies. At the first ultrasound date, any heifer that had been detected in estrus between d 17 to 22, after the TAI protocol, and inseminated again was not ultrasounded. After this ultrasound all heifers that were determined pregnant were sent to summer pasture and not diagnosed of a pregnancy again. At the second ultrasound date (d 55) all remaining heifers were checked for TAI pregnancy diagnosis.

Experiment 2

Experiment 2 consisted of 449 *Bos taurus* heifers located in Crook, Colorado. All heifers were housed in feedlot style pens during the time that MGA was being fed (d 0 to 14). After d 14, all heifers were moved to summer pasture. The estrous synchronization protocol consisted of all heifers being fed MGA 0.5 mg/head/day for 14 d starting on d 0. After heifers were no longer being fed MGA they were moved from feedlot pens to pasture. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had Estroject (Estroject, Spring Valley, WI) patches applied to their tailhead. At 72 h post PGF_{2α}, all heifers had their Estroject patches characterized into 4 scores: 1 = 0% activated, 2 = 50% activated, 3 = 100% activated, and 4 = missing. Heifers with a patch status of 1 or 2 were considered to be inactivated and a patch status of 3 was considered to be activated. Heifers with a patch status of 4 (missing) were removed from the analysis. Heifers were then sorted by patch status and assigned to treatments.

Heifers with activated patches (n = 250) were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) and TAI 72 h post PGF_{2α} injection. The delayed treatment consisted of 98 heifers with inactivated patches. These heifers were processed through the chute at 72 h post PGF_{2α} and given 100 µg GnRH and delayed TAI 8 h. The remaining heifers with inactivated patches (n = 101) were given 100 µg of GnRH and AI 72 h after the PGF_{2α} injection. Pregnancy determination was performed at 35 to 55 d post TAI using an Ibex pro ultrasound machine with a 5.0-MHz linear-array transducer (E.I. Medical Imaging, Loveland, CO) to determine AI pregnancies.

Experiment 3

Experiment 3 had 74 *Bos taurus* heifers synchronized using a 7-d CO-Synch + CIDR estrous synchronization protocol. All heifers had a CIDR (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) i.m. on d 0. On d 7, all heifers had the CIDR removed and were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) i.m. and aerosol adhesive was sprayed onto the tailhead where Estrotect patches (Estrotect, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all heifers were given 100 µg of GnRH, and approximately half of the heifers (37 heifers) were inseminated at 58 h post PGF_{2α} and the remaining half of the heifers were TAI at 76 h (37 heifers). At 58 h post PGF_{2α}, all heifers had their Estrotect patches characterized into 4 scores: 1 = 0% activated, 2 = 50% activated, 3 = 100% activated, and 4 = missing. Heifers with a patch status of 1 or 2 were considered to be inactivated and a patch status of 3 was considered to be activated. Heifers with a patch status of 4 (missing) were removed from the analysis. Heifers were randomly designated to the 58 or 76 h group by ear tag number or randomly selected by chute order. Pregnancy determination was

performed at 35 to 55 d post TAI using an Ibex pro ultrasound machine with a 5.0-MHz linear-array transducer (E.I. Medical Imaging, Loveland, CO) or an Aloka 500V equipped with a 5.0-MHz linear-array transducer (Aloka, Wallingford, CT) to determine AI pregnancies.

RESULTS

Experiment 1

There was no difference in BCS between treatments (table 3.1). Difference in pregnancy rates were examined comparing sires across treatments. Pregnancy rates overall for each bull were reported in table 3.3 . Overall there was a difference in pregnancy rates in sires with bull 2 having the greatest pregnancy rates and bull 1 having the lowest pregnancy rates with no differences in pregnancy rates between other bulls ($P < 0.05$). When looking at pregnancy rates throughout treatments between bulls, there were differences between bulls in the inactivated no delay treatment, inactivated 18 h delay treatment, and overall ($P < 0.05$) and can be seen in Table 3.3. Bull 2 had consistently higher pregnancy rates across all treatments with inactivated patches ($P < 0.05$). Bull 2 (43.6%) had significantly higher pregnancy rates in the inactivated 18 h delay treatment than bull 3 (15.4; $P < 0.05$) There were numerical differences between treatments within bulls. There was an increase in pregnancy rates ($P < 0.0001$) when comparing activated to inactivated patches table 3.2. In the statistical model used there were differences in pen, treatment, and a difference in weight gain between the start of MGA and the $\text{PGF}_{2\alpha}$ injection, which is a 33-d timespan, with P values of $P = 0.001$, $P < 0.0001$, and $P = 0.05$ respectively. The activated treatment had greater pregnancy rates compared to all other inactivated treatments ($P < 0.05$). There was no difference in pregnancy rates comparing heifers in the inactivated

treatments ($P > 0.05$). Furthermore, there was no difference in pregnancy rates ($P > 0.05$) between the missing treatment and all other treatments.

Experiment 2

Heifers with activated patches had greater pregnancy rates than heifers with inactivated patches (table 3.5; $P < 0.0001$). The heifers in activated treatment had greater pregnancy rates than heifers in the inactivated recommended treatment and the inactivated delayed treatment (table 3.6; $P < 0.0001$). No differences in pregnancy rates were seen between heifers with inactivated patches that were delayed insemination to 80 h post PGF_{2α} (25.3%) and those heifers with inactivated patches that were inseminated 72 h post PGF_{2α} injection (28.0%); ($P = 0.68$). Heifers that were delayed with inactivated patches had numerically lower pregnancy rates than heifers that were not delayed and inseminated 72 h after the PGF_{2α} injection.

Experiment 3

No differences were seen comparing BCS between each treatment (table 3.7; $P = 0.82$). However, a decrease in pregnancy rates was observed when BCS was added to the statistical model ($P = 0.01$). Pregnancy rates were not different between activated and inactivated patches (table 3.8; $P > 0.05$). When examining the factorial the interaction was considered to be a trend ($P = 0.08$; table 3.9). Since the interaction was just a trend, main effects of the factorial were examined. Pregnancy rate did not differ between activated and inactivated patches (52.7% vs. 50.5%) ($P = 0.85$). Pregnancy rates for each interval heifers were inseminated on was not different between the 58 h vs. 76 h interval (54.8% vs. 48.4%) ($P = 0.65$). A contrast statement was used to compare the ideal insemination time which consisted of heifers having activated patches inseminated at 58 h and heifers with inactivated patches inseminated at 76 h post PGF_{2α}

injection compared to the overall mean. There was a tendency for the ideal mean (85.9%) to have greater pregnancy rates than the overall mean (51.5%) ($P = 0.08$). There were no differences in pregnancy rates when examining the ideal insemination time compared to the overall 58 h interval pregnancy rates (54.8%) ($P > 0.05$) as well as the ideal insemination time compared to the overall 76 h interval pregnancy rates (48.4%) ($P > 0.05$).

DISCUSSION

Experiment 1

On average all heifers were of adequate BCS for breeding. The factors pen and weight gain go together because of the fact that some pens of cattle came from the same producer while others were of a different producers. These differences could be from breed or age of the heifers. The variation that can be seen by breed or age could explain some of the differences that were seen in the model. The differences seen between sires within each treatment is an indication that different sires work better at different times of insemination. This can be seen in table 3.3 by the fact that bull 2 has consistently higher pregnancy rates across the treatments with inactivated patches. Furthermore, some bulls showed numerical increases or decreases among the inactivated time treatments, while others showed increases or decreases among the activated time treatment. These data showed differences in pregnancy rates of different bulls according to treatment. This could be of significance to show that some bulls are more fertile at different times of insemination than others and could impact the effect on how the bull is used and when the proper time of insemination is for heifers with inactivated patches. These differences also show the general variability from bull to bull. Bull to bull variation can be categorized into 2 categories, compensable and uncompensable (Saacke, 2013). Compensable differences are when

seminal deficiencies impact pregnancy rates but can be overcome by adjusting the total number of sperm cells per AI dose (Saacke, 2013). Uncompensable differences are those that result in subfertility to AI regardless of sperm cell numbers in a dose of semen (Saacke, 2013). Bulls 3 and 4 would be considered bulls that were compensable while bull 1 would be a bull that is considered to be uncompensable. However, there are a number of different factors that should be examined to fully ensure these differences, such as number of sperm cells in each dose of semen, company, collection methods, etc. Further research is needed to determine if differences in bull to bull variability do exist in TAI.

It was reported by Busch et al. (2008) that heifers that had signs of estrus activity had increased pregnancy rates compared to heifers that did not show estrus activity. Pregnancy rates only showed a numerical increase for those heifers with inactivated patches and delayed 12 or 18 h compared to heifers with inactivated patches and inseminated at the time of GnRH. Furthermore, for heifers that were delayed there was a numerical increase in pregnancy rates for those heifers that were delayed 18 h compared to the 12 h delay. This numerical increase is similar to the data seen by Thomas et al. (2014) that cows with inactivated patches that were delayed had increased pregnancy rates compared to cows with inactivated patches and were inseminated at the time of the GnRH injection. Additionally Thomas et al. (2014) used a 20 h delay compared to the 12 or 18 h delay here.

During the time heifers were processed through the chute, heifers were worked very fast to ensure proper timing and to get as many heifers through the chute as fast as possible. Being in this feedlot type environment and working heifers through rapidly could potentially cause difficulties accepting a pregnancy. With this being said the added stress that was put on these

heifers could have had an impact on the decreased pregnancy rates. The protocol these heifers were on was a 14 – d MGA - PG and TAI protocol.

Experiment 2

Heifers with activated patches showed increased pregnancy rates similar to those by Busch et al. (2008). This is due to the fact that those heifers with activated patches have most likely shown estrus and the heifers with inactivated patches that did not show estrus. This is also indicates that these heifers have already ovulated a follicle and the timing of sperm survivability and oocyte maturation overlap each other for an optimal time of accompaniment. Unlike the results seen in Exp. 1 where the longer the delay the greater the numerical pregnancy rate was seen, Exp. 2 showed no differences in pregnancy rates when comparing heifers with inactivated patches to heifers with inactivated patches and delayed insemination. Although not significantly different it was shown that heifers with inactivated patches had numerically greater pregnancy rates than heifers with inactivated patches and were delayed insemination 8 h post GnRH injection. This is unlike data seen by Thomas et al. (2014) in that pregnancy rates were seen to increase with cows that were delayed AI 20 h after the GnRH injection. This is explained by previous research by Twagiramungu et al. (1995) stating that heifers that received a GnRH shot had estrus expression suppressed. This is because exogenous GnRH will induce the hypothalamus to cause the anterior pituitary to secrete LH. This LH surge will suppress estrogen levels which control the expression of estrus and estrus will not be exhibited and ovulation will occur. Those heifers with activated patches during the delayed TAI interval of 12 or 18 h, were most likely coming into estrus already from elevated estrogen levels and LH secretion from exogenous GnRH would have had no effect on estrogen and the exhibition of estrus.

Additionally, it is harder to ensure that each individual heifer consumed an adequate amount of MGA during their time in the feedlot. If some heifers consumed more MGA and some heifers consumed less than adequate amounts the potential for error in the project is increased. It is noteworthy that the heifers at this location were moved midway through the experiment from feedlot pens to pasture. This change in environment could also play a key role in the reason why pregnancy rates were lower than expected.

Experiment 3

On average, all heifers were of adequate BCS for this project. However, the difference in pregnancy rate was influenced by BCS of the heifers. According to our model, heifers with lower BCS had increase pregnancy rates compared to heifers with greater BCS. When examining the pregnancy rates by patch status it was interesting to see that pregnancy rates were numerically highest for heifers with a patch status of 2. It is thought that heifers with patch status of 3 were lower because these heifers were inseminated at a longer PGF_{2α} to TAI interval than recommended by the Beef Reproductive Task Force. The Beef Reproductive Task Force recommends inseminating heifers on a 7 d CO – Synch plus CIDR protocol at 54 h post PGF_{2α} injection. Heifers on the current experiment were inseminated at 58 h interval which could possibly be too long for heifers that have already shown estrus. Those heifers with partially activated patches most likely ovulated at a later time than those with fully activated patches and were inseminated closer to the time of ovulation. Inseminating heifers with a patch status of 2 would more closely overlap the lifespan of the sperm and the oocyte. High embryo quality (grades of excellent or good) and lower fertilization rates were seen when insemination early after the onset of estrus but greater fertilization rates and lower embryo quality (grades of fair or poor) were seen when inseminating late after the onset of estrus (Dalton et al., 2001), indicating

that there was a compromise in the optimal time to AI heifers. Since there was no difference in the pregnancy rates between 58 and 76 h post PGF_{2α}, it can be stated that heifers could be inseminated on either time and still achieve similar pregnancy rates. Only a numerical increase was seen in pregnancy rates when heifers with inactivated patches were delayed TAI until 76 h post PGF_{2α}. This is different from data reported by Thomas et al. (2014), where pregnancy rates were increased by delaying insemination of cows by 20 h. This may be because they inseminated at 86 h post PGF_{2α}, which was 20 h after GnRH. With ovulation occurring 24 to 32 h after the second GnRH injection of a TAI protocol, heifers may have been inseminated too late for those heifers that had already shown estrus activity (Pursley et al., 1995). The ideal situation of inseminating heifers with activated patches at 58 h post PGF_{2α} and heifers with inactivated patches at 76 h post PGF_{2α} shows a tendency for increased pregnancy rates compared to the overall mean. This supports our hypothesis, indicating that by delaying the insemination of heifers with inactivated patches at 76 h post PGF_{2α} and inseminating heifers with activated patches at 58 h post PGF_{2α} overall pregnancy rates can be increased.

If producers could separate females out in TAI protocol there is the ability for that producer to manage them differently. By delaying insemination time after GnRH injection it is thought that the female is preparing a more suitable environment for the oocyte that would not have been present if AI was performed at the time of GnRH injection (Dalton et al., 2001). Although the exogenous GnRH injection will induce ovulation of a physically mature oocyte, estrus expression does not always occur (Twagiramungu et al., 1995). Estrus expression does not occur because estradiol levels have not reached a peak yet to act on the brain of the heifer to express estrus. If these levels have not reached a peak before the GnRH injection, estrus will be suppressed by the LH surge and ovulation will occur. This GnRH induced ovulation is

associated with decreased estradiol concentrations in the peripheral circulation and spontaneous estrus is inhibited (Twagiramungu et al., 1995). Because of the fact that semen only has a limited life span during its time in the female reproductive tract, it is essential that insemination is done at a time where its lifespan overlaps with the time of ovulation of the female oocyte (Saacke et al., 2013). Inseminating too early would result in less viable sperm at the time of ovulation but increased embryo quality, while inseminating too late would result in a greater number of viable sperm cells to reach the oocyte for greater fertilization rates while resulting in poorer quality embryos (Dalton et al., 2001).

Across all experiments it was shown that there was an increase in pregnancy rates for heifers with activated patches compared to heifers with inactivated patches. Furthermore, no differences in pregnancy rate were seen by delaying TAI on heifers of either activated or inactivated patches. As a result, further research is needed to examine the consistency of pregnancy rates for delayed TAI in heifers.

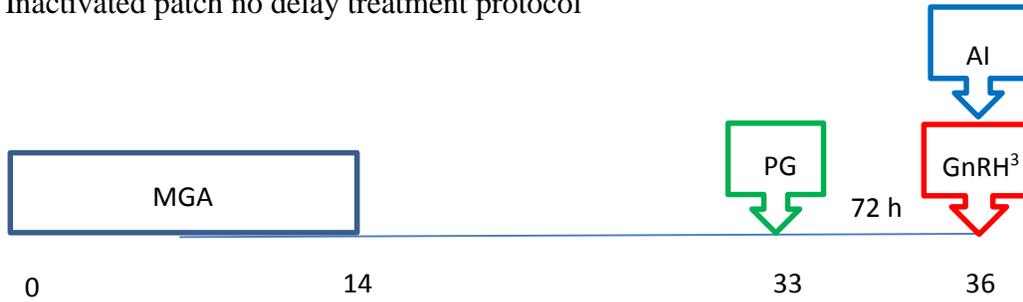
IMPLICATIONS

By delaying TAI of those females with inactivated patches, pregnancy rates could possibly increase. This increase in pregnancy rates can potentially offset the cost of synchronization and result in an overall increase in pregnancy rates. Seeing as how it is the heifers that have not exhibited estrus are the females that are causing the result in decreased overall pregnancy rates, it would be applicable for producers to use a delayed TAI on those heifers with inactivated patches.

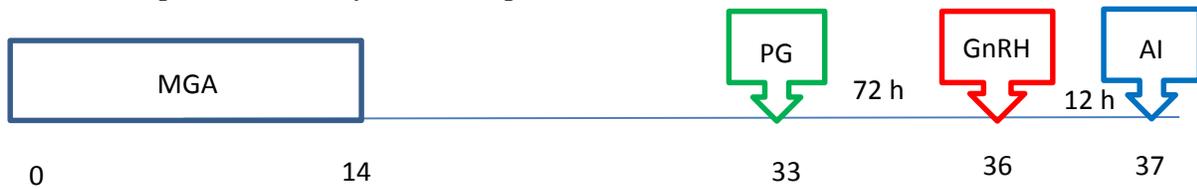
Activated and Missing patch treatment protocol



Inactivated patch no delay treatment protocol



Inactivated patch 12 h delay treatment protocol



Inactivated patch 18 h delay protocol

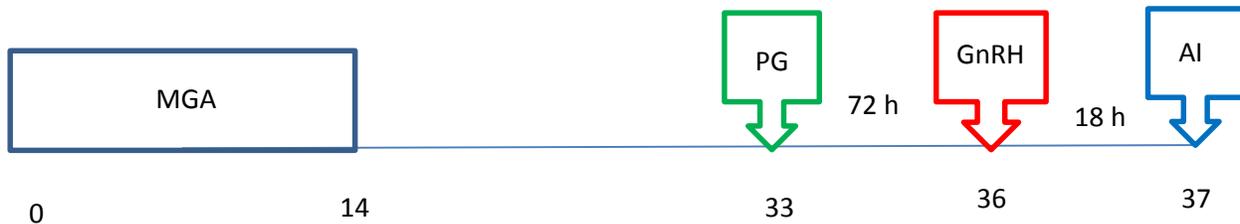


Figure 3.1 Estrous synchronization protocols for beef heifers in Exp. 1

¹Melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis)

²PG: ProstaglandinF_{2α} 25 mg (Lutalyse, Dinoprost tromethamine, Zoetis)

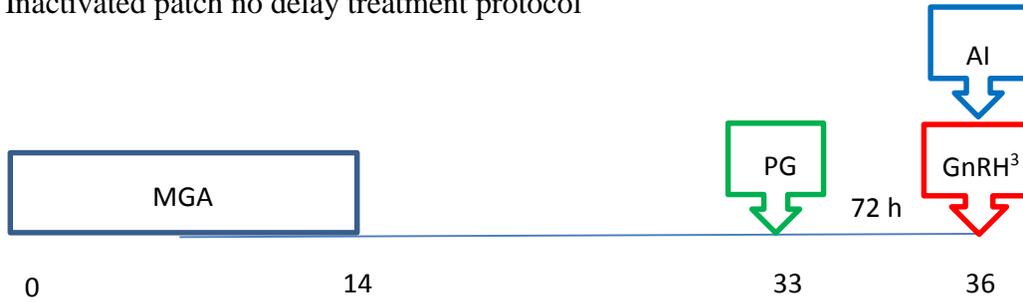
³GnRH: 100 µg (Factrel, Gonadorelin, Zoetis)

⁴TAI: timed AI

Activated patch treatment protocol



Inactivated patch no delay treatment protocol



Inactivated patch 9 h delay treatment protocol



Figure 3.2 Estrous synchronization protocols for beef heifers in Exp. 2

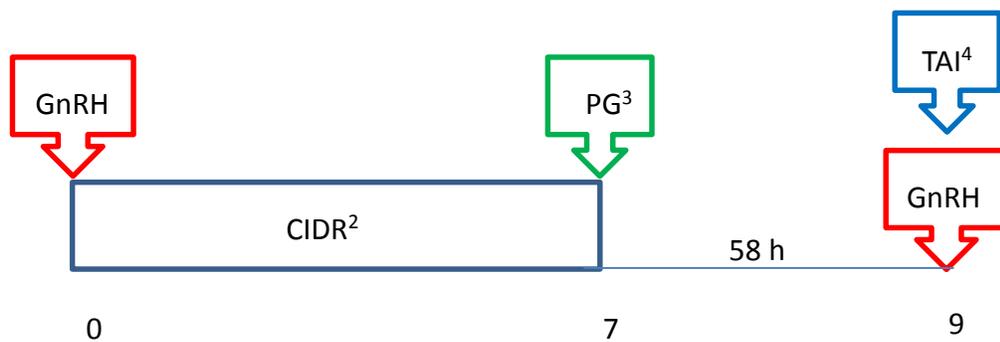
¹Melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis)

²PG: ProstaglandinF_{2α} 25 mg (Lutalyse, Dinoprost tromethamine, Zoetis)

³GnRH: 100 µg (Factrel, Gonadorelin, Zoetis)

⁴TAI: timed AI

58 h Insemination interval



Delayed 76 h Insemination interval

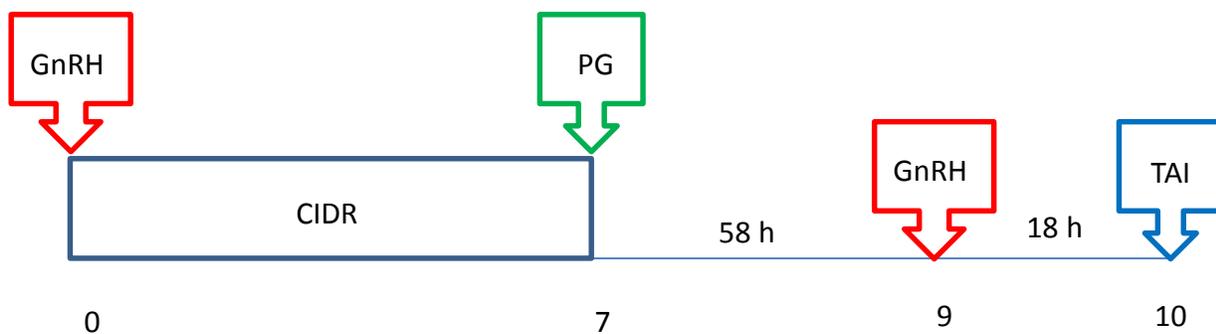


Figure 3.3 Estrous synchronization protocols for beef heifers in Exp. 3

¹GnRH: 100 µg (Factrel, Gonadorelin, Zoetis)

²Controlled Internal Drug Releasing device, (CIDR; EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ)

³PG: ProstaglandinF_{2α} 25 mg (Lutalyse, Dinoprost tromethamine, Zoetis)

⁴TAI: timed AI

Table 3.1. Average BCS among treatments and overall (Exp. 1)

Treatment	n	BCS ¹	SE
Inactivated No delay	271	5.7	0.04
Inactivated 12 h delay	105	5.6	0.06
Inactivated 18 h delay	130	5.6	0.05
Activated	615	5.6	0.02
Missing	38	5.6	0.10
Overall	1159	5.6	0.02

¹BCS on 1 to 9 scale (Wagner et al., 1988)

^{a-e} Among treatments, means within a column lacking common superscripts differ ($P < 0.05$).

Table 3.2. Distribution of Estroject™ patch status at 72 h post prostaglandin injection and pregnancy rate by patch status (Exp.1) ^{1, 2}

Patch status ³	Number of heifers	Percent of heifers	Pregnancy rate (%)	SE
Activated	615	53.1	46.2 ^a	2.0
Inactivated	505	43.6	31.7 ^b	2.1
Missing	38	3.3	44.7 ^{ab}	8.1

¹n = 1159

² All heifers received fed melengestrol acetate (MGA, 0.5 mg/head/day, Zoetis) for 14 d starting on d 0. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had Estroject (Estroject, Spring Valley, WI) patches applied to their tailhead. Seventy two h after PGF_{2α} all heifers were sorted by patch status. Heifers with activated or missing patches were not given GnRH and AI 72 h after PGF_{2α} (n = 615). Approximately half of the heifers with inactivated patches (n = 271) were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) and AI 72 h after PGF_{2α}. The remaining heifers with inactivated patches were randomly sorted into 2 treatments and delayed TAI 12 (n = 105) or 18 h (n = 130) after the GnRH injection.

³ Activated = 100% activated, Inactivated = < 100% patch activation

^{a-b} Means within a column lacking common superscripts differ ($P < 0.05$).

Table 3.3. Pregnancy rates to timed AI and percentages within each treatment by bull (Exp. 1)^{1,2}

Bull	n	Percent of heifers	Pregnancy rate by Breeding Interval ³					Overall	SE
			Inactivated No delay	Inactivated 12 h delay	Inactivated 18 h delay	Activated	Missing		
1	406	35.0	20.5 ^b	36.2	27.3 ^{ab}	43.2	31.6	36.2 ^b	2.4
2	215	18.6	37.3 ^a	50.0	43.6 ^a	50.5	50.0	45.6 ^a	3.4
3	414	35.7	36.6 ^a	33.9	15.4 ^b	44.8	60.0	39.9 ^{ab}	2.4
4 ⁴	124	10.7	21.2 ^{ab}	.	34.4 ^{ab}	55.4	66.7	41.4 ^{ab}	4.4

¹n = 1159

² All heifers received fed melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis) for 14 d starting on d 0. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had Estrotect (Estrotect, Spring Valley, WI) patches applied to their tailhead. Seventy two h after PGF_{2α} all heifers were sorted by patch status. Heifers with activated or missing patches were not given GnRH and AI 72 h after PGF_{2α} (n = 615). Approximately half of the heifers with inactivated patches (n = 271) were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) and AI 72 h after PGF_{2α}. The remaining heifers with inactivated patches were randomly sorted into 2 treatments and delayed TAI 12 (n = 105) or 18 h (n = 130) after the GnRH injection.

³Inactivated = < 100 0% activated, Activated = 100% activated

⁴Bull 4 was not used in the inactivated 12 h treatment

^{a-b} Means within a column lacking common superscripts differ ($P < 0.05$).

Table 3.4. Distribution of pregnancy rate among treatments (Exp. 1)^{1, 2}

Treatment ³	Number of heifers	Percent of heifers	Pregnancy rate (%)	SE
Inactivated No delay	271	23.4	29.3 ^a	2.9
Inactivated 12 h delay	105	9.1	31.1 ^a	4.8
Inactivated 18 h delay	130	11.2	32.7 ^a	4.5
Activated	615	53.1	45.5 ^b	2.3
Missing	38	3.3	44.0 ^{ab}	8.2
Overall	1159	100	39.7	1.4

¹n = 1159

²All heifers received fed melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis) for 14 d starting on d 0. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had Estroprotect (Estroprotect, Spring Valley, WI) patches applied to their tailhead. Seventy two h after PGF_{2α} all heifers were sorted by patch status. Heifers with activated or missing patches were not given GnRH and AI 72 h after PGF_{2α} (n = 615). Approximately half of the heifers with inactivated patches (n = 271) were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) and AI 72 h after PGF_{2α}. The remaining heifers with inactivated patches were randomly sorted into 2 treatments and delayed TAI 12 (n = 105) or 18 h (n = 130) after the GnRH injection.

³Activated = 100% activated , Inactivated = < 100% activated

^{a-b} Means within a column lacking common superscripts differ ($P < 0.05$).

Table 3.5. Distribution of EstroTECT™ patch status at 72 h post prostaglandin injection and pregnancy rate among patch status (Exp.2)^{1,2}

Patch status ³	Number of heifers	Percent of cows	Pregnancy rate (%)	SE
Activated	250	55.7	50.7 ^a	5.9
Inactivated	199	44.3	26.1 ^b	4.8

¹n = 449

²All heifers received fed melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis) for 14 d starting on d 0. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had ESTROTECT (EstroTECT, Spring Valley, WI) patches applied to their tailhead. Seventy two hours after PGF_{2α} all heifers were sorted by patch status. Heifers with inactivated patches were randomly sorted and 101 of the heifers with inactivated patches were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) and AI 72 h post PGF_{2α}. The remaining 98 heifers with inactivated patches were delayed TAI 9 h after the GnRH injection. Heifers with activated or missing patches were given GnRH and TAI 72 h after PGF_{2α} (n = 250).

³ Activated = > 50% activated , Inactivated = < 50% activated

^{a-b} Means within a column lacking common superscripts differ ($P < 0.0001$).

Table 3.6. Distribution of heifers and pregnancy rate by treatment (Exp. 2)^{1,2}

Treatment ³	Number of Heifers	Percent of heifers (%)	Pregnancy rate (%)	SE
Inactivated delayed insemination 81 h	98	21.8	25.3 ^a	5.6
Inactivated insemination 72 h	101	22.5	28.0 ^a	6.2
Activated insemination 72 h	250	55.7	51.7 ^b	6.2

¹n = 449

²All heifers received fed melengestrol acetate (**MGA**, 0.5 mg/head/day, Zoetis) for 14 d starting on d 0. On d 33 all females were given 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis) and had ESTROTECT (Estrotect, Spring Valley, WI) patches applied to their tailhead. Seventy two hours after PGF_{2α} all heifers were sorted by patch status. Heifers with inactivated patches were randomly sorted and 101 of the heifers with inactivated patches were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis) and AI 72 h post PGF_{2α}. The remaining 98 heifers with inactivated patches were delayed TAI 9 h after the GnRH injection. Heifers with activated or missing patches were given GnRH and TAI 72 h after PGF_{2α} (n = 250).

^{a-b} Means within a column lacking common superscripts differ ($P < 0.0001$).

Table 3.7. Average BCS among each patch status and time interval combination (Exp. 3)^{1, 2}

Treatment	n	BCS ¹	SE
58 h Activated	18	5.1	0.12
76 h Activated	20	5.2	0.11
58 h Inactivated	19	5.1	0.11
76 h Inactivated	17	5.1	0.12
Overall	74	5.1	0.06

¹BCS on 1 to 9 scale (Wagner et al., 1988)

²Cows received a controlled internal drug release (**CIDR**) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis, Florham Park, NJ) i.m. on d 0. On d 7 cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis, Florham Park, NJ) and Estroject patches (Estroject, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, half of the cows received AI at 58 h post PGF_{2α} while the remaining cows received AI 76 h post PGF_{2α} injection

Table 3.8. Distribution of EstroTECT™ patch status at 58 h post prostaglandin injection and pregnancy rate among patch status(Exp. 3) ^{1,2}

Patch status ³	Number of heifers	Percent of heifers (%)	Pregnancy rate (%)	SE
Activated	38	51.3	53.6	8.4
Inactivated	36	48.7	53.1	8.4

¹n = 74

²Cows received a controlled internal drug release (**CIDR**) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis, Florham Park, NJ) i.m. on d 0. On d 7 cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis, Florham Park, NJ) and EstroTECT patches (EstroTECT, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, half of the cows received AI at 58 h post PGF_{2α} while the remaining cows received AI 76 h post PGF_{2α} injection

³ Activated = patch status of a 3 (100% activated); inactivated = patch status of a 1 or 2 (0% activated or 50% activated, respectively) evaluated at 58 h post PGF_{2α}.

Table 3.9. Effect of time interval (58 vs. 76 h) from PGF_{2α} to timed AI and Estroject™ patch status at 58 h post PGF_{2α} on pregnancy rate (Exp. 3) ^{1,2}

	58 h ³	76 h ³	58 h ³	76 h ³	<i>P</i> values		
	Activated ⁴	Activated ⁴	Unactivated ⁴	Unactivated ⁴	Interaction	Interval	Patch status
Pregnancy Rate (%)	66.6	38.4	42.4	58.5	0.08	0.65	0.86

1n = 74

2Cows received a controlled internal drug release (**CIDR**) (EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ) inserted intravaginally and were given 100 µg of GnRH (Factrel, Gonadorelin, Zoetis, Florham Park, NJ) i.m. on d 0. On d 7 cows had the CIDR removed and 25 mg of PGF_{2α} (Lutalyse, Dinoprost tromethamine, Zoetis, Florham Park, NJ) and Estroject patches (Estroject, Spring Valley, WI) were then applied. Fifty-eight hours after the PGF_{2α} injection, all cows were given 100 µg of GnRH, half of the cows received AI at 58 h post PGF_{2α} while the remaining cows received AI 76 h post PGF_{2α} injection

3 Prostaglandin to timed AI interval.

4 Activated = patch status of a 3 (100% activated); inactivated = patch status of a 1 or 2 (0% activated or 50% activated, respectively) evaluated at 58 h post PGF_{2α}.

5 Least Squares Means are reported in this table.

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APPENDIX

SAS Code for Chapter II

Code for analyzing TAI pregnancy rates:

```
proc glimmix;
class heat1 daybred ranch sire tech breed;
model preg(Ref=first)= bcs ppi heat1|daybred/dist=binary solution;
random ranch sire(ranch) tech(ranch);
lsmeans heat1|daybred/pdiff ilink;
lsmestimate heat* daybred "ideal vs opposite" 1 -1 -1 1/ilink;
run;
```

Code for analyzing pregnancy rates by patch status:

```
proc sort;
by daybred;
proc glimmix;
class daybred patch1 heat1;
model preg (ref=first)= patch1/dist=binary solution;
lsmeans patch1/ilink pdiff;
run;
proc sort;
by patch1;
proc glimmix;
by patch1;
class daybred patch1 heat1;
model preg (ref=first)= daybred/dist=binary solution;
lsmeans daybred/ilink pdiff;
run;
```

SAS Code for Chapter III

Code for analyzing TAI pregnancy rates in Exp. 1:

```
proc glimmix;
class pen bredcode gain;
model preg(ref=first)=pen bredcode gain/dist=binary solution;
lsmeans bredcode/ pdiff ilink;
run;
```

Code for analyzing pregnancy rate by patch status in Exp. 1:

```
proc glimmix;
class patch;
model preg= patch/solution;
lsmeans patch/ pdiff ilink;
run;
```

Code for analyzing pregnancy rate by bull in Exp. 1:

```
proc sort;
by bredcode;
proc glimmix;
class pen bredcode gain;
model preg(ref=first)= bull pen bredcode gain/dist=binary solution;
lsmeans bull/ pdiff ilink;
run;
proc glimmix;
by bredcode;
class pen bredcode gain;
model preg(ref=first)= bull pen bredcode gain/dist=binary solution;
lsmeans bull/ pdiff ilink;
run;
```

Code for analyzing TAI pregnancy rates in Exp. 2:

```
proc glimmix;
class bredcode heat;
model preg(Ref=first)= bredcode/solution dist=binary;
random sire tech;
lsmeans bredcode/pdiff ilink;
run;
```

Code for analyzing pregnancy rate by patch status in Exp. 2:

```
proc glimmix;
class bredcode heat;
model preg(Ref=first)= heat/solution dist=binary;
random sire tech;
lsmeans heat/pdiff ilink;
run;
```

Code for analyzing TAI pregnancy rates in Exp. 3:

```
proc glimmix;
class heat daybred bull tech day1patch;
model preg (Ref=first)= bcs heat|daybred/dist=binary solution;
random tech;
lsmeans heat|daybred/pdiff ilink;
lsestimate heat* daybred "ideal vs opposite" 1 -1 -1 1/ilink;
run;
```

Code for analyzing pregnancy rate by patch status in Exp. 3:

```
proc glimmix;
class heat daybred bull tech day1patch;
model preg (Ref=first)= day1patch/dist=binary solution;
lsmeans day1patch/pdiff ilink;
run;
```