THESIS

EVALUATION OF PREGNANCY RATES FOLLOWING TIMED AI IN BEEF HEIFERS AFTER SYNCHRONIZATION OF FOLLICULAR WAVES USING A 14-D CONTROLLED INTERNAL DRUG RELEASE INSERT, AND THE LIFETIME PRODUCTIVITY OF BEEF HEIFERS CONCEIVING TO, OR SIRED BY, AI

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ABSTRACT

EVALUATION OF PREGNANCY RATES FOLLOWING TIMED AI IN BEEF HEIFERS AFTER SYNCHRONIZATION OF FOLLICULAR WAVES USING A 14-D CONTROLLED INTERNAL DRUG RELEASE INSERT, AND THE LIFETIME PRODUCTIVITY OF BEEF HEIFERS CONCEIVING TO, OR SIRED BY, AI

Three studies were conducted to evaluate the efficacy of a timed AI (**TAI**) estrous synchronization protocol that used a 14-d controlled internal drug release (**CIDR**) insert and a GnRH injection on d 9 to force ovulation of potentially persistent follicles and induce a second wave of follicle growth.

In the first study 710 yearling heifers across 4 locations were assigned to 1 of 3 treatments: 1) 14-d GnRH-9 heifers (n = 242) received CIDR (1.38 g progesterone) and 100 μg GnRH on d 0, 100 μg GnRH on d 9, and 50 mg $PGF_{2\alpha}$ on d 14 concurrent with CIDR removal, 2) 14-d 6 h PG heifers (n = 233) were identical to 14-d GnRH-9 except that on d 14, 2 25 mg injections of $PGF_{2\alpha}$ were given 6 h apart, and 3) 5-d CO-Synch + CIDR heifers (n = 235) received 100 μg GnRH and CIDR on d 9 and a single 25 mg $PGF_2\alpha$ at CIDR removal. All 3 treatments received 100 μg GnRH with TAI at 72 ± 2 h after CIDR removal. The 14-d GnRH-9 TAI pregnancy rate (54.5%) did not differ (P = 0.57) from the 14-d 6h PG TAI pregnancy rate (53.6%). The TAI pregnancy rate of 14-d protocols combined was 54.1%, and was not different (P = 0.20) from the 5-d CO-Synch + CIDR TAI pregnancy rate of 46.4%.

The following year 319 yearling heifers across 4 locations were assigned to 1 of 3 treatments: 1) 14-d GnRH-9 (n = 107; as described earlier), 2) 14-d PG (n = 107) was identical to 14-d GnRH-9 except instead of receiving GnRH on d 0 they received 25 mg of $PGF_{2\alpha}$ and, 3) 5-d CO-Synch + CIDR (n = 104; as described earlier). All treatments received 100 μg GnRH at TAI 72 ± 2 h after CIDR removal. Heifers' ovaries (n = 120) were ultrasounded at 2 locations on d 0, 9, 14, and 17 of the estrous synchronization protocol to determine ovarian structures and response.

The 14-d GnRH-9 TAI pregnancy rate (52.3%) was not different ($P = 0.82$) than 14-d PG (47.6%), nor was the TAI pregnancy rate of both 14-d treatments combined (50.0%) different ($P = 0.66$) from 5-d CO-Synch + CIDR (47.1%). Based on ultrasonography, the 14-d GnRH-9 treatment induced a second wave of follicular growth in 25.9% of heifers while 14-d PG heifers had larger ($P = 0.01$) follicle size on d 9 but did not reduce ($P > 0.10$) corpora lutea at TAI compared to 14-d GnRH-9 or 5-d CO-Synch + CIDR.

That same year 453 heifers at another location were assigned to 1 of 3 treatments: 1) 14-d GnRH-9 (n = 150; as described earlier), 2) 14-d GnRH-7 (n = 150) received 100 μg GnRH and CIDR on d 0, 100 μg GnRH on d 7, 25 mg $PGF_{2\alpha}$ on d 14 at CIDR removal, and 100 μg GnRH at TAI 63 ± 3 h after CIDR removal, and 3) 7-d CO-Synch + CIDR received 100 μg GnRH and CIDR on d 7, 25 mg $PGF_{2\alpha}$ at CIDR removal, and 100 μg GnRH at TAI 63 ± 3 h after CIDR removal. Pregnancy rate to TAI of 14-d GnRH-9 (51.3%) was not different ($P = 0.75$) than 14-d GnRH-7 treatment (48.0%), nor was the TAI pregnancy rate of both 14-d treatments (49.6%) different ($P = 0.83$) from 7-d CO-Synch + CIDR (48.6%).

These data indicate that the 14-d CIDR estrous synchronization protocol with d 9 GnRH produces comparable pregnancy rates to TAI compared to the industry utilized 5-d and 7-d CO-Synch + CIDR estrous synchronization protocols. However, the additional labor and pharmaceutical cost of handling heifers on d 9 raises the question whether it's a viable TAI estrous synchronization alternative.

The final experiment evaluated the lifetime productivity of heifers conceiving to AI or natural service (**NS**), and heifers sired by AI vs. NS. Calving and breeding records (n = 6,693) at one location for 1,173 Angus females and were obtained from 1991 to 2010. Lifetime weight weaned, calves weaned, and revenue produced was determined and analyzed.

Heifers that conceived to AI had greater ($P < 0.0001$) lifetime weight weaned, lifetime calves weaned, lifetime revenue, and greater ($P < 0.05$) average annual weaning weight than heifers that conceived to NS. There was no difference ($P > 0.10$) in average annual weaning weight, lifetime weight weaned, lifetime calves weaned, or lifetime revenue produced between heifers sired by AI or NS. Estrous synchronization and AI can be a valuable tool to produce replacement heifers that conceive earlier, and in doing so increase their lifetime productivity.

Key Words: Artificial insemination, Estrous synchronization, Follicular waves, heifers

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CHAPTER I

REVIEW OF LITERATURE

Development of Artificial Insemination (AI) and Estrous Synchronization

The use of AI in cattle in the United States began in the 1930's and continued to grow in the 1940's as it became more widely used in the dairy industry (Foote, 2002). Estrous synchronization involves manipulating the estrous cycle in order to cause as many females as possible to enter estrus at a specific time. The first efforts to do so began in the late 1960's by administering oral progestins and an estrogen injection (Wiltbank and Kasson, 1968). Numerous estrous synchronization protocols continue to be developed in order to facilitate the use of AI and improve the reproductive efficiency of today's beef and dairy herds.

Utilizing AI and estrous synchronization offers many benefits to beef producers. Artificial insemination allows access to elite genetics that would not otherwise be available for use. Using AI in combination with estrous synchronization can produce females that conceive earlier in the breeding season, which can then raise heavier calves and have increased postpartum recovery time (Dunn and Kaltenbach, 1980). Artificial insemination can also create a more uniform calf crop, a more concentrated calving season, and reduce bull maintenance and purchase costs (Johnson and Jones 2004; Ellis

2005). Synchronizing estrous has also been shown to produce calves that were 13 d older and 9.5 kg heavier than females that were not synchronized (Schafer et al., 1990).

Comparison of using AI to Natural Service

Several studies have conducted an economic analysis comparing AI to natural service (**NS**). Estrous synchronization and AI increased the percentage of cows calving in the first 30 d of the calving season the following year relative to NS. It also increased short-term revenue by $70 per head over NS by increasing weaning weight and reducing bull costs (Anderson and Deaton, 2003).

Cost per pregnancy resulting from AI or NS has also been evaluated. This value is affected by several factors including, but not limited to, bull purchase price, bull to cow ratio, estrous synchronization protocol, pregnancy rates to AI, and labor costs. Natural service produced a lower cost per pregnancy than any estrous synchronization and AI protocol combination (Johnson and Jones, 2004). However, increased revenue resulting from AI sired calves must also be considered when comparing AI to NS. When increased weaning weight is accounted for in AI-sired calves, many estrous synchronization protocols with AI produce greater returns than NS (Johnson and Jones, 2004).

One study created a model to compare AI and NS and found AI pregnancies were less expensive versus NS when producers used a bull to cow ratio of 1:20. Receiving premiums for superior genetics and managing semen costs had the largest effect on whether AI systems were more profitable than NS (Johnson and Jones, 2008).

The importance of producers capturing the value of superior genetics was reiterated by data that showed retaining ownership through finishing produced greater net income in AI sired calves than NS sired calves. Retaining ownership of AI sired calves

had a $142 to $214 per head premium compared to calves marketed at weaning (Miller et al., 2004). While these findings explain the benefits of estrous synchronization and AI in producing marketable calves, relatively few studies have evaluated the long-term impact of AI on replacement females and their lifetime productivity.

Current use of AI

Despite these benefits, use of estrous synchronization and AI within the beef industry remains low. According to a 2009 National Animal Health Monitoring Service survey, use of estrous synchronization and AI increased as herd size increased, but use of estrous synchronization and AI in 2008 was only 7.9% and 7.6%, respectively (USDA, 2009a). This use of AI is only a slight increase from the 7.1% of producers that utilized AI in 1997 (USDA, 1997), and is dramatically lower than the 76.3% of dairy producers who used AI on cows for first service (USDA, 2009b) and 76.1% of swine producers who used AI in 2006 (USDA, 2007).

The lack of implementation of AI is not a result of producer skepticism as only 2.3% and 1.6% of producers believed estrous synchronization and AI, respectively, do not work. Time and labor were the primary reasons that producers chose not to utilize these technologies (USDA, 2009a).

Progestin use within Estrous Synchronization

A significant development in estrous synchronization history was when progestins became available. Progestin exposure was shown to induce pre-pubertal heifers into cyclicity (Gonzalez-Padilla et al., 1975; Patterson et al., 1990). Progestins increase secretion of luteinizing hormone (**LH**) which reduces the negative feedback produced by

estradiol on GnRH, thus helping non-cycling heifers reach puberty (Anderson et al., 1996). The ability of progestins to induce pre-pubertal heifers into cyclicity is a major benefit of using estrous synchronization as it can cause heifers to become pregnant earlier, thus allowing for older and heavier calves to be weaned in addition to having increased post-partum recovery as a 2 yr old. This induction of puberty should be appealing to all beef producers, particularly those raising late maturing cattle.

Two commonly used progestin products today are melengestrol acetate (**MGA**) and controlled internal drug releasing (**CIDR**) inserts. Melengestrol acetate is an oral progestin that will prevent ovulation and estrus (Imwalle et al., 2002) and CIDRs are vaginal inserts that release progesterone. While plasma progesterone concentrations (**PPC**) varied between cows treated with CIDRs, amount of progesterone released from the CIDR was consistent and the average PPC of 9 cows treated with a CIDR for 15 d was 4.4. ng/ml (Macmillan and Peterson, 1993).

Estrus synchrony was more uniform and pregnancy rate to AI was higher in heifers given CIDRs rather than MGA (Kojima et al., 2004). This increases the appeal of utilizing CIDRs over MGA, however the cost of a 14 d treatment with CIDR is $9.47 compared to the $0.50 cost per d of using MGA (Johnson and Jones, 2008).

Prostaglandin $F_{2\alpha}$ use within Estrous Synchronization

Prostaglandin $F_{2\alpha}$ was developed and found to cause regression of the corpus luteum (**CL**) in the bovine (Lauderdale, 1972). This was important as it allowed prostaglandin to be used with progestins to better manipulate the estrous cycle by extending the luteal phase through the progestin, and then abruptly ending the luteal by

regressing the CL through administration of $PGF_{2\alpha}$. Unfortunately, these protocols did not address follicular development, as follicular dynamics had not been characterized.

The MGA - PG protocol uses MGA and $PGF_{2\alpha}$ to synchronize estrus in beef heifers. Melengestrol acetate is fed for 14 d and $PGF_{2\alpha}$ is given 19 d after the end of MGA feeding. Heifers are then detected for estrus and inseminated over a 6-d period following the $PGF_{2\alpha}$ injection. This protocol has produced conception rates (number of females pregnant/number of females inseminated) to AI between 61 to 68% (Brown et al., 1998; Deutscher, 2000). This protocol's benefits of low cost and high pregnancy rate to AI must be weighed against the drawbacks of increased labor requirements for estrus detection and increased time required for protocol completion.

Impact of Ultrasonography and Gonadotropin-Releasing Hormone on Follicular Growth

The use of transrectal ultrasonography was critical in the evolution of estrous synchronization because it provided valuable information about the growth pattern of follicles. Heifers can have 2 or 3 waves of follicular growth within their estrous cycle. Heifers with 2 waves of follicular growth began a new wave around d 2 and 11 (estrus = d 0) of the estrous cycle, while heifers with 3 waves started a new wave on d 2, 9, and 16 (Sirois and Fortune, 1988).

This understanding of follicular growth paired GnRH has allowed estrous synchronization protocols to influence follicular waves. Giving GnRH to cattle causes LH to be secreted and causes most dominant follicles to be ovulated, thus initiating a new wave of follicular growth (Garverick et al., 1980; Twagiramungu et al., 1995; Sartori et al., 2001). This led to the creation of the Select Synch protocol that included GnRH on d 0 and $PGF_{2\alpha}$ 7 d later with estrus detection and AI occurring between d 6 and 13.

Conception rate to AI for this protocol was 85% (Twagiramungu et al., 1992). While this conception rate to AI is high, this protocol requires increased estrus detection, especially given that 5 to 15% of cows come into estrus before the $PGF_{2\alpha}$ injection (Twagiramungu et al., 1995).

The Ovsynch protocol sought to further synchronize follicular waves by giving $PGF_{2\alpha}$ 7 d after GnRH, then GnRH 2 d after $PGF_{2\alpha}$, with AI occurring 16 to 24 h after the second GnRH injection. This allowed for ovulation to be synchronized and for cattle to receive timed AI (**TAI)** instead of being inseminated following estrus detection. This protocol produced pregnancy rates to TAI of up to 45% in dairy cows (Pursley et al., 1998), however pregnancy rates to TAI in heifers were only 35% (Pursley et al., 1997).

Progesterone exposure through CIDRs was then evaluated in beef heifers through a protocol that used a CIDR from d 0 to 7 with $PGF_{2\alpha}$ given on d 6, and required estrus detection and AI after d 7. Overall pregnancy rates to AI (number of heifers pregnant/number of heifers treated) were 39% for this protocol compared to 14% for heifers receiving a single injection of $PGF_{2\alpha}$ (Lucy et al., 2001). The major benefit of this protocol was for prepubertal heifers as the pregnancy rate to AI in prepubertal heifers receiving the CIDR was 28% compared to 6% for prepubertal heifers only receiving $PGF_{2\alpha}$.

This protocol was further developed when the $PGF_{2\alpha}$ injection was moved to d 7 to align with CIDR removal, and GnRH was given on d 0. The addition of GnRH was given to initiate a new wave of follicular growth in attempt to better control follicular waves. However, there was no difference in pregnancy rate to AI between heifers that received GnRH and those that didn't (Lamb et al. 2006). Thus, putting into question the

benefit of d 0 GnRH and revealing the challenge of synchronizing follicular waves in heifers. This difficulty was confirmed when only 45 to 60% of heifers were shown to respond to GnRH by intiating a follicular wave (MacMillan and Thatcher, 1991; Moreira et al., 2000; Atkins et al., 2008) compared to a 64 to 75% response rate in mature cows (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004).

Development of Timed AI Estrous Synchronization Protocols

The Lamb study (2006) also compared pregnancy rates to AI using TAI on the previously mentioned 7 d CIDR protocols with or without GnRH on d 0 vs. using estrus detection on the 7 d CIDR protocol with or without GnRH on d 0. Both protocols that utilized estrus detection produced numerically but not statistically higher pregnancy rates than the TAI protocols (Lamb et al., 2006).

Although the TAI protocols did not produce higher pregnancy rates, this study helped move estrous synchronization research toward more TAI protocols. These protocols have been developed in an effort to reduce the labor associated with estrous synchronization protocols. This is done through reducing the number of times cattle must be handled, and by removing the need for estrus detection by using TAI.

The CIDR Select protocol is one that uses TAI and has produced encouraging results in beef heifers. It requires a CIDR from d 0 to 14, GnRH on d 23, $PGF_{2\alpha}$ on d 30, and GnRH with TAI occurring 72 h after $PGF_{2\alpha}$. It produced pregnancy rates to TAI of 62%, which was significantly higher than the 47% pregnancy rate to TAI given by the 7 day CO-Synch + CIDR (Busch et al., 2007). While the pregnancy rate to TAI is relatively high for this protocol, producers must account for the cost of 2 additional chute trips required by CIDR Select relative to the 7-d CO-Synch + CIDR.

Heifers have a higher response rate to GnRH at the initiation of an estrous synchronization protocol if they are at d 5 of their estrous cycle compared to d 10 of their estrous cycle (Atkins et al., 2008). This is because on d 5 there is likely to be a dominant follicle growing, whereas on d 10 the growth of the largest follicle has stopped and response rate to GnRH is lower. Because of this, the CIDR Select and other protocols have utilized a CIDR for the first 14-d to cause heifers to enter estrus following CIDR removal. This allows them to be on d 9 or less of their cycle which increases the chance that they will respond to GnRH given on d 23 and initiate a new follicular wave.

However, results from previous studies (Lamb et al., 2006) about the value of GnRH in synchronizing follicular waves caused researchers to question whether GnRH on d 23 was necessary in the CIDR Select protocol. The Show-Me-Synch estrous synchronization protocol uses a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 30, and GnRH with TAI 66 h after $PGF_{2\alpha}$. Show-Me-Synch's pregnancy rate to TAI of 62% tended to be greater than CIDR Select's pregnancy rate to TAI of 51% (Mallory et al., 2011). This increased pregnancy rate is undoubtedly appealing to producers, especially since it requires one less chute trip. This protocol also offers the benefit of extended progestin exposure to help induce prepubertal heifers. One clear drawback of the protocol is its length, as it takes 33 days to complete.

However, prolonged progestin exposure impedes follicular waves through development of persistent follicles (Sirois and Fortune, 1990). The concentration of progestins used for estrous synchronization are effective in producing negative feedback and preventing ovulation; however, they do not prevent LH pulses to the extent of progesterone from a CL (Kinder et al., 1996). Increased LH pulses can increase 17ß-

estradiol secretion and lead to persistent follicle formation (Kinder et al., 1996) if a CL is not present to produce additional progesterone to reduce LH secretion (Savio et al., 1993).

Rationale for Current Experiment

This research led to the creation of a TAI estrous synchronization protocol that utilized extended progestin exposure while also employing multiple GnRH injections to prevent persistent follicles and create more synchronized follicular growth.

Administration of $PGF_{2\alpha}$ prior to GnRH and CIDR improves response to GnRH and reduces variation of follicle 6 d later (Grant et al., 2011). Progestins used for estrous synchronization also allow increased secretion of LH if a natural CL is not present (Kinder et al. 1996; Savio et al., 1993). Expression of mRNA's encoding for LH receptors were first found in granulosa cells 36 h after the first follicular wave and increased with follicular size and stage of follicular wave (Bao et al., 1997). Increased LH receptors were found on granulosa and theca cells of persistent follicles compared with healthy dominant follicles (Cupp et al., 1993). The increase in LH receptors could have been due to the increased frequency of LH release in cow with persistent follicles (Kinder et al., 1996).

Because of this potential relationship between LH pulses and LH receptors, $PGF_{2\alpha}$ was given on d 0 of our 14-d CIDR protocol to lyse any pre-existing CL and reduce progesterone levels to allow for increased LH pulses. This increased exposure to LH would hypothetically increase the number of LH receptors present on granulosa cells which would allow for a faster luteinization of granulosa cells when GnRH was given on

d 9. A more completely luteinized CL would then be more responsive to $PGF_{2\alpha}$ given on d 14 and could improve pregnancy rates to TAI.

Prostaglandin $F_{2\alpha}$ Doses Required at CIDR Removal

The 5 Day CO-Synch + CIDR protocol has been a popular TAI method for mature beef cows, but it has also been used on beef heifers. It involves a CIDR from d 0 to 5, GnRH on d 0, $PGF_{2\alpha}$ on d 5, and GnRH with TAI 72 h post CIDR removal. In a smaller experiment involving 74 heifers it produced a 63.5% pregnancy rate to TAI (Bridges and Lake, 2011). This pregnancy rate to TAI is attractive to producers as it requires only 4 trips through the chute. However relatively little data exist regarding the efficacy of this protocol on beef heifers.

Recent research has evaluated the timing and dosage of $PGF_{2\alpha}$ given in shorter estrous synchronization protocols that utilize a CIDR and administer $PGF_{2\alpha}$ when CIDR's are removed. Thirty mg of $PGF_{2\alpha}$ has been shown to cause luteolysis of the CL when cattle are between d 6 and 16 of their estrous cycle (Lauderdale, 1972; Rowson et al., 1972; Odde, 1990). Because of this, a single dose of 25 mg of $PGF_{2\alpha}$ is given upon CIDR removal on d 7 of the 7 Day CO Synch + CIDR protocol because hypothetically heifers should have ovulated on d 0 thus creating a CL that would be 7 d old and responsive to PGF on d 7.

However recommendations began to change with the advent of the 5-d CO Synch + CIDR protocol in beef cows. This protocol produced a TAI pregnancy rate of 80% which was 13.3 percentage points higher than the 7 Day CO Synch + CIDR in experiment 1 (Bridges et al., 2008). Because the decreased age of the CL, 1 dose of

$PGF_{2\alpha}$ was given at CIDR removal and another was given 12 h later to ensure complete luteal regression (Bridges et al., 2008).

Initially, research looked at the interval between $PGF_{2\alpha}$ doses within the 5-d CO Synch + CIDR. No difference in pregnancy rate was found between a 6 and 12 h interval between injections (Peel et al., 2010), but beef cows receiving their second injection of $PGF_{2\alpha}$ 6 h after the first injection had higher pregnancy rates than those receiving the second injection 2 h after the first (Whittier et al., 2010). The effect on pregnancy rate between 1 and 2 doses of $PGF_{2\alpha}$ was then evaluated due to the benefits to producers of requiring one less time handling cattle.

Research confirmed the original hypothesis that 2 doses of $PGF_{2\alpha}$ were needed in the 5-d CO Synch + CIDR when beef and dairy cows receiving 2 doses produced higher pregnancy rates to TAI than those receiving 1 dose (Kasimanickam et al., 2009; Chebel et al., 2008). Beef heifers in the 5 day protocol that received 2 doses of $PGF_{2\alpha}$ also tended ($P = 0.06$) to have higher pregnancy rates to TAI than those given 1 dose (Peterson et al., 2011).

Another study with beef heifers receiving 2 doses of $PGF_{2\alpha}$ in the 5-d CO-Synch + CIDR protocol resulted in pregnancy rates to TAI of 62.5% (Bridges and Lake, 2011). Although this was not compared to another $PGF_{2\alpha}$ treatment, the high pregnancy rates produced by heifers receiving 2 doses is noteworthy. However, in dairy heifers there was no difference in pregnancy rates or CL regression between heifers receiving 1 or 2 doses of PGF (Rabaglino et al., 2010). Pregnancy rates to TAI were also comparable between dairy heifers in the 5-d protocol receiving one injection of $PGF_{2\alpha}$ compared to the 7 day CO Synch + CIDR (Colazo and Ambrose, 2011).

These varying results regarding the timing and number of $PGF_{2\alpha}$ doses significantly impacts today's beef producer as it affects the cost and pregnancy rate associated with using the 5-Day CO-Synch + CIDR protocol. Because of this importance, further research is needed to evaluate the requirements of future estrous synchronization protocols to ensure pregnancy rate is not compromised while still keeping estrous synchronization protocols relatively inexpensive.

These results also led us to compare 2 $PGF_{2\alpha}$ treatments in our 14-d CIDR protocol. Because GnRH was given on d 9 to force ovulation of any persistent follicles and create a new CL, the CL present on d 14 would likely be similar to the CL produced by the 5-d CO-Synch + CIDR estrous synchronization protocol. The discrepancy in results regarding adequate $PGF_{2\alpha}$ dosage in the 5-d CO-Synch + CIDR protocol led us to give a single dose of 50 mg of $PGF_{2\alpha}$ at one injection site and two 25 mg doses of $PGF_{2\alpha}$ 6 h apart concurrent with CIDR removal on d 14 of our 14-d CIDR protocol.

Timed AI protocols that produce reasonable pregnancy rates while minimizing chute trips are important to encourage the adoption of AI within the beef industry. Continued research regarding manipulation of follicular dynamics should allow for improved TAI protocols that minimize time and labor requirements for producers while keeping pregnancy rates high to ensure the benefits of AI are realized.

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CHAPTER II

Timed artificial insemination pregnancy rates in beef heifers after synchronization of follicular waves and ovulation using a 14-d controlled internal drug release insert*

SUMMARY: Long-term exposure to progestins is effective for synchronizing estrus in beef cows, but can cause persistent follicles with defective oocytes. This study examined the effect on pregnancy rates to timed AI (**TAI)** for a 14-d controlled internal drug release (**CIDR**) with d 9 GnRH in beef heifers. Heifers at 4 locations received 3 treatments. The 14-d 50 PG treatment (n = 242) received 100 μg GnRH im and CIDR (1.38 g progesterone) on d 0, 100 μg GnRH im on d 9, and 50 mg of $PGF_{2\alpha}$ on d 14 at CIDR removal. The 14-d 6h PG treatment (n = 233) was identical to 14-d 50 PG but received $PGF_{2\alpha}$ in two 25 mg injections 6 h apart at CIDR removal. The 5-d CO-Synch +

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CIDR control treatment (n = 235) received 100 μg GnRH im and CIDR on d 9, and 25 mg of $PGF2_{\alpha}$ at CIDR removal on d 14. All heifers received TAI and 100 ug GnRH 72 ± 2 h after $PGF_{2\alpha}$. Pregnancy rates were determined on d 41 to 60 by ultrasonography. Pregnancy rate to TAI in 14-d 50 PG heifers (54.5%) did not differ ($P = 0.57$) from 14-d 6 h PG heifers (53.6%). The TAI pregnancy rate in heifers of 14-d protocols combined was 54.1%, and was not different ($P = 0.20$) from the 5-d CO-Synch + CIDR TAI pregnancy rate of 46.4%. Results indicate one 50 mg dose of $PGF_{2\alpha}$ is sufficient for the 14-d CIDR treatment, but the 14-d CIDR protocol with a second GnRH injection did not increase pregnancy rates to TAI when compared to the 5-d control.

Key Words: Artificial insemination, Estrous synchronization, Follicular waves, Heifers

INTRODUCTION

Oral progestin feeding is effective at inducing cyclicity in prepubertal heifers (Patterson et al., 1990). Unfortunately, heifers under the influence of long-term oral progestins can develop persistent follicles due to a continued period of follicular growth (Sirois and Fortune, 1990; Kinder et al., 1996). The controlled internal drug release (**CIDR**) Select estrous synchronization protocol utilizes long-term progestin exposure for 14-d to cause heifers to ovulate following CIDR removal. This protocol improves ovulation when GnRH is administered on d 23, estrus synchrony (Leitman et al., 2008), and increases pregnancy rate to timed AI (**TAI**) compared to the 7-d CO-Synch + CIDR

protocol (Busch et al., 2007). The Show-Me-Synch protocol was similar to the CIDR Select protocol but without d 23 GnRH (Mallory et al., 2011). One drawback of both protocols is that they require 33 d to complete and are time intensive for beef producers.

Numerous studies have examined $PGF_{2\alpha}$ treatments at CIDR removal within the 5-d CO-Synch + CIDR protocol. In beef cows, a single 25 mg dose of $PGF_{2\alpha}$ reduced pregnancy rates to TAI compared to two 25 mg doses given 7 h apart (Kasimanickam et al., 2009). Beef heifers receiving two 25 mg doses (at CIDR removal and 6 h later) tended ($P = 0.06$) to produce higher pregnancy rates to TAI vs. heifers receiving a single $PGF_{2\alpha}$ dose at CIDR removal (Peterson et al., 2011). However, no differences in pregnancy rate to TAI were found in dairy heifers receiving one 25 mg dose of $PGF_{2\alpha}$ or 2 25 mg doses given 12 h apart (Rabaglino et al., 2010).

Our hypothesis was that synchrony of follicular waves could be improved by giving GnRH on d 9 of a 14-d CIDR-based estrous synchronization TAI protocol. The GnRH injection would cause ovulation of potentially persistent follicles that may have formed due to sustained progestin exposure, and create a second synchronized wave of follicular growth. This would create a TAI protocol with prolonged progestin exposure and synchronized follicular growth while being completed in 17 d. The objectives of this study were to determine the effects of increased CIDR duration (14 vs. 5 d) with GnRH administered on d 9 and interval of $PGF_{2\alpha}$ administration at CIDR removal on pregnancy rate to TAI in beef heifers.

MATERIALS AND METHODS

Experimental procedures with animals were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment. Angus and Angus cross heifers (n = 710) at 4 locations (location 1, n = 89; location 2, n = 440; location 3, n = 147; location 4, n = 34) were randomly assigned to 1 of 3 treatments. Heifers assigned to the 14-d 50 PG treatment (n = 242) were given CIDR (EAZI-BREED™ CIDR®, Pfizer Animal Health, New York, NY; 1.38 g of progesterone) and GnRH analog im (100 μg Factrel, Fort Dodge Animal Health, Fort Dodge, IA) on d 0 (Figure 1). On d 9 they received another injection of Factrel im. When CIDRs were removed on d 14, heifers received 50 mg im of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) at one injection site. These heifers were then given 100 μg GnRH and inseminated 72 ± 2 h after CIDR removal. Heifers in the 14-d 6 h PG treatment (n = 233) similarly received GnRH and CIDR on d 0, 100 μg im GnRH on d 9, 25 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, another 25 mg im of $PGF_{2\alpha}$ 6 h later, and 100 μg im GnRH when inseminated 72 ± 2 h after CIDR removal. The 5-d CO-Synch + CIDR treatment (n = 235) served as the control. Heifers in this treatment received CIDR and GnRH on d 9, 25 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, and GnRH at TAI 72 ± 2 h after CIDR removal.

Weights were recorded on d 0 of the protocol for all heifers except at location 4 where a scale was not available. All heifers were evaluated by a single evaluator for BCS (1 = thin, 9 = obese: Richards et al., 1986) on d 0 and estrus detection patches

(ESTROTECT, Spring Valley, WI) were applied at CIDR removal on d 14. These patches were then scored on a 3-point scale at breeding on d 17 (1 = patch's film was un-removed, 2 = approximately 50% of patch's film was removed and 3 = all or almost all of patch's film was removed). However, at location 1 patches were only scored as 1 or 3. Pregnancy rates to TAI were determined via transrectal ultrasonography (5 MHz microconvex transducer on an EI Medical Ibex console, Loveland, CO) 41 to 60 d after TAI.

Statistical Analyses. Differences in TAI pregnancy rate were analyzed using the GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC) which fits generalized linear mixed models. All significant factors ($\alpha = 0.05$) were used in the final statistical model to analyze pregnancy rate. Factors that were available to the model included location, treatment, BCS, BW, sire, technician, and their first order interactions. There was ($P < 0.001$) a location effect, but there was no ($P = 0.88$) treatment × location interaction term; this term was retained in the model in order to obtain TAI pregnancy rates among treatments across locations. Differences in estrus patch scores were also analyzed using the GLIMMIX procedure. All significant ($\alpha = 0.05$) factors were used in the model and included location, treatment, BCS, BW, and their first order interactions.

Differences in BCS and BW across treatments and locations were analyzed using the GLM procedure in SAS. Sire was not significant ($P = 0.20$) in the model analyzing pregnancy rate to TAI; however, differences in pregnancy rate to TAI among sires at locations were analyzed using the GLIMMIX procedure.

RESULTS AND DISCUSSION

Information on mean BW and BCS are reported by location (Table 2.1) and treatment (Table 2.2). Body condition scores differed ($P < 0.05$) across locations; however, BCS was not different ($P > 0.10$) by treatment pooled across all locations.

Overall pregnancy rates to TAI pooled across all treatments for each location are reported in Table 2.1. Overall pregnancy rates at locations 1 and 3 were lower than expected. While BCS was not significant ($P > 0.10$) in the model for pregnancy rate to TAI, location 1 had a lower ($P < 0.05$) average BCS than the other locations. Given the positive relationship between BCS and reproductive performance (Richards et al., 1986), it can be hypothesized that low pregnancy rates to TAI at location 1 could be attributed to lower BCS.

Pregnancy rate to TAI at location 3 may be partially explained by the lower fertility of sire 1 relative to the other 2 sires used at that location. Differences in each sire's pregnancy rate to TAI within each location were analyzed in a separate model. The only location with sires producing different ($P < 0.05$) pregnancy rates to TAI was location 3, with sire 1 having lower ($P < 0.05$) pregnancy rates to TAI than sires 2 and 3. The reduced fertility of sire 1 is important given 75 heifers were bred to sire 1, whereas only 44 and 28 heifers were bred to sires 2 and 3, respectively. There was no treatment × sire interaction ($P > 0.10$). Mating choices were made by each ranch and were outside the control of this experiment.

Estrous response by treatment is shown in Table 2.3. Both 14-d CIDR protocols had a lower ($P < 0.05$) percentage of non-activated heat patches (patch score = 1) and a higher ($P < 0.05$) percentage of completely rubbed patches (patch score = 3) than the 5-d CO-Synch + CIDR treatment. The higher ($P < 0.05$) percentage of activated and lower ($P < 0.05$) percentage of non-activated Estrotect patches in the 14-d treatments compared to 5-d CO-Synch + CIDR indicates the 14-d protocol caused more heifers to display estrus behavior than 5-d CO-Synch + CIDR. However, since heifers were not actually observed for behavioral estrus following administration of $PGF_{2\alpha}$ and CIDR removal, neither the interval from $PGF_{2\alpha}$ to estrus nor the conception rate of heifers displaying behavioral estrus are known.

Timed AI pregnancy rates by patch score across treatment and location are shown in Table 2.4. Pregnancy rate to TAI increased ($P = 0.03$) as patch score increased. This indicates patch score adequately measured estrus response and heifers were more likely to become pregnant if they showed estrus and had a higher patch score.

Timed AI pregnancy rates by treatment and location are shown in Table 2.5. Because there was no treatment × location interaction ($P = 0.88$), TAI rates were combined across locations so that overall pregnancy rate could be compared across treatments. There was no difference between 14-d 50 PG treatment and 14-d 6 h PG treatment at location 1 ($P = 0.40$), location 2 ($P = 0.97$), location 3 ($P = 0.72$), location 4 ($P = 0.69$), or when locations were pooled ($P = 0.57$).

The similarity in pregnancy rate to TAI between 14-d protocols could be attributed to a CL that was older and more responsive to $PGF_{2\alpha}$ which was produced by the 14-d protocols. However, CL characteristics and progesterone production was not

measured in this study. Administration of GnRH on d 0 should force ovulation in heifers with a follicle that is responsive to GnRH and as a result form a CL that will remain until CIDR removal and $PGF_{2\alpha}$ administration 14-d later. This CL should regress in response to the 50 mg of $PGF_{2\alpha}$ given in the 14-d 50 PG protocol because a single dose of $PGF_{2\alpha}$ can produce luteolysis if given between d 6 and 16 of the estrous cycle (Lauderdale, 1972; Rowson et al., 1972; Odde, 1990).

Two injections of $PGF_{2\alpha}$ given 12 h apart were initially recommended for the 5-d CO-Synch + CIDR protocol to ensure complete luteal regression of a younger CL produced by the shorter 5-d interval between GnRH and $PGF_{2\alpha}$ (Bridges et al., 2008). The need for 2 doses of $PGF_{2\alpha}$ (25 mg each) in the 5-d CO-Synch + CIDR protocol was confirmed when cows receiving two 25 mg $PGF_{2\alpha}$ injections produced higher pregnancy rates to TAI relative to those receiving one dose (Kasimanickam et al., 2009). A similar study in beef heifers showed heifers that received 2 doses of $PGF_{2\alpha}$ 6 h apart tended to produce higher pregnancy rates to TAI than those receiving a single dose (Peterson et al., 2011). However, research form Rabaglino et al., (2010) indicated no difference in pregnancy rates to TAI between dairy heifers receiving 1 or 2 doses of $PGF_{2\alpha}$.Therefore providing the support for one dose of $PGF_{2\alpha}$ in the current study.

The similar pregnancy rates to TAI of the 14-d protocols supports the hypothesis that a more mature CL was produced and that complete luteal regression occurred in both 14-d protocols. The hypothesis about CL responsiveness within the 14-d protocol could be further tested in the future by comparing pregnancy rates to TAI between protocols that received a single dose of 25 or 50 mg of $PGF_{2\alpha}$ at CIDR removal. Future

progesterone analysis would also aid in determining the rate of luteal regression between protocols.

These results have practical benefits for the beef producer facing time and labor constraints as it eliminates the need for an additional handling of heifers due to an additional $PGF_{2\alpha}$ injection in the 14-d 6 h treatment. However, in order for this finding to positively impact producers, pregnancy rates to TAI of the 14-d protocol must be higher than for other protocols in order to justify an additional handling of heifers on d 9.

The combined 14-d CIDR protocols were not different from the 5-d CO-Synch + CIDR at location 1 ($P = 0.86$), location 3 ($P = 0.89$), or location 4 ($P = 0.36$). However, at location 2 the combined 14-d CIDR protocols had greater ($P = 0.02$) pregnancy rates to TAI than the 5-d CO-Synch + CIDR treatment (63% vs. 51%). The treatment × location interaction term was not significant ($P = 0.88$) therefore data were pooled across locations and the combined 14-d CIDR protocols TAI pregnancy rate (54.1%) were not different ($P = 0.20$) from the 5-d CO-Synch + CIDR TAI pregnancy rate of 46.4%.

The 14-d CIDR protocols produced encouraging results at location 2 – the location with the largest sample size (n = 440). The ability of these treatments to produce pregnancy rates to TAI above 60% in a protocol that takes 17 d to complete is important given the fact that producers cite time and labor as the primary reasons they choose not to implement AI (USDA, 2009). However, any improvement in pregnancy rate produced by the 14-d CIDR treatment must be weighed against the additional labor and drug cost required by working heifers an additional time on d 9. Results from location 2 also indicate that the d 9 GnRH may be causing a new wave of follicular growth and could aid in synchronizing follicular waves but this was not directly measured in the current study.

One potential explanation for the success of the 14-d protocols relative to the 5-d CO-Synch + CIDR protocol at location 2 was induction of puberty. Administration of progestins induces prepubertal heifers into cyclicity by increasing LH production (Anderson et al., 1996). Treatment of anestrous beef cows with a CIDR improves pregnancy rates to TAI (Lamb et al., 2001). The 14-d CIDR exposure used in the CIDR Select estrous synchronization protocol also produced higher progesterone levels on the day that $PGF_{2\alpha}$ was given and higher estradiol 17-ß levels 48 h after $PGF_{2\alpha}$ compared to the Select Synch protocol, which uses a CIDR for 7 d (Leitman et al., 2008).

The higher pregnancy rate in the 14-d vs. 5-d protocols at this location could be due to the ability of the 14-d protocols to initiate puberty due to an extended period of progestin exposure relative to the 5-d CO-Synch + CIDR. However, cycling status at the initiation of estrous synchronization was not evaluated.

Effectively synchronizing follicular waves may be more difficult in heifers than cows, as only 45 to 58% of heifers respond to GnRH by ovulating or initiating a new wave of follicular development due to being in different points within the estrous cycle (MacMillan and Thatcher, 1991; Moreira et al., 2000; Atkins et al., 2008). Giving GnRH concurrent with CIDR in the CIDR + PG and Select Synch + CIDR protocols did not improve pregnancy rate or estrus synchrony (Lamb et al., 2006). The authors hypothesized their results could have been due to variation in GnRH responsiveness depending upon day of the estrous cycle and ability to begin a new follicular wave (Lamb et al., 2009).

Only 38% of heifers on d 18 of their estrous cycle responded to GnRH as opposed to a 95% response rate for heifers receiving GnRH on d 5 of their estrous cycle (Moreira

et al., 2000; Atkins et al., 2008). The 14-d 50 PG and 14-d 6 h PG treatments addressed this potential problem through GnRH given on d 9. Heifers that began 14-d protocols on d 18 of their estrous cycle would still have been able to ovulate a fertile follicle at TAI because the d 9 GnRH would have been administered on d 6 of their estrous cycle when they would be more responsive to GnRH.

The 14-d CIDR treatments used in the current study also sought to address this difficulty in synchronizing follicular waves by adding GnRH on d 9. Heifers typically have 2 or 3 waves of follicular growth in each estrous cycle (Sirois and Fortune, 1988). Giving GnRH on d 0 and 9 should effectively mimic natural follicular growth and cause 2 waves of follicular growth while also providing heifers with 2 opportunities to respond to GnRH. This differs from the 5-d CO-Synch + CIDR protocol which only initiates one follicular wave and gives heifers one chance to respond to GnRH given on d 0.

Prolonged progestin exposure impedes follicular waves through development of persistent follicles (Sirois and Fortune, 1990). The concentration of progestins used for estrous synchronization are effective in producing negative feedback and preventing ovulation; however, they do not prevent LH pulses equivalent to progesterone from a CL (Kinder et al., 1996). Increased LH pulses can increase 17ß-estradiol secretion and lead to persistent follicle formation (Kinder et al., 1996) if a CL is not present to produce additional progesterone to reduce LH secretion (Savio et al., 1993). It is likely that our 14-d protocols prevented persistent follicles due to d 9 GnRH. This could have produced a new follicle that ovulated a fertile oocyte.

Previous studies have found the 5-d CO-Synch + CIDR protocol to be an effective TAI protocol on beef heifers, therefore it was selected as the control for this

experiment. For instance, Peterson et al. (2011) reported pregnancy rates to TAI with 2 25 mg doses of $PGF_{2\alpha}$ given 6 h apart across 6 locations were 53.4% (n = 101), 54.3% (n = 127), 58.2% (n = 68), 60.4% (n=91), 66.2% (n = 130), and 83.7% (n = 45) at different locations. A separate study produced pregnancy rates to TAI of 63.4% (n = 64; Bridges and Lake, 2011).

A potential explanation for the discrepancy in TAI pregnancy rates in this study (46.4%) and previous studies could be the difference $PGF_{2\alpha}$ given at CIDR removal. Our study only gave a single 25 mg injection of $PGF_{2\alpha}$ as opposed to the 2 doses given in previous studies. Therefore, incomplete luteal regression may have occurred in this study and impaired TAI pregnancy rates. One injection of 25 mg of $PGF_{2\alpha}$ was used in this experiment due to the similarity in pregnancy rates in dairy heifers receiving 1 or 2 doses of $PGF_{2\alpha}$ at CIDR removal (Rabaglino et al., 2010). Data indicating the tendency for increased pregnancy rates to TAI in heifers receiving 2 doses of $PGF_{2\alpha}$ at CIDR removal in the 5-d CO-Synch + CIDR was not published when this experiment was designed and implemented.

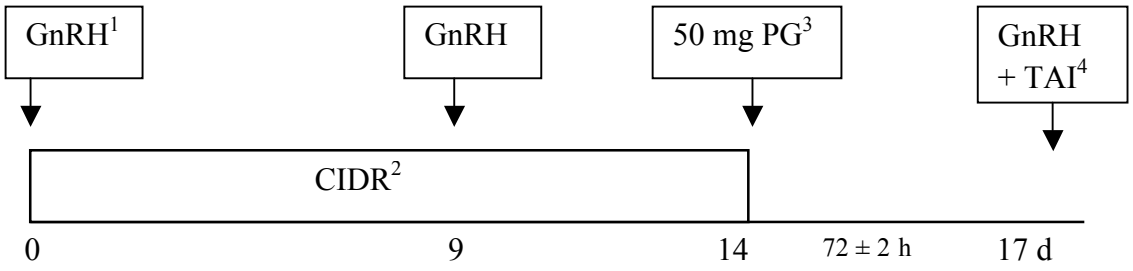
IMPLICATIONS

The success of the 14-d protocol relative to the 5-d CO-Synch + CIDR control offers support that it could be viable timed artificial insemination estrous synchronization protocol for beef heifers. However, further research that incorporates ultrasonography is needed to evaluate the ability of GnRH to synchronize follicular waves if given in the

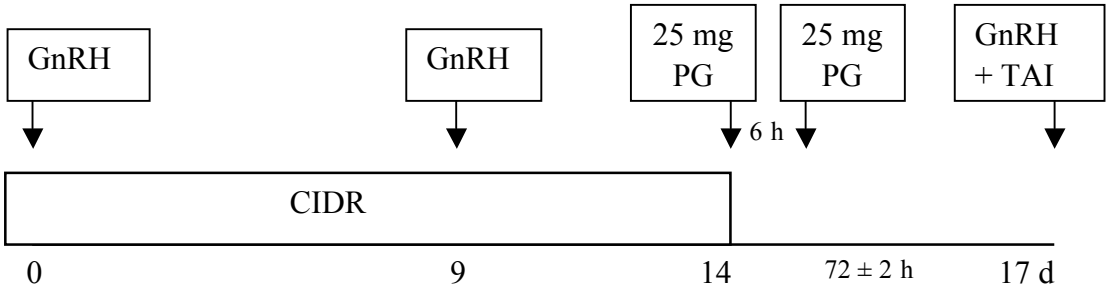
middle of a 14-d CIDR protocol. Such studies will help to clarify mechanisms that cause ovulation of a fertile oocyte. Additional research evaluating the effectiveness of the 14-d CIDR protocol will also help in determining its viability as a TAI protocol for beef heifers.

Figure 2.1. Estrous synchronization treatments administered to beef heifers

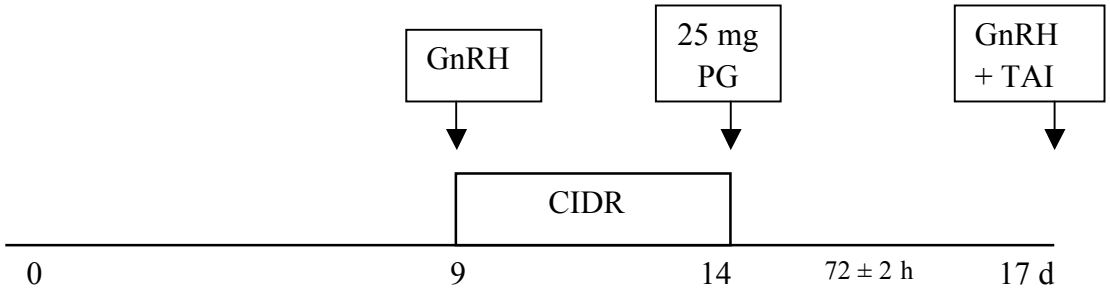
Treatment: 14-d 50 PG



Treatment: 14-d 6 h PG



Treatment: 5-d CO-Synch + CIDR



[1] GnRH: 100 μg given im of the GnRH analogue, Factrel (Fort Dodge Animal Health),
[2] CIDR: Controlled Internal Drug Release Device, 1.38 g of progesterone (EAZI-BREED™ CIDR®, Pfizer Animal Health.
[3] PG: 25 mg of Prostaglandin $F_{2\alpha}$ given im (Lutalyse, Pfizer Animal Health)
[4] TAI: Timed AI

Table 2.1. Number, BW, BCS, and overall pregnancy rate to timed AI (TAI) of beef heifers by location (LS means ± SE**)**

Location	n =	BW (kg)	BCS [1]	Overall percent pregnant to TAI
1	89	311 [a] ± 3.6	3.2 [w] ± 0.04	35.6 [a] ± 0.05
2	440	306 [a] ± 1.7	4.9 [x] ± 0.02	58.9 [b] ± 0.02
3	147	323 [b] ± 2.8	5.1 [y] ± 0.03	34.7 [a] ± 0.04
4	34	-	5.8 [z] ± 0.07	72.0 [b] ± 0.08

[1] Body condition was evaluated using the 9-point scale (1 = thin, 9 = obese; Richards et al., 1986)
[ab] within a column, means without common superscripts differ ($P < 0.05$)
[w-z] within a column, means without common superscripts differ ($P < 0.01$)

Table 2.2. Number, BW, and BCS of beef heifers by estrous synchronization treatment (LS squares means ± SE)

	Treatment		
	14-d 50 PG[1]	14-d 6 h PG[2]	5-d CO-Synch + CIDR[3]
n =	242	233	235
BW (kg)	310 ± 2.3	310 ± 2.4	312 ± 2.4
BCS	4.7 ± 0.03	4.8 ± 0.03	4.8 ± 0.04

No differences ($P < 0.05$)
[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[2] Heifers received a CIDR from d 0 to 14, GnRH on d 0 and d 9, $PGF_{2\alpha}$ (two 25 mg im injections of lutalyse given 6 h apart) on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.

Table 2.3. Estrotect patch scores among estrous synchronization treatments

Treatment					Contrast statistics[1] (P =)	
Patch score as a percentage of total treatment[2]	14-d 50 PG[3] %	14-d 6 h PG[4] %	5-d CO-Synch+CIDR[5] %	SE	14-d vs. 5-d	50 PG vs. 6 h PG
1	9.9	11.2	26.4	4.23	0.01	0.45
2	7.8	9.9	14.9	4.46	0.06	0.43
3	71.9	71.7	54.0	5.73	<0.01	0.96

[1]Contrast statements: Combined 14-d 50 PG and 14-d 6 h PG treatments compared to 5-d CO-Synch + CIDR (14-d vs. 5-d) and 14-d 50 PG compared to 14-d 6 h (PG Effect).
[2] Patch scores: 1 = patch's film was unremoved, 2 = approximately 50% of patch's film was removed and 3 = all or almost all of patch's film was removed).
[3] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[4] Heifers received a CIDR from d 0 to 14, GnRH on d 0 and d 9, $PGF_{2\alpha}$ (two 25 mg im injections of lutalyse given 6 h apart) on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[5] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.

Table 2.4. Pregnancy rates to timed AI (TAI) across estrotect patch scores

Estrotect patch score [1]	Pregnancy rate to TAI across all locations, %	SE
1	30.3 [a]	4.21
2	48.2 [b]	5.54
3	58.4 [b]	2.39

[1] Patch scores: 1 = patch's film was unremoved, 2 = approximately 50% of patch's film was removed and 3 = all or almost all of patch's film was removed).
[ab] Within a column, means without common superscripts differ ($P < 0.05$)

Table 2.5. Pregnancy rates to timed AI in beef heifers by estrous synchronization treatment within location and overall

	Treatment				Contrast statistics[1] (*P* =)	
Location	14-d 50 PG[2] %	14-d 6 h PG[3] %	5-d CO-Synch + CIDR[4] %	SE	14-d vs. 5-d	50 PG vs. 6 h PG
1	41.2 (14/34)	31.0 (9/29)	34.6 (9/26)	5.13	0.86	0.40
2	62.6 (94/150)	62.5 (90/144)	51.3 (75/146)	2.36	0.02	0.97
3	33.3 (16/48)	36.7 (18/49)	34.0 (17/50)	3.92	0.89	0.72
4	80.0 (8/10)	72.7 (8/11)	61.5 (8/13)	7.97	0.36	0.69
Overall[5]	54.5 (132/242)	53.6 (125/233)	46.8 (110/235)	3.84	0.20	0.57

[1]Contrast statements: Combined 14-d 50 PG and 14-d 6 h PG treatments compared to 5-d CO-Synch + CIDR (14-d vs. 5-d) and 14-d 50 PG compared to 14-d 6 h (PG Effect).
[2] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 0 to 14, GnRH on d 0 and d 9, $PGF_{2\alpha}$ (two 25 mg im injections of lutalyse given 6 h apart) on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[4] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[5]There was no treatment × location interaction ($P = 0.88$), therefore data were pooled across locations.

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CHAPTER III

Ovarian response and timed artificial insemination pregnancy rates in beef heifers after synchronization of follicular waves and ovulation using a 14-d controlled internal drug release insert*

SUMMARY: Synchronizing follicular waves in beef heifers to utilize timed AI (**TAI**) is difficult due to a reduced response to GnRH. Therefore, our objectives were to 1) evaluate the effect of extended progestin exposure with 2 GnRH injections on TAI pregnancy rates and ovarian response, 2) evaluate the effect of giving GnRH versus $PGF2_{\alpha}$ at CIDR insertion on TAI pregnancy rates and ovarian response, and 3) evaluate d 7 vs. d 9 GnRH in the midst of a 14-d CIDR protocol on TAI pregnancy rates. In Exp. 1 Angus cross beef heifers (n = 319) approximately 12 to 15 mo old across 4 locations were assigned to 1 of 3 treatments: 1) 14-d GnRH-9 (n = 107) included 100 μg GnRH im and CIDR (1.38 g progesterone) on d 0, 100 μg GnRH im on d 9, and 50 mg $PGF_{2\alpha}$ on d 14 at CIDR removal, 2) 14-d PG (n = 107) was identical to 14-d GnRH-9 but heifers received $PGF_{2\alpha}$ on d 0 instead of GnRH, and 3) 5-d CO-Synch + CIDR (n = 104)

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included 100 μg GnRH im and CIDR on d 9, and 25 mg $PGF2_{\alpha}$ at CIDR removal on d 14. All heifers received TAI and 100 ug GnRH 72 ± 2 h after $PGF_{2\alpha}$. Ovarian structures were determined via ultrasound at locations 1 and 2. Ultrasound occurred at these locations on d 0, 9, 14, and 17, and heifers at location 1 only were ultrasounded on d 3. In Exp. 2 Angus cross beef heifers (n = 452) at 1 location were assigned to 1 of 3 treatments: 1) 14-d GnRH-9 (n = 150) as described earlier, 2)14-d GnRH-7 (n = 150) which was identical to 14-d GnRH-9 but heifers received GnRH on d 7 instead of d 9 and received 25 mg instead of 50 mg of $PGF_{2\alpha}$ on d 14, 3) 7-d CO-Synch + CIDR (n = 152) included 100 μg GnRH im and CIDR on d 7, and 25 mg $PGF2_{\alpha}$ at CIDR removal on d 14. The 14-d GnRH-7 and 7-d CO-Synch + CIDR treatments received TAI and 100 μg GnRH 63 ± 3 h after $PGF_{2\alpha.}$ The 14-d GnRH-9 TAI pregnancy rate (52.3%) was not different ($P = 0.82$) than 14-d PG (47.6%), nor was the TAI pregnancy rate of both 14-d treatments combined (50.0%) different ($P = 0.66$) from 5-d CO-Synch + CIDR (47.1%). Pregnancy rate to TAI of 14-d GnRH-9 (51.3%) was not different ($P = 0.75$) than 14-d GnRH-7 treatment (48.0%), nor was the TAI pregnancy rate of both 14-d treatments (49.6%) different ($P = 0.83$) from 7-d CO-Synch + CIDR (48.6%). Ultrasound analysis showed 14-d GnRH-9 induced a second wave of follicular growth in only 25.9% of heifers while the 14-d PG had larger ($P = 0.01$) follicle size on d 9 but did not reduce ($P = 0.37$; $P = 0.97$) presence of corpora lutea at TAI compared to 14-d GnRH-9 or 5-d CO-Synch + CIDR, respectively. These ultrasound results combined with TAI pregnancy rates indicate the 14-d treatment is not a consistent or suitable TAI option compared to available estrous synchronization protocols.

Key Words: Artificial insemination, Beef heifers, Estrous synchronization, Follicular waves

INTRODUCTION

Timed AI (**TAI**) estrous synchronization protocols that eliminate the need for estrus detection are beneficial for beef producers as time and labor are the primary reasons given for not utilizing AI (USDA, 2009). Progestins can be beneficial to TAI estrous synchronization protocols by inducing cyclicity in prepubertal heifers (Patterson et al., 1990), and 14-d progestin exposure can produce greater estradiol 17-ß levels 48 h after $PGF_{2\alpha}$ compared with 7-d exposure (Leitman et al., 2008). Unfortunately, synchronizing follicular waves to facilitate TAI is more difficult in heifers than cows due to a reduced response to GnRH (Moreira et al., 2000; Atkins et al., 2008).

Therefore, to effectively synchronize follicular waves in heifers it appears that multiple administrations of GnRH would be beneficial in order to create 2 follicular waves. Further, it is advisable to concurrently incorporate a progestin to help induce cyclicity in prepubertal heifers. Two injections of GnRH on d 0 and 9 would create 2 waves of follicular growth with the d 9 injection forcing ovulation of any potentially persistent follicles that may form due to extended progestin exposure.

Administration of $PGF_{2\alpha}$ prior to GnRH and CIDR insertion improves response to GnRH and reduces variation of follicle size 6 d later (Grant et al., 2011). Progestins used for estrous synchronization also allow increased secretion of LH if a natural corpus luteum (**CL**) is not present (Kinder et al. 1996; Savio et al., 1993). Our hypothesis was

that increased LH exposure would increase LH receptors present on granulosa cells which would allow for a more complete lutenization of the ovary on d 9 if GnRH is given. A more luteinized, older CL would then be more responsive to $PGF_{2\alpha}$ and potentially improve pregnancy rate to TAI.

Therefore, the objectives of Exp.1 were to determine the effect of GnRH or $PGF_{2\alpha}$ at the beginning of a long-term progestin administration on pregnancy rates to TAI and ovarian structures; determine the effect of long-term progestin exposure and short-term progestin exposure on pregnancy rates to TAI and ovarian structures.

Objectives for Exp. 2 were to determine the effect of d 7 or d 9 GnRH within a long-term progestin administration on pregnancy rates to TAI, and determine the effect on TAI pregnancy rate of a long-term progestin exposure estrous synchronization protocol compared to the Beef Reproduction Task Force recommended 7-d CO-Synch + CIDR estrous synchronization protocol (Johnson et al., 2011).

MATERIALS AND METHODS

Experimental procedures with animals were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment.

Experiment 1

Angus, Angus cross, and Hereford heifers (n = 319) at 4 locations (location 1, n = 89; location 2, n = 31; location 3, n = 161; location 4, n = 38) were randomly assigned to 1 of 3 treatments: 1) 14-d GnRH-9, 2) 14-d PG, and 3) 5-d CO-Synch + CIDR. Heifers in 14-d GnRH-9 (n = 107) included CIDR (EAZI-BREED™ CIDR®, Pfizer Animal Health,

New York, NY: 1.38 g of progesterone) and GnRH analog (100 μg Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0. On d 9 they received another injection of GnRH im. When CIDRs were removed on d 14, heifers received 50 mg im of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) at 1 injection site. These heifers were then given 100 μg GnRH and inseminated 72.1 ± 0.93 h after CIDR removal. Heifers in the 14-d PG treatment (n = 107) received CIDR and 25 mg im of $PGF_{2\alpha}$ on d 0, 100 μg GnRH on d 9, 50 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, and 100 μg GnRH when inseminated 72.1 ± 1.3 h after CIDR removal. The 5-d CO-Synch + CIDR treatment (n = 105) served as the control. Heifers in this treatment received CIDR and GnRH on d 9, 25 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, and GnRH at TAI 72.1 ± 2.1 h after CIDR removal (Figure 3.1). All heifers were evaluated for BCS (1 = thin, 9=obese; Richards et al., 1986) by 1 evaluator throughout the experiment and weighed on d 0. Pregnancy rates to TAI were determined via transrectal ultrasonography (5 MHz microconvex transducer on an EI Medical Ibex console, Loveland, CO) 43 to 60 d after TAI.

Ovarian structures were determined via transrectal ultrasonography (5 MHz microconvex transducer on an EI Medical Ibex console, Loveland, CO) on d 0, 3, 9, 14, and 17 at location 1 and on d 0, 9, 14, and 17 at location 2. Structures ≥ 5 mm were recorded. A successful response to d 9 GnRH was defined as a d 9 follicle ≥ 5 mm that was absent on d 14 and replaced by a new CL.

Response to d 9 GnRH for the 14-d GnRH-9 treatment at location 1 was evaluated using ultrasound data from d 0 and 3. Non-ovulatory dominant follicles can be from 10.2 to 12 mm in 3-wave heifers (Sirois and Fortune, 1988; Knopf et al., 1989) and 15.8 to 17.1 mm in 2-wave heifers (Ginther et al., 1989; Knopf et al., 1989). These follicles can

remain static without changing in size for 5 to 6 d before slowly decreasing in diameter (Ginther et al., 1989). Therefore, a successful response was when ≥ 10 mm follicle is present on d 0 and absent on d 3. Heifers were categorized as having no response to d 0 GnRH if follicles increase in size or remained the same size from d 0 to 3.

Heifers at locations 1 and 2 were observed for estrus twice daily for 1 h starting 21 d prior to protocol initiation to determine cycling status. A heifer was considered cycling if she was observed in estrus prior to protocol initiation or if there was a CL present on either ovary on d 0 of the protocol. Heifers observed in estrus prior to protocol initiation were classified into 1 of 4 cycle groups; the 0-5 cycle group were heifers on d 0 to 5 of their estrous cycle upon protocol initiation, the same criteria followed for cycle groups 6-10, 11-16, and 17-21.

Statistical Analyses. Differences in TAI pregnancy rate were analyzed using the GLIMMIX procedure in SAS (SAS Institute, Cary, NC) which fits generalized linear mixed models. All significant factors ($\alpha = 0.05$) were used in the final model to analyze pregnancy rate. Factors that were available to the model included location, treatment, BCS, BW, sire, technician, and their first order interactions. The final model included location, treatment, and the treatment × location interaction term, which was not significant ($P = 0.32$). Differences in BCS, BW, and follicle size were analyzed using the GLM procedure in SAS. Differences in response to GnRH were also analyzed using the GLIMMIX procedure in SAS.

Experiment 2

Angus and Angus cross heifers (n = 452) at one location were randomly assigned to one of 3 treatments. Heifers in the 14-d GnRH-9 treatment (n = 150) were given CIDR

(1.38 g of progesterone) and GnRH analog im (100 μg Factrel, Fort Dodge Animal Health) on d 0. On d 9 they received another injection of GnRH im. When CIDRs were removed on d 14, heifers received 50 mg im of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) in 1 injection. These heifers were then given 100 μg GnRH and inseminated 69.5 ± 3.50 h after CIDR removal. Heifers in the 14-d GnRH-7 (n = 150) received GnRH and CIDR on d 0, 100 μg GnRH on d 7, 25 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, and 100 μg GnRH when inseminated 63.5 ± 1.42 h after CIDR removal. The 7-d CO-Synch + CIDR treatment (n = 152) served as the control. Heifers in this treatment received CIDR and GnRH on d 7, 25 mg im of $PGF_{2\alpha}$ at CIDR removal on d 14, and GnRH at TAI 63.2 ± 1.81 h after CIDR removal (Figure 3.2).

All heifers were evaluated for BCS (1 = thin, 9 = obese; Richards et al., 1986) by 1 evaluator on d 0 and given estrus detection patches (ESTROTECT, Spring Valley, WI) at CIDR removal on d 14. These patches were then subjectively scored on a 3-point scale at TAI on d 17 (1 = patch film was not removed, 2 = approximately 50% of the patch film was removed, and 3 = all or almost all of the patch film was removed). Pregnancy rates to TAI were determined via transrectal ultrasonography (as described earlier) 45 d after TAI.

Statistical Analyses. Body condition score differences were analyzed using the GLM procedure in SAS. Differences in TAI pregnancy rate were analyzed using the GLIMMIX procedure in SAS. Factors that were available to the model included treatment, BCS, sire, technician, and their first order interactions. The final model included treatment and technician ($P < 0.01$). The technician × treatment interaction term was not significant ($P = 0.88$) and was not included in the final model.

RESULTS AND DISCUSSION

Experiment 1

Mean BW and BCS for Exp. 1 are reported by location (Table 3.1) and treatment (Table 3.2). Mean BCS differed ($P < 0.05$) across all locations; however, BCS was not different ($P > 0.10$) by treatment across all locations in Exp. 1. Mean BCS for Exp. 2 for are reported by treatment (Table 3.3). Body condition score was not different ($P > 0.10$) by treatment in Exp. 2.

The 6-10 cycle group had higher ($P = 0.02$) pregnancy rate to TAI (84.6%) than the 11-16 (41.2%) and 17-21 (30.0%) cycle groups but did not have a higher ($P = 0.18$) pregnancy rate to TAI compared to the 0-5 cycle group (63.6%; Table 3.4). The improved pregnancy rates of the 6-10 cycle group could be due to an improved response to d 0 GnRH. Heifers on d 5 of their cycle had a 95% response rate to GnRH (Moreira et al., 2000; Atkins et al., 2008), and is likely due to the growing first wave dominant follicle's ability to respond to GnRH by ovulating.

Ten of the 13 heifers in the 6-10 cycle group were on d 6-8 of their estrous cycle when the protocol began. These heifers would likely have a growing dominant follicle that could respond to GnRH, thus synchronizing follicular growth to allow for improved response to TAI.

The 30.0% pregnancy rate to TAI in the 17-21 cycle group could be attributed to heifers with 3 waves of follicular growth. The growth of these heifer's second dominant follicle will cease by d 18 (Atkins et al., 2008), thus reducing their ability to ovulate to d

0 GnRH and initiate a fresh wave of follicular growth. Unfortunately, follicular growth patterns in heifers were not characterized prior to initiation of the experiment.

The 14-d PG treatment had a larger (P = 0.01; Table 3.5) average follicle size on d 9 than 14-d GnRH-9 but not (P = 0.31) 5-d CO-Synch + CIDR. The 14-d PG treatment had the most (P = 0.01) ≥ 10 mm follicles among treatments on d 9 (Table 3.4). This could be due to the absence of a CL from d 0 to 9, creating a lower progesterone environment with increased secretions of LH (Kinder et al., 1996) that caused increased secretion of17-ß Estradiol which led to increased follicular growth.

Giving $PGF_{2\alpha}$ to beef heifers 3 d prior to GnRH and CIDR created larger follicles 2 d later and increased levels of estradiol and increased LH pulse frequency (Grant et al., 2011). Unfortunately LH levels were not measured in this study.

Progesterone levels under 3 ng/ml increased LH pulse frequency and mean LH levels compared with greater progesterone concentrations (10.2 ng/ml) in the mid luteal phase (Kojima et al., 1992). Expression of mRNA's encoding for LH receptors were first found in granulosa cells 36 h after the first follicular wave and increased with follicular size and stage of follicular wave (Bao et al., 1997). Increased LH receptors were found on granulosa and theca cells of persistent follicles compared with healthy dominant follicles (Cupp et al., 1993). The increase in LH receptors could have been due to the increased frequency of LH release in a cow with persistent follicles (Kinder et al., 1996). Luteinizing hormone pulses are also required for adequate luteal development in cattle (Niswender et al., 2000).

Because of this potential relationship between LH pulses, LH receptors, and luteal development, $PGF_{2\alpha}$ was given on d 0 to lyse any pre-existing CL and reduce

progesterone levels facilitate increased LH pulses. This increased exposure to LH would hypothetically increase the number of LH receptors present on granulosa cells which would allow for a faster luteinization of granulosa cells when GnRH was given on d 9. A more completely luteinized CL would then be more responsive to $PGF_{2\alpha}$ given on d 14.

We hoped response to d 9 GnRH would clarify the effects of giving $PGF_{2\alpha}$ on d 0. However, the percentage of 14-d PG heifers with a successful response to d 9 GnRH (36.6%) was not different ($P = 0.38$; $P = 0.39$) from the successful responses of the14-d GnRH-9 and 5-d CO-Synch + CIDR treatments, respectively. These results differ from previous data showing $PGF_{2\alpha}$ administration prior to GnRH and CIDR increases initiation of a new follicular wave due to GnRH 3 d later (Grant et al., 2011).

It is possible more heifers responded to d 9 GnRH and initiated a new wave of follicular growth than indicated by the successful response category of response to d 9 GnRH. Because heifers were not ultrasounded more frequently throughout the study, the absence of a d 9 follicle combined with the presence of a new CL on d 14 on the same ovary was used instead of comparing follicle sizes on d 9 and 14 to determine response to d 9 GnRH.

The 14-d PG treatment produced smaller ($P = 0.01$) follicles on d 14 than the 14-d GnRH-9 treatment and tended ($P = 0.07$) to produce smaller d 14 follicles than the 5-d CO-Synch + CIDR treatment. The 14-d PG treatment's variation in d 14 follicle size tended to be lower ($P = 0.11$; $P = 0.35$) than the 14-d GnRH-9 and 5-d CO-Synch + CIDR treatment, respectively. Variation in follicular size on d 6 was lower ($P = 0.03$) in heifers receiving $PGF_{2\alpha}$ on d 0 (Grant et al., 2011) and could be due to the increased response to GnRH causing a more synchronized wave of follicular growth.

In the 5-d CO-Synch + CIDR treatment, 96.6% of heifers formed a CL by d 14, compared to 90.6% and 84.4% in the 14-d PG and 14-d GnRH-9 treatments respectively. There were no differences ($P > 0.10$) in d 14 CL presence between treatments. The 14-d PG group was expected to have the highest incidence of corpora lutea by d 14 in this comparison, due to a faster rate of luteinization on d 9, however 90% of heifers with a CL does support the idea that complete luteinization did occur.

Ten percent of heifers in the 14-d PG and 5-d CO-Synch + CIDR treatments had a CL present at TAI compared with only 4.9% of 14-d GnRH-9 heifers. The numerically higher ($P = 0.37$) incidence of corpora lutea in the 14-d PG treatment relative to 14-d GnRH-9 contradicts our hypothesis that a faster rate of lutenization on d 9 due to increased LH exposure would create a more responsive CL to $PGF_{2\alpha}$ on d 14.

Follicles < 10.7 mm and > 15.7 mm did not result in more pregnancies compared to 12.5 mm follicles (Perry et al., 2007). In the current study, 14-d PG had a lower ($P < 0.01$) percentage of 11-15 mm follicles than the 5-d CO-Synch + CIDR treatment and tended to have a lower ($P = 0.07$) percentage of these follicles than the 14-d GnRH-9 treatment (Table 3.6). Interestingly, the pregnancy rate to TAI of 14-d PG follicles ≥ 16 mm was greater ($P = 0.05$) than the 14-d GnRH-9 treatment and tended to be greater ($P = 0.11$) than the 5-d CO-Synch + CIDR. Typically, the fertility of larger follicles is lessened because of an extended period of dominance (Mihm et al., 1996).

Giving $PGF_{2\alpha}$ on d 0 of a 14 d CIDR treatment likely increased LH secretions which created larger follicles on d 9 but did not improve response to d 9 GnRH compared to the other 2 treatments. Pregnancy rate to TAI for the 14-d PG treatment was not different ($P = 0.82$) from the 14-d GnRH-9 treatment across all locations. However, the

high number of corpora lutea at TAI questions whether quicker luteinization and a more responsive CL to $PGF_{2\alpha}$ resulted from this treatment. Further hormone analysis that measures progesterone levels before and after d 9 GnRH is required to more fully understand this treatment.

Sixty six percent (19/30) of the 14-d GnRH-9 treatment at location 1 responded to d 0 GnRH and initiated a new wave of follicular growth. Past research indicates response to d 0 GnRH ranging from 48 to 58% (Moreira et al., 2000; Atkins et al., 2008). However, only 25.9% of 14-d GnRH-9 heifers successfully responded to d 9 GnRH by ovulating a d 9 follicle and creating a new CL by d 14. This poor response would mean persistent follicles were present and pregnancy rates to TAI would be impaired. However, the combined pregnancy rate to TAI at locations 1 and 2 for 14-d GnRH-9 was 53.6% (22/41), indicating that more heifers may have truly responded to d 9 GnRH than indicated by our successful response category in this experiment.

Pregnancy rates to TAI for 14-d GnRH-9 was not different from 14-d PG at location 1 ($P = 0.34$), location 2 ($P = 0.53$), location 3 ($P = 0.89$), location 4 ($P = 0.35$), or across all locations ($P = 0.82$; Table 3.7). Similarly, combining the 14-d GnRH-9 treatment and 14-d PG treatment did not improve pregnancy rates to TAI compared with the 5-d CO-Synch + CIDR. No difference was present at location 1 ($P = 0.72$), location 2 ($P = 0.89$), location 3 ($P = 0.17$), location 4 ($P = 0.73$), or across all locations ($P = 0.66$).

One reason for the lack of improvement due to 14-d treatments could have been their inability to improve pregnancy rates to TAI in non-cycling heifers relative to the 5-d control. There was no difference ($P > 0.10$) between pregnancy rates to TAI of cycling and non-cycling heifers between treatments combined across locations 1 and 2 (Table

3.8) in Exp. 1. Fourteen d CIDR duration was used in the study to induce puberty in non-cycling heifers because administration of progestins induce cyclicity by increasing LH production (Anderson et al., 1996). But, pregnancy rates to TAI of non-cycling heifers were not greater ($P = 0.59$) in the 14-d treatments relative to the 5-d CO-Synch + CIDR. However, sample sizes across treatments and cycling groups were small, which could have impeded the ability of the experiment to detect small differences.

Another potential explanation for the lack of improvement in pregnancy rates to TAI in the 14-d GnRH-9 treatment could have been due to follicle size on breeding day. Only 17.1% of 14-d GnRH-9 heifers across locations 1 and 2 had a follicle in the ideal size range of 11-15 mm, according to Perry et al (2007). This small proportion of desirable follicles could have impaired pregnancy rates. Nevertheless, the 14-d PG and 5-d CO-Synch + CIDR treatments only had 5.0% and 17.9% respectively of their breeding follicles between 11 and 15 mm.

Prolonged progestin exposure impedes follicular waves through development of persistent follicles (Sirois and Fortune, 1990). Therefore the rationale behind the d 9 GnRH in the 14-d GnRH-9 treatment was to force ovulation of any potentially persistent follicles that may have formed as well as to create a second synchronized wave of follicular growth. Only 25.9% of 14-d GnRH-9 heifers had a successful response to d 9 GnRH across locations 1 and 2. This is lower than the 61% (Atkins et al., 2008) ovulatory response to GnRH given 7 d after GnRH and CIDR insertion on d 0. Daily ultrasound throughout the protocol is required in the future to accurately determine the precise response to d 9 GnRH, but given this data, d 9 GnRH appears to be only moderately effective in creating a second wave of follicular growth.

Follicle sizes on d 14 were compared between heifers pregnant to TAI and open heifers (Figure 3.3). Follicles that were 12.5 mm at TAI supported the most pregnancies (Perry et al., 2007), and heifers with 2 waves of follicular growth had a growth rate 1.4 mm/d of the ovualtory follicle (Knopf et al., 1989). Given these figures, we hypothesize that a 7 mm follicle on d 14 will produce a 12.5 mm follicle at TAI and increase pregnancy rates to TAI, and that heifers pregnant to TAI will have a larger proportion of 7 mm follicles on d 14. However, there was no ($P = 0.53$) difference in average follicle size on d 14 between TAI pregnant (10.7 mm) and non-pregnant heifers (10.4 mm) and no ($P = 0.30$) difference in proportion of ≥ 10 mm follicles on d 14 between TAI pregnant and open heifers.

There was large variation in follicle size on d 17, with follicles ranging from 5 to 23 mm. This is consistent with other studies indicating the ovulatory follicle in beef cows at time of AI ranging from 9 to 20 mm (Perry et al., 2005) and 7.7 to 18.2 mm in cycling beef cows (Atkins et al., 2010). In this study the average follicle size at breeding for TAI pregnant heifers across all treatments (15.1 mm) was not different ($P = 0.62$) than the average follicle size at breeding for open heifers (15.5 mm).

Given the previously mentioned research indicating the improvement in pregnancy rates produced by 11-15 mm follicles, we would assume that heifers pregnant to TAI would have a larger proportion of these follicles at TAI. However, there was no difference ($P = 0.13$) in the percentage of ≥ 11 mm follicles at TAI in heifers pregnant to TAI (84.0%) compared to ≥ 11 mm follicles at TAI in heifers that were not pregnant to TAI (74.0%; Figure 3.4). Concentrations of estradiol are mediated through ovulatory follicle size (Perry et al., 2007), therefore follicle size can be a good predictor of fertility,

but in this study follicle size distributions were not different between TAI pregnant and open heifers.

Experiment 2

There was no difference ($P = 0.75$) in pregnancy rate to TAI (Table 3.9) or Estrotect patch score ($P > 0.10$; Table 3.10) between 14-d GnRH-9 and 14-d GnRH-7 treatments in Exp. 2. Moving the second GnRH from d 9 to 7 did not improve pregnancy rates to TAI over the 14-d GnRH-9 treatment, however, the similarity in pregnancy rates to TAI between 14-d treatments in Exp. 2 offers evidence that producers can save pharmaceutical cost by only giving 25 mg of $PGF_{2\alpha}$ at CIDR removal with the 14-d GnRH-7 treatment.

The reason for this similarity might be due to an older CL produced by the 14-d GnRH-7 treatment due to the administration of GnRH 2 d earlier on d 7 instead of d 9. A 25 mg injection of $PGF_{2\alpha}$ causes luteolysis of a CL if given on d 6-16 of the estrous cycle (Lauderdale, 1972; Rowson et al., 1972; Odde, 1990), therefore 25 mg of $PGF_{2\alpha}$ given on d 14 should have effectively lysed the CL formed after d 7 GnRH admistration in 14-d GnRH-7 heifers. This is in contrast to the younger CL in the 14-d GnRH-9 treatment that needs additional $PGF_{2\alpha}$ in order to ensure complete luteal regression.

The combined 14-d treatments resulted in fewer ($P < 0.01$) Estrotect patches with film that was not disturbed and more ($P < 0.01$) Estrotect patches with film that was completely or almost completely removed compared with the 7-d CO-Synch + CIDR treatment (Table 3.10). However, this encouraging estrus response did not correlate to improved pregnancy rates in the 14-d treatments as the pregnancy rate to TAI for the 14-

d treatments combined (49.6%) was not different ($P = 0.83$) from the 7-d CO-Synch + CIDR treatment (48.6%).

The 14-d treatments in Exp. 2 had an increased estrus response than the 7-d CO – Synch + CIDR treatment yet did not produce improve pregnancy rates to TAI. This could be evidence that the interval from CIDR removal to TAI was not ideal. This could be true for heifers in the 14-d GnRH-9 treatment as their mean interval from CIDR removal to TAI was 69.5 ± 3.50 h, lower than the targeted 72 h interval. But breeding interval was not significant ($P = 0.16$) and was not included in the final statistical model.

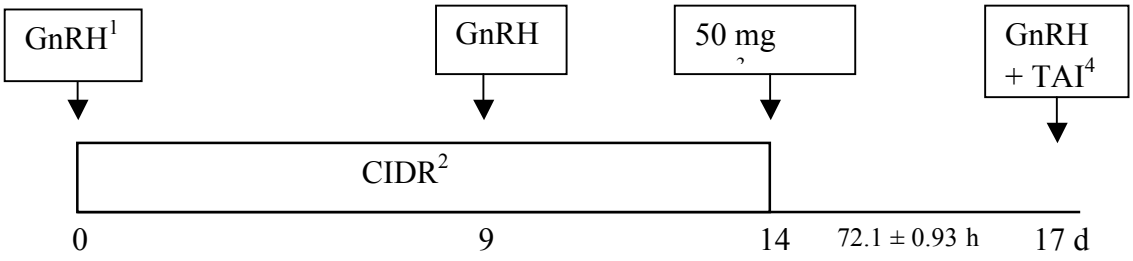
The use of ultrasonography in Exp. 1 showed the 14-d GnRH-9 treatment was marginally effective in inducing a second wave of follicular growth and had the lowest percentage of corpora lutea at TAI, but percentage of ideal 11-15 mm follicles was not increased relative to other treatments. The ability of the 14-d PG treatment to produce larger d 9 follicles is encouraging, however the lack of improvement in response to d 9 GnRH and greater incidence of corpora lutea at TAI indicates the original hypothesis of creating a more responsive CL to $PGF_{2\alpha}$ did not occur. Further progesterone analysis that measured progesterone levels before and after d 9 GnRH and d 14 $PGF_{2\alpha}$ would increase understanding of the luteinization process occurring in the 14-d PG treatment, as well as daily ultrasound analysis for 14-d GnRH-9 to more accurately determine response to d 9 GnRH.

IMPLICATIONS

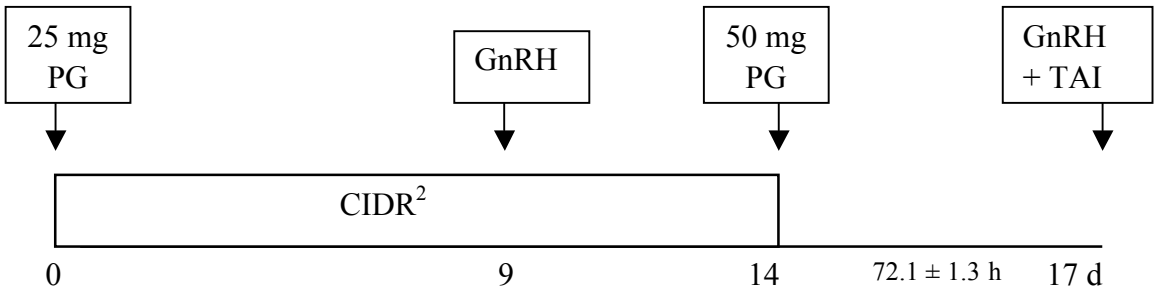
In both experiments, the modified 14-d treatments did not produce more pregnancies resulting from TAI than the 14-d GnRH-9 treatment. Similarly, the combined 14-d treatments in both experiments did not improve pregnancy rates to TAI over either control treatment. Because of the additional cost required by the 14-d treatment to handle heifers on d 9, the 5 or 7-d CO Synch + CIDR remains a better alternative for TAI than the 14-d treatment evaluated in this study.

Figure 3.1. Estrous synchronization treatments administered to beef heifers in Exp. 1

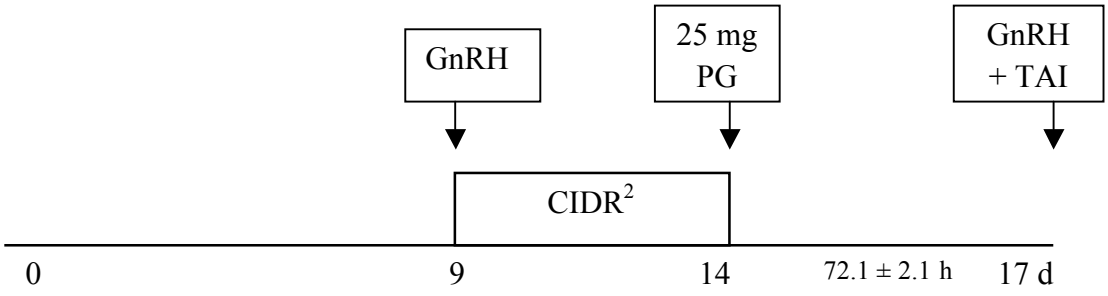
Treatment: 14-d GnRH-9



Treatment: 14-d PG



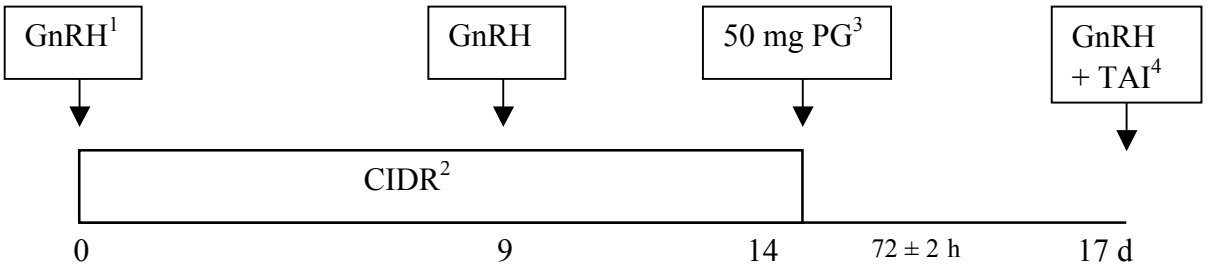
Treatment: 5-d CO-Synch + CIDR



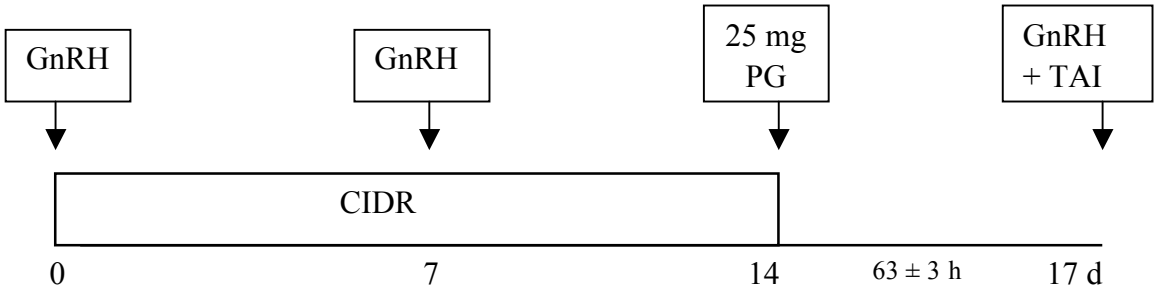
[1] GnRH: 100 μg given im of the GnRH analogue, (Factrel Fort Dodge Animal Health),
[2] CIDR: controlled internal drug release device, 1.38 g of progesterone (EAZI-BREED™ CIDR®, Pfizer Animal Health
[3] PG: prostaglandin $F_{2\alpha}$ given im (Lutalyse, Pfizer Animal Health)
[4] TAI: Timed AI

Figure 3.2. Estrous synchronization treatments administered to beef heifers in Exp. 2

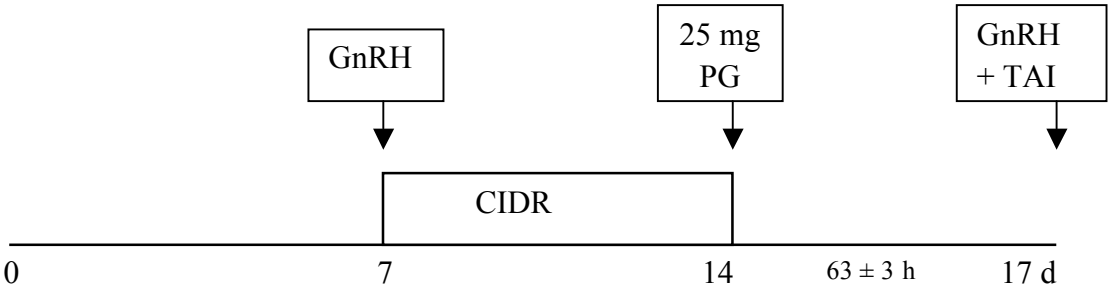
Treatment:14-d GnRH-9



Treatment: 14-d GnRH-7

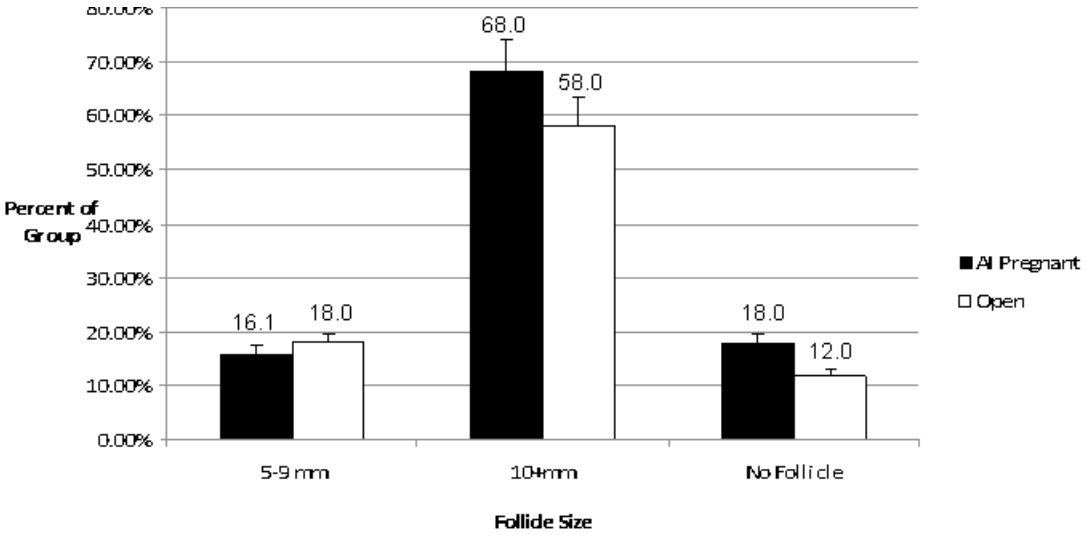


Treatment: 7-d CO-Synch + CIDR



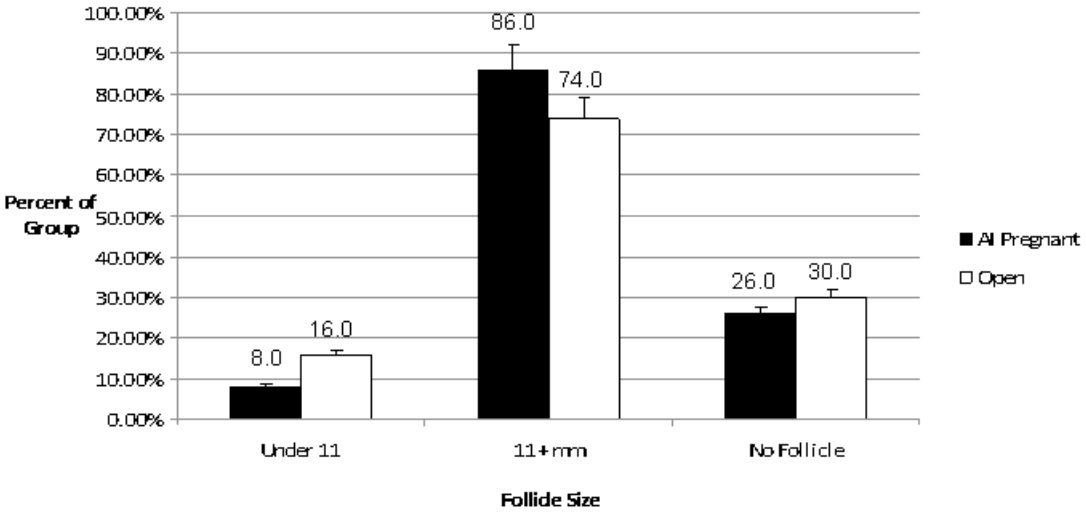
[1] GnRH: 100 μg given im of the GnRH analogue, Factrel (Fort Dodge Animal Health),
[2] CIDR: (Controlled Internal Drug Release Device, 1.38 g of progesterone (EAZI-BREED™ CIDR®, Pfizer Animal Health)
[3] PG: Prostaglandin $F_{2\alpha}$ given im (Lutalyse, Pfizer Animal Health)
[4] TAI: Timed AI

Figure 3.3. Distribution of follicle sizes at CIDR removal (d 14) for heifers that became pregnant or not pregnant to timed AI in Exp. 1.



No differences ($P > 0.10$)

Figure 3.4. Distribution of follicle sizes at timed AI (TAI) in heifers that became pregnant or not pregnant to TAI in Exp. 1



No differences ($P > 0.10$)

Table 3.1.. Number, BW, BCS, and overall pregnancy rate to timed AI (TAI) of beef heifers in Exp. 1 by locations (LS means ± SE)

Location	n =	BW (kg)	BCS [1]	Percent pregnant to TAI
1	89	339 [b] ± 3.4	4.7 [a] ± 0.05	40.9 ± 7.75
2	31	401 [c] ± 5.8	5.8 [d] ± 0.09	54.8 ± 12.75
3	161	318 [a] ± 2.5	5.1 [b] ± 0.04	52.6 ± 5.74
4	38	319 [a] ± 5.2	5.3 [c] ± 0.08	39.6 ± 9.71

[a-d] within a column, means without common superscripts differ ($P < 0.05$)

[1] Body condition was evaluated using the 9-point scale (1 = thin, 9 = obese; Richards et al., 1986)

Table 3.2. Number, BW, and BCS of beef heifers by estrous synchronization treatment in Exp. 1 (LS means ± SE)

	Treatment		
Variable	14-d GnRH-9[1]	14-d PG[2]	5-d CO-Synch + CIDR[3]
n =	107	107	104
BW (kg)	320 ± 3.7	332 ± 3.7	334 ± 3.8
BCS	5.1 ± 0.06	5.1 ± 0.05	5.1 ± 0.06

No differences ($P > 0.10$)

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.

[2] Heifers received a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 0 and d 9, 50 mg $PGF_{2\alpha}$ on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.

[3] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration

Table 3.3. Number, BW, and BCS of beef heifers by estrous synchronization treatment in Exp. 2 (LS means ± SE)

	Treatment		
Variable	14-d GnRH-9[1]	14-d GnRH-7[2]	7-d CO-Synch + CIDR[3]
n =	150	150	152
BCS	4.9 ± 0.03	4.9± 0.02	4.9 ± 0.06

No differences ($P > 0.10$)

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.

[2] Heifers received a CIDR from d 0 to 14, GnRH on d 0 and d 7, and 100 ug GnRH and TAI 60-66 h after $PGF_{2\alpha}$ administration.

[3] Heifers received a CIDR from d 7 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 60-66 after $PGF_{2\alpha}$ administration

Table 3.4. Pregnancy rates to timed AI (TAI) in beef heifers by day of estrous cycle upon protocol initiation in Exp. 1

	Treatment				
Cycle Group[1]	14-d GnRH-9[2] %	14-d PG[3] %	5-d CO-Synch + CIDR[4] %	All treatments %	SEM
0-5	50.0 (3/6)	71.4 (5/7)	66.6 (6/9)	63.6 [ab] (14/22)	10.21
6-10	66.6 (4/6)	100.0 (1/1)	100.0 (6/6)	84.6 [b] (11/13)	10.42
11-16	50.0 (4/8)	50.0 (3/6)	0 (0/3)	41.2 [a] (7/17)	12.30
17-21	50.0 (2/4)	0 (0/2)	25.0 (1/4)	30.0 [a] (3/10)	15.27

[ab] within a column, means without common superscripts differ ($P < 0.05$)

[1] Heifers were observed for estrus for 1 h twice a day, from d -21 to d 0 of the protocol. Cycle group 0-5: heifer was on or between d 0 and 5 of the estrous cycle upon protocol initiation, 6-10: heifer was on or between d 6 and 10 of the estrous cycle upon protocol initiation, 11-16: heifer was on or between d 11 and 16 of the estrous cycle upon protocol initiation, 17-21: heifer was on or between d 17 and 21 of the estrous cycle upon protocol

initiation.
[2] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 0 and d 9, 50 mg $PGF_{2\alpha}$ on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[4] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration

Table 3.5. Ovarian structures and responses between treatments combined across locations 1 and 2 for Exp.1

Variable	Treatment		
	14-d GnRH-9[1]	14-d PG[2]	5-d CO-Synch + CIDR[3]
Ovulation to d 0 GnRH (%)[4]	66.6 (20/30)	-	-
Absence of CL on d 3 (%)	-	66.6 (20/30)	-
Follicles Present on d 9 ≥ 10 mm (%)	57.6 [a] (19/33)	90.6 [b] (29/32)	64.5 [a] (20/31)
Successful response to d 9 GnRH[5] (%)	25.9 (7/27)	36.6 (11/30)	48.0 (12/25)
D 9 follicle size (mm)	10.5 [a] ± 0.64	12.8 [b] ± .61	11.9 [ab] ± 0.68
CL formed by d 14 (%)	84.4 (27/32)	90.6 (29/32)	96.6 (29/30)
D 14 follicle size (mm)	11.4 [a] ± 0.93	10.0 [b] ± 0.77	10.4 [ab] ± 0.83
D 17 CL presence (%)	4.9 (2/41)	10.0 (4/40)	10.2 (4/39)
Follicle Present on d 17 ≥ 12 mm (%)	65.8 (27/41)	57.5 (23/40)	76.9 (30/39)
D 17 follicle size (mm)	14.9 ± 1.22	14.7 ± 1.20	15.2 ± 1.02

[ab]Within a row, means without common superscripts differ ($P < 0.05$)

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[2] Heifers received a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 0 and d 9, 50 mg $PGF_{2\alpha}$ on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration
[4]Data only from location 1
[5]A successful response was defined as a heifer having a d 9 follicle that was absent on d 14 and replaced by a new corpus luteum

Table 3.6. Follicle characteristics and pregnancy rates between treatments combined across locations 1 and 2 for Exp. 1

	Treatment						
	14-d GnRH-9[1]		14-d PG[2]		5-d CO-Synch + CIDR[3]		
Follicle size	% of Treatment	% Pregnant	% of Treatment	% Pregnant	% of Treatment	% Pregnant	SEM
<11 mm Follicle	4.9 (2/41)	0.0 (0/2)	17.5[a] (7/40)	57.1 (4/7)	7.7 (3/39)	0.0 (0/3)	0.67
11-15.9 mm Follicle	31.7 (13/41)	61.5 (8/13)	15.0 [a] (6/40)	66.6 (4/6)	41.0 (16/39)	50.0 (8/16)	0.16
≥ 16 mm Follicle	34.1 (14/41)	35.7 (5/14)	42.5 [b] (17/40)	70.5 (12/17)	35.8 (14/39)	42.8 (6/14)	0.31
No follicle present	29.3 (12/41)	58.3 (7/12)	25.0 [b] (10/40)	40.0 (4/10)	15.4 (6/39)	50.0 (3/6)	-

[ab] within a column, means without common superscripts differ ($P < 0.01$)
[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[2] Heifers received a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 0 and d 9, 50 mg $PGF_{2\alpha}$ on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration

Table 3.7. Pregnancy rates to timed AI by estrous synchronization treatment by location for Exp.1

	Treatment				Contrast statistics[1] (P =)	
Location	14-d GnRH-9[2] %	14-d PG[3] %	5-d CO-Synch + CIDR[4] %	SE	14-d vs.5-d	14-d GnRH vs. 14-d PG
1	46.6 (14/30)	36.6 (11/30)	51.7 (15/29)	7.74	0.72	0.34
2	72.7 (8/11)	60.0 (6/10)	70.0 (7/10)	12.75	0.89	0.53
3	54.9 (28/51)	56.4 (31/55)	40.0 (22/55)	5.75	0.17	0.89
4	42.8 (6/14)	25.0 (3/12)	41.6 (5/12)	9.71	0.73	0.35
Overall[5]	52.3 (56/107)	47.6 (51/107)	47.1 (49/104)	2.78	0.66	0.82

[1]Contrast statements: 14-d vs. 5-d CIDR effect (combined 14-d GnRH-9 and 14-d PG treatments compared to 5-d CO Synch+ CIDR) and 14-d comparison (14-d GnRH-9 compared to 14-d PG).
[2] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 0 and d 9, 50 mg $PGF_{2\alpha}$ on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.
[4] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration
[5]There was no treatment × location interaction (P = 0.32), therefore data were pooled across locations.

Table 3.8. Pregnancy rates to timed AI (TAI) by cycling status between estrous synchronization treatments in Exp. 1

	Treatment						
	14-d GnRH-9[1]		14-d PG[2]		5-d CO-Synch +CIDR[3]		
Location	Cycling,[4] %	Non Cycling, %	Cycling, %	Non Cycling, %	Cycling, %	Non Cycling, %	SEM
1	47.1 (8/17)	46.1 (6/13)	50.0 (6/12)	27.7% (5/18)	58.8 (10/17)	41.6 (5/12)	5.30
2	70.0 (7/10)	100.0 (1/1)	60.0 (6/10)	-	60.0 (6/10)	100.0 (1/1)	8.53
Overall[5]	55.5 (15/27)	50.0 (7/14)	54.5 (12/22)	27.7% (5/18)	59.3 (16/27)	46.1 (6/13)	4.58

No differences ($P > 0.10$)

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.

[2] Heifers received a CIDR from d 0 to 14, $PGF_{2\alpha}$ on d 0 and d 9, 50 mg $PGF_{2\alpha}$ on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration.

[3] Heifers received a CIDR from d 9 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 72 h after $PGF_{2\alpha}$ administration

[4] A heifer was determined to be cycling if she was observed in standing estrus prior to protocol initiation or if a corpus luteum was present on one of her ovaries on d 0

[5]There was no treatment × location interaction ($P = 0.32$), therefore data were pooled across locations.

Table 3.9. Pregnancy rates to timed AI (TAI) in beef heifers by estrous synchronization treatment in Exp. 2

	Treatment						Contrast Statistics[1]	
	14-d GnRH-9[2], %		14-d GnRH-7[3], %		7-d CO-Synch + CIDR[4], %		14-d vs 7-d	GnRH-9 vs GnRH-7
Variable	%	SE	%	SE	%	SE		
% Pregnant to TAI	51.3 (77/150)	4.09	48.0 (72/150)	4.10	48.6 (73/152)	4.06	0.83	0.75

[1] Contrast statements: 14-d vs. 7-d CIDR effect (combined 14-d GnRH-9 and 14-d GnRH-7 treatments compared to 7-d CO Synch+ CIDR) and 14-d comparison (14-d GnRH-9 compared to 14-d GnRH-7).
[2] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[3] Heifers received a CIDR from d 0 to 14, GnRH on d 0 and d 7, and 100 ug GnRH and TAI 60-66 h after $PGF_{2\alpha}$ administration.
[4] Heifers received a CIDR from d 7 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 60-66 after $PGF_{2\alpha}$ administration

Table 3.10. Estrotect patch scores in heifers between estrous synchronization treatments for Exp. 2

	Treatment					Contrast statistics[1] (*P* =)
Patch score[2]	14-d GnRH-9,[3] %	14-d GnRH-7[4], %	7-d CO-Synch+CIDR[5], %	SE	14-d vs. 7-d	GnRH-9 vs. GnRH-7
1	19.3	17.3	28.2	7.66	<0.01	0.65
2	6.0	7.3	11.8	7.17	0.01	0.63
3	62.6	65.4	51.3	7.85	<0.01	0.69

[1]Contrast statements: 14-d vs. 7-d CIDR effect (combined 14-d GnRH-9 and 14-d GnRH-7 treatments compared to 7-d CO Synch+ CIDR) and 14-d comparison (14-d GnRH-9 compared to 14-d GnRH-7).
[2] Patch scores: 1 = patch film was not removed, 2 = approximately 50% of the patch film was removed, 3 = all or almost all of the patch film was removed.
[3] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) from d 0 to 14, 100 ug GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im on d 0 and d 9, 50 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) im on d 14, and 100 ug GnRH and timed AI (TAI) 72 h after $PGF_{2\alpha}$ administration.
[4] Heifers received a CIDR from d 0 to 14, GnRH on d 0 and d 7, and 100 ug GnRH and TAI 60-66 h after $PGF_{2\alpha}$ administration.
[5] Heifers received a CIDR from d 7 to d 14, GnRH on d 0, 25 mg $PGF_{2\alpha}$ im on d 14, and 100 ug GnRH and TAI 60-66 after $PGF_{2\alpha}$ administration

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CHAPTER IV

Comparing the lifetime productivity of beef females initially conceiving to, or sired by, artificial insemination or natural service*

SUMMARY: Artificial insemination offers substantial benefits to beef producers; however, little data exists quantifying the long-term effects of using AI on replacement heifers. Calving and breeding records (n = 6,693) at one location for 1,173 Angus females were obtained from 1991 to 2010. For Objective 1, heifers were classified as conceiving to AI or natural service (**NS**) as a yearling. The heifer's AI date was noted and a 290-d gestation length was added to create a cutoff date. Any heifer that calved before this date was classified as conceiving to AI as a heifer, and any heifer that calved after this cutoff date was classified as conceiving to NS as a heifer. For Objective 2, females were classified into 4 different dam groups if they were the result of: an AI pregnant heifer (H-AI), a NS pregnant heifer (H-NS), an AI pregnant cow (C-AI), or a NS pregnant cow (C-NS). Weaning weights from each female's calf were recorded annually until she left the herd. Historic price data was obtained from the nearest marketing center so that each calf could be assigned an economic value based upon their weaning weight.

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Values of a heifer's calves were summed to give a lifetime revenue value for each heifer. Four different price scenarios were used: actual market prices for each year, average price from 1991 to 2010, minimum prices, and maximum prices. Females that conceived to AI as a heifer had greater ($P < 0.0001$) lifetime weight weaned, lifetime calves weaned, lifetime revenue under all four price scenarios, and higher ($P < 0.05$) average annual weaning weight than females that conceived to NS as a heifer. There was no difference ($P > 0.10$) in average annual weaning weight, lifetime weight weaned, lifetime calves weaned, or lifetime revenue produced between the 4 dam groups. In summary, estrous synchronization and AI can be a valuable tool to produce replacement heifers that conceive earlier, have better genetics and have increased their lifetime productivity.
Key Words: Artificial insemination, Beef heifers, Estrous synchronization

INTRODUCTION

Utilizing AI and estrous synchronization offers many benefits to beef producers. Artificial insemination can provide access to elite genetics that would not otherwise be available to a breeding program, and using AI in combination with estrous synchronization can produce females that become pregnant earlier in the breeding season which in turn results in older and therefore heavier calves the following year, and provides for a longer postpartum recovery period (Dunn and Kaltenbach, 1980). Synchronizing estrous has also been shown to produce calves that were 13 d older and 9.5 kg heavier than females not synchronized (Schafer et al., 1990). Estrous synchronization and AI can result in a more concentrated calving season, in turn creating

a more uniform calf crop. Additionally, AI has potential to reduce bull maintenance and purchase costs (Johnson and Jones 2004; Ellis, 2005).

Several studies have evaluated AI compared to natural service (**NS**) from an economic perspective. Estrous synchronization and AI increased the percentage of cows calving in the first 30 d of the calving season the following year relative to NS. In turn, the program increased short-term revenue by $70 per head over NS by increasing weaning weight and reducing bull costs (Anderson and Deaton, 2003). Additionally, when increased weaning weight is accounted for in AI-sired calves, many estrous synchronization protocols with AI produce greater returns than NS (Johnson and Jones, 2004).

Despite these benefits, use of estrous synchronization and AI within the beef industry remains low, as only 7.6% of beef producers used AI in 2008 (USDA, 2009). One potential reason for this low implementation could be the lack of data documenting the long-term benefits of estrous synchronization and AI. The immediate benefit of early calving females has been well documented; heifers born early in the calving season have higher lifetime productivity than those born later (Lesmeister et al., 1973; Funston et al., 2011) and early calving cows also wean more pounds than late calving cows (Garcia et al., 1992). However, little has been published regarding the lifetime productivity of heifers that conceive to AI or are sired by an AI sire. Therefore, the objectives of the study were to compare lifetime productivity between heifers that conceived to AI and those that conceived to NS as a yearling, and to compare lifetime productivity between females that were the result of an AI mating with those that were the result of a NS mating.

MATERIALS AND METHODS

Data for the study included a historical database from the John E. Rouse Colorado State University Beef Improvement Center near Saratoga, Wyoming. Calving and breeding records were acquired from 1991 to 2010 and resulted in 6,693 records from 1,173 purebred Angus females. Weaning weights were also recorded for each cow annually until she left the herd.

Heifers were classified as conceiving to AI or NS as yearlings using calving and breeding records. The AI date from a given yr was determined and a 290-d gestation length was added to that date to create a cutoff date. Any heifer calving before this date was classified as conceiving to AI, and any heifer calving after this date was classified as conceiving to NS (Figure 4.1). We realize this determination method is robust, however pregnancy diagnosis results were not available throughout the entire dataset.

Females were classified as being sired by AI or NS using the same criteria described above. To determine whether a female was the product of an AI mating, the AI date from the previous year would be determined. A 290-d gestation length was then added to this AI date to create a cutoff date within the calving season. Any female born before this cutoff date was classified as being sired by AI, any female born after this date was classified as being sired by NS (Figure 4.2). For a histogram describing distribution of gestation lengths, see Figure 4.3.

Each year the ranch inseminated their heifers 3 to 4 wk prior to the cows, which led us to further classify heifers into 4 different dam groups. Based upon calving date, a

female could be produced by a heifer that conceived to AI (H-AI), a heifer that conceived to NS (H-NS), a cow that conceived to AI (C-AI), or a cow that conceived to NS (C-NS). This delineation was made to isolate the effects of heifer age on lifetime productivity. Postpartum interval was calculated for each yr a female was in the herd, and was determined as the difference between her calving date and when the estrous synchronization and artificial insemination protocol began.

Economic Analyses. Because the ranch typically weaned in October, average price data for the month of October was collected from the Torrington, Wyoming Livestock Commission from 1991 to 2010. Prices were collected for steers and heifers in the following weight divisions: 136 to 159 kg, 160 to 181 kg, 182 to 204 kg, 205 to 227 kg, 228 to 250 kg, 251 to 272 kg, and 273 to 295 kg. Prices were then multiplied by each calf's weaning weight to produce a value for every calf a heifer weaned. A female's lifetime revenue under the market price scenario used the actual prices for the yr a calf was produced. Lifetime revenue under the average price scenario used the average price from 1991 to 2010 for each weight division. Prices were not adjusted for inflation changes.

Prices were then adjusted to reflect market conditions with a maximum price difference, where there were substantial price differences between weight divisions, and a minimum price difference, where there were small price differences between weight divisions. The average price for the 182 to 204 kg weight division from 1991 to 2010 was used as a base price and did not change under maximum and minimum price difference conditions. Maximum prices were produced by determining the difference between each weight division and the average price for the 182 to 204 kg base price. This difference

was then multiplied by a factor of 2 and added to the base price. Minimum prices were produced the same way except differences were multiplied by a factor of 0.25 instead of 2. Mean, maximum, and minimum prices are presented in Table 4.1.

Statistical Analyses. Female yearling weight, age at first breeding, average calf weaning weight, lifetime weight weaned, lifetime calves weaned, postpartum interval, and lifetime revenue were analyzed as dependant variables using a generalized linear model via the GLM procedure in SAS (SAS Institute, Cary, NC). Fixed effects included the heifer's own yearling weight as a covariate and age at first breeding, as well as conception treatment (whether a heifer conceived to AI or NS as a yearling).

To evaluate productivity across the 4 dam groups, heifer yearling weight, age at first breeding, average calf weaning weight, lifetime weight weaned, lifetime calves weaned, postpartum interval, and lifetime revenue were analyzed as dependant variables using a generalized linear model via the GLM procedure in SAS (SAS Institute, Cary, NC). Fixed effects included the heifer's own yearling weight as a covariate and age at first breeding, as well as dam treatment (whether a heifer was H-AI, H-NS, C-AI, C-NS).

RESULTS AND DISCUSSION

Females that conceived to AI as a yearling were older and heavier ($P = 0.02$) than females that conceived to NS. Females that conceived to AI also had a greater ($P = 0.04$) average weaning weight, weaned more ($P < 0.0001$) pounds and more ($P < 0.0001$) total calves than females that conceived to NS as a yearling (Table 4.2). The average weaning

weight for a female that originally conceived to AI was 5 kg greater than a female that originally conceived to NS. Because heifers were synchronized as part of university research, bulls were exposed approximately 10 d after AI in order to differentiate pregnancies resulting from AI or NS at pregnancy diagnosis. Average daily gain for calves between 106 and 273 d of age was between 0.67 and 0.72 kg per d (Dunn and Kaltenbach, 1980). Therefore, in the current study, the difference in average weaning weight of calves from females that conceived to AI or NS could be attributed to the increased age of AI sired calves relative to NS calves.

One of the facility's goals was to produce seedstock bulls specifically adapted to high altitude environments. Because of this, ranch management inseminated heifers to bulls produced by the ranch and then used these same bulls via NS on their heifers. This facilitated genetic improvement of their herd while also utilizing sires that could perform in their environment. This decision to AI heifers to the same bulls used for NS is noteworthy because it reduces some of the benefit of using elite genetics through AI, but also reduces the risk of introducing genetics that are not adapted to high altitude environments. The average weaning weight of calves from females conceiving to AI would likely be even higher if outside sires with improved genetics for growth had been utilized, but also would have increased the risk of mortality in offspring associated with non-adapted genetics.

Conceiving to AI rather than NS also allowed females to wean an additional 438 additional kg and 2 calves over the course of their productive life (Table 4.2). This difference could be attributed to increased postpartum recovery for the first calf heifer as a 2 yr old resulting from an earlier conception date as a yearling. First calf heifers have

longer postpartum intervals than mature cows (Wiltbank, 1970; Bellows and Short, 1978), therefore the earlier a heifer can conceive and calve the following yr, the higher likelihood she has of cycling and becoming pregnant after calving and remaining in the herd.

The increase in production resulting from AI bred heifers highlights the importance of maximizing the number of females pregnant early in the breeding season. Synchronizing estrous in beef cows produced calves that were 13 d older and 9.5 kg heavier than non synchronized females (Schafer et al., 1990). The increased weaning weight for calves and postpartum recovery for heifers implied by this study illustrates the benefits that estrous synchronization offers beef producers.

Although not significantly different ($P = 0.67$), females that conceived to AI as a heifer had an average postpartum interval 5 d longer over their lifetime compared to those that conceived to NS. This difference could be attributed to earlier conception as a yearling, which results in increased postpartum recovery as a 2 yr old. The increased ($P = 0.67$) postpartum interval relative to females conceiving to NS could also explain the difference in lifetime calves weaned between females originally conceiving to AI or NS.

Females that conceived to AI as a yearling had greater ($P < 0.0001$) lifetime revenue than those that conceived to NS (Table 4.3). The largest revenue difference between female groups occurred under the minimum price difference scenario where revenue for heifers conceiving to AI was $974 greater ($P < 0.0001$) than heifers who conceived to NS. The smallest revenue difference occurred under the actual price scenario where revenue for heifers conceiving to AI was $922 greater ($P < 0.0001$) than heifers who conceived to NS. Because heifers conceiving to AI weaned 438 kg more, we

conclude the increased lifetime revenue under all 4 price scenarios between the 2 heifer groups are attributed to weaning weight differences and are independent of market conditions.

The number of heifers that conceived to AI (n = 871) and heifers that conceived to NS (n = 302) is clearly unbalanced. This was due to management decisions that emphasized selecting as many AI bred heifers as replacements as possible starting in the late 1990's and was beyond our control. Yet every year there were females that conceived to NS, so within every yr both groups were represented.

Heifers from cows that conceived to NS (C-NS) had the lowest ($P < 0.0001$) average age at first breeding and average yearling weight among dam groups. This is logical as these females were born toward the end of the calving season, making them younger and lighter at AI as yearlings. No difference ($P > 0.10$) was found in average weaning weight, lifetime weight weaned, lifetime calves weaned, or postpartum interval between the 4 different dam groups (Table 4.4).

Females from first calf heifers that conceived to AI (H-AI) had the highest ($P > 0.10$) average weaning weight and C-NS females produced the lowest ($P > 0.10$) average weaning weight. This is understandable as females born in the first third of the calving season (H-AI in this study) would be oldest at AI, would have the highest likelihood of cycling at AI, and would have the oldest and heaviest calves at weaning (Funston et al., 2011). However, there was no difference ($P = 0.24$) in average weaning weights between H-AI and C-NS females.

Given these data it is hypothesized that H-AI females would have the greatest lifetime weight weaned and calves weaned and C-NS females would have the lowest

lifetime weight weaned and fewest calves weaned, indicating a positive relationship between heifer age and lifetime production. However, there no differences ($P > 0.10$) in lifetime weight weaned or calves weaned between the 4 dam groups. No differences ($P > 0.10$) in lifetime revenue were present between dam groups in any of the 4 price scenarios (Table 4.5). This is likely due to the lack of difference in lifetime weight weaned between dam groups.

Pre-breeding rate of gain has a reduced impact on heifer pregnancy rate (Funston and Deutscher, 2004) which suggests the importance of heifer age in pre-breeding cycling status and ultimate pregnancy rate. Interestingly, in this study, yearling weight rather than heifer age was significant ($P < 0.01$) and was included in the final model for analyzing differences in weaning weight and revenue. Though not confirmed by this study, the importance of heifer age on pregnancy rate could encourage utilization of estrous synchronization to produce earlier born (older) replacement heifers. Further data regarding cycling status and pregnancy rates between the 4 dam groups would be valuable in supporting previous research on the effect of heifer age on lifetime productivity.

IMPLICATIONS

The use of estrous synchronization and AI can produce calves with superior genetics and replacement heifers with increased postpartum recovery periods as 2 yr olds to prevent fallout, thus significantly increasing lifetime production. Estrous synchronization with AI should be considered an effective management tool to produce

replacements that are older at breeding, who become pregnant early in the breeding season, and consist of superior genetics.

Figure 4.1. Determination of whether a heifer conceived to AI or natural service (NS)

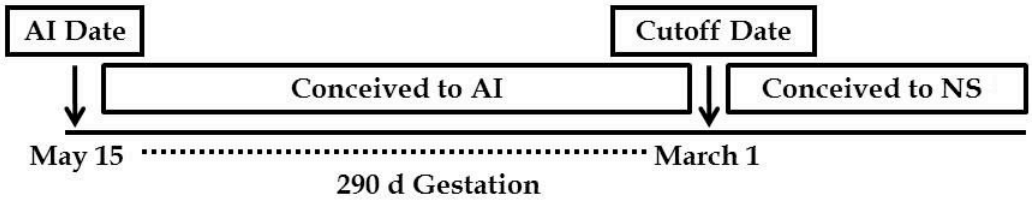

Figure 4.2. Determination of whether a female was sired by AI or natural service (NS)

Figure 4.3. Distribution of gestation length for all females

Figure includes data from 1,173 females from 1991 to 2010

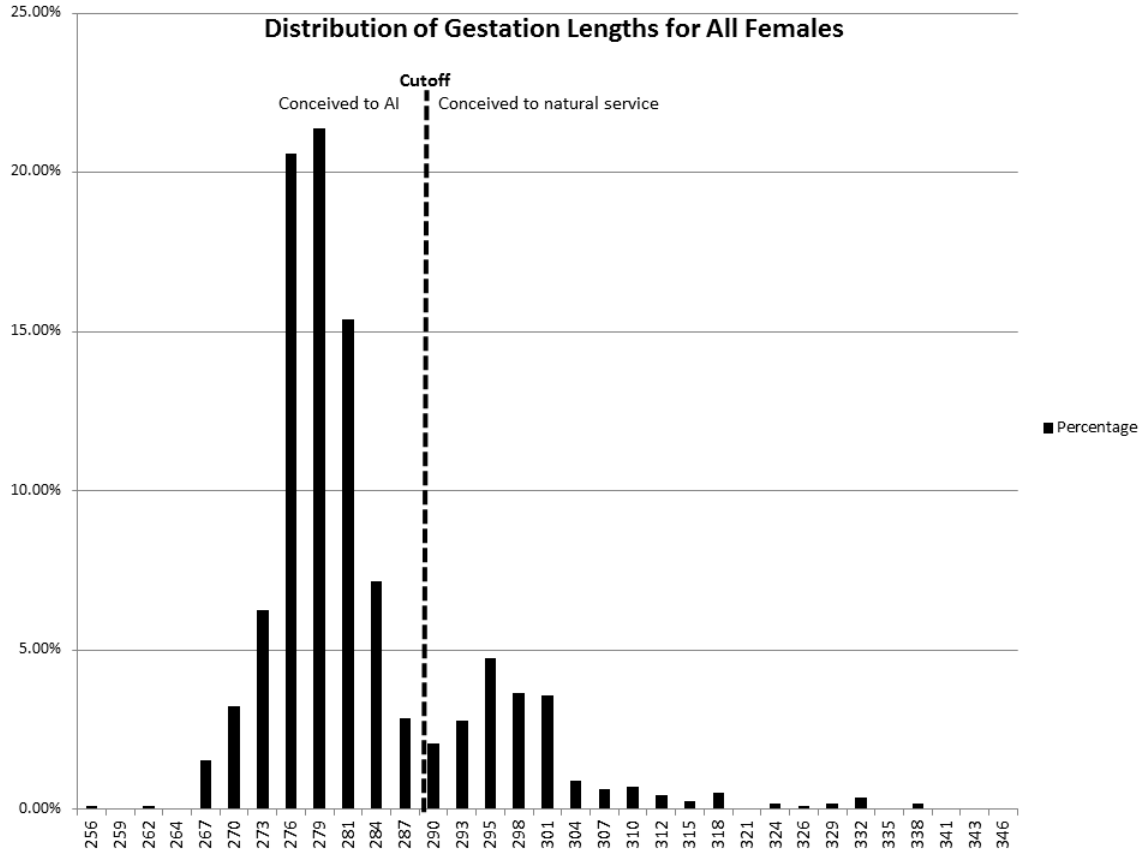

Table 4.1. Minimum, maximum, and mean prices used to calculate lifetime revenue

	Steer prices ($/cwt)			Heifer prices ($/cwt)		
Weight Division (kg)	Min price	Avg. price	Max. price	Min price	Avg. Price	Max. price
136-159	109.00	120.18	135.07	97.70	106.18	117.49
160-181	107.75	115.15	125.03	96.75	102.38	109.88
182-204	106.84	111.51	117.74	95.72	98.23	101.57
205-227	105.28	105.28	105.28	94.88	94.88	94.88
228-250	104.03	100.28	95.28	94.14	91.91	88.94
251-272	102.23	93.10	80.92	93.65	89.98	85.08
273-295	101.36	89.62	73.96	92.76	86.40	77.92

Price data obtained from Torrington, WY Livestock Commission from 1991 to 2010

Table 4.2. LS Means ± SE for weaning weight, lifetime weight weaned, calves weaned, and postpartum interval for females that conceived to AI or natural service (NS)

	n =	Average yearling weight (kg)	Average age at 1st AI (d)	Average weaning weight (kg)	Lifetime weight weaned (kg)	Lifetime calves weaned	Average postpartum interval[1] (d)
Conceived to AI	871	309[b] ± 1.8	429[b] ± 1.6	210[b] ± 1.0	1,072[f] ± 23.8	5.2[f] ± 0.11	92 ± 5.1
Conceived to NS	302	300[a] ± 3.2	418[a] ± 2.7	205[a] ± 1.8	634[e] ± 43.1	3.0[e] ± 0.20	87 ± 10.2

[a-d] Means within a column without a common superscript differ ($P < 0.05$)

[ef] Means within a column without a common superscript differ ($P < 0.0001$)

[1] Postpartum interval defined as the number of days between a female's calving date, and when AI occurred

Table 4.3. LS Means ± SE for lifetime revenue produced from females that conceived to AI or natural service (NS)

		Lifetime revenue produced ($) per female			
	n =	Actual price	Average price	Maximum price	Minimum price
Conceived to AI	871	2,483[a] ± 56.6	2,334[a] ± 51.3	2,302[a] ± 50.4	2,359[a] ± 52.0
Conceived to NS	302	1,561[b] ± 96.9	1,376[b] ± 92.8	1,364[b] ± 91.2	1,385[b] ± 94.3

[ab] Means within a column without a common superscript differ ($P < 0.0001$)

Table 4.4. LS Means ± SE for weaning weight, lifetime weight weaned, calves weaned, and postpartum interval for heifers that were sired by AI or natural service (NS)

Dam group [1]	n =	Average yearling weight (kg)	Average age at 1st AI (d)	Average weaning weight (kg)	Lifetime weight Weaned (kg)	Lifetime calves weaned	Average postpartum interval (d)
H-AI	195	$308^{a} \pm 3.9$	$450^{a} \pm 3.2$	210 ± 2.4	974 ± 57.3	4.6 ± 0.26	88 ± 10.2
H-NS	40	$299^{ab} \pm 8.1$	$421^{b} \pm 7.1$	209 ± 4.6	870 ± 111.7	4.2 ± 0.54	88 ± 22.9
C-AI	618	$314^{a} \pm 1.0$	$427^{b} \pm 1.8$	209 ± 1.2	966 ± 29.7	4.7 ± 0.14	87 ± 5.8
C-NS	320	$293^{b} \pm 2.9$	$403^{c} \pm 2.5$	207 ± 1.8	989 ± 43.0	4.7 ± 0.20	84 ± 8.3

[1] H-AI – females out of a heifer who conceived t
o AI, H-NS – females out of a heifer who conceived to NS, C-AI – females out of a cow that conceived to AI, C-NS – females out of a cow who conceived to NS

[a-c] Means within a column without a common superscript differ ($P < 0.0001$)

Table 4.5. LS Means ± SE for lifetime revenue produced for females in Exp. 4 that were sired by AI or natural service (NS)

		Lifetime revenue produced ($) per female			
Dam group[1]	n =	Actual price	Average price	Maximum price	Minimum price
H-AI	195	2,223 ± 136.5	2,124 ± 124.0	2,083 ± 121.8	2,155 ± 125.9
H-NS	40	1,949 ± 265.5	1,901 ± 240.6	1,878 ± 236.3	1,917 ± 244.3
C-AI	618	2,253 ± 70.9	2,092 ± 64.4	2,068 ± 63.2	2,110 ± 65.4
C-NS	320	2,313 ± 102.2	2,168 ± 92.5	2,139 ± 90.9	2,188 ± 93.9

[1] H-AI – females out of a heifer who conceived to AI, H-NS – females out of a heifer who conceived to NS, C-AI – females out of a cow that conceived to AI, C-NS – females out of a cow who conceived to NS

No differences ($P < 0.05$)

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APPENDIX I

SAS Code for Chapter II

Code for analyzing TAI pregnancy rates:

```
proc glimmix noclprint=20 data = complete2;
class id loc trt;
model preg_(ref=first) = loc|trt / dist=binary link=logit;
lsmeans loc|trt slicedif = loc ilink adj=tukey;
ods output lsmeans = trt123;
run;
ods output close;
```

Code for analyzing TAI pregnancy by sire:

```
Proc glimmix noclprint = 20 data=complete2;
Class id loc trt sire breeder hp;
Model preg(ref=first) loc|trt /  weight hp bcs sire(loc) breeder dist=binary link=logit;
Lsmeans loc*trt/ slicediff = loc ilink adj=tukey;
Lsmeans loc trt / ilink adj=tukey;
slice sire(loc)/sliceby loc diff ilink adj=tukey;
run;
```

SAS Code for Chapter III

Code for analyzing TAI pregnancy rates in Exp. 1:

```
Proc glimmix data = complete2;
Class loc trt12_3 tech sire;
Model preg(ref=first) = loc trt12_3 /solution d=binary;
Random tech sire;
Lsmeans loc trt12_3/pdiff ilink
Run;
```

Code for analyzing TAI pregnancy rates in Exp. 2:

```
Proc glimmix data = complete2;
Class loc trt12_3 tech sire;
Model preg(ref=first) = trt12_3  tech /solution d=binary;
Random sire;
Lsmeans trt12_3/pdiff ilink
```

```
Run;
```

SAS Code for Chapter IV

Code for analyzing age and yearling weight differences between conception treatments:

```
proc glm;
class conceivetx;
model age=conceivetx;
lsmeans conceivetx/pdiff;
run;

proc glm;
class conceivetx;
model yw=conceivetx;
lsmeans conceivetx/pdiff;
run;
```

Code for analyzing lifetime revenue differences between sire treatments:

```
proc glm;
class siretx;
model mkt_value=siretx;
lsmeans siretx/pdiff;
run;

proc glm;
class siretx;
model maxslidevalue=siretx;
lsmeans siretx/pdiff;
run;

proc glm;
class siretx;
model minslidevalue=siretx;
lsmeans siretx/pdiff;
run;
```