EVALUATION OF THE 5-DAY VS. 7-DAY CO-SYNCH + CIDR PROTOCOL IN DAIRY HEIFERS USING TIMED AI

by

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Abstract

Our objectives were to determine: the effectiveness of upfront $PGF_{2\alpha}$ injection to regress the corpus luteum; ovulation response to GnRH; and pregnancy outcomes. Dairy heifers (n = 545) from three locations (Florida, Kansas, and Mississippi) were assigned randomly to each of two treatments: 1) 25 mg of $PGF_{2\alpha}$ injection and insertion of previously used autoclaved CIDR on d -7 followed by 100 µg of GnRH administered on d -5, and a 25 mg $PGF_{2\alpha}$ injection at CIDR removal (7D) on d 0; 2) 100 µg of GnRH and insertion of previously used autoclaved CIDR on d -5 and 25 mg of $PGF_{2\alpha}$ injection at CIDR removal (5D) on d 0. Artificial insemination (AI) occurred after detected estrus from d 0 to 3. Those heifers not detected in estrus were inseminated on d 3 and given a second 100 µg of GnRH. Blood collected on d -7 and -5 was assayed to determine concentrations of progesterone, presence of a CL (progesterone ≥1 ng/mL) on d -7, and whether luteolysis occurred in 7D heifers. Blood progesterone concentration from d 0 and 3 determined if luteolysis occurred in all heifers. Ovarian structure maps on d -5 and 0 were used to determine ovulation in response to GnRH on d -5. Pregnancy was determined on d 32 and 60 and intervening pregnancy loss was calculated. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on d -7, the proportion having progesterone <1 ng/mL 2 d later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment (43.0 vs. 22.9%, respectively). Total proportion of follicles that ovulated per heifer was numerically greater in the 7D treatment but only differed ($P < 0.05$) between locations. A treatment x location interaction was detected for pregnancy rates per AI. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced greater ($P < 0.05$) pregnancy rates in the first replicate of the Florida location and at the Mississippi location. We concluded that the 5D protocol was not

effective in producing acceptable luteolysis, pregnancy, and ovulation rates in comparison with the modified 7D protocol.

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Dedication

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Chapter 1 - General Review of Literature

INTRODUCTION

Over time, animal breeding as we know it has evolved from natural service to artificial insemination (AI). The first successful AI was performed in canines during the late 16th century by Spallanzani and the first AI reported in cattle was in the early 20th century in Japan (Foote, 2002). During the late 1930s, AI was reported in cattle in New York, Minnesota, and Wisconsin. With the development of the New York Artificial Breeders Cooperative, Inc., (now known as Genex), a successful partnership was formed between producers and researchers, which led to an enormous amount of artificially inseminated cattle and published research that established techniques worldwide for the use of AI (Foote, 2002). Most cattle producers are now adopting this technology for use in breeding heifers. When producers were asked why they were not using AI, their responses included: "heifers not at a convenient location, inadequate heat detection, and lack of time to supervise AI" (Rivera et al., 2004). Although using AI can increase the genetic merit of a particular location by using superior, progeny-tested bull semen, some producers do not feel that the added cost and labor are of much benefit to their production.

Around 2003, the commercial application of gender-biased (sexed) semen began in America. Use of gender-biased semen allows the selection of a particular sex, usually female, to be inseminated with the desired outcome occurring approximately 85 to 90% of the time. The greatest application of this technology is for AI of dairy heifer replacements, although some dairy producers use sexed semen in their most fertile cows at first services. Because heifers are more fertile than lactating cows, using sexed semen generally allows location size to grow while maintaining a closed herd without having to purchase new cattle. In addition, because X-

chromosome sorted sperm produces nearly all heifers that have smaller birth weights, this application helps to prevent dystocia and other problems that can occur during parturition. The estimates of fertility using sex sorted semen within the herd indicated that conception rates will be 75 to 80% of those achieved when using conventional semen (DeJarnette et al., 2009). With the increased cost and decreased sperm per AI dose (2×10^6), producers tend to use the product on heifers for the first services only and those that do not conceive will be re-inseminated with conventional semen thereafter.

It is important to note that not all bulls are good candidates for producing sex-sorted semen because their sperm must go through multiple procedures before they are packaged and frozen and not all bulls have sufficient sperm quality to meet the rigors of sperm sorting. Initially, semen quality and fertility of a bull's semen conventionally dosed for AI, served as criteria for whether a given bull would be a good candidate for sexed semen, but that relationship has rarely held true. Once the semen is sorted not many bulls have sperm of sufficient quality, motility, and fertility to meet the standards for good sex-sorted semen because the process affects each bull differently.

Sperm from those bulls that are not negatively affected by the sorting process can be used to decrease the cost of progeny testing. Sex-sorted sperm can be inseminated into high-fertility females and more daughters can be produced in less time in order to evaluate a bull's worth. Sexed semen is still a new technology with the potential for a large upside to its acceptance. The pitfalls of increased cost per service and decreased conception rates are obvious, but the possibility of more replacement heifers and less time associated with progeny testing is what the future holds.

Recently, a variety of estrus- and ovulation-synchronization protocols have been developed to increase pregnancies per AI in dairy cattle. These protocols involve using progestogens, prostaglandins ($PGF_{2\alpha}$), and gonadotropin-releasing hormone (GnRH) alone or in various combinations (Rivera et al., 2004). Performing AI at a fixed time is the goal of most protocols because it increases AI submission rate (proportion of females submitted for AI per unit of time) of females with limited or no observation of estrus. These protocols are recommended to those raising replacement heifers because their application can facilitate genetic and economic advantages when using AI (Rivera et al., 2004).

The first successful timed AI protocol developed to synchronize ovulation of dairy cattle was known as Ovsynch (Pursley et al., 1997). Ovsynch involves the injection of GnRH 7 d before and 48 to 56 h after $PGF_{2\alpha}$, with AI administered approximately 16 h after the second GnRH injection. Later it was determined that giving the second injection of GnRH at the time of AI would produce nearly similar fertility (CO-Synch), but required less individual cow handling (Geary and Whittier, 1998). Use of an intravaginal progesterone-impregnated controlled intravaginal drug release (CIDR) insert was added to the protocol in order to suppress premature estrus in females whose corpus luteum might spontaneously regress before the scheduled $PGF_{2\alpha}$ injection. Dairy heifers, however, inseminated as part of an Ovsynch protocol generally have reduced conception rates compared with those inseminated after detected estrus (Pursley et al., 1997).

Timed AI protocols that produce consistently acceptable pregnancy rates in dairy heifers seem to be lacking in the reproductive network. The objective of this review is to examine the factors involved in an effective ovulation control program and summarize results of various research efforts to date.

PHYSIOLOGY OF THE ESTROUS CYCLE

Stages of the Estrous Cycle

An understanding of the estrous cycle in cattle is essential to identifying the different phases or stages of the cycle, how waves of follicles develop before estrus and ovulation, and the optimal time for AI in various estrus- or ovulation-synchronization protocols. The cycle is composed of 2 separate phases: follicular and luteal, which make up unequal portions of the cycle. The follicular phase comprises approximately 20% of the cycle and is known as the time of corpus luteum (CL) regression and final follicular maturation before estrus and ovulation. During this phase, the dominant follicle is the primary structure and secretes estradiol (E_2). Estradiol is a steroid hormone that is produced by the follicle and provides positive feedback to the surge center of the hypothalamus. This positive feedback increases gonadotropin-releasing hormone (GnRH) production which in turn increases luteinizing hormone (LH) production and facilitates growth of the follicle. The other 80% of the cycle (luteal phase) is under control of the CL, which occurs after the dominant follicle ovulates and forms the CL, the dominant structure in the ovaries that is responsible for progesterone (P_4) production. Progesterone is a steroid hormone that inhibits GnRH production and also provides negative feedback to the anterior pituitary, thus altering characteristics of LH secretion. The luteal phase encompasses the period immediately after the end of estrus, including ovulation, and formation and maturation of the CL.

Within each of the 2 phases of the estrous cycle 2 stages are defined. The follicular phase includes proestrus and estrus, and the luteal phase includes metestrus and diestrus. Proestrus ranges from 2 to 5 d and signals the beginning of follicle maturation. At the onset of proestrus the CL begins to regress and progesterone concentrations return to baseline, while E_2 becomes the dominant hormone and eventually triggers behavioral estrus. Estrus is the second stage of

the cycle in the follicular phase and is best known for the sexual receptivity and willingness to mate by the female. Estradiol is the dominating hormone and its increasing titer in blood initiates signs of estrus including increased movement and activity, vocalization, investigative sniffing, nervousness, mucous secretion, and attempts to mount (Senger, 2003). In cattle, estrus lasts from 6 to 24 h and ovulation occurs after the end of estrus at the beginning of metestrus. Synchronization of this period is the goal of an estrus-synchronization program because more females display estrus (lordosis) during a shorter period of time, thus allowing less time to observe for signs of estrus before AI.

Metestrus follows estrus and includes ovulation and CL formation. Limited amounts of E_2 and P_4 are secreted, but are present at minimal concentrations. Luteinization of the theca and granulosa cells of the ruptured follicle occurs during formation of the CL at the site of the ruptured follicle. Development of the CL requires 2 to 5 d before significant amounts of progesterone are secreted and become detectable in blood.

Diestrus is the longest and final stage of the estrous cycle, lasting 10 to 14 d in cattle. At this time the CL is fully functional and produces large amounts of P_4 in the blood. No sexual receptivity occurs during this stage regardless of conception status. If the female is not pregnant, $PGF_{2\alpha}$ is secreted by the uterus to initiate luteolysis or death of the CL as a prelude to the subsequent onset of a new cycle. Prostaglandin $F_{2\alpha}$ is a fatty acid that is produced by the uterine endometrium that binds to the CL and initiates a cascade of intracellular events that culminate in a nonfunctional regressed CL or the corpus albicans (Senger, 2003).

Follicular Dominance

Follicular dynamics occur throughout the estrous cycle giving rise to either 2 or 3 waves per cycle. A wave begins when a cohort of small antral follicles respond to follicle-stimulating

hormone (FSH) secretion and emerge in the ovaries (Senger 2003). Later, when one of these emerging antral follicles is selected, it deviates in size from the next largest follicle (subordinate follicle). The selected follicle then responds to LH secretion and continues to grow as the dominant follicle.

Both FSH and LH are glycoproteins that are secreted by the anterior lobe of the pituitary. When hypothalamic pulses of GnRH are released, GnRH causes FSH and LH to be released from the anterior pituitary to cause recruitment and growth of follicles in the ovary. Four growth stages describe all follicular waves to allow the dominant follicle(s) to undergo final maturation before ovulation. The stages include: recruitment, selection, dominance, and atresia. During recruitment, a cohort of small antral follicles begins to grow in size and secrete E_2. Those follicles that do not enter the selection phase then become atretic. Follicles that are selected may become dominant follicles as they continue to grow. Once a follicle enters the dominance stage it increases production of estradiol until a single follicle ovulates. Inhibin production by the dominant follicle increases to inhibit FSH secretion and cause other subordinate follicles to undergo atresia. Atresia is the destiny of more than 90% of follicles in the ovary (Senger, 2003).

During a normal estrous cycle, the dominant follicle will ovulate after the end of estrus. Thereafter, luteinization of follicular cells lining the ruptured follicular cavity occurs to form a corpus hemorrhagicum (CH; central cavity of blood and fluid in the ruptured follicle) and later to be called the CL. The CL is maintained throughout pregnancy or until luteolysis in the absence of pregnancy. When follicles fail to ovulate after estrus or in the presence of low concentrations of supplemental P_4 after luteolysis, they persist and become aged follicles leading to decreased fertility in affected females.

Persistent or aged follicles can develop when supplemental P_4 is included during a synchronization protocol where females are enrolled at different stages of the estrous cycle. When duration of the estrous cycle was increased from 21.6 ± 0.4 to 30.2 ± 0.2 d by inclusion of a controlled intravaginal progesterone-releasing insert (CIDR), pregnancy rates decreased from 83% to 17%, respectively (Stock and Fortune 1993). Increasing duration of follicle dominance from 4.1 ± 0.2 d in the control to 8.6 ± 0.2 d and 12.1 ± 0.2 d in various treatments, decreased pregnancy rates from 87.5% to 57.8% and 0%, respectively, in a separate study (Mihm et al., 1994).

Because not all females in a treatment may be at similar stages of the estrous cycle, pre-synchronization of estrous cycles may be a useful tool especially in heifers to gain control over follicular dynamics and to obtain optimum fertility in a specific treatment. To determine which stage of the estrous cycle is optimal during which the Ovsynch protocol should be initiated, research was conducted to identify those days of the cycle that yielded the greatest fertility. It was concluded that treating heifers during the early luteal phase (before d 10 of the cycle) would yield the most acceptable pregnancy rates per AI (PR/AI) when using the Ovsynch protocol (Moreira et al., 2000; Stevenson, 2008).

In all synchronization programs follicular dynamics should be considered especially when using exogenous progestogens that extend the dominance period of an ovulatory follicle. Studies show the optimum duration of dominance of a particular follicle should be ≤8 d (Mihm et al., 1994). Austin et al. (1999) concluded, however, that fertility in heifers is greatest when duration of follicle dominance is ≤5 d.

During synchronization protocols, the timing of the final $PGF_{2\alpha}$ injection relative to the beginning of the hormone sequence is an important component when determining the

effectiveness of a protocol. Bridges et al. (2010) attempted to determine the optimal duration of proestrus for an effective hormone-based ovulation control program. Using beef cows synchronized with a 7-d CIDR insert and $PGF_{2\alpha}$ injection at withdrawal, they determined fertility after 2.2 d versus 1.2 d of proestrus. They concluded that decreasing the duration of proestrus before ovulation resulted in decreased PR/AI and a shorter subsequent luteal phase. Under optimal conditions, a longer proestrus may be beneficial because it gives the follicle time to produce increasing amounts of E_2 and to increase fertility.

ARTIFICIAL INSEMINATION BREEDING PROGRAMS

Progesterone Insert + $PGF_{2\alpha}$

When P_4 inserts are used in synchronizing protocols they are combined with $PGF_{2\alpha}$, GnRH, or a combination of both. Combining a P_4 insert with $PGF_{2\alpha}$ at the end of the insert period in dairy heifers allowed for synchronized estrus and ovulations rates (Smith et al. 1984; Lucy et al., 2001). Smith et al. (1984) observed an increase in the interval to onset of estrus when $PGF_{2\alpha}$ was administered 24 h before removal of the insert than when the injection was concurrent with insert removal (75 ± 2 vs. 66 ± 2 h, respectively). Lucy et al. (2001) reported an increased proportion of dairy heifers treated with a P_4 insert and $PGF_{2\alpha}$ showing signs of estrus (84%) compared with applying only $PGF_{2\alpha}$ (57%). A positive response to the CIDR insert and $PGF_{2\alpha}$ treatment also was observed in dairy heifers with estrus-detection rates of 85.5% compared with beef heifers (89.1%), and greater pregnancy rates in dairy heifers (59%) vs. beef heifers (40.6%; Richardson et al., 2002). Comparable pregnancy rates also resulted when a CIDR+ $PGF_{2\alpha}$ (45%) protocol was compared with $PGF_{2\alpha}$ alone (37%; Lucy et al., 2001). Earlier research using a P_4-releasing intravaginal device (PRID) produced greater pregnancy rates (72%

when $PGF_{2\alpha}$ was administered 1 d before PRID removal and 82% when injection was concurrent with PRID removal; Smith et al., 1984) than those reported in later studies using the CIDR insert (Richardson et al., 2002; Lucy et al., 2001). Uses of progesterone inserts have become more commonplace because of their ability to suppress estrus with no reduction in PR/AI (Rivera et al., 2005). Further, they improve synchronized estrus and increase PR/AI to first AI during increased temperatures of summer (Alnimer et al., 2009).

GnRH + Progesterone Insert (7 to 14 d) + $PGF_{2\alpha}$

Previously used CIDRs have been used in studies to reduce costs of the program. Zuluga et al. (2008) noted that previously used, autoclaved CIDRs may be best for synchronization because they increase P_4 concentrations immediately after insertion. The authors also found that mean P_4 in blood serum of cattle treated with an autoclaved CIDR almost doubled that of previously used CIDRs that were disinfected, and autoclaved CIDRs also produced serum concentrations 1.3 to 1.4 times greater than produced by new intravaginally placed CIDRs. Increased conception rates after applying used (47.7%) compared with new CIDRs (39.2%) were observed in heifers in addition to shorter intervals to estrus (Júnior et al., 2010).

Combining $PGF_{2\alpha}$ with the time of CIDR insert removal is done to initiate luteolysis in heifers for which the CL has not already regressed before $PGF_{2\alpha}$ is administered. Giving $PGF_{2\alpha}$ at the time of CIDR removal in a 7-d protocol shortened the onset of estrus for the group administered $PGF_{2\alpha}$ at CIDR removal (49.3 ± 6.2 h) compared with those injected with $PGF_{2\alpha}$ the day after insert removal (77.5 ± 9 h; Hittinger et al., 2004). Ambrose et al. (2008) also found that administering $PGF_{2\alpha}$ 24 h before CIDR removal resulted in a consistent PR/AI that was greatest when the protocol was initiated in diestrus (57%) than at other stages of the estrous cycle (34.8%).

Presynchronization of estrus can occur to allow a group of females to initiate a timed AI program at a specific and more favorable stage of the cycle to facilitate greater pregnancy outcomes to the timed AI. Those females to which a protocol is applied that do not become pregnant are sometimes resynchronized for subsequent AI. Presynchronization did not affect dairy heifers expressing estrus nor did it improve synchronization or fertility (Rivera et al, 2003). In contrast, Stevenson et al. (2008) observed that presynchronization with GnRH increased ovulation to the first GnRH injection at the start of the CO-Synch + CIDR protocol and led to increased ovulatory follicle size and decreased period from estrus to ovulation. The difference between these 2 studies was that the Rivera et al. (2006) study did not use CIDR inserts, whereas CIDRs were used in the Stevenson et al. (2008) study; however, both reported an average PR/AI of approximately 50%. Busch et al. (2007) reported increased synchronous estrus and greater PR/AI using the CIDR-Select protocol (heifers received a CIDR from d 0 to 14, GnRH-1 on d 23, and $PGF_{2\alpha}$ on d 30 with GnRH-2 and TAI 72 h after $PGF_{2\alpha}$ injection; 62%) compared with the CO-Synch + CIDR (47%).

Previously inseminated heifers were treated with CIDR insert between 14 and 20 d after AI or no insert. More heifers showed estrus by 72 h after CIDR removal (78%) and conceived (47%) than control heifers (50 and 36%; respectively; Rivera et al., 2005).

The goal of all synchronization protocols is to have a homogenous group of females in estrus that can be submitted to AI. It is well known that virgin heifers often fail to have acceptable synchronization and pregnancy rates because of a variety of factors. Pursley et al. (1997) stated that a fixed TAI protocol is not an effective tool for use in heifers because of the lack of synchronization. Pregnancy outcomes in control heifers inseminated after $PGF_{2\alpha}$-induced estrus (74.4%) were greater than in heifers treated with Ovsynch (35.1%; Pursley et al. 1997).

Tenhagen et al. (2005) determined that premature estrus and ovulation probably caused a failure of synchronization in dairy heifers. Further, Saldarriaga et al. (2007) concluded that beef cattle had poor conception because 40% fail to develop a synchronized follicular wave. As stated previously, if the synchronization protocol is started at a less opportune stage of the cycle, most females do not respond properly, resulting in less than acceptable PR/AI. Using GPG (GnRH d 0, $PGF_{2\alpha}$ d 6, GnRH and TAI d 8) in dairy heifers decreased PR/AI where 38.3% of treated heifers conceived compared with a control of 46.5% (Rivera et al., 2004). Lamb et al. (2006) obtained acceptable PR/AI and eliminated the need for estrus detection in beef heifers with the control (CIDR d 0 to 7, $PGF_{2\alpha}$ at CIDR withdrawal, estrus detection and AI for 72 h plus AI 84 h after $PGF_{2\alpha}$ for heifers not detected in estrus) achieving 54.7% PR/AI compared with the treatment (GnRH on d 0, CIDR d 0 to 7, $PGF_{2\alpha}$ at CIDR withdrawal, GnRH and TAI 60 h later; 53.1%).

Besides GnRH, researchers also have used estradiol benzoate (EB), estradiol cypionate (ECP), and porcine LH (pLH) in combination with the CIDR and $PGF_{2\alpha}$ to synchronize estrus and ovulation in heifers. Xu et al. (1999) increased reproductive performance of dairy heifers using EB and a 10-d CIDR insert. Achieving PR/AI of 72.4% (d -12 CIDR inserted with a 10 mg capsule of EB, d -6 $PGF_{2\alpha}$ injection, d -2 CIDR removed) compared with 67.8% in the control (no treatment, AI preformed each morning in those heifers detected in estrus during previous 24 h. Using ECP, Peeler et al. (2004) also achieved acceptable PR/AI with 63% in the ECP treatment (CIDR and 1 mg ECP d 0, CIDR removal and $PGF_{2\alpha}$ d 7, 0.5 mg ECP 24 h after CIDR removal) and 57.1% in the GnRH treatment (CIDR and 1 mg ECP d 0, CIDR removal and $PGF_{2\alpha}$ d 7, GnRH 48 h after CIDR removal). They reported the interval from CIDR removal to ovulation occurred soon after ECP (63.8 ± 3 h) than after GnRH (71.6 ± 2.3 h). In 2002,

Martinez et al. concluded that acceptable PR/AI could be achieved in beef heifers using GnRH, pLH, or EB in combination with exogenous P_4 given either intravaginally or orally. The authors observed an increased estrus response from heifers treated with EB (92%) compared with those receiving GnRH or pLH. Pregnancy rates were not different between treatments but heifers observed in estrus (62.6%) obtained greater pregnancy rates than heifers not observed in estrus (51.9%).

GnRH+CIDR(5 d) +$PGF_{2\alpha}$

A study by Bridges et al. (2008) in suckled beef cows was first to reduce the interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 d with AI at 60 h in the 7-d treatment and 72 h in the 5-d treatment. Reducing the CIDR insert from 7 to 5 d also required a second $PGF_{2\alpha}$ injection (given 12 h after the first $PGF_{2\alpha}$ injection) to ensure luteolysis occurred in cows that formed a new CL in response to the GnRH injection administered at the time of CIDR insert. The PR/AI in the 7 d treatment was 59.9% and increased to 70.4% in the 5 d treatment (Bridges et al., 2008). In lieu of 2 injections of $PGF_{2\alpha}$ administered 12 h apart, Kasimanickam et al. (2009) administered $PGF_{2\alpha}$ 7 h apart and reported greater PR/AI after 2 injections (69%) than after a single injection (52%). Ahmadzadeh et al. (2010) observed differences between the 7- and 5-d programs in beef heifers with a tendency for greater PR/AI in the 5-d treatment (62.5%) compared with the 7-d treatment (52%).

In dairy heifers, where a similar 5-d CIDR program was administered, 1 injection of $PGF_{2\alpha}$ yielded acceptable PR/AI (46.4%) when compared with 2 injections of $PGF_{2\alpha}$ given 12 h apart (48.6%; Rabaglino et al., 2010). In a study done to show the effects of GnRH at initiation of the 5-d timed AI program in dairy heifers, GnRH was administered on the first day of the 5-d protocol and resulted in poor ovulation incidence and no improvement in PR/AI when heifers

received a single dose of $PGF_{2\alpha}$ (Lima et al., 2010). The authors also noted that the combination of an upfront GnRH injection given 5 d before the 2 $PGF_{2\alpha}$ injections improved PR/AI compared with no GnRH upfront and a single injection of $PGF_{2\alpha}$.

SUMMARY

In heifer management, the 7-d and 5-d Co-Synch or Ovsynch protocols have shown great potential in beef heifers, but with more mediocre results in dairy heifers. Thus far in dairy heifers, the best protocol favors the 7-d program. This protocol offers a greater acceptance rate with increased PR/AI than the 5-d program. Use of the 5-d protocols has several advantages: 1) decreased handling; 2) increased duration of proestrus, and 3) increased PR/AI in certain cases (mostly cows and beef heifers). Increased duration of proestrus allows a follicle to mature longer before ovulation and produce sufficient E_2 concentrations that may lead to greater PR/AI. It is necessary to continue testing both types of protocols to get a proper time and hormone combination to be used effectively across locations in order to receive acceptable PR/AI. The hypothesis for the current study is that the 5-d CO-Synch + CIDR protocol will increase PR/AI in treated dairy heifers and synchronization of estrus and ovulation will provide another useful management tool to facilitate TAI in heifers. The objectives of the present study were to: 1) determine the effectiveness of upfront $PGF_{2\alpha}$ injection to regress the corpus luteum (CL); 2) determine the ovulation response to the first GnRH injection of the treatments; and 3) determine pregnancy outcomes of the two treatments.

REFERENCES

Ahmadzadeh, A., D. Gunn, J. B. Hall, and J. B. Glaze. 2010. Evaluation of 5-day versus 7-day CIDR treatment on reproductive outcomes of beef heifers using a modified timed-AI protocol. J. Anim. Sci. 88(E-Suppl. 2) (Abstract T271).

Alnimer, M. A. 2009. The effect of a progesterone intravaginal device (CIDR) on resynchronization of oestrus and embryonic loss in previously timed inseminated dairy heifers. Animal 3:9, pp 1271-1278.

Ambrose, D. J., D. G. V. Emmanuel, M. G. Colazo, and J. P. Kastelic. 2008. Pregnancy rates to timed artificial insemination in Holstein heifers given prostaglandin $F_{2\alpha}$ twenty-four hours before or concurrent with removal of an intravaginal progesterone-releasing insert. J. Dairy Sci. 91:2678-2683.

Austin, E. J., M. Mihm, M. P. Ryan, D. H. Williams, and J. F. Roche. 1999. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. J. Anim. Sci. 77:2219-2226.

Bridges, G. A., L. A. Hesler, D. E. Grum, M. L. Mussard, C. L. Gasser, and M. L. Day. 2008. Decreasing the interval between GnRH and PGF from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 69:843-851.

Bridges, G. A., M. L. Mussard, C. R. Burke, and M. L. Day. 2010. Influence of length of proestrus on fertility and endocrine function in female cattle. Anim. Repro. Sci. 117:208-215.

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson, 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933-1939.

DeJarnette, J. M., R. L. Nebel, and C. E. Marshall. 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology 71: 49-58.

Foote, R. H. 2002. The history of artificial insemination: Selected notes and notables. J. Anim. Sci. 80:1-10.

Geary, T. W., and J. C. Whittier. 1998. Effects of a timed insemination following synchronization of ovulation using the Ovsynch or CO-synch protocol in beef cows. The Professional Anim. Sci. 14:217-220

Hittinger, M. A., J. D. Ambrose, and J. P. Kastelic. 2004. Luteolysis, onset of estrus, and ovulation in Holstein heifers given prostaglandin $F_{2\alpha}$ concurrent with, or 24 hours prior to, removal of an intravaginal, progesterone-releasing device. Can. J. Vet. Res. 68:283-287.

Júnior, I. C., O. G. Sá Filho, R. F. G. Peres, F. H. S. Aono, M. L. Day, and J. L. M. Vasconcelos. 2010. Reproductive performance of prepubertal Bos *indicus* heifers after progesterone-based treatments. Theriogenology 74:903-911.

Kasimanickam, R., M. L. Day, J. S. Rudolph, J. B. Hall, and W. D. Whittier. 2009. Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone-based synchronization protocol in beef cows. Theriogenology 71: 762-767

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:3000-3009.

Lima, F. S., H. Ayres, M. Favoreto, R. S. Bisinotto, L. F. Greco, E. S. Souza, R. S. Marsola, C. A. Risco, W. W. Thatcher, and J.E.P. Santos. 2010. Effects of GnRH at initiation of the 5-d timed AI program and timing of induction of ovulation relative to AI on ovarian dynamics and fertility of dairy heifers. Reproduction in Domestic Animals VII. Soc. Reprod. Fertil. 67:616 (Abstr.).

Lucy, M. C., H. J. Billings, W. R. Butler, L. R. Ehnis, M. J. Fields, D. J. Kesler, J. E. Kinder, R. C. Mattos, R. E. Short, W. W. Thatcher, R. P. Wettemann, J. V. Yelich, and H. D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of $PGF_{2\alpha}$ for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. J. Anim. Sci. 79:982-995.

Martinez, M. F., J. P. Kastelic, G. P. Adams, and R. J. Mapletoft. 2002. The use of a progesterone-releasing device (CIDR-B) or melengesterol acetate with GnRH, LH, or estradiol benzoate for fixed-time AI in beef heifers. J. Anim. Sci. 80:1746-1751.

Mihm, M., A. Baguisi, M. P. Boland, and J. F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. Journal of Reproduction and Fertility. 102: 123-130.

Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. 78:1568-1576.

Peeler, I. D., R. L. Nebel, R. E. Pearson, W. S. Swecker, and A. Garcia. 2004. Pregnancy rates after timed AI of heifers following removal of intravaginal progesterone inserts. J. Dairy Sci. 87:2868-2873.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation of synchronized estrus. J. Dairy Sci. 80:295-300.

Rabaglino, M. B., C. A. Risco, M. J. Thatcher, I. H. Kim, J. E. P. Santos, and W. W. Thatcher. 2010. Application of one injection of prostaglandin $F_{2\alpha}$ in the five-day Co-Synch + CIDR protocol for estrous synchronization and resynchronization of dairy heifers. J. Dairy Sci. 93:1050-1058.

Richardson, A. M., B. A. Hensley, T. J. Marple, S. K. Johnson, and J. S. Stevenson. 2002. Characteristics of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, $PGF_{2\alpha}$, and progesterone. J. Anim. Sci. 80:2792-2800.

Rivera, H., H. Lopez, and P. M. Fricke. 2004. Fertility of Holstein dairy heifers after synchronization of ovulation and timed AI or AI after removed tail chalk. J. Dairy Sci. 87:2051-2061.

Rivera, H., H. Lopez, and P. M. Fricke. 2005. Use of intravaginal progesterone-releasing inserts in a synchronization protocol before timed AI and for synchronizing return to estrus in Holstein heifers. J. Dairy Sci. 88:957-968.

Rivera, H., R. A. Sterry, and P. M. Fricke. 2006. Presynchronization with gonadotropin-releasing hormone does not improve fertility in Holstein heifers. J. Dairy Sci. 89:3810-3816.

Saldarriaga, J. P., D. A. Cooper, J. A. Cartmill, J. F. Zuluaga, R. L. Stanko, and G. L. Williams. 2007. Ovarian, hormonal, and reproductive events associated with synchronization of ovulation and timed appointment breeding of Bos *indicus*-influenced cattle using intravaginal progesterone, gonadotropin-releasing hormone, and prostaglandin $F_{2\alpha}$. J. Anim. Sci. 85: 151-162.

Savio, J. D., L. Keenan, M. P. Boland, and J. F. Roche. 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J. Reprod. Fertil. 83:663-671.

Schenk, J. L., D. G. Cran, R. W. Everett, and G. E. Seidel Jr. 2009. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure, and sperm storage before sorting. Theriogenology 71:717-728.

Smith, R. D., A. J. Pomerantz, W. E. Beal, J. P. McCann, T. E. Pilbeam, and W. Hansel. 1984. Insemination of Holstein heifers at a preset time after estrous cycle synchronization using progesterone and prostaglandin. J. Anim. Sci. 58:792-800

Stevenson, J. L., J. C. Dalton, J. E. P. Santos, R. Sartori, A. Ahmadzadeh, and R. C. Chebel. 2008. Effect of synchronization protocols on follicular development and estradiol and progesterone concentrations of dairy heifers. J. Dairy Sci. 91:3045-3056.

Stevenson, J. S. 2008. Progesterone, follicular, and estrual responses to progesterone-based estrus and ovulation synchronization protocols at five stages of the estrous cycle. J. Dairy Sci. 91:4640-4650.

Stock, A. E., and J. E. Fortune. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 132:1108-1114

Tenhagen, B. A., S. Kuchenbuch, and W. Heuwieser. 2005. Timing of ovulation and fertility of heifers after synchronization of oestrus with GnRH and prostaglandin $F_{2\alpha}$. Reprod. Domest. Anim. 40: 62-67.

Xu, Z. Z., and L. J. Burton. 1999. Reproductive performance of dairy heifers after estrus synchronization and fixed-time artificial insemination. J. Dairy Sci. 82:910-917.

Zuluaga, J. F., and G. L. Williams. 2008. High-pressure steam sterilization of previously used CIDR inserts enhances the magnitude of the acute increase in circulating progesterone after insertion in cows. Anim. Repro. Sci. 107: 30-35.

Chapter 2 - Evaluation of the 5D vs. 7D CIDR protocol in dairy heifers using Timed AI

ABSTRACT

Our objectives were to determine: the effectiveness of upfront $PGF_{2\alpha}$ injection to regress the corpus luteum; ovulation response to GnRH; and pregnancy outcomes. Dairy heifers (n = 545) from three locations (Florida, Kansas, and Mississippi) were assigned randomly to each of two treatments: 1) 25 mg of $PGF_{2\alpha}$ injection and insertion of previously used autoclaved CIDR on d -7 followed by 100 µg of GnRH administered on d -5, and a 25 mg $PGF_{2\alpha}$ injection at CIDR removal (7D) on d 0; 2) 100 µg of GnRH and insertion of previously used autoclaved CIDR on d -5 and 25 mg of $PGF_{2\alpha}$ injection at CIDR removal (5D) on d 0. Artificial insemination (AI) occurred after detected estrus from d 0 to 3. Those heifers not detected in estrus were inseminated on d 3 and given a second 100 µg of GnRH. Blood collected on d -7 and -5 was assayed to determine concentrations of progesterone, presence of a CL (progesterone ≥1 ng/mL) on d -7, and whether luteolysis occurred in 7D heifers. Blood progesterone concentration from d 0 and 3 determined if luteolysis occurred in all heifers. Ovarian structure maps on d -5 and 0 were used to determine ovulation in response to GnRH on d -5. Pregnancy was determined on d 32 and 60 and intervening pregnancy loss was calculated. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on d -7, the proportion having progesterone <1 ng/mL 2 d later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment (43.0 vs. 22.9%, respectively). Total proportion of follicles that ovulated per heifer was numerically greater in the 7D treatment but only differed ($P < 0.05$) between locations. A treatment x location interaction was detected for pregnancy rates per AI. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced greater ($P < 0.05$) pregnancy rates in the first replicate of the

Florida location and at the Mississippi location. We concluded that the 5D protocol was not effective in producing acceptable luteolysis, pregnancy, and ovulation rates in comparison with the modified 7D protocol.

INTRODUCTION

Since 1997, it has been known that dairy heifers do not respond as well as lactating dairy cows to GnRH + $PGF_{2\alpha}$ protocols to synchronize estrus, ovulation, or both. For example, a multi-site study demonstrated that heifers treated after a timed AI, Ovsynch-like protocol averaged 35% conception compared with non-treated heifers inseminated after estrus (74%; Pursley et al., 1997).

A study by Bridges et al. (2008) in suckled beef cows was first to reduce the interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 d. They hypothesized that reducing the interval between injections would allow the maturing ovulatory follicle to develop during a longer proestrus, low progesterone environment. The pregnancy rate per AI (**PR/AI**) in the 7 d treatment was 59% and increased to 70% in the 5 d treatment (Bridges et al., 2008). Similarly, Ahmadzadeh et al. (2010) observed differences between the 7- and 5-d programs in beef heifers with a tendency for greater PR/AI in the 5-d treatment (62.5%) compared with the 7-d treatment (52%).

When GnRH induced ovulation of follicles in a 5-d treatment, the resulting corpus luteum (**CL**) may not undergo luteolysis when a $PGF_{2\alpha}$ injection is administered 5 d after GnRH. In dairy heifers where a similar 5-d CIDR program was administered, however, 1 injection of $PGF_{2\alpha}$ yielded acceptable PR/AI (46.4%) when compared with 2 injections of $PGF_{2\alpha}$ given 12 h apart (48.6%; Rabaglino et al., 2010).

Some differences in fertility responses between heifers and cows may be associated with poor ovulation responses to GnRH in heifers (Stevenson, 2008) compared with that in lactating

dairy cows (Vasconcelos et al., 1999). Although GnRH injection at initiation of the 5-d timed AI (TAI) program resulted in poor ovulation incidence and no improvement in PR/AI when heifers received a single dose of $PGF_{2\alpha}$, the combination of an upfront GnRH injection given 5 d before 2 $PGF_{2\alpha}$ injections (1 d apart) did improve PR/AI compared with no GnRH injection upfront and a single $PGF_{2\alpha}$ injection (Lima et al., 2010).

The hypothesis for the current study was that the 5-d CO-Synch + CIDR protocol will increase PR/AI in treated dairy heifers and synchronization of estrus and ovulation will serve as a useful management tool to facilitate TAI in heifers. The objectives were to: 1) determine the effectiveness of upfront $PGF_{2\alpha}$ injection to regress the CL; 2) quantitate the ovulation response to the first GnRH injection in both treatments; and 3) assess pregnancy outcomes.

MATERIALS AND METHODS

This experiment was conducted at 3 locations: 1) Kansas State University Dairy Teaching and Research Center in Manhattan; 2) University of Florida, Marianna, FL; and 3) Mississippi State University Dairy Center. Heifers were assigned randomly to receive either a 5 or 7-d treatment, both of which incorporated an intravaginal controlled internal drug release (CIDR) insert (Pfizer Animal Health, New York, NY; Figure 2.1). The CIDR inserts had been used once previously for 7 d, were cleaned, and autoclaved as described by Zuluaga et al. (2008).

On d −7 of the experiment, heifers in the 7 d treatment (7D) received an i.m. injection of 25 mg of $PGF_{2\alpha}$ (5 mL Lutalyse, Pfizer Animal Health). Then on d −5, heifers received i.m. 100 µg injection of GnRH (2 mL Factrel, Fort Dodge Animal Health, Overland Park, KS). In the 5-d treatment (5D), heifers received the first GnRH injection at the time of CIDR insertion on d −5. The CIDR insert was removed from heifers in both treatments on d 0 concurrent with a 25-mg

injection of $PGF_{2\alpha}$. On d 3, a second GnRH injection was administered at the time of fixed time AI (72 h after CIDR insert removal).

Heifers were inseminated artificially using frozen-thawed semen based on either standing estrus or at 72 h post CIDR removal. Detection of estrus occurred from d 0 to 3 and those heifers showing definitive signs of estrus were inseminated before the scheduled TAI. Heifers inseminated based on standing estrus did not receive a second GnRH injection. Pregnancy was diagnosed 32 d later by transrectal ultrasonography based on the presence of uterine fluid, a large CL without a cavity, presence of a viable embryo, or both. Pregnancy was reconfirmed 4 wk later.

Kansas

Holstein heifers enrolled in the experiment had an average age at first breeding of 423 ± 53 d and BW was 402 ± 55 kg. Heifers were fed a TMR consisting of prairie hay, corn, soybean meal, corn silage, minerals, and vitamins, with water provided *ad libitum.* Feed was delivered to feed bunks twice daily and heifers were housed in dirt lots with a concrete apron next to the feed bunk. The experiment was conducted in 19 replicates (n = approximately 10 heifers/replicate) between October 2009 and January 2011. Those heifers detected not pregnant were re-enrolled in the same treatment up to 2 more times. Heifers at this location were inseminated with gender-biased semen.

Florida 1

Jersey heifers were enrolled in the experiment based on same criteria as Kansas heifers. At enrollment, BCS of heifers averaged 3.01 ± 0.03. The experiment was conducted in 3 replicates between January and April 2010. Heifers at this location were randomly assigned to receive either conventional or gender-biased semen.

Florida 2

Jersey heifers were enrolled in the experiment based on same criteria as Kansas heifers. The experiment was conducted in 3 replicates between December 2010 and March 2011. Heifers at this location were inseminated using conventional semen.

Mississippi

Holstein and Jersey heifers were enrolled in the experiment at an average age of 476 ± 7 d and average BW of 338 ± 5 kg. The experiment was conducted in 3 replicates between December 2010 and January 2011. Heifers at this location were inseminated using gender biased semen.

Blood Sampling

Blood samples were collected from all heifers via coccygeal venipuncture before administration of injections in both treatments on d −7, −5, 0, and 3. Blood samples were allowed to clot for 24 h at 4°C, centrifuged (1,000 x g for 15 min), and serum was harvested and stored at −20°C until assayed for progesterone using a solid-phase, no-extraction radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA; Stevenson et al., 2011). Blood collected on d −7 and −5 was used to verify the functional presence of a corpus luteum (**CL**; when concentrations of progesterone exceeded 1 ng/mL) at the onset of treatments. Samples from d −7 and −5 were used to verify luteolysis when concentrations of progesterone exceeded 1 ng/mL on d −7 and were less than 1 ng/mL on d −5. Samples collected on d 0 and 3 were used to determine whether luteolysis occurred in heifers as defined previously for d −7 to −5. The sensitivity of the assay was 0.004 ng/mL, and the intra- and interassay coefficients of variation were 5.2 and 7.4%, respectively.

Ovarian Structures

At 2 locations (Kansas and Mississippi), ovarian structures were examined by using transrectal ultrasonography on d −5 and 0. On d −5, ovaries were scanned to determine the number of ovarian follicles >8 mm and the diameter of all CL present. On d 0, ultrasound was performed to determine whether ovulation occurred in response to GnRH administered on d −5. Ovulation was verified when the presence of a new CL was detected in the same ovarian location where a follicle was observed previously.

Luteolysis

To determine whether luteolysis occurred in all heifers in the 7D treatment between d −7 and −5 during which CIDR inserts were applied at the same time as the $PGF_{2\alpha}$ injection, some assumptions were made. For heifers that had concentrations of P4 <1 ng/mL (likely had no CL) on d −7 and were treated with a CIDR insert, an average progesterone concentration on d −5 was calculated for each location. This concentration represented the systemic progesterone concentration after a 48-h exposure to the CIDR. For heifers to have had luteolysis, progesterone concentration must have been ≥1 ng/mL on d −7 regardless of what occurred 48 h later. Luteolysis between study day −7 and −5 occurred when the concentration on d −5 was <60% of that on d −7 and the P4 concentration on d −5 did not exceed the location average progesterone concentration calculated previously.

Statistical Analyses

Discrete, binomial variables (pregnancy diagnoses at d 32 and 60; pregnancy loss; presence of a CL at d −5 and 0; and ovulation response to GnRH; presence of high (≥1 ng/mL) or low (<1 ng/mL) progesterone on d −7; high or low progesterone d −5; and luteolysis between

d –7 and –5 and between d 0 and 3) were analyzed using logistic regression (procedure LOGISTIC; SAS Institute Inc., Cary, NC). The model included treatment, location, and their interaction.

Continuous variables (number of CL on d –5; CL diameter, cavity, and volume; number of follicles; largest and second largest follicle diameter; number of CL on d 0; original CL diameter, cavity, and volume; new CL diameter, cavity, and volume; number of follicles on d 0; largest and second largest follicle d 0; and blood progesterone concentrations on d –7, –5, 0, and 3) were analyzed by ANOVA (procedure GLM, SAS Inst. Inc.). The model included treatment, location, and treatment x location interaction.

RESULTS

Ovarian Characteristics and Responses to GnRH

Ovarian characteristics of heifers 5 d before the common $PGF_{2\alpha}$ injection and responses to the first GnRH injection are presented in Table 2.1. Heifers in the 5D treatment had more ($P = 0.026$) CL per heifer and a greater ($P = 0.043$) proportion of the total had a CL than in the 7D treatment. These results were not consistent between locations (treatment x location interaction, $P = 0.001$). In the Kansas location, more 5D than 7D heifers had a CL (87 vs. 59%), whereas the reverse was true for the Mississippi location (89 vs. 94%), respectively. Likewise, the number of CL per heifer was greater for the 5D than 7D heifers in the Kansas location (0.9 ± 0.04 vs. 0.6 ± 0.04 CL) compared with the Mississippi location (0.9 ± 0.07 vs. 0.9 ± 0.07 CL), respectively. A greater volume of luteal tissue was also detected ($P < 0.05$) in the 5D than in the 7D treatment.

Neither number of follicles >8 mm in diameter nor size of the largest follicle per heifer differed between treatments. At the Kansas and Mississippi locations, more ($P < 0.05$) heifers in the 7D than 5D treatment ovulated their largest follicle (47.2 vs. 27.6%).

Small treatment by location differences were detected for size of the second largest follicle. At the Kansas location, the 7D had greater follicle size than the 5D treatment (10.8 ± 0.3 vs.9.4 ± 0.3 mm), whereas the reverse was detected at the Mississippi location (9.2 ± 0.6 vs.10.1 ± 0.6 mm). Total proportion of follicles that ovulated per heifer was greater ($P < 0.05$) in the 7D treatment (51.1%) than in the 5D (30.4%).

Ovarian characteristics at the onset of $PGF_{2\alpha}$ injection (study d 0) are presented in Table 2.2. Heifers in the 5D treatment had more ($P = 0.001$) heifers with their original CL (from d --5), and greater original CL volume ($P = 0.001$) than the 7D treatment. Neither proportion of heifers having a new CL nor its volume differed between treatments. An interaction, however, was detected ($P = 0.020$) for the presence of a new CL. At the Kansas location, more 5D than 7D heifers had a new CL (90 vs. 79%), whereas at the Mississippi location, the reverse was true (43 vs. 58%). Although number of follicles > 8 mm in diameter tended ($P = 0.11$) to be greater in the 7D treatment, diameter of the largest and second largest follicles did not differ between treatments.

Blood Progesterone

Concentrations of serum progesterone on d −7, −5, 0, and 3 are represented in Figure 2.2. At the onset of treatments, progesterone concentrations did not differ on d −7 or d −5. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on d −7, the proportion having progesterone <1 ng/mL 2 d later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment

(43.0 vs. 22.9%, respectively). The 7D treatment also had decreased ($P < 0.05$) progesterone concentrations on d 0 compared with the 5D treatment, but this difference did not persist on d 3 at TAI.

Luteolysis in response to $PGF_{2\alpha}$ calculated from serum concentrations on d 0 and 3 indicated that luteolysis occurred in 90.1% of heifers in the 7D treatment and did not differ from that of 88.6% of heifers in the 5D treatment.

Pregnancy and Loss

Pregnancy rates were determined 32 d post AI and are illustrated for the 3 locations in Figure 2.3. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced greater ($P < 0.05$) pregnancy rates in the first replicate of the Florida location and at the Mississippi location (Figure 3).

With estrus detection being performed from d 0 until TAI, 166 heifers (30.6%) were inseminated before TAI and 39.2% of early inseminated heifers became pregnant in the current study. The remainder of the 376 heifers submitted to TAI on d 3 of the protocol and 26.2% of them became pregnant. The PR/AI for heifers inseminated at estrus and for those receiving the timed AI differed ($P = 0.006$).

Pregnancy rates in heifers having high (≥1 ng/mL) progesterone on d –7 were 36% (n = 345) and were different ($P < 0.05$) from those having low progesterone (<1 ng/mL; 20%, n = 194). This difference also existed for heifers having high progesterone on d –5 (33%, n = 415 vs. 20%, n = 105). For heifers having low vs. high progesterone at TAI, PR/AI was increased ($P = 0.006$) from 12% (n = 51) to 32% (n = 460). At 60 d post AI, the PR/AI was consistent with that for d 32. Pregnancy loss calculated between 32 d and 60 d post AI was minimal (between

2.7 and 4.4%) and did not differ between treatments, although insufficient numbers of observations precluded detection of any differences.

DISCUSSION

Success of a synchronization protocol is a very important aspect of location management for producers who retain their own heifers and also for those that grow and sell heifers. Although heifers are usually the most fertile females in a production system, some multi-location studies (Pursley et al., 1997) have reported decreased fertility after synchronization protocols.

In all synchronization programs follicular dynamics should be considered especially when using exogenous progestogens that extend the dominance period of an ovulatory follicle. Studies show the optimum duration of dominance of a particular follicle should be ≤8 d (Mihm et al., 1994). Austin et al. (1999), however, observed that fertility in heifers was greatest when duration of dominance is ≤5 d.

During synchronization protocols, the timing of the final $PGF_{2\alpha}$ injection relative to the beginning of the hormone sequence is an important component when determining the effectiveness of a protocol. Bridges et al. (2010) synchronized ovulation in beef cows with a 7-d CIDR and $PGF_{2\alpha}$ injection at withdrawal to determine fertility after 2.2 d versus 1.2 d of proestrus. They observed that decreasing the duration of proestrus before ovulation resulted in decreased PR/AI and a shorter subsequent luteal phase. Under optimal conditions, a longer proestrus may be beneficial because it gives the follicle time to produce increasing amounts of estradiol and to increase fertility.

The first objective of the study was to determine effectiveness of an upfront $PGF_{2\alpha}$ injection in the 7D treatment. On d −5 at the second blood sample, serum progesterone concentrations were approximately 4.28 ng/mL (Figure 2.2). Comparing blood progesterone

samples on d −7 and −5 to determine luteolysis, we calculated that only 43% of heifers given $PGF_{2\alpha}$ on d −7 had luteolysis by d −5. These data indicate that in this experiment, $PGF_{2\alpha}$ was not effective in causing luteolysis in heifers at random stages of the estrous cycle in the 7D treatment.

In most synchronization protocols, the ability of a female to respond to GnRH and cause ovulation is the most important step in achieving a successful PR/AI. Only 30% of heifers in the 5D treatment were observed to have ovulated to the first GnRH given on d −5. This finding is similar to 35.4% in heifers using a similar protocol (Lima et al. (2010). Bisinotto et al. (2010) reported a similar ovulation rate (34.8%) in resynchronized lactating Holstein cows after the first GnRH of a similar 5-d Co-Synch protocol that applied 2 $PGF_{2\alpha}$ injections after CIDR insert removal and the second GnRH administered 16 h before TAI. In contrast, heifers in the 7D treatment had a greater ovulation response of 50% which is more than reported by Stevenson (25.1%; 2008) and Stevenson et al. (2008) who reported 30.7%. What is important to note, but may not be critical to the comparisons, is that the ovulation response in the Stevenson (2008) study was calculated 48 h post GnRH, whereas those from the present study were determined 5 d after GnRH.

Serum concentrations of progesterone were greater in heifers of the 5D treatment across this study, but this was only significant on d 0 at CIDR removal and at the final $PGF_{2\alpha}$ injection. Although heifers in the 7D treatment received the CIDR insert 2 d earlier than the 5D treatment, first $PGF_{2\alpha}$injection failed to cause luteolysis and may have allowed for increased blood progesterone because of the combination of supplemental (CIDR) and endogenous (CL) sources contributing to the serum pool of systemic progesterone concentrations. As expected on d 3 after $PGF_{2\alpha}$ injection, heifers in both treatments produced less progesterone at TAI. Rate of luteolysis

just before AI was not different between the 7D (90%) and the 5D treatments (88%). The relationship between progesterone concentrations at the initiation of a synchronization protocol (d −7) and pregnancy is interesting because 36% (345) of the total heifers that became pregnant in this study had serum concentration ≥1 ng/mL. Usually progesterone concentrations at the initiation of a successful protocol are <1 ng/mL because the upfront $PGF_{2\alpha}$ injection causes luteolysis and initiates a new follicular wave. In the current study only 20% of heifers that became pregnant had decreased (<1 ng/mL) progesterone concentrations on d −5.

It is important to note mainly gender-biased (sexed) semen was used in the present study, whereas other studies used conventional semen. In recent years, the use of gender-biased (sexed) semen has been gaining popularity because of its high genetic merit, typical calving ease, and increased odds of producing a heifer. First-service conception rates in heifers, however, are only 80% of what is achieved with conventional semen (DeJarnette et al., 2009). There also is an added cost per insemination, but some producers feel the pros outweigh the cons.

A study by Bridges et al. (2008) in suckled beef cows was one of the first to reduce the interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 d with AI at 60 h in the 7-d treatment and 72 h in the 5-d treatment. Reducing the CIDR insert from 7 to 5 d also required a second $PGF_{2\alpha}$ injection (given 12 h after the first $PGF_{2\alpha}$ injection and CIDR insert removal) to ensure luteolysis occurred in cows that formed a new CL in response to the first GnRH injection administered at the time of CIDR insert. The PR/AI in the 7 d treatment was 59.9% and increased to 70.4% in the 5 d treatment (Bridges et al., 2008).

Reduced ovulation responses to GnRH were not expected but their effects on PR/AI were an important factor to have a successful pregnancy outcome. At 32 d post AI, the 5D treatment averaged 28% conception rate, which was quite reduced from earlier reports. For example, when

using a 5-d CO-Synch protocol with an upfront GnRH injection and conventional semen, Rabaglino et al. (2010) reported 46.1% conception after only 1 $PGF_{2\alpha}$ injection at CIDR insert removal in a 5-d CO-Synch + CIDR protocol compared with 48.6% after 2 injections of $PGF_{2\alpha}$. Using a similar protocol to the current study but with conventional semen, Lima et al. (2010) obtained 54.1% conception in dairy heifers with an upfront GnRH injection at CIDR insertion and 1 $PGF_{2\alpha}$ injection. Without the upfront GnRH injection, Lima et al. (2010) still observed 52% conception with dairy heifers in the 5-d protocol. Using beef heifers, Ahmadzadeh et al. (2010) reported 62.5% conception during a 4-yr period administering no upfront hormone injections with the 5 d Co-Synch + CIDR protocol and conventional semen.

The 7D treatment did slightly better with a 32% conception rate on d 32 post AI. Results from the present study are consistent with Pursley et al. (1997) who obtained 35.1% in an Ovsynch protocol without a CIDR (conventional semen). Lamb et al. (2006) achieved 53.1% conception in beef heifers, which is more than 20% greater than what was obtained in the present study. The first GnRH injection of the Lamb et al. (2006) study was given at CIDR insertion and conventional semen was used instead of sexed semen. Using a similar protocol to the Lamb study, Busch et al. (2007) reported a comparable conception rate of 47% in beef heifers. In addition, Ambrose et al. (2008), with the exception of the second GnRH injection given 16 to 20 h before TAI, reported a conception rate of 55.6% in dairy heifers, both of which are still greater than that of the present study. With the use of sexed-semen in the present study we obtained 75% of the conception rates of what was achieved with conventional semen of comparable studies.

Estrus detection was applied in the current study to determine if it could be used as an effective tool in the current synchronization protocols. Of the 166 heifers that displayed signs of

estrus, 39.2% became pregnant. This value was significantly greater than those that were submitted to TAI on d 3 (n = 376) and had a pregnancy rate of 26.2%. This pregnancy rate was less than that reported by Lamb et al. (2006) who obtained about 60% on AI at estrus and over 35% on TAI.

We concluded that treatment of dairy heifers with the 5-d Co-Synch + CIDR protocol failed to increase PR/AI and total ovulation of follicles in comparison with the modified 7D protocol used in the current study. The PR/AI was similar in the Florida and Kansas locations, but favored the 7D treatment in the Mississippi location. With the majority of semen used in the study being gender-biased, the decreases in PR/AI were expected, but not to the extent reported in other studies. It is probable that the combination of synchronization and use of sexed semen are at fault for the decreased PR/AI because previous studies used conventional semen at TAI. The potential for increased pregnancy rates with the use of the 5-d CIDR program has been shown in previous studies, but various protocols have produced mixed results. This variability indicates that further studies are required to identify a reliable TAI program for dairy heifers.

REFERENCES

Ahmadzadeh, A., D. Gunn, J. B. Hall, and J. B. Glaze. 2010. Evaluation of 5-day versus 7-day CIDR treatment on reproductive outcomes of beef heifers using a modified timed-AI protocol. J. Anim. Sci. 88(E-Suppl. 2) (Abstr.).

Ambrose, D. J., D. G. V. Emmanuel, M. G. Colazo, J. P. Kastelic. 2008. Pregnancy rates to timed artificial insemination in Holstein heifers given prostaglandin $F_{2\alpha}$ twenty-four hours before or concurrent with removal of an intravaginal progesterone-releasing insert. J. Dairy Sci. 91:2678-2683.

Austin, E. J., M. Mihm, M. P. Ryan, D. H. Williams, J. F. Roche. 1999. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. J. Anim. Sci. 77:2219-2226.

Bisinotto, R. S., E. S. Ribeiro, L. T. Martins, R. S. Marsola, L. F. Greco, M. G. Favoreto, C. A. Risco, W. W. Thatcher, and J. E. P. Santos. 2010. Effect of interval between induction of ovulation and artificial insemination (AI) and supplemental progesterone for resynchronization on fertility of dairy cows subjected to a 5-d timed AI program. J. Dairy Sci. 93:5798-5808.

Bridges, G. A., L. A. Hesler, D. E. Grum, M. L. Mussard, C. L. Gasser, M. L. Day. 2008. Decreasing the interval between GnRH and PGF from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 69:843-851.

Bridges, G. A., M. L. Mussard, C. R. Burke, M. L. Day, 2010. Influence of length of proestrus on fertility and endocrine function in female cattle. Anim. Repro. Sci. 117:208-215.

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933-1939.

DeJarnette, J.M., R. L. Nebel, and C. E. Marshall. 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology 71:49-58.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:3000-3009.

Lima, F. S., H. Ayres, M. Favoreto, R. S. Bisinotto, L. F. Greco, E. S. Souza, R. S. Marsola, C. A. Risco, W. W. Thatcher, and J.E.P. Santos. 2010. Effects of GnRH at initiation of the 5-d timed AI program and timing of induction of ovulation relative to AI on ovarian dynamics and fertility of dairy heifers. Reproduction in Domestic Animals VII. Soc. Reprod. Fertil. 67:616 (Abstr.).

Mihm, M., A. Baguisi, M. P. Boland, J. F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fertil. 102:123-130.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson, 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation of synchronized estrus. J. Dairy Sci. 80:295-300.

Rabaglino, M. B., C. A. Risco, M. J. Thatcher, I. H. Kim, J. E. P. Santos, and W. W. Thatcher. 2010. Application of one injection of prostaglandin F2A in the five-day Co-Synch + CIDR protocol for estrous synchronization and resynchronization of dairy heifers. J. Dairy Sci. 93:1050-1058.

Stevenson, J. S. 2008. Progesterone, follicular, and estrual responses to progesterone-based estrus and ovulation synchronization protocols at five stages of the estrous cycle. J. Dairy Sci. 91:4640-4650.

Stevenson, J. S., S. L. Pulley, and H. I. Mellieon, Jr. 2011. Prostaglandin $F_{2\alpha}$ and GnRH administration improve progesterone status, luteal number, and proportion of ovular and anovular dairy cows with corpora lutea before a timed AI program. Unpublished data.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Zuluaga, J. F., and G. L. Williams. 2008. High-pressure steam sterilization of previously used CIDR inserts enhances the magnitude of the acute increase in circulating progesterone after insertion in cows. Anim. Repro. Sci. 107: 30-35.

Table 2.1. Ovarian characteristics 5 d before $PGF_{2\alpha}$ and ovulation response to the first GnRH injection

Item	Treatment[1]		*P* values		
	5D	7D	Treatment	Location	T x L
Heifers with a CL, %	87.3 (150)[2]	68.6 (137)	0.043	0.001	0.002
CL per heifer, no.	0.9 ± 0.04 (150)	0.8 ± 0.04 (137)	0.026	0.001	0.001
Total luteal volume, cm^3	4.6 ± 0.3 (120)	2.4 ± .3 (87)	0.001	0.292	0.110
Follicles >8 mm, no.	1.4 ± 0.07 (148)	1.4 ± 0.07 (134)	0.738	0.353	0.955
Largest follicle (LF)	12.1 ± 0.3 (144)	12.7 ± 0.3 (126)	0.225	0.463	0.146
Ovulation of LF, %	27.6 (145)	47.2 (127)	0.001	0.112	0.289
Second largest follicle (SLF)	9.8 ± 0.3 (56)	10.0 ± 0.3 (56)	0.567	0.582	0.031
Ovulation of SLF, %	10.9 (55)	16.1 (56)	0.886	0.939	0.206
Total ovulation[3], %	30.4 (148)	51.1 (129)	0.0013	0.258	0.714

[1] Both treatments consisted of a GnRH (G1) injection at d -d -5 with $PGF_{2\alpha}$ injection on d 0. The 7D treatment also included a controlled internal drug release (CIDR) insert on d -d -7 concurrent with a $PGF_{2\alpha}$ (PG) injection, whereas the 5D treatment included a CIDR on d -d -5 at G1.

[2] Number of heifers.

[3] Proportion of total follicles that ovulated after G1 injection.

Table 2.2. Ovarian characteristics at onset of $PGF_{2\alpha}$ treatment

	Treatment[1]		*P* values		
Item	5D	7D	Treatment	Location	T x L
CL per heifer, no.	1.1 ± 0.06 (150)[2]	1.0 ± 0.06 (136)	0.206	0.018	0.397
Presence of original CL, %	75.3 (150)	44.1 (136)	0.001	0.270	0.881
Original luteal volume, cm^3	3.8 ± .2 (98)	2.3 ± .3 (51)	0.001	0.290	0.519
Presence of new CL[3], %	78.6 (150)	73.5 (132)	0.660	0.001	0.020
New luteal volume, cm^3	2.1 ± 0.3 (44)	2.3 ± 0.3 (63)	0.659	0.232	0.217
Follicles >8 mm, no.	1.4 ± 0.07 (146)	1.6 ± 0.07 (134)	0.109	0.145	0.313
Largest follicle, mm	11.7 ± 0.2 (142)	11.9 ± 0.2 (132)	0.685	0.914	0.384
Second largest follicle, mm	9.8 ± 0.3 (64)	9.7 ± 0.3 (59)	0.685	0.982	0.395

[1] Both treatments consisted of a GnRH (G1) injection at d -5 with $PGF_{2\alpha}$ injection on d 0. The 7D treatment also included a controlled internal drug release (CIDR) insert on d -7 concurrent with a $PGF_{2\alpha}$ (PG) injection, whereas the 5D treatment included a CIDR on d -5 at G1.

[2]Number of heifers.

[3]Determine if animal has new CL present on the ovary after ovulation.

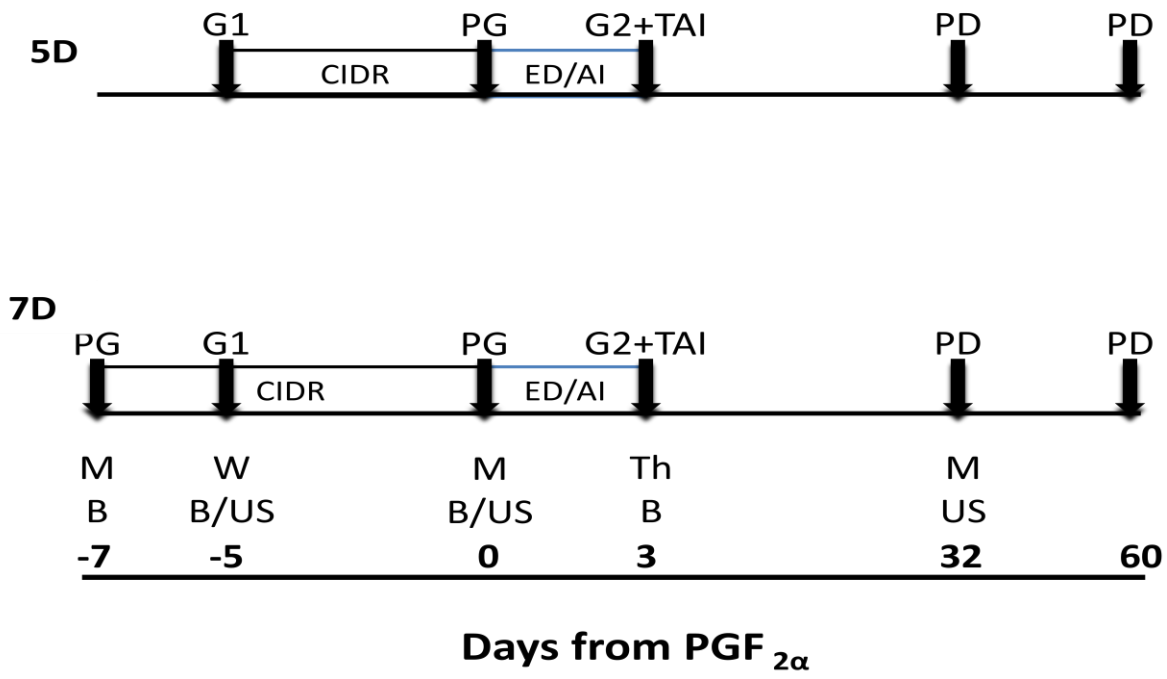

Figure 2.1. Experimental design of treatments. Both treatments consisted of a GnRH (G1) injection at d –5 with $PGF_{2\alpha}$ injection on d 0. The 7D treatment also included a controlled internal drug release (CIDR) insert on d –7 concurrent with a $PGF_{2\alpha}$ (PG) injection, whereas the 5D treatment included a CIDR on d –5 at G1. Estrus detection (ED) and AI at detected estrus occurred from d 0 to 3. Those heifers not detected in estrus were time inseminated (TAI) on d 3 (72 h after PG) and concurrent with the second GnRH (G2) injection. Pregnancy was diagnosed on d 32 by ultrasound and heifers detected not pregnant, in some cases, were re-treated up to 2 times on the same treatment. A second pregnancy diagnosis was conducted 4 wk later to calculate pregnancy loss since the first positive pregnancy diagnosis. GnRH = 100 μg of GnRH; PG = 25 mg of $PGF_{2\alpha}$; US = transrectal ultrasonography; and B = blood collection.

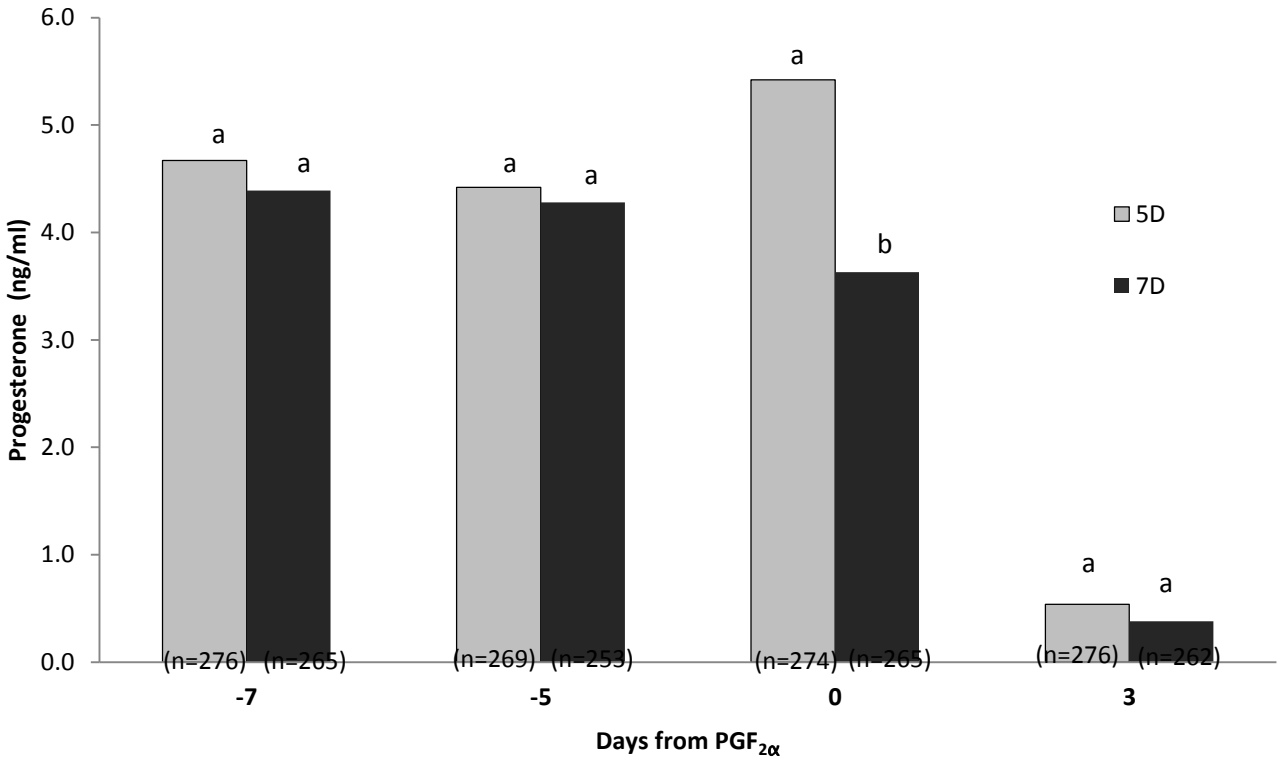

Figure 2.2. Concentrations of serum progesterone in heifers from day of common $PGF_{2\alpha}$ treatment (d 0) in the 5D and 7D treatments. Dairy heifers (n = 545) from three locations (Florida, Kansas, and Mississippi) were assigned randomly to each of two treatments: 1) 25 mg of $PGF_{2\alpha}$ injection and insertion of previously used autoclaved CIDR on d -7 followed by 100 µg of GnRH administered on d -5, and a 25 mg $PGF_{2\alpha}$ injection at CIDR removal (7D) on d 0; 2) 100 µg of GnRH and insertion of previously used autoclaved CIDR on d -5 and 25 mg of $PGF_{2\alpha}$ injection at CIDR removal (5D) on d 0. Artificial insemination (AI) occurred after detected estrus from d 0 to 3.

[a-b]Means within experimental day having different letters differ ($P < 0.05$).

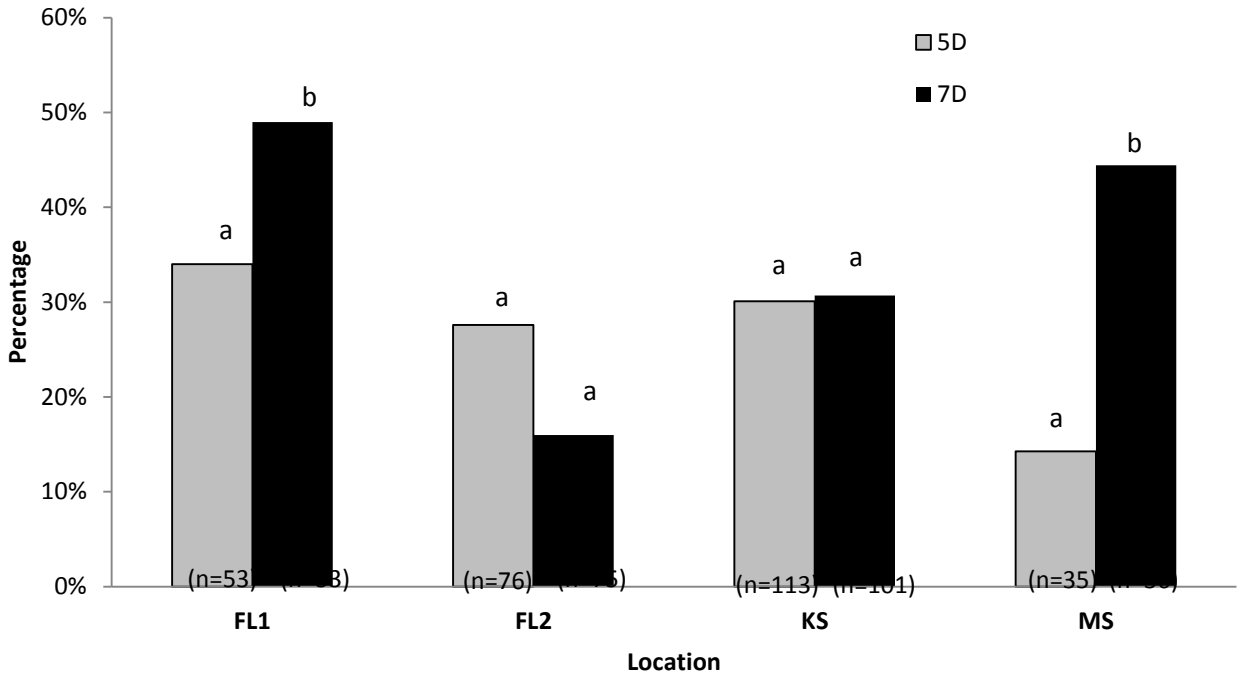

Figure 2.3. Pregnancy rates of heifers at 32 d post AI. Dairy heifers (n = 545) from three locations (Florida, Kansas, and Mississippi) were assigned randomly to each of two treatments: 1) 25 mg of $PGF_{2\alpha}$ injection and insertion of previously used autoclaved CIDR on d -7 followed by 100 µg of GnRH administered on d -5, and a 25 mg $PGF_{2\alpha}$ injection at CIDR removal (7D) on d 0; 2) 100 µg of GnRH and insertion of previously used autoclaved CIDR on d -5 and 25 mg of $PGF_{2\alpha}$ injection at CIDR removal (5D) on d 0. Artificial insemination (AI) occurred after detected estrus from d 0 to 3.

[a-b]Means within location having different letters differ ($P < 0.05$).