PRESYNCHRONIZING INJECTIONS OF PROSTAGLANDIN $F_{2\alpha}$ OR PROSTAGLANDIN $F_{2\alpha}$ + GONADOTROPIN-RELEASING HORMONE BEFORE A FIXED TIME ARTIFICIAL INSEMINATION CO-SYNCH + CIDR PROGRAM IN SUCKLED BEEF COWS

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ABSTRACT

We hypothesized that pregnancy outcomes may be improved by inducing luteal regression, ovulation, or both before a control CO-Synch + CIDR program (100 mcg GnRH i.m. [GnRH-1] and insertion of a progesterone-impregnated intravaginal controlled internal drug release [CIDR] insert on d -10, 25 mg PGF2alpha (PG) i.m. and CIDR insert removal on d -3, and 100 mcg GnRH i.m. [GnRH-2] and timed AI [TAI] on d 0) in suckled beef cows. This hypothesis was tested in 2 experiments, in which cows were treated with either PG or PG + GnRH before initiating a control CO-Synch + CIDR program to increase the proportion of cows starting the program in a low (< 1 ng/mL; Exp. 1) or high (≥ 1 ng/mL; Exp. 2) progesterone status, respectively. Blood was collected before each injection for later progesterone analyses. In Exp. 1, cows at 9 locations (n = 1,537) were assigned to either: (1) control or (2) PrePG (same as control with a PG injection on d -13). The PrePG cows had larger ($P < 0.05$) follicles on d -10 and more ($P < 0.05$) ovulated after GnRH-1 than controls (60.6 vs. 36.5%). Incidence of estrus between d -3 and 0 was greater ($P < 0.05$) for treated multiparous cows than multiparous controls and treated and control primiparous cows (74.1 vs. 64.3, 58.6, and 59.1%, respectively). In Exp. 2, cows at 4 locations (n = 803) were assigned to: (1) control (same as Exp. 1) or (2) PrePGG (same as control with PG injection on d -20 and GnRH injection on d -17. Cows with BCS > 5.0 or ≥ 70 d postpartum at TAI were more ($P < 0.05$) likely to become pregnant than thinner cows or those with fewer days postpartum. Treated cows in both experiments were more ($P < 0.05$) likely than controls to have luteolysis after initial PG injections and reduced ($P < 0.05$) serum progesterone. In both experiments, pregnancy rates at d 35 did not differ between treatment and control; however, cows classified as anestrous before d -10, but with elevated progesterone on d

-10, had increased ($P < 0.05$) pregnancy outcomes than remaining anestrous cows with low progesterone concentrations. In summary, luteal regression and ovulation were enhanced by treatments before the 7 d CO-Synch + CIDR program; however, pregnancy per TAI was not improved.

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My parents have not only provided me with a solid background growing up in one of the truly unique places in America, but they also showed me the importance of a faith in God, the support of a family, and the value of hard work. I will always be indebted to them and hope that I honor them with my life.

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Dedication

The research summarized herein is dedicated to my friends, neighbors, and former colleagues that have undertaken the challenge of providing the world with high-quality beef protein. The Grimm's fable of Rumpelstiltskin tells how a miller's daughter spins straw into gold. The task of the American cattleman is similar as he works to turn low-quality forage into the best protein source in the world. The tools to accomplish the work of spinning gold are elusive at best; it is my goal to add in some small way to that toolbox.

Chapter 1 - Literature Review

INTRODUCTION

Agriculturalists who focus on raising excellent-quality beef are currently utilizing artificial insemination (AI) to improve the concentration of desirable genetics in their herds. The majority of cattle breeders, however, still rely on the inconsistency of semi-planned matings associated with natural-service sires. Specifically only 5.2% of beef cows in the United States were inseminated artificially in 2007-2008 (NAHMS, 2009). A greater percentage of heifers (16.3%) were artificially inseminated. Artificial insemination in domestic farm animals has been practiced since the beginning of the twentieth century (Foote, 2002). Developments, including the use of semen extenders, artificial vagina, frozen semen, and modern AI equipment, made possible rapid expansion in the use of AI in dairy cattle when the potential for genetic progress was recognized. Factors that have prevented beef producers from utilizing the same potential include tradition, economics, and labor. The developments of sex-sorted semen (DeJarnette, 2009), applying genomics for sire selection (VanRaden, 2009), and fixed-timed AI all have the potential to change the paradigm of natural service in beef cattle.

Reproductive performance is the most important measurement of success in cow-calf operations (Wiltbank, 1990). Missed breeding opportunities not only result in reduced calving percentages, but also account for smaller, more variable-sized calves at weaning. Increasing the percentage of calves born in the first 21 d of the breeding season can improve weaning weight of the calf crop. Synchronizing estrus before AI is an effective tool to increase the number of cows calving early in the season. One study demonstrated that cows calving from a synchronized AI weaned calves 9.5 kg heavier than their counterparts weaned from nonsynchronized cows

(Schafer et al., 1990). Increasing the number of pregnancies per AI beyond the currently applied synchronization protocols might lead to wider acceptance of AI among beef producers.

REVIEW OF BOVINE ESTROUS CYCLE

Knowledge of the estrous cycle of cattle is a prerequisite for understanding and improving estrus- and ovulation-synchronization protocols. One method of studying the estrous cycle is to categorize it into the luteal and follicular phases (Senger, 2005). The luteal phase usually lasts about 13 d and is associated with the lifespan of the corpus luteum (CL). The luteal phase is divided into two stages: (1) metestrus and (2) diestrus. The follicular phase is much shorter, approximately 8 d in duration. The follicular phase, characterized by the absence of the CL and presence of a maturing dominant follicle, consists of two stages: (1) proestrus and (2) estrus.

Metestrus

When a cow no longer exhibits sexual receptivity metestrus begins. Shortly after the beginning of metestrus the cow ovulates. After ovulation, the ruptured follicle contains theca and granulosa cells that in the presence of LH are transformed into small luteal cells and large luteal cells , respectively (Niswender et al., 1986; Garverick et al., 1992). Progesterone is synthesized in the most simplistic pathway of the steroid hormones (Niswender et al., 2000). Cholesterol, the substrate for progesterone, is converted to pregnenolone in the inner mitochondrial membrane of small and large luteal cells utilizing P-450 side-chain cleavage enzyme. Pregnenolone is transported out of the mitochondria and converted to progesterone by 3β-hydroxysteroid dehydrogenase. Progesterone then appears to diffuse from the luteal cells. The shift in production of steroid hormones from estradiol to progesterone is accomplished by an increase in the expression of P-450 side-chain cleavage and 3β-hydroxysteroid dehydrogenase

and a decrease in the expression of the enzymes necessary for conversion of progesterone to estrogens. The combination of the small and large luteal cells makes up the functional part of the newly developed CL.

Diestrus

When the CL is fully functional, the cow's cycle enters the diestrus phase. This early part of the luteal phase (diestrus) is characterized by decreasing plasma concentrations of LH and increasing concentrations of progesterone (Garverick et al., 1971). The large luteal cells account for the majority (80%) of the progesterone production in the ovary. The small luteal cells produce a comparatively small quantity of progesterone. During diestrus, significant production of progesterone suppresses the secretion of LH and estradiol which is beneficial to the maintenance of pregnancy if it has occurred following ovulation (Hansel and Convey et al., 1983). If a conceptus is not present, then the uterine endometrium up regulates oxytocin receptors in response to oxytocin released by the aging CL. The oxytocin triggers prostaglandin F2α (PG) production by the uterine endometrium (McCracken et al., 1996). The utero-ovarian vascular plexus via the countercurrent exchange initiates a positive feedback loop between CL-produced oxytocin and uterine produced PG. Ultimately PG concentration is sufficient to cause vascular constriction in the CL. The intracellular signaling that results in apoptosis of capillary endothelial is initiated by PG binding to specific receptors on large luteal cells. The receptors are seven-transmembrane G protein coupled receptors. Progesterone binding with these high affinity receptors induces activation of membrane-bound Phospholipase C (PLC). The presence of PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP3) and 1,2-diacyglycerol (DAG). Elevated IP3 concentrations in the cytoplasm result in the release of free Ca2+ that stimulate the catalytic activity of Ca2+-dependent protein

kinase (PKC). PKC is directly involved in the reduction of steroidogenesis in large luteal cells and is likely responsible for the activation of proteins that cause apoptosis in luteal cells. Destruction of luteal tissue or luteolysis is caused by the loss of blood flow and results in a rapid reduction in progesterone production by the CL. Luteolysis marks the end of diestrus.

Proestrus

The beginning of the follicular phase is termed proestrus and is characterized by a maturing dominant follicle. Theca and granulosa cells in the antral follicle are responsible for the production of estradiol. Dominant follicles acquire receptors for LH in both theca and granulosa cells (Fortune et al., 1988). LH stimulates the conversion of cholesterol to pregnenolone, however only theca cells convert pregnenolone to androstenedione through the delta-5 pathway. Androstenedione diffuses across cell membranes and in the granulosa cell is converted to testosterone by the enzyme 17 β-hydroxysteroid dehydrogenase. Testosterone is then converted to estradiol-17β via P450 aromatase. Reduction of progesterone concentrations during luteolysis removes the negative feedback of progesterone on the preovulatory GnRH surge center of the hypothalamus. A positive feedback loop is established between the estradiol production of the maturing follicle and pituitary gonadotrophs that secrete FSH and LH. The circulating estradiol increases the sensitivity of the gonadotrophs to GnRH. Pulsatile GnRH release from the stalk median eminence causes a corresponding release of FSH and LH from the hypothalamus. The rapidly growing follicle produces increasing concentrations of estradiol that culminate in the expression of sexual behavior and estrus.

Estrus

Estrus is defined as the period of sexual receptivity lasts between 12 and 18 h (Wiltbank et al., 1967). During this stage of the estrous cycle, estradiol concentrations increase and ultimately reach a peak. When a threshold concentration is achieved in the hypothalamus, a preovulatory surge of GnRH from the surge center of the hypothalamus is released, resulting in the secretion of LH from the anterior pituitary.

POSTPARTUM ANESTRUS

Involution

Following calving, beef cows enter a time devoid of estrous behavior. This period of anestrus is characterized first by the absence of pulsatile LH pulses and later an increase in LH pulses (Short et al., 1990). Uterine involution is the most immediate barrier to fertilization following parturition; however, uterine involution is complete in healthy cattle by 40 d postpartum. Inseminations following estrus in the first 20 d postpartum are not likely to be successful. The non-involuted uterus provides a barrier to sperm transport. In most cases, estrus will not be initiated until uterine involution is complete; therefore, involution is not normally a factor in reduced fertility. Ovarian steroids decrease the sensitivity of the hypothalamus during gestation (Yavas et al., 2000). By 7 d postpartum, FSH secretion resumes, producing sufficient concentrations to enable follicle recruitment and growth (Crowe et al., 2008). The surge center of the hypothalamus, however, remains inactive for a longer period of time and insufficient circulating concentrations of LH exist to enable ovulation of a dominant follicle. Duffy et al. (2000) demonstrated that exogenously administering small-pulse concentrations of LH would result in ovulation. In the anestrous cow, GnRH pulses have insufficient frequency to support the dominant follicle to mature and initiate ovulation (Stevenson et al., 1997). Sufficient stores of

LH are replenished by 30 d postpartum; however, the inability of the surge center to generate a preovulatory LH surge remains the most common reason for anestrus in the postpartum beef cow.

Short Cycles

Beef cows that return to estrus in the first 30 to 40 d after parturition often exhibit a sub-fertile, abnormally short, estrous cycle (Foote and Hunter, 1964). The first ovulation following parturition is not preceded by elevated progesterone. The uterus without exposure to progesterone is more responsive to oxytocin secreted by the newly formed CL. A prematurely and abnormally high concentration of PG is released by the uterus. Early luteolysis of the CL precedes maternal recognition of the newly fertilized oocyte. The result is reduced pregnancy maintenance and an abnormally rapid return to estrus. The next estrous cycle is usually of normal duration. Inseminating cows artificially or natural mating preceding the short cycle estrus has no probability of success. Exposing the animal to progesterone before the first ovulation will reduce the number of short cycles (Ramirez-Godinez et al., 1981. In a breeding program that has a large percentage of anestrous cows, the addition of a progestin into the protocol will reduce the infertility associated with short cycle estrus.

Suckling

The suckling of beef calves is a major contributor to the reduction in LH pulses. Physical presence, olfactory stimulation, and tactile stimulation along with actual suckling have been shown to increase the production of endogenous opioid peptides (EOP) such as β-endorphin. Endogenous opioid peptides likely reduce GnRH production by the hypothalamus (Williams,

1990). Temporary (24 h) calf removal has been shown to increase LH pulse frequency and estrus when applied in conjunction with an estrus-synchronization protocol (Geary et al., 2001).

Nutrition

It is firmly established that nutrition is a factor in altering the duration of postpartum anestrus (Wiltbank et al.,. 1962; 1964). What is not clearly defined is the mechanism by which nutrition exerts control over the hormonal mechanisms of reproductive function. Body condition score (BCS) has been used as a measure of nutritional fitness of beef cows; however, energy balance is not accurately evaluated by a one-time BCS evaluation (Short et al., 1988). Partitioning of nutrients to meet the requirements of lactation, growth, basal metabolism and reproduction could be important in understanding variable results in nutrition studies (Short et al., 1990). Adequate glucose concentrations are necessary for maintaining neural function. Blood glucose concentrations have a positive relationship with GnRH production by the hypothalamus. Most nutritional work has focused on energy as the limiting nutrient in establishing estrous cycles in the postpartum cow. It is possible that other nutrients such as protein, minerals or vitamins have an equally significant role. The prevalence of research on nutritional controls of anestrus has declined in the last two decades.

Other Factors

Numerous other factors exist that have been identified as having an impact on the duration of anestrus following parturition. Cows experiencing dystocia have prolonged postpartum intervals to first estrus. (Brinks et al., 1973; Laster et al., 1973). Parity also has an impact on the incidence of estrus. Nutrient partitioning results in a larger percentage of available nutrients supporting growth in primiparous than in multiparous cows. Primiparous cows have

longer intervals to first estrus after calving than multiparous cows. There are also numerous minor factors such as breed, twin births, or presence of a bull that have been identified as having some impact on the interval to first estrus.

SYNCHRONIZATION OF ESTRUS AND OVULATION

Artificially Prolonging the Luteal Phase

Synchronizing estrus and the subsequent opportunity to schedule the time of insemination has been pursued by researchers during the past 60 y with varied results (Beal, 1988). The first attempts at synchronization of estrus were based on research that showed progesterone prevented estrus and ovulation (Christian and Casida, 1948; Trimberger and Hansel, 1955). Progestin treatment delayed ovulation resulting in a synchronized estrus once the progestin treatment was withdrawn. After prolonged exposure to progestins subsequent fertility of inseminations was very poor. Shorter exposure to progesterone coupled with a pharmacological dose of estrogen at the initiation of the treatment to induce luteolysis was reported (Wiltbank and Kasson, 1968). Response to estrogen was dependent on the stage of the estrous cycle and produced inconsistent results. Approval of exogenous estrogen for use in beef cattle in the United States has not been granted by the USDA Food and Drug Administration.

Currently available progestins are the oral feed additive melengestrol acetate (MGA) and an intravaginal controlled internal drug release insert (Eazi-Breed CIDR, Pfizer Animal Health) that contains 1.38 g progesterone per device. Use of MGA artificially extends the luteal phase by supplementing progestin during CL function, or after spontaneous luteolysis, supplemental progestin prevents ovulation in the bovine. Beal (1998) found, that extending the luteal phase longer than normal resulted in reduced fertility from the ovulation that followed the removal of MGA from the diet. Reduction in oocyte quality associated with extending the luteal phase

demonstrated that controlling follicular dynamics also are important in successful synchronization protocols.

Ending the Luteal Phase

The inconsistency of progestin-based synchronization and the inability to use estrogens for synchronization of estrus led to the search for a luteolytic agent. The discovery of PG (McCracken et al., 1972) provided for an effective luteolysin. Within 4 h after injection PG decreased the progesterone production of large luteal cells and decreased the blood flow to a mature CL (Niswender et al., 2000). Prostaglandin F2α also triggered a reduction in the size of the CL, a reduction in the ratio of small to large luteal cells, and a reduction in the enzyme 3β-HSD (essential for progesterone production). Exogenously administered PG was metabolized rapidly; 66% of PG was metabolized in one passage through the lungs (Davis et al., 1985). Prostaglandin F2α wass effective at inducing luteolysis from the 5th d of the estrous cycle (Hansel and Convey, 1983) until spontaneous luteolysis occurred on approximately d 17 of the estrous cycle. Shortening or ending the luteal phase subsequently led to estrus and ovulation; however, timing of estrus varied significantly to prevent acceptable pregnancy outcomes after AI at a predetermined fixed time.

Ovarian Follicular Waves

The wavelike growth and atresia of ovarian follicles in heifers was suggested after post-mortem observation of ovaries on known days of the estrous cycle (Rajakoski, 1960). Use of transrectal ultrasonography (Ginther et al., 1989) demonstrated that follicular waves were present in heifers before puberty (Adams et al., 1994). Waves of follicle growth were observed as early as 14 d of age. Neonatal heifers had their full complement of oocytes at the time of parturition.

The prepuberal heifer showed a positive linear relationship between age and size of her largest follicle (Rawlings et al., 2003). Hypothalamic changes in response to small concentrations of ovarian secreted estradiol allowed for increasing follicular size as puberty approached. Sexual maturation occurred when the hypothalamus secreted sufficient LH in a pulsatile manner to trigger ovulation. The post ovulatory spike in FSH observed in cycling beef cattle was associated with the recruitment of the first cohort or first wave of follicles during the estrous cycle (Adams et al., 1992). The rapid decline of estradiol following estrus resulted in an increase in serum FSH that recruited the next group of follicles to develop in a wavelike fashion. These waves of follicle growth were followed by atresia or ovulation preceding the next wave of follicle growth. Similarly, early postpartum cows demonstrated regular follicular waves that were preceded by surges in FSH. In both cases, the follicular wave continued despite the absence of ovulation.

Growth and atresia of follicles has a profound impact on synchronization of estrus and ovulation. There are essentially two unique phases, the luteal and follicular, that exist somewhat independent of one another. In a spontaneously ovulating animal, the two phases are synchronized at estrus and a large ovulatory size follicle is available at the time of insemination. Manipulation of the luteal phase via exogenous hormonal control needs to account for the timing of the follicular phase for successful synchrony of the follicular maturation at the time of insemination.

Control of Follicular Waves

Gonadotropin-Releasing Hormone

Administration of GnRH will induce ovulation in bovine females that have a dominant follicle of sufficient size (Ryan et al., 1998). Follicles that are at least 10 mm in diameter will ovulate in response to a GnRH-induced LH surge. Administering GnRH will induce ovulation in

about 66% of cows; however, the problems associated with physiologically immature follicles need to be addressed for optimal pregnancy outcomes (Geary et al., 2000). The fertility of insemination in beef cows after GnRH-induced ovulation varies according to the size of follicle at GnRH injection (Perry et al., 2005). When follicles less than 13 mm are induced to ovulate, pregnancies per insemination are decreased. After GnRH-induced ovulation of these smaller follicles, an increase in the percentage of embryonic loss occurs after inseminations. In addition, follicles induced to ovulate in the presence of high progesterone concentrations associated with a viable CL also are less likely to result in pregnancies when inseminated (Colazo et al., 2008).

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is commercially available for use in cattle. It has gonadotrophic characteristics that make it a viable option for inducing ovulation in synchronization protocols (Fricke et al., 1993). It is most effective at inducing ovulation during early (4 to 7 d) and late (14 to 16 d) luteal phase (Price and Webb, 1989). Administration of hCG is as effective at ovulating follicles as GnRH; however, substituting hCG for GnRH at the beginning of a fixed time AI protocols has produced fewer pregnancies per AI compared with protocols that use GnRH (Geary et al., 2001; Burns et al., 2008;). The reduced pregnancy percentage following hCG treatment compared to GnRH causes hCG to be an inferior ovulation synchronization choice in TAI.

CONTEMPORARY TIMED AI PROTOCOLS

Seven-Day CO-Synch + Controlled Internal Drug Release (CIDR) Insert

Variability of ovulation timing associated with artificially prolonging or ending the luteal phase led researchers to combine elements of previous synchronization methods. Use of GnRH

at the onset of a protocol turned over the dominant follicle in 66% of treated animals. Administering PG 7 d later induced luteolysis for the newly developed CL, the original CL, or both, which allowed the new dominant follicle that formed subsequent to GnRH injection to grow to preovulatory size in a reduced progesterone environment. Inducing ovulation 48 h later with a second GnRH injection coupled with AI resulted in satisfactory pregnancy outcomes (Geary and Whittier, 1997). Modifications to this protocol (CO-Synch) include the use of a progestin to improve pregnancy percentages in anestrous cows (Stevenson et al., 2003; Larson et al., 2006) and adjustments to the period between second GnRH and AI of 56, 64, and 72 h (Dobbins et al., 2009). Pregnancy outcomes in numerous trials have been 50% or better (Geary et al., 2001, Larson et al., 2006). Attempts to optimize oocyte quality at TAI have led researchers to try both 5 and 6 d protocols.

Five-Day CO-Synch + CIDR

The goal of higher-quality oocytes at ovulation led researchers to examine follicle characteristics associated with timed AI. Dairy cows with larger preovulatory follicles had greater concentrations of estradiol in their follicular fluid and also had improved pregnancy rates to AI than dairy cows with smaller dominant follicles (Lopes et al., 2007). Examination of beef cow follicles (Perry et al., 2005) revealed that follicle size was important for maximal fertility only when follicles underwent induced ovulation. Spontaneously-ovulated follicles attain physiological maturity, necessary for sufficient estradiol production. The goal of younger, growing follicles proposed by Valdez et al. (2005) is balanced by the need for physiologically-mature follicles at induced ovulation. One attempt at reaching the optimum follicle maturity at timed AI is the 5-d CO-Synch + CIDR protocol (Bridges et al., 2008). The 5-d protocol shortens the time between first GnRH injection and PG with the CIDR administered for only 5 d. When

combined with 2 injections of PG 12 h apart and AI at 72 h post-CIDR removal, pregnancy outcomes were greater than the control 7-d protocol. Additional research has shown that the 2 injections of PG can be administered at the same time as CIDR removal, thus reducing the number of processing events per cow without negatively impacting pregnancies per AI (Bridges et al., 2012).

Six-Day CO-Synch + CIDR

For optimal AI pregnancy outcomes at a fixed time, it is necessary to have consistent ovulation of follicles that are at the peak of their reproductive potential. By adding a PG injection before the start of the traditional 6 d CO-Synch protocol, researchers hypothesized that follicles at insemination would be more uniform by resetting the follicular wave associated with the PG-induced estrus (Perry et al., 2012). The researchers also tested whether a 6-d exposure to progesterone from a CIDR would give better pregnancy results than the 5-d exposure. Cows that were injected with PG were more likely to ovulate after the second GnRH injection administered at timed AI and more likely to become pregnant at timed AI. Having a synchronized follicular wave at the beginning of a timed AI protocol could improve pregnancy results. Dairy cows at 4 herds in Kansas had improved pregnancy outcomes when they were treated with PG and GnRH prior to beginning a 7-d Ovsynch protocol compared to cows that were not presynchronized (Stevenson et al., 2012). A similar response might be measured in lactating beef cows treated with PG and GnRH , 20 and 17 d respectively, prior to breeding using timed AI and a 7 day CO-Synch + CIDR protocol.

SUMMARY

Reluctance of cattle producers to incorporate AI into their ranch management is at least partly because of labor requirements to detect estrus. Improving the percentage of cows that

conceive to a timed AI has the opportunity to influence a paradigm shift in which producers are more likely to experience the benefits of controlled breeding programs. Timed AI success depends on the success of managing the follicular wave so that at the chosen time of insemination a follicle with optimum potential for reproductive success is available for ovulation. Two areas that seem to be exploitable in protocols are: 1) improving the follicular wave synchrony of cycling cows before initiating the breeding protocol (i.e., presynchronization), and 2) increasing the percentage of anestrous cows that will possess a preovulatory sized follicle and a conditioned uterine environment to facilitate conception. By treating cows with PG or PG + GnRH prior to the start of a 7-d CO-Synch protocol, more cows could enter the protocol with low progesterone. It is also possible that ovulating dominant follicles in anestrus cows could cause formation of a CL followed by initiation of a fertile estrus cycle. We hypothesize that pretreating cows with PG or PG + GnRH before initiating a 7-d CO-Synch + CIDR protocol will: 1) improve follicular wave synchrony, and 2) improve pregnancy outcomes.

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Chapter 2 - Presynchronizing $PGF_{2\alpha}$ or $PGF_{2\alpha}$ + GnRH before a Fixed Time Artificial Insemination (TAI) CO-Synch + CIDR Program in Suckled Beef Cows

ABSTRACT

We hypothesized that pregnancy outcomes may be improved by inducing luteal regression, ovulation, or both before a control CO-Synch + CIDR program (100 mcg GnRH i.m. [GnRH-1] and insertion of a progesterone-impregnated intravaginal controlled internal drug release [CIDR] insert on d -10, 25 mg PGF2alpha (PG) i.m. and CIDR insert removal on d -3, and 100 mcg GnRH i.m. [GnRH-2] and timed AI [TAI] on d 0) in suckled beef cows. This hypothesis was tested in 2 experiments, in which cows were treated with either PG or PG + GnRH before initiating a control CO-Synch + CIDR program to increase the proportion of cows starting the program in a low (< 1 ng/mL; Exp. 1) or high (≥ 1 ng/mL; Exp. 2) progesterone status, respectively. Blood was collected before each injection for later progesterone analyses. In Exp. 1, cows at 9 locations (n = 1,537) were assigned to either: (1) control or (2) PrePG (same as control with a PG injection on d -13). The PrePG cows had larger ($P < 0.05$) follicles on d -10 and more ($P < 0.05$) ovulated after GnRH-1 than controls (60.6 vs. 36.5%). Incidence of estrus between d -3 and 0 was greater ($P < 0.05$) for treated multiparous cows than multiparous controls and treated and control primiparous cows (74.1 vs. 64.3, 58.6, and 59.1%, respectively). In Exp. 2, cows at 4 locations (n = 803) were assigned to: (1) control (same as Exp. 1) or (2) PrePGG (same as control with PG injection on d -20 and GnRH injection on d -17. Cows with BCS > 5.0 or ≥ 70 d postpartum at TAI were more ($P < 0.05$) likely to become pregnant than thinner cows or those with fewer days postpartum. Treated cows in both experiments were more ($P < 0.05$) likely than controls to have luteolysis after initial PG injections and reduced ($P < 0.05$) serum

progesterone. In both experiments, pregnancy rates at d 35 did not differ between treatment and control; however, cows classified as anestrous before d -10, but with elevated progesterone on d -10, had increased ($P < 0.05$) pregnancy outcomes than remaining anestrous cows with low progesterone concentrations. In summary, luteal regression and ovulation were enhanced by treatments before the 7 d CO-Synch + CIDR program; however, pregnancy per TAI was not improved.

INTRODUCTION

Widespread acceptance of artificial insemination (AI) in beef cattle partly depends on the success of programs that facilitate insemination at a predetermined time. Several ovulation synchronization protocols that utilize exogenous GnRH at the time of insertion of a progesterone-impregnated intravaginal controlled internal drug release (CIDR) insert have been developed (Lamb et al., 2006; Larson et al., 2006). Beef herds that have more than 50% anestrous cows at the start of breeding season may benefit from protocols that promote presynchronization ovulation induction in response to GnRH. Dominant follicles are present in noncycling cows (Stagg et al., 1995) and exogenous GnRH will initiate ovulation of a dominant follicle (Bo et al., 1995; Twagiramungu et al., 1995).

Follicles induced to ovulate before reaching 11 mm in diameter were less likely to result in pregnancy than ovulation of larger follicles in beef cows (Perry et al., 2005). Replacement beef heifers were more likely to become pregnant when follicles induced to ovulate with GnRH ranged between 10.7 and 15.7 mm in diameter (Perry et al., 2007). Efficacy of GnRH to ovulate follicles depended on the maturity of the follicle exposed to GnRH (Bridges et al., 2010). Follicles > 10 mm ovulated in response to GnRH (Ryan et al., 1998); however, only 66% of beef cows ovulated after a single injection of GnRH because of variation in follicle size (Geary et al.,

2000). Using $PGF_{2\alpha}$ to synchronize the follicular wave before the start of a 6-d CO-Synch timed AI (TAI) protocol improved pregnancy outcomes in beef cows compared with a 5-d CO-Synch+ CIDR (GnRH injection plus CIDR insertion 5 d before and 66 to 70 h after $PGF_{2\alpha,}$ with GnRH concomitant with TAI; Perry et al., 2012). Likewise, a larger proportion of heifers exhibited a new follicular wave at the start of a TAI protocol when they were injected with $PGF_{2\alpha}$ 3 d before the initiation of the protocol (Grant et al., 2011). Lactating dairy cows had better pregnancy outcomes during the summer when they were treated with $PGF_{2\alpha}$ and GnRH (3 d after $PGF_{2\alpha}$) 7 d before the start of the Ovsynch protocol than cows whose estrous cycles were presynchronized with 2 $PGF_{2\alpha}$ injections 14 d apart, with the second $PGF_{2\alpha}$ injection administered 10 d before Ovsynch (Stevenson and Pulley, 2012a).

We hypothesized that using $PGF_{2\alpha}$ or $PGF_{2\alpha}$ combined with GnRH 3 d later before the start of the 7-d CO-Synch + CIDR protocol would improve pregnancy outcomes. Our objectives were to determine if presynchronization treatments would increase the proportion of cows beginning a 7-d CO-Synch + CIDR protocol either in a low (Exp. 1) or high (Exp. 2) progesterone status and consequently improve pregnancy outcomes compared with the 7-d CO-Synch + CIDR control.

MATERIALS AND METHODS

Experiment 1

Experimental Design

A total of 1,537 primiparous and multiparous cows at 9 locations in 4 states (Florida, Georgia, Kansas, and South Dakota) were enrolled in Experiment 1. Characteristics of suckled beef cows enrolled by location are summarized in Table 1 including breed, percentage 2-year-old

cows, days postpartum at TAI, body condition score, and percentage cycling status at the onset of the 7-d CO-Synch + CIDR program. Cows were stratified by breed, days postpartum, and parity and then assigned randomly to 2 treatments (Figure 1). Control cows received the standard CO-Synch + CIDR program (100 μg GnRH [2 mL Factrel, Pfizer Animal Health, Whitehouse Station, NJ] 7 d before and 72 h after 25 mg $PGF_{2\alpha}$ [5 mL Lutalyse; Pfizer Animal Health]). A new CIDR insert (Pfizer Animal Health) containing 1.38 g progesterone was placed intravaginally at the time of the first GnRH injection (d –10). Experimental cows (PrePG) received 25 mg $PGF_{2\alpha}$ 3 d before (d –13) the CO-Synch + CIDR program began.

Body condition scores (BCS; 1 = thin; 9 = very fat) were assigned on d –13. Estrus-detection patches (Estrotect, Rockway, Inc. Spring Valley, WI) were affixed to all cows. Estrus-detection patches were removed on d –10 and scored (0 = not colored, 1 = partially colored, and 2 = completely colored). On d –3 CIDR inserts were removed, a second estrus-detection patch was applied and $PGF_{2\alpha}$ was administered to all cows in both treatments. Only 3.6% of patches were scored as 1 (partially colored) by d –10 (3 d after the treatment $PGF_{2\alpha}$ injection) and 5.5% of patches were scored as 1 at timed AI. Therefore, we eliminated patch score information from cows with patch scores of 1 and assumed that cows having patch scores of 0 did not show estrus. We further assumed that cows with completely colored patches (scored as 2) had been mounted and were in estrus sometime after the $PGF_{2\alpha}$ injections.

Artificial insemination was performed 72 h after CIDR insert removal on d 0 and estrus-detection patches were removed and scored. Cows were either exposed to cleanup bulls 10 to 12 d later or re-inseminated at subsequent estrus (KS-P location; Table 1). At 35 d after TAI, pregnancy was confirmed by transrectal ultrasonography (Aloka 500V, 5 MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required presence of a corpus

luteum and uterine fluid or an embryo with a heartbeat. A final pregnancy diagnosis was determined via transrectal ultrasonography 35 d after the end of the breeding season (removal of natural service sires). Embryonic losses in cows that conceived to the TAI were determined at that time.

Cycling Status

Blood samples were collected via caudal vessel puncture from cows at 7 of the 9 locations (excepting FL-1 and FL-2; Table 1) at d −23, −13, −10, −3, and 0. Progesterone concentrations were categorized as high (≥ 1 ng/ml unless a CIDR was in place then ≥ 2.0 ng/ml) or low (all other samples). The samples collected on d −13 and −23 were used to determine cycling status. Cows with high progesterone status at d −23, and −13 were defined as cycling. Additional cows that had no blood samples but had a fully colored patch (score 2) were deemed cycling on d −13. Any cow that had low progesterone status on d −23 and −13 was deemed anestrus. The sample collected at d −3 reflected progesterone concentrations resulting from the CIDR insert, a functional corpus luteum, or both.

Ovarian Structures

Ovaries in a subset of cows (n = 188) at 2 locations (GA and KS-P; Table 1) were scanned by transrectal ultrasonography at d −10 and −3. The largest follicle ≥ 6 mm in diameter was measured using the internal calipers of the ultrasound machine. Follicle size was determined by averaging the diameter of the follicle at the widest point and the diameter at a right angle to the first measurement. Presence of all luteal structures was recorded on both days and new luteal structures observed on d −3 were noted.

Radioimmunoassay

Serum progesterone concentration was measured in all blood samples by direct quantitative (nonextracted) RIA using Coat-A-Count progesterone kits (catalog # TKPG; Siemens Medical Solutions Diagnostics, Los Angeles, CA) previously validated for bovine serum (Stevenson et al., 2012b). Intra- and interassay CV for progesterone were 6.5 and 9.3%, respectively. Assay sensitivity was 7.8 pg/mL.

Experiment 2

Experimental Design

A total of 803 primiparous and multiparous cows at 4 locations in 2 states (Florida and Kansas) were enrolled in this study. Characteristics of suckled beef cows enrolled by location are summarized in Table 1 including breed, percentage 2-year-old cows, days postpartum at TAI, body condition score, and percentage cycling status at the onset of the CO-Synch + CIDR program. Cows were stratified by breed, days postpartum, and parity, and then assigned randomly to 2 treatments (Figure 1). Control cows received the standard CO-Synch + CIDR protocol as defined in Exp.1. Treated cows (PrePGG; Figure 1) received 25 mg $PGF_{2\alpha}$ 10 d before (d −20) followed by 2 mL Factrel 7 d before the CO-Synch + CIDR program was initiated. Body condition scores (BCS; 1 = thin; 9 = very fat) were assigned to all cows at the time $PGF_{2\alpha}$ was administered to the experimental group on d −20.

Cycling Status

Blood samples were collected via caudal vessel puncture at d −20, −17, −10, −3, and 0. As in Exp. 1 progesterone concentrations were categorized as either high or low. The samples from d −20, −17, and d −10 were used to determine cycling status. Cows in the high group at d

−20 and −17 were determined to be cycling. Any cow that had a low progesterone designation at d −20, −17, and −10 was deemed anestrus. The sample collected at d −3 reflected progesterone concentrations resulting from the CIDR insert, a functional corpus luteum, or both.

Artificial insemination was performed 60 to 72 h after CIDR insert removal on d 0 and estrus-detection patches were removed and scored. Cows were either exposed to cleanup bulls 10 to 12 d later or reinseminated at subsequent estrus (KS-P location; Table 1). Pregnancy 35 d after AI, pregnancy 35 d after the end of the breeding season, and embryo loss were determined by transrectal ultrasonography as in Exp. 1.

Ovarian Structures

Ovaries of a subset of cows (n = 169) at 1 location (FL; Table 1) were scanned using transrectal ultrasonography at d −17, −10, and −3. All follicles ≥ 6 mm in diameter were measured using the internal calipers of the ultrasound machine as described in Exp. 1. All luteal structures also were measured. Volume of each luteal structure was calculated [4/3 × r3 x π, where r = (average of the diameter measured)/2, and π = 3.14159]. If a fluid-filled cavity was present in the luteal structure, volume of the cavity was calculated using the same procedure and cavity volume was subtracted from the calculated total luteal volume.

Radioimmunoassay

As in Exp. 1, serum progesterone concentration was measured in all blood samples. Intra- and interassay CV for progesterone were 3.4 and 7.6%, respectively. Assay sensitivity was 1.9 pg/mL.

Statistical Analysis

Experiment 1

Concentrations of progesterone for all sampling days were analyzed by ANOVA using a general linear model (procedure GLM, SAS Institute Inc., Cary, NC). The model included treatment (n = 2), location (n = 6), parity (primiparous vs. multiparous), median BCS (≤ 5 vs. > 5), all 2-way interactions with treatment, and median days postpartum (< 70 vs. ≥ 70 d) at TAI. Treatment differences were detected by F-test. Binomial fertility responses were analyzed separately for pregnancy rate 35 d after TAI (PR per TAI), final pregnancy rate at the end of the breeding season, and pregnancy loss using the GLIMMIX procedure (SAS Institute Inc., Cary, NC), with location as a random effect and treatment, parity, BCS, days postpartum, and all 2-way interactions with treatment as fixed effects.

Effects of cycling status at the onset of CO-Synch + CIDR after PrePG and the concentration of progesterone at CIDR-insert removal on subsequent PR per TAI assessed on d 35 after TAI were examined. We assumed that cows having progesterone ≥ 2 ng/mL (luteal) at CIDR insert removal most likely had a functional CL, whereas those with progesterone < 2 ng/mL (non-luteal) reflected endogenous concentrations resulting from the CIDR insert. The binomial pregnancy responses for TAI were examined using the GLIMMIX procedure. The model include location as a random effect and treatment, parity, BCS, days postpartum, cycling status at the trial onset, progesterone at CIDR insert removal (luteal or non-luteal), all 2-way interactions, and the 3-way interaction of cycling status, progesterone concentration, and treatment as fixed effects.

Cows with low (< 1 ng/mL) serum progesterone concentrations at the time of the first GnRH injection and had high (≥ 1 ng/mL) progesterone concentrations at the subsequent sample

collection were assumed to have been in estrus. Similarly, cows that had high progesterone at the time of either of the $PGF_{2\alpha}$ injections and then had low progesterone at the following collection were assumed to have had luteolysis. Treatment response was examined in relationship to the determined estrus and luteolysis measures using the GLIMMIX procedure with location as a random effect and parity, BCS, and days postpartum, and 2-way interactions of treatment with parity and BCS as fixed effects.

The largest follicle diameter was measured by transrectal ultrasonography at 2 different times (d –3, and –10) in cows at 2 locations (GA and KS-P). Measurements were analyzed using ANOVA; the statistical model included treatment as the response variable and location, parity, breed (n = 6), BCS, cycling status, and days postpartum as fixed effects. Two-way interactions between treatment and location, parity, BCS and cycling status also were examined. Variance between the largest follicle diameters on d–3 and –10 between treatment and control cows were compared using the Levene's test (Levene, 1960), assuming that less variance of follicle diameters would serve as a measure of improved follicular synchrony.

Experiment 2

Progesterone concentrations in blood collected from cows at 5 times during the protocol were analyzed using the GLM procedure and the same model as in Exp. 1. Cycling status was determined as defined earlier and all pregnancy responses were examined using the GLIMMIX procedure model explained in Exp. 1.

The diameter of the largest follicle present at d –17, –10, and –3 on the ovaries of a subset of cows (FL) was analyzed using the GLM procedure with the following model: treatment as the response variable and of parity, median BCS (≤ 5 vs. > 5), median days postpartum (< 70 vs. ≥ 70 d), and the 2-way interaction of treatment by parity as fixed effects. The calculated

volume of luteal tissue measured at d –17, –10, and –3 was examined using the same model. Treatment differences were detected by F-test. Variance of treatment follicle diameters on d –17, –10, and –3 were compared as in Exp. 1.

Combined Results

Serum progesterone concentrations on d –10 for cows in both experiments were combined and analyzed to determine the effect of either high or low serum progesterone concentration on subsequent pregnancy outcomes. A logistic model (GLIMMIX; SAS Inst. Inc.) was employed to determine the fixed effects of treatment, year, cycling status, BCS, days postpartum, parity, progesterone concentration at d –10 (≥ 1 ng/mL [high] or < 1 ng/mL [low]) and the 2-way interaction between cycling status and high vs. low progesterone concentration at d –10 on PR per TAI.

RESULTS

Experiment 1

In Exp. 1, blood samples (n = 1,162) were assayed for progesterone at 5 times during the protocol. Progesterone concentrations did not differ between treatments at d –13 ($P = 0.51$), −3 ($P = 0.35$), or 0 ($P = 0.37$; Figure 2). In contrast, cows in PrePG treatment had lesser ($P < 0.001$) progesterone concentrations on d –10 (3 d post PrePG treatment) than the control (0.5 ± 0.1 vs. 1.4 ± 0.1 ng/ml, respectively). In cows (n = 294) in which luteolysis could occur (progesterone ≥ 1 ng/mL) after the PrePG injection, the PrePG treatment produced more ($P < 0.001$) luteolysis than the control (82 vs. 40 %, respectively). Cows (n = 184) that were examined by transrectal ultrasonography were more ($P < 0.01$) likely to have new luteal tissue after PrePG (60 %) than

the untreated control (35 %). The $PGF_{2\alpha}$ treatment in all cows on d –3 produced luteolysis in 98 % of cows (n = 910) that were assumed to have had a corpus luteum on d −3.

Cycling status at the beginning of the experiment is reported by location in Table 1. Across locations cycling status ranged from 16.4 % (SD-CT) to 69.5 % (KS-P). The calculated percentage of 1,496 cows in the experiment assumed to be cycling based on progesterone concentrations, for which cycling status could be determined, was only 45.5 %.

Follicles ≥ 6 mm in diameter on d –10, –3, and 0 in 152 cows at 2 locations were analyzed. The PrePG cows had larger (P = 0.02) follicles on d –10 than controls (12.6 ± 0.5 vs. 11.0 ± 0.4 mm, respectively). No treatment difference in follicle size at d –3 (P = 0.55) or d 0 (P = 0.37) was detected. Differences in follicle size were detected between the 2 locations; however, no interaction was detected between location and treatment. Levene's Test of Residuals detected no treatment difference in the variance of follicle sizes on d –10, –3, and 0. Multiparous cows treated with PrePG on d −13 had a greater (P < 0.05) incidence of estrus between d –13 and –10 than multiparous controls (32.3 vs. 15.4 %, respectively). In contrast, lesser but similar proportions of treated and control primiparous cows were detected in estrus (16.8 and 16.2%, respectively).

Pregnancy rates per TAI (PR per TAI) across locations and treatments ranged from 48 to 67% (Figure 3). Neither treatment nor BCS influenced pregnancy outcomes (Table 2). Multiparous cows were more (P < 0.05) likely to be pregnant at d 35 after TAI (59.4 vs. 48.2%) and at the end of the season (96.1 vs. 92.3 %) than primiparous cows. Cows that were cycling at the beginning of the experiment were more (P = 0.004) likely to be pregnant at d 35 than anestrous cows (60.5 vs. 49.5 %, respectively) as were (P = 0.033) cows that were > 70 d postpartum at TAI than later-calving cows (56.7 vs. 51.0 %, respectively). Final pregnancy rates

(95.1 vs. 93.7 % for PrePG and control, respectively) and pregnancy loss (1.4 vs. 1.4 % for PrePG and control, respectively) did not differ between treatments.

Pregnancy rate per TAI was summarized for cows according to their cycling status at the onset of the CO-Synch + CIDR program and according to the concentration of progesterone at CIDR insert removal (Table 3). No differences were detected between treatments in either the cycling ($P = 0.22$) or anestrous ($P = 0.39$) classifications. Likewise, when cycling and anestrous cows were grouped by progesterone status at CIDR insert removal as high ($P = 0.17$ and $P = 0.50$ for cycling and anestrous cows, respectively) or low ($P = 0.46$ and $P = 0.57$ for cycling and anestrous cows, respectively), no differences between treatments were observed.

Experiment 2

Progesterone concentrations were less ($P = 0.02$) in PrePGG cows on d –17 after the presynchronizing injection of $PGF_{2\alpha}$ on d –20 than in controls (0.37 ± 0.10 vs. 0.71 ± 0.10 ng/mL, respectively). Concentrations of progesterone did not differ between treatments on d –20 ($P = 0.51$), d –10 ($P = 0.39$), d –3 ($P = 0.45$), or d 0 ($P = 0.36$; Figure 4). Cycling status of cows by location ranged from 62.0 % (KS-P) to 32.3 % (KS-H) and the mean percentage of cycling cows was only 49.9 % (Table 1).

Cows in 1 location (FL) were analyzed for differences in ovarian structures on d –17, –10, and –3. No differences in follicular diameter were detected between treatments at any of the time points examined. Neither BCS nor parity affected follicle size; however, cows that were at ≥ 70 d postpartum at TAI had more ($P = 0.007$) total follicles ≥ 10 mm in diameter at d –17 than cows < 70 d postpartum (1.1 ± 0.10 vs. 0.8 ± 0.1 per cow, respectively). Although total volume of luteal tissue was similar between treatments at all time points in cows with at least one corpus luteum (CL), more (P = 0.006) control cows had at least 1 CL on d –17 than PrePGG cows (18.6

vs. 4.8 %, respectively) 3 d after the PrePG was administered. On d −10 (7 d after PreGnRH was administered) more ($P = 0.002$) PrePGG cows had at least 1 CL than controls (63.9 vs. 41.9 %, respectively). In addition, more ($P = 0.022$) PrePGG cows had at least 1 CL than controls (62.7 vs. 45.3 %, respectively) on d −3.

Pregnancy rate per TAI across locations and treatments ranged from 39 to 67% (Figure 5). Across 4 locations, PR per TAI did not differ ($P = 0.95$) between treatments (Table 4). Neither cycling status nor parity influenced pregnancy outcome. Cows ≥70 d postpartum at TAI were more ($P < 0.001$) likely to be pregnant at d 35 after TAI (52.6 vs. 36.1%, respectively) and also ($P < 0.05$) at the end of the season (85.6 vs. 79.1 %, respectively) than cows less than 70 d postpartum. Likewise, cows that had BCS > 5 were more ($P < 0.001$) likely to become pregnant than thinner cows (49.8 vs. 39.6 %, respectively). Final pregnancy rates (82.6 vs. 82.6 %) and pregnancy loss (5.4 vs. 3.6 %) did not differ between PrePGG and control, respectively.

As in Exp. 1, PR per TAI was summarized for cows according to their cycling status at the onset of the CO-Synch + CIDR program and according to the concentration of progesterone at CIDR insert removal (Table 5). Similar to Exp. 1 no treatment differences were detected between cycling or anestrous classifications in either the high or low progesterone designations.

Combined Results

Results of the two experiments were combined to examine the relationship between progesterone status at the onset of the 7-d CO-Synch + CIDR program (d – 10). Progesterone status had no effect ($P = 0.47$) on pregnancy outcomes of cycling cows (Figure 6). In contrast, anestrous cows with low progesterone status at the onset of the program had less ($P < 0.05$) PR per TAI than cycling cows with low progesterone status and anestrus cows with high

progesterone status. The cycling cows with high progesterone were intermediate (53.0 %) and did not differ from any of the other 3 progesterone-cycling statuses.

DISCUSSION

Ovulation synchronization protocols that utilize exogenous GnRH 10 d before TAI followed in 7 d with $PGF_{2\alpha}$ and a second GnRH either 24 h before (Ovsynch) or at TAI (CO-Synch) are effective methods of using fixed TAI in suckled beef cows without any detection of estrus (Geary et al., 2001a,b). Addition of a progestin to the protocol improved the response in anestrous cows (Stevenson et al., 2000, Lamb et al., 2001). These studies have shown pregnancy success in anestrous cows to be more than 50%; however, variability in follicle size at AI and variability in follicle maturity at AI (Geary et al., 2000, Perry et al., 2005) probably limit response to TAI protocols. Presynchronization with $PGF_{2\alpha}$ before the initiation of a TAI protocol in dairy cows improved oocyte quality (Cerri et al., 2009) and improved pregnancy rates (Moreira et al., 2001; El-Zarkouny et al., 2004). In beef cows, presynchronization ($PGF_{2\alpha}$ administered 3 d before starting the TAI protocol) coupled with a 6-d CO-Synch + CIDR protocol improved follicle turnover and subsequent pregnancy success compared with a 5 d CO-Synch + CIDR treatment (Perry et al., 2012). In addition, heifers were more likely to display estrus and had better follicle turnover when presynchronized (Grant et al., 2011).

Ovarian characteristics at the initiation of a TAI protocol seem to be a critical factor in treatment success. Varying proportions of cycling beef heifers on d 2, 5, 9, 13, and 18 of their estrous cycle ovulated in response to GnRH (0, 75, 67, 50, and 58%, respectively; Atkins et al., 2010). A large percentage of cows on d 15 through 17 of their estrous cycle failed to ovulate in response to GnRH (Geary et al., 2000). Injection of a single dose of $PGF_{2\alpha}$ will cause CL regression in the majority of cows that are between d 6 and 16 of the estrous cycle and will cause

them to exhibit estrus (Lauderdale, 2009). In the current study, cows in Exp. 1 that responded to the presynchronization injection of $PGF_{2\alpha}$ will be at d 0 of their cycle 3 d later (i.e., at the beginning of the CO-Synch program). Cows that did not respond will either be between d 1 and 9 of their estrous cycle or they will spontaneously ovulate near the beginning of the CO-Synch program. As such, a greater proportion of cows in Exp. 1 that received the experimental treatment should be in a low progesterone condition when compared to the control cows. In Exp. 2, cows that responded to $PGF_{2\alpha}$ 10 d before the synchronization program should have been in d 7 of their estrous cycle whereas cows that did not respond should have been in d 8 to 16 of their estrous cycle. Consequently, a greater proportion of cows in Exp. 2 that received the experimental treatment should have been in a high-progesterone condition when compared to the control cows. Elevated progesterone concentrations at the start of the Ovsynch protocol was associated with greater PR per TAI in dairy cows (Silva et al., 2007); however, Perry et al. (2012) suggested that low-progesterone concentrations in beef cattle at the beginning of the TAI protocol may be beneficial to pregnancy success.

We hypothesized that using $PGF_{2\alpha}$ with or without GnRH before the start of the 7-d CO-Synch + CIDR protocol would improve pregnancy outcomes. In the current studies, the presynchronization regimen was designed to increase the percentage of the cows that started the 7-d CO-Synch + CIDR protocol in either a low progesterone (Exp. 1) or a high progesterone status (Exp. 2). In both studies, more than 50% of the cows were anestrous. Anestrous cows are more likely to become pregnant when both a GnRH injection and a progestin are included in the program (Stevenson et al., 2003). The addition of a third GnRH injection in Exp. 2 examined the effect on anestrous cows. The cycling cows in Exp. 1 were more likely to become pregnant to TAI than anestrous cows; however, the plurality of anestrous cows enrolled in the study limited

our ability to test the treatment on cycling cows. In contrast, cycling status had no effect on PR per TAI in Exp. 2 with the addition of a GnRH injection to the $PGF_{2\alpha}$ presynchronization treatment. When the progesterone status on d –10 for both studies was examined, anestrous cows with high progesterone and cycling cows with low progesterone had better pregnancy outcomes than anestrous cows with low progesterone. In both experiments, blood serum progesterone concentrations were lower in all treated cows at the sample subsequent to the presynchronizing $PGF_{2\alpha}$ injection compared with the control.

Lamb et al. (2001) noted that body condition was linked to reduced pregnancy outcomes in TAI protocols. In that study, 1 unit decrease in BCS resulted in a 22.9% decrease in pregnancy success. Conversely, we found no difference in PR per TAI of cows with BCS > 5 compared with their thinner herd mates in Exp. 1. In Exp. 2, in the subsequent year to Exp. 1, which was characterized by severe drought and poor pasture conditions in Kansas locations, better conditioned cows had greater PR per TAI than their thinner herd mates. It is possible that a poorer BCS can be overcome by a more positive energy balance in postpartum cows (Short et al., 1990).

Both experiments were interpreted to suggest that an increased postpartum interval to TAI was a significant factor contributing to pregnancy success in suckled beef cows. This result disagrees with Perry et al. (2012); however, all cows in that study were at least 30 d postpartum compared with the present studies where cows were as short as 16 d postpartum at the initiation of the experiments.

In summary, presynchronization treatments changed serum concentrations of progesterone before initiating a TAI protocol. It appears beneficial for anestrous cows to start the 7 d CO-Synch + CIDR protocol in a high progesterone status. This can only occur if they can be

induced to ovulate sometime before the TAI protocol begins. Cows have better pregnancy outcomes when they are ≥ 70 d postpartum at the TAI. Overall pregnancy rates were not improved with presynchronization; however, individualized treatments depending on cycling status should be explored. Treatments that induce cycling status in anestrous cows seem to have the potential to improve PR per TAI, whereas elevated progesterone concentrations before TAI in cycling cows had no benefit in terms of pregnancy success, as long as they were cycling at the beginning of the TAI protocol. If cycling status could be determined or estimated very simply before initiating a TAI protocol, treatment could be adjusted to reduce costs and perhaps improve PR per TAI in anestrous cows.

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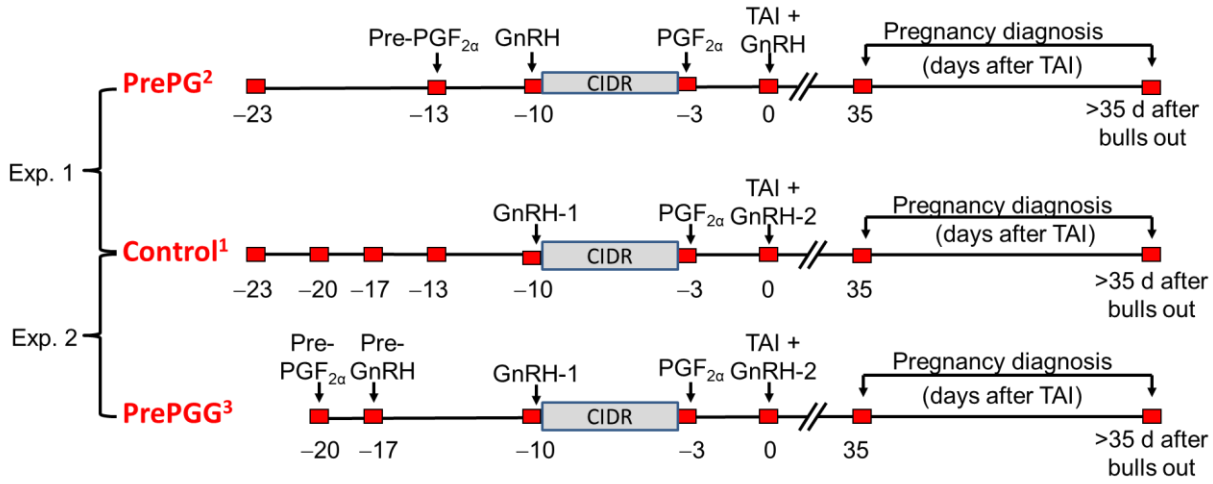

Figure 1. Design of Experiments 1 and 2. PrePG cows received an injection of GnRH and insertion of a controlled internal drug-release (CIDR) insert on d –10, an injection of $PGF_{2\alpha}$ and CIDR removal on d –3, and an injection of GnRH and AI on d 0. Control cows received an injection of $PGF_{2\alpha}$ on d –13, an injection of GnRH and insertion of a CIDR on d –10, an injection of $PGF_{2\alpha}$ and CIDR removal on d –3, and an injection of GnRH and AI on d 0. PrePGG cows received an injection of $PGF_{2\alpha}$ on d –20, an injection of GnRH on d –17, an injection of GnRH and insertion of a CIDR on d –10, an injection of $PGF_{2\alpha}$ and CIDR removal on d –3, and an injection of GnRH and AI on d 0. Blood samples were collected on d –23, –13, –10, –3, and 0 (Exp. 1) and d –20, –17, –10, –3, and 0 (Exp. 2).

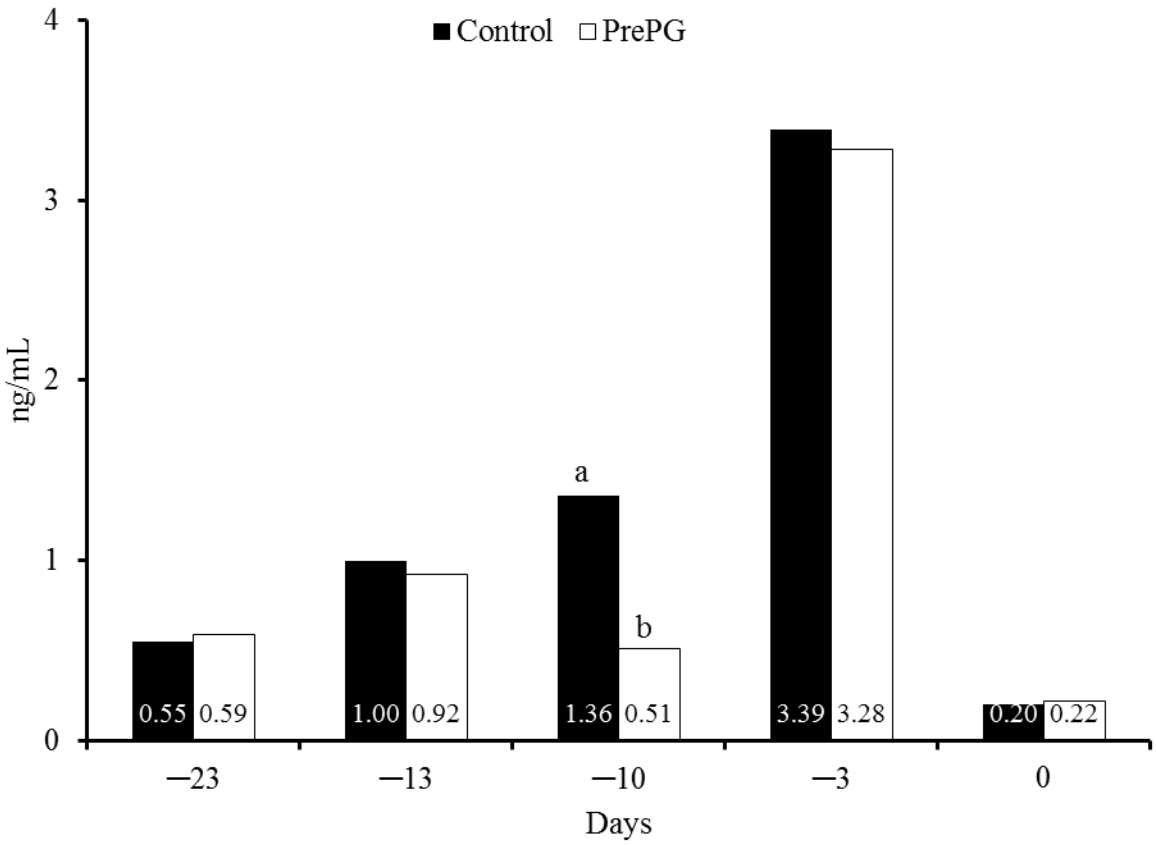

Figure 2. Serum concentrations of progesterone in Exp. 1 on selected days relative to TAI (d 0) by treatment (see Figure 1). Serum concentrations on d -3 also included progesterone supplied by controlled internal drug release (CIDR) insert, which was assumed to be approximately 1 ng/mL after 7 d in situ. [a,b] Means within day without a common letter differ ($P < 0.05$).

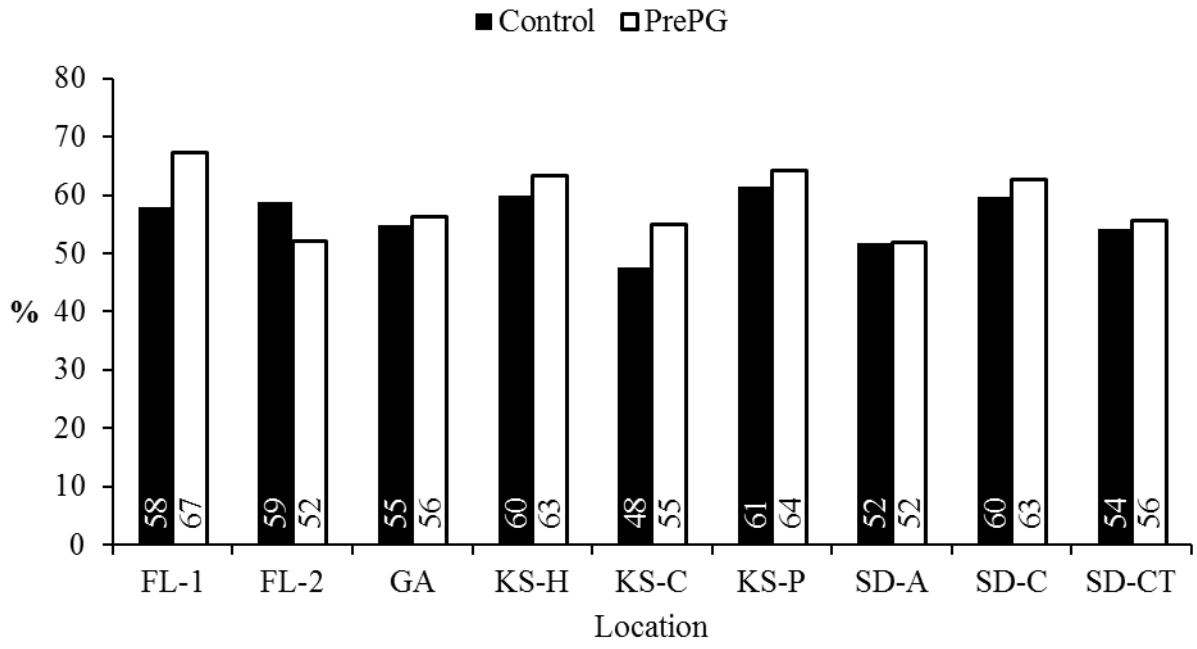

Figure 3. Experiment 1 pregnancy rates per TAI (%) at 9 locations in 4 states by treatment (see Figure 1).

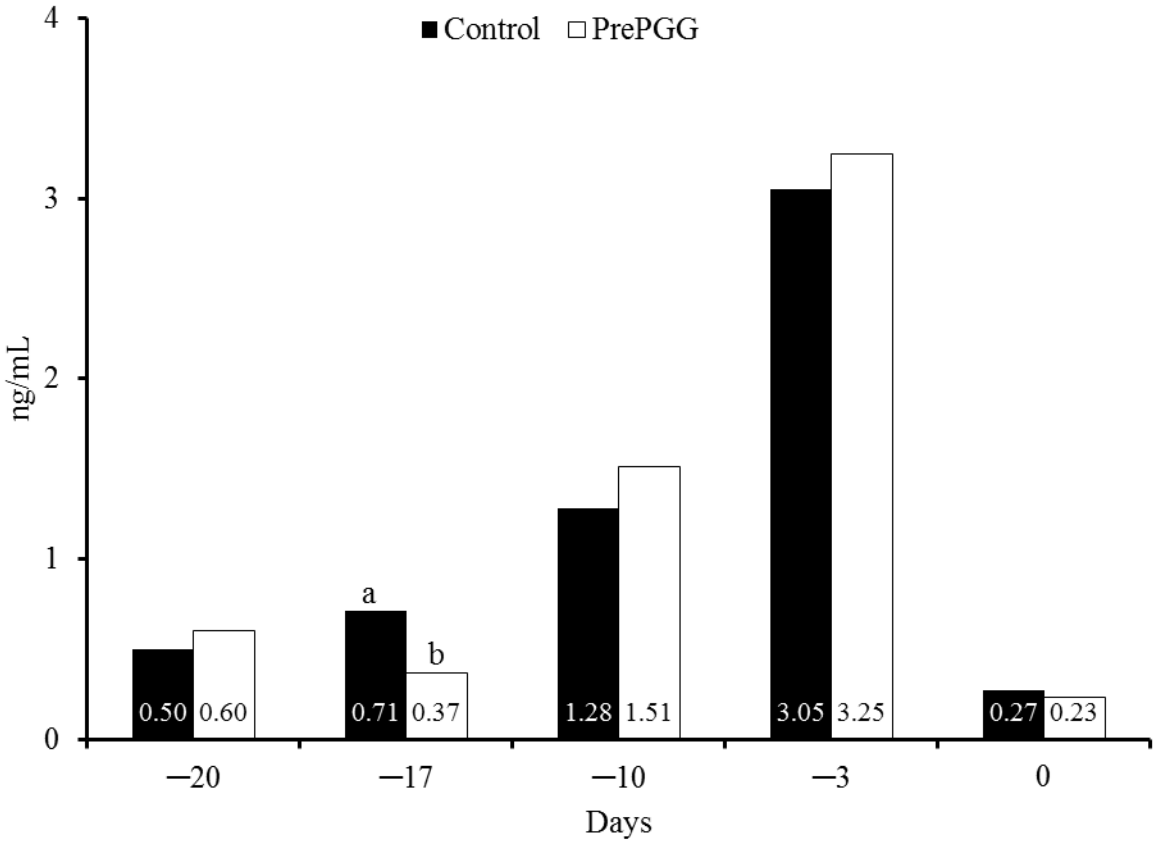

Figure 4. Serum progesterone concentrations in Exp. 2 on selected days relative to TAI by treatment (see Figure 1). Serum concentrations on d –3 also included progesterone supplied by controlled internal drug –release (CIDR) insert, which was assumed to be approximately 1 ng/mL after 7 d in situ. Means within day without a common letter differ ($P < 0.05$).

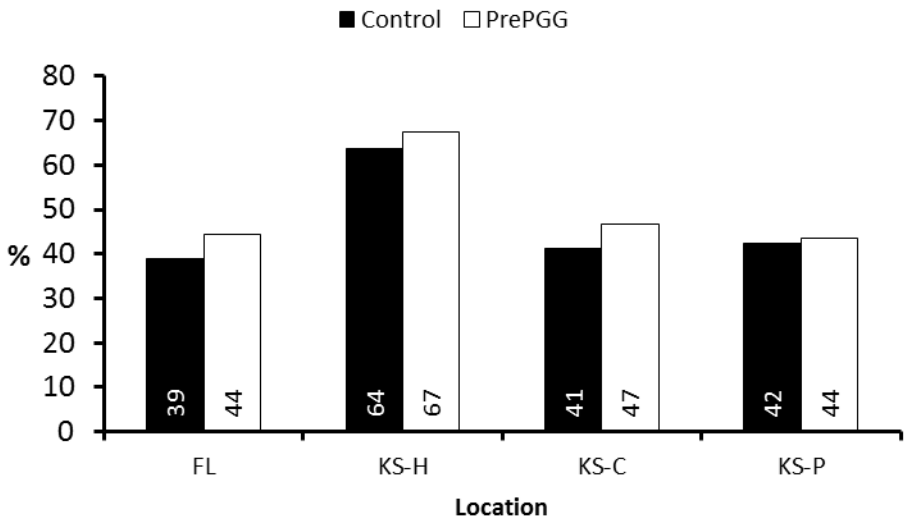

Figure 5. Experiment 2 pregnancy rates per TAI (%) at 4 locations in 2 states by treatment (see Figure 1).

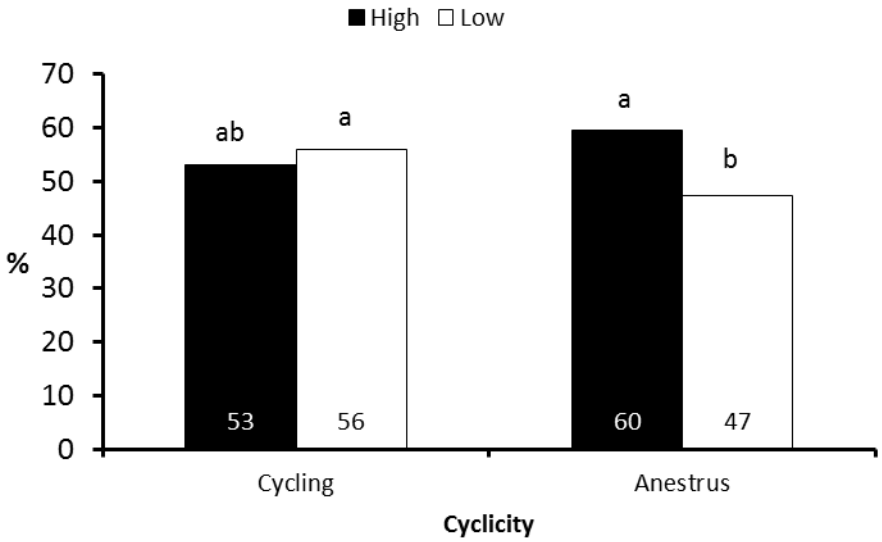

Figure 6. Combined pregnancy rates per TAI (%) for Exp. 1 and 2 based on serum progesterone concentration at d −10 relative to TAI and cycling status at the onset of the TAI protocol: high (≥ 1 ng/mL) and low (< 1 ng/mL). Means within cycling status without a common letter differ (P < 0.05).

Table 1. Selected characteristics of suckled beef cows enrolled in Exp. 1 and 2.

Location[1]	Breed	No. of cows	2 yr. old, %	Mean days postpartum at AI	Mean BCS	Cycling status, %
		Exp. 1				
FL-1	Angus, Charolais, Brangus	228	10.5	69	5.0	30.3[2]
FL-2	Angus, Charolais, Brangus	146	8.2	54	5.3	20.5[2]
GA-1	Angus	126	21.4	75	5.0	65.1[3]
KS-H	Angus x Hereford	195	25.1	80	5.7	53.3[3]
KS-C	Angus x Hereford	205	27.8	71	6.0	50.2[3]
KS-P	Angus, Hereford, Simmental	167	27.0	69	5.2	69.5[3]
SD-A	Angus x Hereford	222	37.8	74	4.4	22.7[3]
SD-C	Angus x Hereford	104	31.1	75	4.9	36.5[3]
SD-CT	Angus x Hereford	144	0.7	67	4.3	16.4[3]
		Exp. 2				
FL	Angus, Hereford, Brangus	169	16.6	69	5.6	56.2[4]
KS-H	Angus x Hereford	195	37.4	80	5.5	32.3[4]
KS-C	Angus x Hereford	261	16.9	71	5.5	50.6[4]
KS-P	Angus, Hereford, Simmental	184	24.5	69	4.9	62.0[4]

[1] In Exp. 1 cows located in 4 states at 9 locations were enrolled and in Exp. 2 cows in 2 states and 4 locations were enrolled.

[2] Cycling status computed from estrus detection only.

[3] Cycling status computed from serum progesterone concentrations and estrus detection.

[4] Cycling status computed from serum progesterone only.

Table 2. Pregnancy rates per TAI in suckled beef cows (Exp. 1).

Item	%	n	*P* value
Treatment[1]			0.257
PrePG	55.5	765	
Control	52.2	770	
Cycling status			0.004
No	49.5	612	
Yes	60.5	500	
Parity			<0.001
Primiparous	48.2	331	
Multiparous	59.4	1204	
Days postpartum at AI			0.033
< 70	51.0	682	
≥ 70	56.7	854	
Body condition score			0.685
≤ 5	54.4	918	
>5	53.3	617	

[1] See experimental design of treatments (Figure 1).

Table 3. Pregnancy rates per TAI based on cycling status before onset of CO-Synch + CIDR and progesterone concentration at CIDR insert removal (Exp. 1).

Cycling status	Progesterone at CIDR insert removal[2]	Treatment[1]	
		Control	PrePG
	(ng/mL)	----------% (n) ----------	
Cycling	≥ 2	55.1 (199)	63.1 (201)
	< 2	57.8 (50)	65.6 (50)
	Total	56.5 (249)	64.3 (251)
Anestrus	≥ 2[3]	48.0 (149)	52.1 (146)
	< 2	47.3 (157)	50.7 (160)
	Total	47.6 (306)	51.4 (306)

[1]See experimental design of treatments (Figure 1).

[2]Cows with progesterone concentration ≥ 2 ng/mL were assumed to have a functional corpus luteum (luteal), whereas those with < 2 ng/mL were assumed to have no corpus luteum (non-luteal; progesterone supplied predominately by the CIDR insert).

[3]Assumed to have ovulated in response to GnRH-1 (See Figure 1)

Table 4. Pregnancy rates per TAI in suckled beef cows (Exp. 2).

Item	%	n	*P* value
Treatment[1]			0.951
PrePGG	44.4	402	
Control	44.0	401	
Cycling status			0.459
No	42.6	404	
Yes	49.1	399	
Parity			0.412
Primiparous	42.4	190	
Multiparous	46.0	613	
Days postpartum at AI			<0.001
< 70	36.1	263	
≥ 70	52.6	543	
Body condition score			0.008
≤ 5	39.6	340	
>5	49.8	463	

[1]See experimental design of treatments (Figure 1).

Table 5. Pregnancy rates per TAI based on cycling status before onset of CO-Synch + CIDR and progesterone concentration at CIDR insert removal (Exp. 2).

Cycling status	Progesterone at CIDR insert removal[2]	Treatment[1]	
		Control	PrePGG
	(ng/mL)	----------% (n)----------	
Cycling	≥ 2	47.9 (135)	44.0 (161)
	< 2	52.5 (53)	51.9 (50)
	Total	50.2 (188)	48.0 (211)
Anestrus	≥ 2[3]	39.4 (104)	42.1 (94)
	< 2	45.2 (109)	43.6 (97)
	Total	42.3 (213)	42.8 (191)

[1]See experimental design of treatments (Figure 1).

[2]Cows with progesterone concentration ≥ 2 ng/mL were assumed to have a functional corpus luteum (luteal), whereas those with < 2 ng/mL were assumed to have no corpus luteum (non-luteal; progesterone supplied predominately by the CIDR insert).

[3]Assumed to have ovulated in response to GnRH-1 (See Figure 1).