ABSTRACT

HAGGERTY, JULIA ALLISON. Comparison of two CIDR based Estrous Synchronization Programs in Beef Heifers. (Under the direction of Dr. C. S. Whisnant).

Artificial Insemination (AI) and estrous synchronization reproductive management techniques have been developed for use in beef heifers and cows and have allowed for beef producers to enhance the efficiency of their cow herd. Despite these attributes, beef producers have been reluctant to adopt thesis management tools due to time and labor costs. For this reason, research has focused on developing estrous synchronization protocols that facilitate fixed-time AI (FTAI). Fixed time AI protocols have worked well with beef cows and they have reduced the time and labor required for heat checks. These protocols have not been as successful in beef heifers. Additionally, no published research has compared the effectiveness of these two protocols in beef heifers. For this study, two fixed time artificial insemination protocols known as 5 day CO-Synch + CIDR and 14 day CIDR Select were compared for effectiveness in beef heifers. The second objective of this study was to determine if the reproductive condition of beef heifers described in terms of reproductive tract scoring and pubertal status influences the success rate of these two fixed time AI protocols.

The pregnancy rates were higher (P<0.05) for the heifers in the 5-day CIDR group (n=80) than in the 14-day CIDR Select group (n=82). Across the research locations the average pregnancy rate of heifers in the 5-day CIDR treatment group was 66.9% and in the 14-day CIDR Select treatment group was 55.7%. This is a difference of (P<0.05) by Chi Square. These results suggest that the 5-day CO-Synch + CIDR protocol may facilitate fixed-time AI more effectively in beef heifers than the CIDR Select protocol.
Heifer Reproductive Tract Scores (RTS) ranged from 2-4 with all but two heifers being either a 2 or 3. There was no difference in RTS between treatment groups. In the 14 day CIDR group 50% of the heifers with RTS of 2 became pregnant and 66% of heifers with RTS of 3 became pregnant. For the 5 day CIDR group 75% of heifers with RTS of 2 became pregnant and 58% of heifers with RTS of 3 became pregnant. These results suggest that the 5-day CO-Synch + CIDR protocol may also be more effective in mixed herds with a range of RTS scores from 1-5 and with beef heifers of relatively low pubertal status with RTS scores of 2-3.
Comparison of two CIDR based Estrous Synchronization Programs in Beef Heifers

by
Julia Allison Haggerty

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APPROVED BY:

______________________________
Dr. C. Scott Whisnant
Committee Chair

______________________________
Dr. Gary Hansen

______________________________
Dr. Daniel Poole
DEDICATION

This thesis is dedicated to my parents, Libby and Gavin Smith and Steve and Karen Haggerty, who instilled in me the values of hard-work and responsibility, and encouraged me to pursue my dreams; and to my siblings Katie and Jack for their friendship and support; to Dr Whisnant for providing me the opportunity to continue my education and to achieve my true potential: and to Justin for his unwavering support. All of you have inspired and challenged me to become the person I am today and have played a pivotal role in helping me accomplish this goal – for that I will forever be grateful.
BIOGRAPHY

Julia Allison Haggerty was born June 2, 1988 to Elizabeth Smith and Steve Haggerty. She was raised most of her life in Raleigh, North Carolina. Her interest in agriculture and passion for showing horses formed a desire to pursue a career in the animal agriculture industry.

After graduating from William B. Enloe High School she knew the only college that would further develop her interest and opportunities in agriculture would be the College of Agriculture and Life Sciences at North Carolina State University. As an undergraduate, Julia continued to follow her passion of raising and showing horses on a State and National level and participated in classes and clubs that furthered her education in agriculture.

While completing her Bachelor of Science she became increasingly interested in reproductive physiology. She completed an internship at a horse-breeding farm where she was first exposed to advanced reproductive techniques. She became fascinated with reproduction and assisted reproductive techniques. After completing her Bachelor of Science in Animal Science and a minor in Genetics in the Spring of 2010 she decided to pursue a Master of Science to further her education in reproductive physiology. Julia will receive a Master of Science degree in Animal Science in the fall of 2012. She began her career in Embryology in November, 2012 and she is keeping the possibility of continuing her education in reproductive physiology and theriogenology.
ACKNOWLEDGMENTS

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CHAPTER 1

REVIEW OF LITERATURE

As research on the endocrinology of the estrous cycle in beef cattle has progressed, several estrous synchronization programs have been developed for use in beef heifers and cows. Fixed time artificial insemination (AI) protocols facilitate the mass breeding of all animals at a predetermined time rather than after the time of heat detection. Fixed-time AI protocols have reduced the time and labor required for heat checks in beef herds. These protocols have been less successful in beef heifers. For this study, two fixed time artificial insemination protocols known as 5 day CIDR and 14 day CIDR Select were compared for effectiveness in beef heifers. The second objective of this study was to determine if the reproductive condition of beef heifers described in terms of reproductive tract scoring and pubertal status influences the success rate of these two fixed time AI protocols. To understand research previously assembled in the field of study of these experiments this review of literature was conducted.

Reproductive Physiology of Beef Heifers

Beef heifers reach sexual maturity and experience their first estrous cycle between 12 and 15 months of age. This time to puberty can be delayed by genetic predisposition to late maturity or by poor nutrition. The hypothalamus releases Gonadotropin Releasing Hormone (GnRH), which causes the release of gonadotropins from the pituitary. Prior to puberty, heifers produce increasing amounts of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary. These hormones act on the
reproductive system of the heifer and stimulate the follicular development of the ovaries. As the ovaries develop, they produce and secrete estrogen and house the developing ovum. Before the heifers reach puberty these follicles that are being produced in response to LH and FSH concentrations grow and regress without ovulating an oocyte.

Puberty is not achieved in a heifer until she responds to the estrogen produced by the follicle. This response made by the heifer to the increasing estrogen is expressed as estrus and the production of a surge of LH leading to ovulation and the first ovulation is generally considered as time of puberty (Williams and Amstalden, 2010). For fertility to be increased the heifer should exhibit two to three estrous cycles prior to breeding. The bovine estrous cycle is typically 19-23 days in mature cows and 19-22 days in heifers (Nellor and Cole, 1956). When compared to heifers bred on their first (pubertal) estrus, heifers bred on their third cycle had a 20% higher conception rate (Byerley et al. 1987).

**Puberty in Beef Heifers**

The timing in which replacement beef heifers reach puberty in relation to the breeding season is imperative to the success of a cow-calf operation. The goal is to have replacement heifers cycling at the same time as the cows in the herd. Research has found that fertility in heifers increases by 21% if bred on their third estrus compared to their first “pubertal estrus” (Perry, 2011). Radioimmunoassays are used to measure the amount of progesterone cycling at various times collected from heifers, which can help determine the pubertal status of heifers. A significant increase in the plasma concentration of progesterone occurs prior to puberty in heifers (Donaldson et al., 1970). The pattern of progesterone concentrations in plasma prior to the first ovulation in heifers has been mapped out and two
period of significant increase have been identified. The first increase occurs between days -18 and -11 and the second occurs between -9 and 0, on which ovulation occurs (Berardinelli et al., 1979). The source of the two increases in the concentrations of the plasma progesterone in heifers prior to puberty has been found to be the product of ovaries (Berardinelli et al., 1979). It has been suggested by Gonzalez-Padilla et al, 1975, that the role of progesterone is to sensitize the ovaries to LH. The presence of estradiol causes the secretion of LH in prepubertal heifers but without exposure to progesterone, ovulation does not occur (Gonzalez et al., 1975). Anderson et al. (1991) suggested that progesterone could also increase hypothalamic sensitivity to get more LH. Progesterone exposure is needed for estrous behavior.

The endocrine events involving gonadotropins that lead to the establishment of puberty in heifers have been studied to determine the role of the relationship between the hormones of the hypothalamus and the pituitary and its effect on the onset of puberty in heifers. As heifers reach puberty, the negative feedback relationship of estradiol on gonadotropin secretion by the pituitary declines as the hypothalamus becomes desensitized to estradiol (Schillo et al., 1982). The exact mechanism responsible for the decreased feedback action of estradiol on LH secretion in developing heifers is unknown. Day et al. (1987) evaluated the number of estradiol receptors present of the hypothalamus and the pituitary in prepubertal heifers during the time in which the negative feedback between estradiol and LH secretion declines.

Fifty prepubertal heifers were selected by standardization of size to be used in this study. Progesterone concentration was analyzed from blood samples collected from all
heifers three times per week. The concentration of progesterone present in the heifers was used to determine the day puberty was achieved. Blood samples were analyzed from serum concentrations of LH using radioimmunoassay.

Luteinizing hormone serum concentrations increased in a linear fashion during the period leading up to puberty. The amplitude of the LH pulses declined as puberty approached. The concentration of estradiol receptors located in the hypothalamus and the anterior pituitary decreased as the heifers reached puberty. The concentration of LHRH receptors located on the anterior pituitary showed no change as the animals approached puberty. Day et al. (1987) found that the increases in the frequency of the pulses of LH, 50 days prior to the onset of puberty accompanied the decline in the amplitude of the LH pulses they noted in their study. The increase in the frequency of the LH pulses is the result of the decrease in the sensitivity of the estradiol negative feedback system.

Endocrinology of Estrous Cycle in Cattle

Several hormones work to regulate the bovine estrous cycle. Changes in the concentrations of these different hormones control the recruitment and growth of the follicular waves, timing of ovulation, and the length of the estrous cycle. Estrous synchronization protocols are designed to utilize naturally occurring hormones in a synthetic application to control ovulation and the follicular waves that occur on the ovary during the 21-day estrous cycle of beef cattle. Manipulation of these hormones can aid the producer in efficiently controlling and altering the naturally occurring reproductive processes of cattle to create a greater gain. Utilizing these hormones such as progesterone, gonadotropin releasing
hormone, and prostaglandin can synchronize the estrous cycle to time insemination, shorten the calving season, and increase calf uniformity.

**Progesterone**

Progesterone (P4) is a hormone vital for the regulation of the function of the female reproductive system. It is secreted by the corpus luteum (CL) and is the major hormone in the progestogen class of hormones. Progesterone is synthesized and produced in the CL by conversion of pregnenolone. WM Allen and Corner identified one of the functions of this product of the CL as preparation of the uterus for reception of the embryos (Allen, 1928). Later, four independent laboratories claimed to have isolated the crystalline product of the corpus luteum and named it progesterone in 1934 (Butenandt, 1934; Hartmen, 1934; Slotta, 1934; Wintersteiner, 1935). In that same year Butenandt was able to determine the chemical structure of progesterone. Like all steroid hormones, progesterone is derived from cholesterol (Ruzicka, 1936).

**Role of Progesterone in the Female Reproductive Tract**

Progesterone is important throughout the entire estrous cycle and it is vital to the ovulation of follicles. Progesterone prepares the uterus for pregnancy and maintains the pregnancy if conception occurs. It also can function to inhibit the cow from exhibiting signs of standing estrus and ovulation. Progesterone is primarily synthesized by the large luteal cells, which develop from the granulosa cells of the follicle (Hansel and Convey, 1983). In the event of a successful fertilization, the corpus luteum does not regress and continues to produce progesterone throughout the duration of pregnancy in some species or until the
placenta takes over the production of progesterone seen in other mammalian species. In the cow, the adrenals appear to be able to support gestation through secretion of progesterone after 200 days of gestation (Wendorff et al., 1983). If no mating occurs or conception is simply not achieved, the CL will remain and P4 will be high until prostaglandin F2alpha causes it to regress. When the CL has regressed and progesterone levels are low, there is a rise in estradiol and GnRH secretion. This causes the release of LH in a surge to a level high enough that ovulation occurs and the cycle is completed (Hansel et al., 1973).

Progestosterone also plays a key role in the development and onset of puberty. A major increase in the plasma concentration of progesterone occurs preceding the start of puberty in heifers (Donaldson et al., 1970). The diameter of a corpus luteum present on the ovary has a positive correlation to the concentration of serum progesterone between ovulations (Perry et al., 1991). When the corpus luteum is present and producing progesterone ovulation does not occur (Inskeep and Dailey, 2005).

**Fixed Time Artificial Insemination**

For a group of animals that are often in various stages of the estrous cycle, fixed time artificial insemination programs are designed to synchronize ovulation. In order to achieve synchronization, fixed time AI programs were designed to 1) standardize follicular growth 2) induce regression of the CL and 3) stimulate the ovulation of the dominant follicle. In order to facilitate this, fixed time AI protocols utilize GnRH, prostaglandin F2alpha (PGF), and a second administration of GnRH. Gonadotropin releasing hormone induces the ovulation of the dominant follicle that controls the follicular waves and thus the follicular growth patterns
resulting in the presence of a dominant follicle at the time of the second GnRH administration (Twagiramungu et al., 1995). Prostaglandin F2alpha is given to regress the CL and decrease the circulating progesterone concentrations. Once the CL regresses the second GnRH administration is conducted to induce ovulation. Only 45-60 percent of heifers ovulate after injection of GnRH at random stages of the estrous cycle (MacMillan and Thatcher, 1991). Human chorionic gonadotropin (hCG) is a hormone secreted by human trophoblast cells starting at the 7 days stage of embryo development that when administered to beef heifers instead of GnRH can increase the proportion of heifers that ovulate (Dahlen et al. 2011). Human chorionic gonadotropin mimics LH and when administered to cows can increase ovulation. Estradiol is also effective in synchronization of follicular waves but is not licensed for use in many countries (Martinez et al., 2011).

**Controlled Intravaginal Drug Releasing Device (CIDR) in Cattle**

The CIDR is made of silicone rubber saturated with progesterone and molded over a T-shaped spine. The CIDR folds up into its applicator to be inserted into the vagina of a cow. A nylon extension from the CIDR reaches out from the reproductive tract to allow for easy removal of the device. Progesterone is released from the CIDR into the blood stream and absorbed through vaginal epithium to suppress estrus and ovulation during its time in the reproductive tract. Originally developed in New Zealand in the late 1980’s, it was not approved for use in the United States until its approval by the Food and Drug Administration in May of 2002. The CIDR device developed by Pfizer Animal Health has progesterone content of 1.38g. The CIDR differs from other intravaginal devices such as Bovine
Intravaginal Device (DIB) and Medroxiprogesterone (MAP) – impregnating sponges, in the concentration of progesterone they possess. Bovine Intravaginal Device contains 1 g of progesterone and MAP has a progesterone content of 350 mg. CIDR used in New Zealand and other countries aside from the US contain 1.9 g of progesterone.

There are several protocols for CIDR use in beef and dairy cattle breeding programs. In general, the CIDR is inserted into the reproductive tract and remains for 7 to 8 days later when it is removed and an injection of PGF2alpha is administered to initiate the regression of any CL that may be present on the ovaries. Some CIDR protocols utilize either GnRH or estradiol benzoate (EB) administered prior to the CIDR insertion to initiate follicular growth. CIDR administration increases the size of the dominant follicle at the time of ovulation (Martinez et al., 2011). Previous studies have found that an increase in the diameter of the pre-ovulatory dominant follicle is linked to increased fertility (Vasconcelos et al., 1999; Lamb et al., 2001; Peters and Pursley, 2003; Perry et al., 2007; and Busch et al., 2008). A diameter of 12.8 mm was found to be an ideal size of the preovulatory follicle to result in optimal fertility and any < 12 mm in diameter resulted in decreased fertility (Perry et al., 2007). When progesterone is added to a synchronization protocol, it can increase the response to the program in anestrous cows and heifers. Progesterone secretion is required for the onset and achievement of puberty. Microscopic evaluation of ovaries from heifers that had experienced one to two increases in progesterone demonstrated areas of compact luteal tissue (Berardinelli et al., 1979). Located within the ovary were small luteal tissues that were 1.5 to 6mm in diameter. These were assumed to be the luteinization of small follicles and not palpable from the exterior of the ovary. Polat et al. (2009) found that progesterone
supplementation in heifers with a progesterone releasing intravaginal device (PRID) for 12 days, that had delayed onset of puberty resulted in 93.9% of the heifers being cyclic (P4 ≥ 1 ng/ml) within 72 hours of the removal of the device. Artificial insemination at 48 and 72 hours after removing the PRID produced a pregnancy rate of 54.6% at 60 days post-insemination.

Reproductive Tract Scoring in Beef Heifers

Reproductive tract scoring (RTS) is a 5-point scale that identifies the pubertal status of beef heifers. The reproductive tract scoring system was developed by Anderson et al. (1991) to identify the pubertal status of beef heifers to estimate the breeding potential of the heifers. Anderson et al. (1991) determined the following reproductive tract scoring scale detailed below. Heifers with a RTS of 1 have immature tracts that have no uterine tone and have no ovarian structures. A RTS of 2 has a no uterine tone and a uterine horn diameter of 20-25 mm with follicles present on the ovaries that are less than 8 mm in diameter. A RTS score of 3 shows some uterine tone and follicles 8-10 mm in size present on the ovaries. A 4 has a toned uterus with a horn diameter of 30 mm and follicles greater than 10 mm. A heifer with a score of 5 has a palpable corpus luteum present. Heifers with a score of 1, 2, or 3 are identified as prepubertal and heifers with a score of 4 or 5 are considered pubertal (Rosenkrans and Hardin, 2002).

The reproductive tract score is a two-part examination of heifers that includes a rectal palpation exam with ultrasonography and a blood serum test to determine the progesterone concentrations present in the heifer. The rectal exam consists of ultrasonography, which aid
in indentifying the size and location of ovarian structures including follicles and corpus lutea. Heifers with follicles greater than 10 mm in diameter or heifers with corpus lutea present are classified as pubertal (Rosenkrans and Hardin, 2002). Serum progesterone is tested on the day of the rectal exam and 10 days later. Serum progesterone can be determined by radioimmunoassay. Heifers are classified as prepubertal if their serum progesterone level is less than 1 ng/ml in both samples acquired and classified as pubertal if their progesterone level is greater than 1 ng/ml in at least one of the samples collected (Rosenkrans and Hardin, 2002).

**Exogenous Progesterone and Puberty**

The establishment of puberty can be accelerated by the addition of progesterone. Administration of progesterone or progestin in prepubertal heifers can induce puberty in heifers (Gonzalez-Padilla, 1975). The effectiveness of this is influenced by age, breed and body weight of the heifer. Previous research indicates that on average, 11 to 33% of heifers are prepubertal at the start of the breeding season (Lynch et al., 1997; Lammoglia et al., 2000; Lamb et al., 2006). Anderson et al. (1996) designed an experiment to determine the control of progesterone on the development of the prepubertal heifer’s reproductive tract. They found that follicle development was not altered during progestin administration but that the uterus experienced dramatic changes after the removal of the progesterone source. They attributed the increase in uterine weight to the increase in the estradiol concentrations that is associated with the pubertal follicular phase (Anderson et al., 1996). Anderson et al. (1996) saw the greatest effect of exogenous progesterone/progestin on the secretion of LH. The
frequency of LH pulses increased with the addition of progesterone up to a limit after which LH secretion was suppressed. Thus, the addition of progesterone could be useful in a program for synchronization of heifers.

5 Day CO-Synch+ CIDR Program

CO-Synch is a commonly used fixed time artificial insemination protocol for beef cattle (Geary, 1998). The CO-Synch program uses both GnRH and PGF2alpha to control the follicular function of the ovary and the interval between the onset of luteolysis and the LH surge induced by the GnRH. The first GnRH injection is given to reset follicular growth so that at the time of the second GnRH administration ovulation can be induced. The interval between GnRH and PGF2alpha administration is necessary for a new follicular wave to occur and the selection and development of a dominant follicle to occur for a successful ovulation to follow (Thatcher et al., 1989). The success of the CO-Synch programs was only marginal in synchronizing estrus because only 5-15% of cycling cows expressed estrus before the PGF was administered (Twagirimungu et al., 1995). The addition of fixed time artificial insemination with the use of CIDR to the CO-Synch program causes the cow to not go into estrus prior to the time of the prostaglandin F2alpha injection and thus enhances fertility (Lamb et al., 2006). The interval between GnRH and prostaglandin F2alpha administrations was reduced from a 7 day to 5 day CO Synch + CIDR protocol when it was hypothesized that shortening the duration of CIDR treatment with the removal coinciding with optimal follicular development would cause greater fertility rates after fixed time artificial insemination (Bridges et al., 2008).
The timing of fixed time artificial insemination following the prostaglandin F2alpha injections differ between the 7 day and 5 day CIDR protocols. Fixed time artificial insemination occurring 72 hours after prostaglandin GF2alpha administration in the 5 day CO Synch CIDR protocol increased pregnancy rates by 10.5% over the rates seen in 7 day CO Synch protocols with fixed time artificial insemination occurring 60 hours after the prostaglandin F2 alpha injections (Bridges et al. 2008). No difference in pregnancy rates were seen between the 5 and 7 day protocols in cows artificially inseminated 66 hours after one injection of PGF2alpha in the 7 day protocol (Wilson et al., 2010). Busch et al. (2008) compared 66 hour and 54 hour protocols for fixed time artificial insemination in the 7 day protocol and found 66 hours to cause increased pregnancy rates and to be the near optimal time of insemination for 7 day CO Synch CIDR programs.

The variability of luteolysis that can be seen in 5-day CIDR protocols that use only a single dose of prostaglandin F2alpha has been shown to improve with the inclusion of a second prostaglandin F2alpha dose administration on day 5 of the protocol. A comparison of a protocol utilizing a single dose of prostaglandin F2alpha and one including 2 doses of prostaglandin F2alpha in a the 5 day CO-Synch + CIDR program was conducted by Kasimanickam et al. (2008) at Washington State University. The study found that when prostaglandin F2alpha is administered in two doses the pregnancy rate is 15-17% higher than similar timed artificial insemination protocols that only include one prostaglandin F2alpha administration. Kasimanickam et al. (2008) compared the two versus one prostaglandin F2alpha dose at CIDR removal on day 5 in beef heifers in a 5-day CIDR CO-Synch program. Cows were randomly selected from within a herd to receive a single dose of prostaglandin
F2alpha at the time of CIDR removal and others to receive a first dose of alpha F2alpha at CIDR removal and a second 6 hours later. All of the heifers were inseminated 72 hours following CIDR removal.

Cows were checked for pregnancy 50-70 days following AI through rectal palpation and ultrasonography to determine the time of conception. In this study Kasimanickam et al. (2008) saw a higher fixed time artificial insemination pregnancy rate in the heifers receiving 2 doses of prostaglandin F2alpha than those that receive a single dose of prostaglandin F2alpha at CIDR removal on day 5. The 5-day protocol requires two injections of prostaglandin F2alpha to effectively regress the CL (Kasimanickam et al., 2008).

14 Day CIDR Select Program

It has been postulated that heifers would benefit from longer exposure to the progesterone delivered by CIDR. Rather than the traditional 7-day CO-Synch CIDR protocol usually implemented to synchronize the estrous cycle and ovulation timing, heifers benefit from the prolonged exposure of the 14-day CIDR Select protocol. Low pregnancy rates in heifers can add to the length of the calving season and increase labor costs and can be detrimental to producing a uniform calf crop. The 14-day CIDR Select protocol creates a greater more synchronized estrous response and a more synchronized return to estrus after fixed-time artificial insemination (Busch et al., 2007). Researchers traded the 14-day MGA (melengestrol acetate) protocol and substituted with a CIDR and compared pregnancy rates with the 7-day CIDR protocol. In 2006, 217 heifers were assigned a reproductive tract scorings of 1 representing an immature tract to 5, indicating the luteal phase of the estrous
cycle were allocated to receive either a CIDR insert from Day 0 to day 14 or the 7-day CO-sync CIDR protocol (Busch et al., 2007). The 108 heifers in the 14-day CIDR Select group had a CIDR inserted on Day 0 and removed on Day 14 followed by an injection of GnRH on Day 23, nine days after the CIDR was removed and a prostaglandin injection 7 days after the GnRH, Day 30. Fixed-time artificial insemination occurred 72 hours after the prostaglandin administration along with a second injection of GnRH.

The findings indicated a difference in pregnancy rates between the two protocols. It was concluded that heifers that receive the 14-day CIDR Select protocol are 1.86 times more likely to conceive during fixed-time artificial insemination than those that receive the 7-day CO-Synch + CIDR protocol (Busch et al., 2007). The pregnancy rate of the heifers that received the 14-day CIDR Select protocol was 63% compared to the 43% pregnancy rate seen in the 7-day CO-Synch + CIDR protocol heifers. One of the drawbacks to the CIDR Select protocol compared to that of the 7-day CO-Synch program is that the heifers have to be run through the chute five times in order to perform all the steps whereas the shorter practices only require three times to complete. This increases the time and work required to complete the process and increases the stress to the animal.

MGA Estrous Synchronization in Beef Heifers

Melengesterol acetate (MGA) is an orally administered feed additive that contains progestin, that when incorporated with artificial insemination can yield successful conception rates without the need to detect heat. Melengesterol acetate is one of the most effective synchronization systems for beef heifers when cost is a key variable. Incorporating MGA to
improve AI rates can cost about $0.02 per heifer per day to feed. Melengesterol acetate works to suppress heat and prevent ovulation. MGA can also be used to trigger the onset of puberty in heifers in the herd members that have not started cycling. A disadvantage of MGA protocols is the lengthy time of the program, at least one month in duration, and the specific feed requirements that can be hard to manage in large herds.

Melengesterol Acetate is fed for 14 days at a rate of 0.5 mg/heifer/day. Heifers that respond best to this protocol are at least 65% of their mature body weight and have a reproductive score of at least a three or higher. Melengesterol acetate paired with Prostaglandin requires the administration of prostaglandin F2alpha 19 days following the last day of feeding the MGA. The interval between the last MGA dose and the PGF administration was increased from 17 days to 19 days with increased efficacy (Lamb et al., 2000). The 14-day treatment with MGA brings all the heifers into the late luteal stage of their cycle so that when the prostaglandin is administered it can shorten the synchronization period to maximize the conception rate. If any non-cycling heifers experience a false heat after the MGA feeding it does require a second prostaglandin injection. The heifers are then bred 1-7 days following the prostaglandin F2alpha injection.

Gonadotropin releasing hormone, GnRH, is also incorporated in some estrous synchronization protocols that utilize MGA in order to facilitate a new follicular wave and further control the timing of ovulation (Johnson and Day, 2004). Synchronization of the follicular growth in heifers was improved with an injection of GnRH occurring 7 days before the prostaglandin F2alpha injection in the MGA protocol with PGF (Wood et al., 2001). A second injection of GnRH occurring 48 hours following the prostaglandin F2alpha injection
in concurrence with time artificial insemination can further increase conception rates in heifers (Burke et al., 1996).

After reviewing the literature on estrous synchronization management techniques and fixed timed AI in beef heifers and the 5 day CIDR protocol it was found that no direct comparison had been made between the 5 day CIDR and the 14 day CIDR Select protocols and it was decided to compare the two in beef heifers.
COMPARISON OF TWO CIDR BASED ESTROUS SYNCHRONIZATION PROGRAMS IN BEEF HEIFERS
Introduction

Estrous synchronization and artificial insemination enables beef producers to maximize the reproductive potential of their herd by shortening the calving season, increasing calf uniformity and the ability to incorporate superior genetics into their herd. Beef producers are reluctant to incorporate these management techniques because of the time and labor intensive aspect of some protocols. Therefore, the development of estrous synchronization protocols that decrease the demands of time and labor may increase the use of these new technologies.

The addition of CIDRs into estrous synchronization management techniques has successfully been used to facilitate fixed-time artificial insemination (FTAI) in beef heifers (Busch et al., 2007). The CIDR Select protocol has been studied in comparison to the shorter 7-day CO-Synch + CIDR protocol and was found to yield significantly higher pregnancy rates. Drawbacks to the CIDR Select are the lengthy time requirement of 33 days to implement this protocol and the number of times the heifers need to be handled. The 7-day CO-Synch + CIDR has also been compared to the abbreviated 5-day CO-Synch + CIDR and shown to have a 10.5% increase in pregnancy rate when shortened to the 5-day protocol. To date, no comprehensive studies have been conducted in beef heifers comparing the 14-day CIDR Select and the 5-day CO-Synch + CIDR and their potential for facilitating the successful use of FTAI. The hypothesis tested was that the 14-day CIDR Select would yield higher pregnancy rates than the 5-day CO-Synch CIDR because the longer exposure to progesterone in the 14-day protocol would induce more prepubertal heifers to start cycling at
the time of FTAI. The objective of this study was to compare these two estrous
synchronization protocols and to identify if the pubertal status of the heifers described by
RTS would influence the success of these two management techniques.
Materials and Methods

The North Carolina State University Institutional Animal Care and Use Committee approved all of the procedures described in the following experiments.

Animals

Beef heifers from the Butner Beef Cattle Field Laboratory, Upper Piedmont Beef Research Center and the Tidewater Research Center were used in this study. Heifers were 416 ± 14 days of age and 322.5 ± 10.2 kg body weight. The heifers were evaluated and assigned a score of 1–5 based on the RTS scale in which 1 indicates an immature tract with no palpable ovarian structures and 5 signifying heifers with a palpable CL on the ovaries. The heifers were assigned to treatment groups so that breed and RTS score was balanced across the treatments. Scoring was done on the day heifers were to start the 14 day CIDR Select protocol. There were 62 heifers with an RTS of 2, 78 with an RTS 3, and two heifers with an RTS of 4 (figure 6). At some locations, tail vein samples were taken for measurement of serum progesterone to determine if heifers were cycling or not at time of the protocol initiation. Body condition scoring was also performed at this time and all heifers scored between five and seven on a scale of one to nine with one being extremely thin and nine being extremely obese.
Treatment Groups

Heifers were randomly assigned to receive one of two timed AI synchronization protocols. One group was given a new Eazi-Breed CIDR™ (Pfizer, New York) for 14 days (n = 82) (CIDR Select). Nine days after removal of the CIDR heifers were given 100 ug Cystorelin® (Merial, Duluth, GA) im. Seven days after the injection of Cystorelin® heifers were injected im with 25 mg of Lutalyse® (Pfizer, New York). Seventy-two hours after the injection of Lutalyse® heifers were inseminated and given an injection of Cystorelin® im.

The other group received a new Eazi-Breed CIDR™ and was injected with 100 ug Cystorelin® on day one of their treatment (n = 80; 5 day Co-Synch CIDR, figure 1). Five days later the the Eazi-Breed CIDR™ was removed and the heifers were injected with 25 mg of Lutalyse® at the time of CIDR removal and again 8 hours later. Heifers were injected with 100 ug Cystorelin® and inseminated at 72 hours after the first injection of Lutalyse®. The timing of the treatments was set up so that all heifers in both groups would be inseminated on the same day at that location. At one location all inseminations were performed by one inseminator. At the other locations two inseminators performed AI and heifers were randomly allotted to each inseminator (figure 5).

Pregnancy Diagnosis

Heifers not detected as returning to estrus after the timed AI were examined by ultrasonography or rectal palpation between 40 and 50 days after AI for pregnancy
determination. Calving dates were used to help distinguish between the first and second AI for heifers that delivered a live calf.

Sample Collection

Blood samples were taken by venipuncture from the tail vein and collected into Vacutainer (Becton, Dickinson, and Company) tubes and blood was stored at 4°C overnight before centrifugation at 2500 rpm. Serum was stored at -20°C until assayed for progesterone concentrations. Blood samples were collected seven days before and on the day of CIDR insertion.

Progesterone Analysis

Serum progesterone concentrations were determined using a commercial radioimmunoassay kit (Coat-A-Count, Siemens Medical. Los Angeles, CA) validated for use with bovine serum in our laboratory (Whisnant and Burns, 2002). Intra and inter-assay coefficients of variation were 4.8% and 6.9%, respectively. Serum progesterone concentrations of $\geq 1$ng/ml were used as the indication that heifers were pubertal.

Statistical Analysis

Pregnancy rates were compared between treatments using the Chi Square option of PROC FREQ of SAS (SAS 9.2, Cary, NC). Pregnancy rates were also compared between locations and no location effect was noted. Also no differences between inseminators were noted at the locations with more than one AI technician.
Results

The pregnancy rates were higher (P<0.05) for the heifers in the 5-day CIDR group than in the 14-day CIDR Select group (figure 3). Across the research locations, the average pregnancy rate of heifers in the 5-day CIDR treatment group was 66.9 ± 2.1% and in the 14-day CIDR Select treatment group was 55.7 ± 1.8%. This is a difference of (P<0.05) by Chi Square. There was no location effect on pregnancy or location by treatment interaction as tested by PROC MIXED of SAS. The P4 status rates at the Tidewater location for low P4 was 66 ± 1.4% and for the high P4 was 68.5 ±2.1%. The P4 status rate for the Riedsville location was 66 ± 1.3% and was 69 ± 2.7% for high P4. There were no differences between groups in number of heifers with serum P4 concentrations > 1ng/ml.

Heifer RTS ranged from 2-4 with all but two heifers being ether a 2 or 3 (figure 6). There was no difference in RTS between treatment groups. There was no difference between heifers with a RTS of 2 and heifers with a RTS of 3 for pregnancy rate and no interaction between RTS and treatment for pregnancy rate although numbers in each treatment are relatively low (figure 4). In the 14 day CIDR group 50 ± 1.4% of the heifers with RTS of 2 became pregnant and 66±1.9% of heifers with RTS of 3 became pregnant. For the 5 day CIDR group 75 ± 1.5% of heifers with RTS of 2 became pregnant and 58 ± 1.8% of heifers with RTS of 3 became pregnant (figure 4).
Discussion

Estrous synchronization management techniques and artificial insemination practices have been available to beef producers for three decades and despite the many benefits, producers have been reluctant to implement these programs on their farms. The benefits of increased calf uniformity and shortened calving seasons afforded by these management techniques are often overlooked for the increased labor and cost. The development of economical protocols to synchronize estrus and ovulation are the focus of the research that looks to facilitate FTAI and thus eliminate the time and cost of labor associated with estrus detection.

Integration of controlled internal drug releasing devices (CIDR) containing progesterone into fixed time artificial insemination protocols for efficacy in heifers had not been directly compared for two recently developed protocols (Five day CO-Synch- CIDR and 14 day CIDR-Select). The objective of this study was to compare the effectiveness of these two protocols for use in beef heifers and to determine if reproductive condition described by reproductive tract scoring and pubertal status had any influences on the success of these two protocols.

The 5-day CO-Synch + CIDR treatment group of beef heifers resulted in a higher pregnancy rate than the heifer group that followed the 14-day CIDR Select protocol. Our hypothesis that the prolonged exposure to progesterone from the 14-day CIDR Select treatment would have greater effectiveness in heifers categorized with low RTS was not
supported by the data collected in this study. In contrast, the shorter progesterone treated program of the 5-day Co-Synch CIDR had a greater overall effectiveness in achieving higher pregnancy rates in heifers and although the differences were not significant the numerical trends if anything indicated that the heifers with RTS of had higher pregnancy rates on the 5 day CIDR protocol.

Wood-Follis et al. (2004) suggested that pubertal status of heifers prior to the initiation of estrous synchronization treatments might influence the degree of synchrony achieved. Reproductive tract scoring is an attempt to evaluate the degree of puberty and estrous cycling a heifer has achieved prior to breeding in order to increase the efficacy of estrous synchronization. Therefore, the effectiveness of an estrous synchronization protocol should be based on the ability to induce estrus in a mixed population of heifers that are both cycling and prepubertal. The inclusion of progesterone into an estrous synchronization program is successful at inducing estrous cyclicity and ovulation in prepubertal heifers (Patterson et al., 2000).

The pregnancy rate for the 14-day CIDR Select was 62% in beef heifers (Busch et al., 2007, Lamb et al., 2006) compared to the 7-day CO-Synch + CIDR protocol with a 47% pregnancy rate (Busch et al., 2007). The pregnancy rate in the 7-day CO-Synch + CIDR study by Lamb et al. in 2001 was 59%. Bridges et al. (2008) reported a 10.5% increase in pregnancy rate in beef heifers when the 7-day CO-Synch + CIDR protocol was decreased to the 5-day CO-Synch + CIDR protocol. The pregnancy rates of heifers in the current study are in the same ranges as these previous studies. Studies testing the success of one prostaglandin F2alpha administration vs. two PGF injections in the 5-day CO-Synch + CIDR protocol
found that one dose had a pregnancy rate of 15-17% less than that of the 2 prostaglandin F2alpha dose pregnancy rate of 69% (Kasimanickam et al., 2008). Our comparison of the 14-day CIDR Select and the 5-day CO Synch + CIDR yielded a pregnancy rate of heifers in the 5-day CIDR treatment group was 66.9% and in the 14-day CIDR Select treatment group was 55.7%. The 5-day CO-Synch + CIDR protocol improved the synchrony of estrus and ovulation compared to the CIDR Select protocol among cycling beef heifers. Based on these results the 5-day protocol is more advantageous than the 14-day protocol even with heifers of relatively low RTS scores of 2-3. The 5-day protocol has the added benefits of being less labor intensive to implement and could allow for the reuse of CIDRs.
Treatment: 5-day CIDR

<table>
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<tr>
<th>Day 0:</th>
<th>Day 5:</th>
<th>6-10 hours later:</th>
<th>72 hours after 1st PGF:</th>
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<tbody>
<tr>
<td>GnRH injection and implant CIDR</td>
<td>Remove CIDR and inject 1st PGF</td>
<td>Inject 2nd PGF</td>
<td>Inject GnRH and Inseminate</td>
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**Figure 1:** 5-day CIDR estrous synchronization protocol. CIDR was inserted vaginally on Day 0 and GnRH injection administered. CIDR was removed on day 5 and two injections of PGF were administered 6-10 hours apart. The heifers were inseminated 72 hours after the first PGF administration and given a second GnRH injection.
Treatment: 14-day CIDR Select

Figure 2: 14-day CIDR Select estrous synchronization protocol. CIDR was inserted vaginally on Day 0. CIDR was removed 14 days later. On Day 30 an injection of PGF was administered. 70–74 hours after PGF injection, GnRH was administered and inseminate occurred at this same time. We also injected GnRH 7 days before PGF in this study. This is a variation on the CIDR-Select that is often used.

14-day CIDR Select vs. 5-day CIDR in Beef Heifers
**Figure 3:** Comparison of pregnancy rates in beef heifers between the 14-day CIDR Select protocol and the 5-day CIDR show a greater effectiveness with the 5-day CIDR treatment in heifers.

**Pregnancy Rates by Treatment and RTS**
Figure 4: Comparison of pregnancy rates in heifers by reproductive tract scores and by the treatment. For the 14-day CIDR Select program the heifers with a RTS of 2 had a pregnancy rate of 50% and the heifers with RTS of 3 had a rate of 66%. For the 5-day CIDR group the heifers with a RTS of 2 had a pregnancy rate of 75% and those with an RTS of 3 had a rate of 58%.
**Figure 5:** Comparison of pregnancy rates by location and inseminator. There was one inseminator at the Tidewater location with a rate of 62%. At the Reidsville location, there were two inseminators, inseminator A had a rate of 68% and inseminator B had a rate of 63% which are not statistically different. At Butner there were two inseminators, inseminator A had a rate of 58.1% and inseminator B had a rate of 60.3%.
Figure 6: In this study the RTS of the heifers ranged from 2-4. There were 62 heifers with an RTS of 2, 78 heifers with an RTS of 3, and two heifers with an RTS of 4.
**Figure 7:** The pregnancy rate of the heifers with a P4 of greater than 1 ng/ml was 64.2% and the pregnancy rate of the heifers with a P4 of less than 1 ng/ml was 59.5%.
REFERENCES


Polat, B., A. Colak, M. Kaya, O. Ucar. 2009. Stimulation of delayed puberty in heifers by using a PRID regime. Ataturk University Faculty of Veterinary Medicine Department of Obstetrics and Gynaecology.


