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Improving Production Efficiency of Beef Cow-Calf Operations

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Abstract

Three studies were conducted to improve and incorporate reproductive management practices into beef cow-calf production. First study evaluated serial use of Estrotect estrous detection patches as a simple, cost-effective reproductive management tool to identify cyclic animals before breeding, distinguish between females conceiving to AI versus natural service, and determine seasonal pregnancy rate after bull removal. Also determined, was effectiveness of altered timing of GnRH treatment (1 d ± CIDR removal) in a modified 14-d CIDR-Select Synch protocol. When evaluated over a 4-wk period, estrous detection patches correctly ($P < 0.01$) identified 79% of cyclic and 86% of non-cyclic heifers. Patches were 96 and 98% accurate in identifying heifers and cows pregnant by AI, respectively, and were 76 and 87% accurate in identifying pregnant heifers and cows at the end of the breeding season ($P < 0.01$). Treatment with GnRH at CIDR removal reduced labor costs and animal handling without compromising estrous response (both ~63.0%) and AI pregnancy rates (~76 and 77%; $P > 0.1$). Second study determined if addition of PGF2alpha treatment on d 7 of a modified 14-d progesterone protocol improved estrous response in beef cows and effect of insemination timing on conception rate when using X-sorted semen. Cows were inseminated with X-sorted semen either 9 to 15, or 16 to 24 h after detected estrus. Percentage of cows exhibiting estrus was similar (76.5 and 71.2%; $P = 0.33$) regardless of treatment. Pregnancy rates after AI were similar ($P = 0.64$) at 63.3 and 66.7% for cows inseminated 9 to 15, or 16 to 24 h after estrus, respectively. Third study compared estrous response and synchrony resulting from administration of PGF2alpha on D 6 of CIDR protocol, with CIDR removal occurring concurrently (D 6) or 1 d later (D 7). Percentage of cows detected in estrus after synchronization was similar between treatments (74.0 and 71.4%, respectively; $P = 0.83$). However, 7 d CIDR treatment resulted in 100% of cows exhibiting

estrus within a 12-h period versus 75% of 6 d treatment cows. Similar AI pregnancy rates were also observed regardless of treatment (65.0 and 60.0%, respectively; P = 0.74).

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Even youths grow tired and weary, and young men stumble and fall;

but those who hope in the Lord will renew their strength.

They will soar on wings like eagles; they will run and not grow weary,

they will walk and not be faint.

Isaiah 40:30-31

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Published papers

A. J. Davis, T. D. Lester, E. A. Backes, and R. W. Rorie. 2015. Sequential use of Estrotect estrous detection patches as a reproductive management tool. Professional Animal Scientist 31:50-56.

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Chapter 1: Literature review

Reproduction in beef cattle

The *2012 Census of Agriculture* reported that approximately 913,246 farms in the United States had inventories of cattle and calves totaling approximately 90 million head of cattle. Of those, 637,293 farms are reported to be small operations (< 50 head) totaling over ten million head (USDA NASS, 2012). Good reproductive rates are critical to the success and profitability of cattle operations regardless of size. In fact, reproduction is the single most important factor affecting gross revenue of cow-calf operations (Anderson, 2009); with benefits including improved economic sustainability, quality of product, genetics, disease control, and convenience (Dziuk and Bellows, 1983).

It is generally accepted that females of reproductive age should produce a calf on an annual basis resulting in a 90% or greater net calf crop. Cows that fail to produce a calf on an annual basis waste valuable resources thereby decreasing productivity. Reproductive management includes all decisions made by a producer resulting in the failure or success of an operation (Dziuk and Bellows, 1983). However, in order for a producer to establish a successful reproductive management program, one must address common factors affecting reproductive efficiency such as nutrition, genetics, environment, development of replacement heifers, epigenetics, etc.

Factors affecting reproduction

Nutrition. Proper nutrition is perhaps the biggest factor affecting reproductive efficiency of beef cattle. Increased cost of feed is one of the predominant factors affecting profitability of cow-calf operations (Ramsey et al., 2005). Although grazing is the preferred type of feed source

for most producers, limited nutrient availability of forages during specific times of the year and during drought conditions require producers to provide supplemental feeding in order to meet herd nutrient requirements.

Prolonged postpartum anestrous periods are a major concern for cow-calf producers due to effects on calf age, weaning weight, and the number of services per conception during a breeding season (Randel, 1990). Extended anestrous periods following parturition are due in part to uterine involution which has been reported to vary as much as 28 to 54 d in cattle (Kiracofe, 1980). Ideally, cows should resume normal estrous cycles by 50 to 60 d postpartum and conceive within 83 d of calving to ensure maximum profitability. Unfortunately for beef producers, many cows have not resumed normal estrous cycles by 83 d postpartum, at least in part, due to a low plane of nutrition (Lamb, 2012).

Body condition in cattle is typically measured on a scale of 1 to 9 as an indicator of fatness. The measurement of body condition is a valuable reproductive management tool and has been referred to as the most important factor influencing early return to estrus and pregnancy in cows following parturition (Richards et al., 1986). A body condition score (BCS) of five or greater is recommended for mature cows at calving, since BCS less than five can result in fewer cows pregnancy after 80 d postpartum (Herd and Sprott, 1986). Due to higher nutritional requirements of heifers compared to cows, it is recommended that heifers reach at least 65% of their mature body weight before start of the breeding season with a BCS of six to seven (Lamb, 2012).

The exact mechanism through which nutrition regulates ruminant reproduction remains largely unknown because no single nutrient, metabolite, or hormone completely mediates reproduction (Hess et al., 2005). Producers are advised to be aware of nutrient values of forages

available to their livestock and supplement with a completely balanced ration during critical periods of fetal development.

Genetics. Another way to improve production efficiency of cow-calf operations is through genetics. Genetic composition of cow-calf operations may be either purebred or crossbred animals, depending upon production and breeding objectives of the producer. Thus, the two methods of increasing genetic merit of a herd include within breed selection and crossbreeding (Abeygunawardena and Dematawewa, 2004). The goal of within breed selection is to genetically improve traits of interest while simultaneously preserving the uniqueness and flexibility of management and environmental conditions. However, selection intensity, genetic variability, accuracy of selection, and generation interval are all factors affecting the rate of genetic gain. Thus, these factors affect the amount of genetic improvement that can be achieved via within breed selection (Abeygunawardena and Dematawewa, 2004). Increased heterosis obtained through crossbreeding has resulted in improved reproductive performance of cows and hastens puberty in heifers and bulls (Dziuk and Bellows, 1983). Genetic improvements may be introduced into a breeding population through planned matings, selection, and culling of nonproductive individuals.

Artificial insemination (AI) is the most rapid way to improve genetic diversity of a population and has been utilized in farm animals worldwide, particularly the dairy industry (Foote, 2002). The use of AI and availability progeny data allows producers to select bulls of high genetic merit thereby improving economic sustainability of cow-calf operations through improved consistency and quality of product (Foote, 2002).

Although heritability of reproductive traits is generally considered to be low in relation to management and environmental effects (Dziuk and Bellows, 1983), improvements in fertility of cows and heifers is essential for improving efficiency of cow-calf operations (Smith et al., 1989). Fertility of beef females has been reported and measured a number of ways including age of puberty, age at first calving, ovulatory follicle size, first service conception rates, pregnancy rates, postpartum interval, and longevity and stayability (Cammack et al., 2009).

Age at puberty is a measure of fertility, in that the most reproductively efficient heifers reach puberty and are capable of being bred early in the breeding season (Cammack et al., 2009). Puberty is typically defined as the period of time leading to increased gonadal activity due to a combination of morphological, physiological, and behavioral events (Krishnamurthy et al., 2001). Although heritability of reproductive traits is low; scrotal circumference in bulls is considered to be highly heritable and positively correlated to age at puberty in heifers (Brinks, 2010). Studies have shown that bulls with larger scrotal circumferences have the ability to sire daughters that reach puberty at an earlier age and exhibit increased milking ability (Smith et al., 1989; Vargas et al., 1998). Although age at puberty is considered as an indicator of fertility, the age at which an animal reaches puberty is dependent on numerous factors aside from genetics including, body weight, nutrition, environment, social and hormonal factors (Abeygunawardena and Dematawewa, 2004; Cammack et al., 2009). Age at puberty also varies greatly among breeds of cattle, as *Bos indicus* heifers typically exhibit a 6 to 12 month delay in puberty compared to *Bos taurus* heifers (Abeygunawardena and Dematawewa, 2004; Warnick, 1965). Another factor associated with heifer fertility is age at first calving, which Gutiérrez et al. (2002) showed to be highly correlated with age at subsequent calving and subsequent calving intervals.

Environment. External physical and biological factors, such as climate and environmental conditions, are all elements effecting an animal's environment with extreme conditions effecting reproduction (Gwazdauskas, 1984). Heat stress is a major factor affecting fertility of cattle (De Rensis and Scaramuzzi, 2003). Dunlap and Vincent (1971) showed that heat stress immediately post breeding affected conception rate of Herford heifers. Rectal temperatures were found to be highly correlated with respiration rate, and both were negatively correlated with conception rate. Heat stress can also affect dry matter intake of lactating dairy cows, contributing to a state of negative energy balance adversely affecting hypothalamic-pituitary gonadotropic axis function, resulting in poor estrus expression and oocyte quality (De Rensis and Scaramuzzi, 2003). Furthermore, heat stress has been shown to compromise embryonic development particularly in *Bos taurus*, compared to *Bos indicus*, embryos (Silva et al., 2013).

Development of replacement heifers. Selection and development of replacement heifers affects the entire cowherd thus affecting producer sustainability. Ideally, heifers should be managed to calve by two years of age in order to maximize lifetime productivity. However, development of replacement heifers can be costly to beef producers. Cleere (2006) determined the cost to developing a replacement heifer from weaning through pregnancy determination to be greater than $500.00. In order to ensure adequate herd replacements, beef producers may retain up to 40% or more heifers than the number of anticipated replacements (Cleere, 2006). Therefore, it is imperative that producers select the most fertile heifers for retention in the cow herd to enhance economic sustainability of cow-calf operations.

A study by Ireland et al. (2011) suggested that circulating anti-Mullerian hormone (AMH) concentration may serve as an indicator of fertility in cattle. Anti-Mullerian hormone, which is produced by small (3 to 5 mm) developing ovarian follicles, has been shown to be highly correlated with antral follicle counts (AFC) and the number of healthy follicles and oocytes present in the ovary, also known as ovarian reserve (Ireland et al., 2011; Ireland et al., 2008; Visser et al., 2006). Newborn heifers have been reported to possess anywhere from 10,000 - 350,000 healthy oocytes and follicles at birth (Erickson, 1966). However, that number may be reduced to as few as 1920 - 40,960 by one year of age (Ireland et al., 2008), thereby reducing a female's original number of healthy oocytes by as much as 80% at one year of age (Erickson, 1966). Since oogenesis occurs in utero when primordial oocytes enter meiosis but are prevented from further development until puberty, it is possible maternal nutritional epigenomics during gestation may affect AFC and size of ovarian follicular reserves in her female offspring.

Epigenetics. Epigenetics is a term that has received much attention in the past fifteen years. Barker (1990) first described maternal epigenetic effects simply as environmental influences that occur during early gestation which impairs embryonic and fetal development, resulting in increased risk of adult onset diseases. Since then that definition has been expanded to include any heritable changes in gene expression, due to altered chromatin structure, which occur without altering the DNA sequence (Funston and Summers, 2013) via DNA methylation, histone modification, or noncoding microRNAs (Canani et al., 2011).

Over the past 15 years, a growing body of evidence has been presented that demonstrates that maternal nutrition during gestation greatly affects offspring postnatal growth and development (Funston et al., 2010). Because the majority of fetal growth occurs within the last

two months of gestation, the low nutrient requirements of a developing ruminant fetus during early gestation may appear as insignificant (Robinson et al., 1977). However, maternal nutrient restriction during early pregnancy can affect placental development and vascularity, fetal organogenesis, and fetal muscle development (Funston et al., 2010). Vonnahme et al. (2007) showed that nutrient restriction from d 30 to 125 of gestation affected placental angiogenesis and the quantity of angiogenic factor mRNA in beef cows.

Bovine fetal organ development begins to occur *in utero* by 25 d of gestation, with testicular development being completed as early as 45 d, and ovarian developments as early as 50 d of gestation (Hubbert et al., 1972). Ireland et al. (2011) conducted a study to investigate effects of maternal nutrient restriction on offspring antral follicle counts (AFC) and ovarian reserve in beef heifers. Data indicated a 60% reduction in AFC of calves born to nutrient restricted beef heifers that were fed at 60% of their maintenance energy requirements during the first trimester of gestation. Because AFC and ovarian reserve are positively correlated, maternal nutrition may play an important role in regulation of the size of ovarian follicular reserves and fertility in cattle (Ireland et al., 2008; Ireland et al., 2011).

Adequate nutrition availability is also critical for skeletal muscle growth and development. Nutrient portioning is of greater importance for organs such as the brain and heart compared to skeletal muscle (Bauman et al., 1982; Close and Pettigrew, 1990). However, adequate nutrient availability during early gestation is vital for optimal skeletal muscle development because there is no net increase in the number of muscle fibers after birth (Greenwood et al., 2000; Nissen et al., 2003). Consequently, reductions in muscle fiber formation during critical periods of fetal development, due to limited nutrient availability of

dams, can have long-term, irreversible consequences for offspring and thus cow-calf producers (Du et al., 2010).

Reproductive technologies

Production efficiency of beef cow-calf operations can be improved through use of the wide variety of reproductive technologies that have become available to producers over the last fifty years. Today reproductive ultrasonography (Pierson and Ginther, 1987) and tools such as reproductive tract scoring (RTS: Anderson et al., 1991) provides producers with an effective means for determining cyclic and pregnancy status of females. Estrous synchronization and AI remain the most readily available biotechnologies allowing beef producers to rapidly improve the genetics of a population (Seidel, 1995). In fact, the use of AI allows producers to predetermine the sex of calves at insemination, through the use of sex-sorted semen, allowing for select market opportunities.

Reproductive tract scoring and ultrasonography. Anderson et al. (1991) developed a 5-point scale for determining the reproductive status of pubertal heifers based on reproductive tract score (RTS). This method utilizes rectal palpation of the uterus and ovarian structures to determine breeding potential of females. Immature heifers (uterine horns < 20 mm in diameter) lacking uterine tone with no palpable ovarian structures were considered to have a RTS of 1. Reproductive tract scores of 2 are reserved for heifers with small follicles (< 8 mm) but lacking uterine tone, whereas heifers displaying slight uterine tone with follicle 8 to 10 mm are classified as RTS of 3. Typically heifers with RTS of 1, 2, and 3 are considered as non-cyclic while heifers with RTS of 4 and 5 are considered cyclic. Heifers exhibiting follicles greater than 10 mm in

diameter and good uterine tone but lacking a CL are classified as RTS of 4. Presence of a CL and good uterine tone correspond to RTS of 5 (Anderson et al., 1991). Reproductive tract scores were also found to be correlated with age of puberty, estrous response, and pregnancy rates in heifers (Anderson et al., 1991). Thus, by evaluating the RTS of heifers prior to breeding, producers are able to distinguish between females with good versus poor breeding potential and manage females accordingly.

In addition to rectal palpation of uteri and ovarian structures, the use of real-time ultrasonography has become a valuable asset for the assessment of bovine reproduction. Early work by Pierson and Ginther (1987) showed that transrectal ultrasonography was an accurate method for determining follicle size and presence of a CL in heifers. Reproductive ultrasonography has also provided valuable insight into complicated reproductive processes including ovarian follicular dynamics, CL formation, and fetal development (Fricke, 2002). Practical on-farm uses of ultrasonography include identification of ovarian structures for determination of cyclic status, early determination of pregnancy, and fetal sexing (Fricke, 2002). Although use of reproductive ultrasonography has become a reproductive management strategy commonly used in the dairy industry (DesCôteaux and Fetrow, 1998), use of reproductive ultrasonography may not be feasible for small scale cow-calf operations due to additional cost associated with veterinarian assessment and animal handling.

Estrous synchronization and detection. The purpose of estrous synchronization is to promote the use of artificial insemination, thus shortening the calving season and increasing calf uniformity (Larson et al., 2006). Prior to selecting an estrous synchronization protocol, producers must consider a number of factors to ensure synchronization of estrous is effective.

Prolonged anestrous periods in lactating beef cows are perhaps the most challenging obstacle for beef producers to overcome when attempting estrous synchronization (Larson et al., 2006). Postpartum anestrous has been defined as the amount of time required, following parturition, for normal resumption of the hypothalamic-pituitary-ovarian-uterine axis to occur (Yavas and Walton, 2000). Extended anestrous periods in beef cows are a major concern for cow-calf producers since cows must be rebred and conceive within 80 to 85 d following calving, in order to produce a calf on an annual basis (Yavas and Walton, 2000). Although factors such as suckling, nutritional status, and age can contribute to prolonged anestrous periods following calving (Yavas and Walton, 2000), cows that are anestrous prior to synchronization can be induced to cycle through use of prostaglandins to hastening uterine involution (Short et al., 1990).

Early estrous synchronization protocols attempted to control the estrous cycle solely through regression of corpus luteum (CL; Lamb et al., 2010). Later protocols targeted the suppression of estrus through use of progesterone containing subcutaneous implants and later exogenous sources of progesterone such as melengestrol acetate (MGA) and controlled internal drug release devices (CIDR), followed protocols which combined used of prostaglandin and progesterone (Lamb et al., 2010). Although these protocols were effective in suppressing ovulation and inducing CL regression, accurate detection of estrus remained a challenge for many producers (Foote, 1975). National Animal Health Monitoring System (NAHMS) survey data indicated that fewer than 6% of small beef producers have ever utilized estrous synchronization or AI because these practices were perceived as time/labor intensive, expensive, and difficult to use (USDA NAHMS, 2011). However, the discovery of follicular wave dynamics and dominant follicle formation (Fortune et al., 1988) prompted the development of

the next generation of estrous synchronization protocols utilizing gonadotropin releasing hormone (GnRH) in an attempt to make estrous synchronization more attractive and practical for producers (Lamb et al., 2010). Administration of GnRH results in synchronization of follicular waves and ovulation through stimulated release of gonadotropins (Pursley et al., 1995). However, follicles must be at ≥ 9 mm in diameter in order to respond to GnRH treatment, thereby triggering massive release of luteinizing hormone (LH) to induce ovulation (Martinez et al., 1999; Sartori et al., 2001). Due to GnRH's ability to tighten synchronization of estrus, much work has been done in recent years to develop protocols which allow cows to be bred at a predetermined time (fixed-time AI) thus shortening or eliminating the amount of time required for estrus detection (Lamb et al., 2010).

Estrous Detection Aids. Because efficiency of cow-calf operations is based on a female's ability to conceive within an allotted time following parturition, efficient and accurate detection of estrus is crucial for artificial insemination and embryo transfer programs (Rorie et al., 2002). Within the last thirty years, a wide variety of estrous detection aids have become commercially available to producers including electronic mount detectors. Estrotect estrous detection patches are an inexpensive self-adhesive estrous detection aid available to producers which function similar to a scratch-off ticket. As intense pressure is applied to the patch, due to mount activity, the outside coating of the patch is rubbed off allowing for visualization of fluorescent patch color indicating estrus activity. HeatWatch (DDx, Inc., Denver, CO) is a computerized mount detection system which transmits radio signals from a transmitter, located on the rump of a cow, to a receiver (Rorie et al., 2002). Data is then broadcast from the receiver to a computer so that each animal's mount information may be viewed using the HeatWatch software (Rorie et al.,

2002). Although HeatWatch systems are highly accurate and efficient at detecting estrus (Stevenson et al., 1998; Stevenson et al., 1996; Walker et al., 1996; Nebel et al., 1995), these systems may not be a practical consideration for small cow-calf producers due to initial purchase expense. Currently the newest generation of HeatWatch systems, HeatWatch II, can be purchased for approximately $3,950.00. Repeaters, which function to improve signal strength, can be purchased for roughly $945.00 and monitors/detectors at $49.00 each. Initial purchase prices for a producer with fifty head of cattle would be at least $7,345.00 plus additional expenses associated with expendable supplies (such as patches and glue). Other less-expensive estrous detection aids commercially available to producers include chalk or tail head paint and chin-ball markers.

Artificial insemination. Foote (2002) described AI as the first great biotechnology improving the genetics of domestic farm animals, thus paving the way of other technologies such as estrous synchronization, gamete and embryo cryopreservation, embryo transfer, sex determination of sperm, and cloning. The history of AI is fascinating, dating back more than 335 years ago. Although Antonie van Leeuwenhoek is best known for his contributions in the development of high powered microscope lenses, it was his discovery of sperm or "animalcules" that earned him the title of Father of Microbiology. Lazzaro Spallanzani reported the first successful AI one hundred years following Leeuwenhoek's discovery of sperm (Spallanzani, 1784), and yet an additional one hundred years before AI was successfully reported in rabbits, dogs, and horses (Heape, 1897; Foote, 2002).

Use of AI in the United States began to occur rapidly in the 1940's. Research from early studies involving AI led to increased awareness of the importance of semen evaluation, semen

extension and storage, frequency and method of semen collection, and sire selection (Foote, 2002). As geneticists were working to improve the genetics of a population through sire selections, biologists were working to preserve cells and tissues through freezing (Foote, 2002). Indeed, biologist began to consider the possibility of cryopreserving cells and tissues as early as the late 19th century (Fuller, 2004). Nevertheless it wasn't until the "accidental" discovery of the protective properties of glycerol in frozen fowl semen (Polge et al., 1949) that scientist began to study the deliberate addition of cryoprotectants to semen in order to protect against freezing damage (Fuller, 2004).

Over the years, the use of AI has continued to increase in the United States, particularly in the dairy industry (Foote, 2002). Although beef cattle greatly outnumber dairy cattle in the United States, management and facilities of dairy operations are more conducive to estrous synchronization and AI (Foote, 2002). While fewer than 6% of small beef producers utilize AI and estrous synchronization (USDA NAHMS, 2011), the use of fixed-time AI protocols (FTAI) have become a popular idea for producers because such protocols reduce labor associated with animal handling and the need for estrus detection (Lamb et al., 2010). Although FTAI protocols make AI more feasible for producers, FTAI often results in lower pregnancy rates in heifers compared to insemination based on detected estrus (Beef Reproduction Task Force, 2006).

Artificial insemination also allows producers to predetermine the sex of offspring by using sex-sorted semen for the production of either herd replacements or market animals. Currently, the only reliable and cost-effective method for predetermining the sex of offspring is the use of sex-sorted semen via flow cytometry (Garner, 2006). Although studies have shown that calves resulting from use of sorted semen are normal without defects (Seidel and Garner, 2002; Tubman et al., 2004), the use of sorted semen is generally associated with reduced fertility

due to damages incurred by spermatozoa during the sorting process (Garner and Seidel Jr., 2008). While sorting, sperm cells are exposed to numerous potential hazards including dilution, incubation, and exposure to DNA stains (Boe-Hansen et al., 2005). Addition damage to sperm occurs due to exposure to elevated pressures, laser light, and prolonged periods of incubation, centrifugation, and freezing-thawing (Boe-Hansen et al., 2005). Although much advancement has been made in recent years to improve the quality of sorted semen, the reduced fertility observed with sorted versus conventional semen remains an issue. The question also remains, why do higher conception rates appear to be observed when AI is performed closer to time of ovulation when utilizing sex-sorted semen. Therefore, in order to make the most economic use of sorted semen, it is essential to ensure inseminations are performed at the appropriate time, based on detected estrus.

Proper timing of insemination is critical for ensuring optimal conception rates in cattle bred by AI (Dorsey et al., 2011). Traditional AI protocols recommend use of classic A.M./P.M. rule allowing insemination to occur approximately 12 h after detected estrus (Trimberger and Davis, 1943; Foote, 2002). Data suggests that optimal time of insemination in dairy cattle occurs approximately 4-12 h following onset of estrus (Dransfield et al., 1998) but that a broader range of insemination times are available in beef cattle (Rorie et al., 2002; Dorsey et al., 2011). Rorie et al. (2002) compared conception rates in beef cows that were inseminated with conventional frozen-thawed semen, at 4-h intervals, ranging from 8 to 24 h after the onset of estrus. Time of insemination had no effect on AI conception rates, indicating there is flexibility in time of insemination in beef cows when using high quality, conventional semen. However, optimal timing of insemination using conventional semen may not be compatible with the use of sex-sorted semen.

Conception rates from sex-sorted semen are often reported to be lower than that achieved with conventional, unsorted semen, due to the reduced number of sperm per insemination dose and potential damage to sperm during the sorting process (Frijters et al., 2009). Preliminary data in beef cattle suggest that conception rates might be improved by delaying insemination a few h later than the usual 12 h after onset of estrus, when using sex-sorted semen (Rorie et al., 2012). Funston and Meyer (2012) directly compared single service conception rates in beef heifers inseminated with either conventional or sex-sorted semen from the same sires. All inseminations occurred approximately 18 to 24 h after detected estrus. Conception rates resulting from insemination with conventional and sex-sorted semen were 58.4 and 41%, respectively. A study in Jersey heifers, synchronized with two doses of PGF2alpha and inseminated with X-sorted semen from 12 to 24 h, indicated higher pregnancy rates occurring from inseminations performed 16 to 24 h following onset of estrus (Filho et al., 2010). Insemination occurring earlier at 12 to 16 h, or later than 24 h after onset of estrus, resulted in reduced conception rates when compared to inseminations occurring from 16 to 24 h after onset of estrus (Filho et al., 2010).

Methods to improve sustainability for small producers

Beef production in the United States consists of a large number of small beef operations (farms which contain fewer than 50 head), that are almost exclusively family owned and operated. Although reproductive management is the single most important factor contributing the economic sustainability of beef production (Anderson, 2009), the vast majority of small beef producers in the United States under-utilize recommended reproductive management practices. According to National Animal Health Monitoring System (NAHMS) survey data, only 1.2% of

small beef producers evaluate the reproductive (cyclic) status of breeding age heifers prior to breeding season (USDA NAHMS, 1994).

Within the last forty years, major advancements have been made in reproductive technologies such as gamete cryopreservation, artificial insemination (AI), estrous synchronization, embryo transfer, and the use of sex-sorted semen. However, less than 6% of small beef producers have ever utilized estrous synchronization or AI while less than 12% of producers check their cows or heifers for pregnancy (USDA NAHMS, 2011). The small-scale U.S. cow-calf operations report (USDA NAHMS, 2011) indicated that small cattle producers were less likely to use management practices such as estrous synchronization, artificial insemination (AI), pregnancy palpation, body condition scoring (BCS), and semen evaluation because these practices were perceived as either time/labor intensive, costly, too difficult to use, or lacked profit potential. However, if the reproductive status of the herd is largely unknown, producers cannot make good management decisions. Small beef producers would be more likely to utilize such reproductive management practices if their application were practical, inexpensive and easy to use.

Enhancements in consistency and quality of beef products are also essential to improve the economic sustainability of small cattle farms. The beef industry has been reported to lose as much as $44.66 per head in opportunity costs due to a lack of consistency in carcass quality (USDA NAHMS, 2011). Estrous synchronization and AI can be used to achieve rapid genetic improvement in beef cattle efficiency, quality and consistency. In addition, the availability of sex-sorted semen allows producers to predetermine the sex of calves born, allowing for increased marketing opportunities.

Three studies were conducted to improve and incorporate reproductive management practices into beef cow-calf production. The first study evaluated the serial use of Estrotect estrous detection patches as a simple, cost-effective reproductive management tool to identify cyclic animals before breeding, to distinguish between cows or heifers conceiving to AI versus natural service, and to determine seasonal pregnancy rate after bull removal. A secondary objective was to determine if altering the timing of GnRH treatment (either at or 1 d after CIDR removal) in a modified 14-d CIDR-Select Synch synchronization protocol compromised protocol effectiveness. The second study was conducted to determine if addition of PGF2alpha treatment on d 7 of a modified 14-d progesterone protocol improved estrous response in beef cows, as well as, the effect of insemination timing on conception rate when using X-sorted semen. The third study was designed to compare estrous response and synchrony resulting from a synchronization protocol where PGF2alpha was given on D 6 of a CIDR protocol, with CIDR removal occurring concurrently (D 6) or 1 d later (D 7).

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Chapter 2: Sequential use of Estrotect estrous detection patches as a reproductive management tool

Abstract

This study investigated whether Estrotect estrous-detection patches could be used as a simple, cost-effective reproductive-management tool to identify cyclic animals before breeding, to distinguish between cows or heifers conceiving to AI versus natural service, and to determine seasonal pregnancy rate after bull removal. A secondary objective was to determine whether altering the timing of gonadorelin (GnRH) treatment in a 14-d progesterone-Select Synch synchronization protocol could reduce labor costs without reducing protocol effectiveness. Compared with cyclic status determined via ultrasonography, Chi-square analysis indicated that estrous-detection patches monitored for a 4-wk period were able to correctly identify 79% of cyclic and 86% of noncyclic heifers ($P < 0.01$). Estrous-detection patches were 96 and 98% accurate in identifying heifers and cows pregnant by AI, respectively. When compared with pregnancy data obtained via ultrasonography, estrous-detection patches were 76% accurate in identifying pregnant heifers and 87% accurate in identifying pregnant cows at the end of the breeding season ($P < 0.01$). Data indicated that accuracy of estrous-detection patches in predicting pregnancy depends upon cyclic status of the herd. Estrus was synchronized in lactating cows using a 14-d CIDR-Select Synch protocol where timing of GnRH administration occurred at time of CIDR removal (d 14) or 24 h later (d 15). In both treatments, prostaglandin F2α was given 7 d after GnRH. Estrous response and AI pregnancy rates were similar ($P > 0.10$), regardless of timing of GnRH treatment. Treatment with GnRH at CIDR removal reduced labor costs and animal handling.

Key words: bovine, estrous detection patch, estrous synchronization, reproductive management

Introduction

Reproductive management is the single-most-important factor contributing to the economic success of beef producers, with benefits including improved economic sustainability, quality of product, genetics, disease control, and convenience (Dziuk and Bellows, 1983). Unfortunately, many small, family-owned beef operations underutilize basic reproductive-management practices because these practices are perceived as either too time or labor intensive, costly, or difficult to use (USDA NAHMS, 1994). Beef producers would be more likely to utilize reproductive-management practices if their application were more practical, inexpensive, and easy to use. Basic reproductive management might be achieved through the serial use of estrous-detection patches for (1) identification of cyclic animals before the breeding season, (2) detection of estrus before insemination, (3) distinguishing between cows or heifers conceiving to AI versus natural service, and (4) determining the seasonal pregnancy rate after bull removal.

Estrous synchronization can be used as a reproductive management tool to facilitate AI and ensure more cows are cyclic at the start of the breeding season. Good estrous response (> 80%) and AI pregnancy rates (> 75%) have been achieved in lactating beef cows synchronized using a 14-d progesterone controlled internal drug-release insert (CIDR) treatment, followed by administration of gonadorelin (GnRH) on d 16 and prostaglandin $F_{2\alpha}$ (PGF) on d 23 (Powell et al., 2011). This estrous synchronization protocol might be simplified, and associated labor costs reduced, if GnRH treatment could be given at the time of CIDR removal, without a loss in treatment effectiveness. Therefore, the objectives of this study were (1) evaluation of a simple, cost-effective reproductive management tool, based on estrous-detection patches, and (2) evaluation of effects of timing of GnRH administration in a modified progesterone-Select Synch protocol on estrous response and AI pregnancy rates of beef cows.

Materials and methods

Angus based cows (n = 149) and heifers (n = 81) from the University of Arkansas Savoy Beef Research Station were used in this study. At the start of the study, cows had a mean BW of 494.8 ± 64.3 kg, had a BCS of 5.5 ± 0.9, and were 57 ± 12.8 d postpartum. Heifers averaged 405.1 ± 12.7 d of age, with a mean BW of 282.1 ± 2.7 kg and BCS of 5.4 ± 0.5. Body condition was scored using a scale from 1 to 9, with a score of 1 being emaciated and 9 being extremely fat (Richards et al., 1986). All animal procedures were approved by the University of Arkansas Animal Care and Use Committee (IACUC protocol # 12010).

Evaluation of pubertal status of heifers before breeding. Thirty days before the start of the estrous synchronization, each heifer received an Estrotect estrous detection patch (Estrotect; Rockway Inc., Spring Valley, WI), which was adhered to the animal for a 4-wk period. In the area where the patch was to be placed, hair was clipped and skin was sprayed with a multipurpose spray adhesive (3M Super 77 Spray Adhesive, 3M Corp., St. Paul, MN) and allowed 30 to 45 s for the adhesive to get tacky. Patches were then placed on the rump, with the front edge of the patch in line with the hipbones. After the 4-wk patch evaluation period, reproductive-tract scores (RTS) were assigned to all heifers based on transrectal ultrasonography (Ibex Pro, E.I. Medical Imaging, Loveland, CO) using the L6.2 transducer (8-5 MHz 66-mm linear array). Criteria for determining RTS are listed in Table 1 (Anderson et al., 1991). Heifers with RTS of 1 through 3 were considered as non-cyclic, whereas heifers with RTS of 4 and 5 were considered as cyclic (Rosenkrans and Hardin, 2003). Accuracy of estrous-detection-patch data was compared to known cyclic status, as determined by RTS performed via ultrasonography.

Patches were evaluated using 2 separate scoring methods: a patch score (**PS**) of 1 to 4 or Yes or No designation based on subjective evaluation of the patch. The PS scoring method used the following scale: 1 = 25% or less of the patch had been activated, including minor scratches; 2 = up to 50% of the patch had been activated; 3 = up to 75% of the patch has been activated; and 4 = fully activated patch. With the Yes or No designation, an estrous-detection patch was considered activated when a minimum of 50% of the center portion of the patch was completely clean. Patches with minor wear due to scratching or environmental conditions were considered nonactivated. Any estrous detection patches missing or torn loose were noted and considered a prediction failure in the analysis. For consistency, the same trained technician evaluated the patch of each individual animal weekly from a vehicle while heifers grazed.

Estrous synchronization and insemination of heifers and cows. Estrous cycles of heifers were synchronized, using a 14-d **CIDR** progesterone treatment, (EAZI-Breed CIDR; 1.38g progesterone, Zoetis, Florham Park, NJ) followed by **GnRH (**100 µg i.m., Factrel, Zoetis) at CIDR removal on d 14, and prostaglandin $F_{2\alpha}$ (**PGF**; 25 mg i.m., Lutalyse, Zoetis) 7 d later on d 21. Cows were stratified across estrous-synchronization treatments based on ovarian ultrasonography (cows identified as having a corpus luteum, follicle >10 mm in diameter, or both were considered cyclic), BCS, postpartum interval, and weight. Cows were synchronized using the same protocol as heifers, except GnRH was administered either at CIDR removal (d 14; **GnRH+0**) or 1 day after CIDR removal (d 15; **GnRH+1**). At the time of GnRH administration to cows, ultrasonography was used to record the diameter (mm) of the largest follicle present on either ovary. Cows then received PGF 7 d after GnRH treatment. All heifers and cows received an Estrotect estrous detection patch at the time of PGF treatment and were visually monitored by

a trained observer for onset of estrus for a minimum of 30 min every 2 h from 0800 until 2000 h, then at 2400 and 0400 h, over a 72-h period. All animals observed in estrus were inseminated with conventional, frozen-thawed semen approximately 12 h after detected estrus. Any cows that failed to exhibit estrus within 72 h of PGF administration were administered an injection of GnRH and time inseminated at 96 h after PGF.

Determination of AI and seasonal pregnancy rates. Ten days after the last insemination, heifers and cows received another estrous-detection patch and were turned out with bulls for a 45-d breeding season. Estrous detection patches were evaluated weekly for 4 wk, using the same 2 scoring methods described above. Approximately 45 d after the last insemination, ultrasonography was used to determine AI pregnancy status and confirm conception date, based on fetal crown-rump length. Upon bull removal at the end of the breeding season, all cows and heifers received another estrous-detection patch that was evaluated weekly for 4 wk, again using the same scoring methods described previously. Approximately 30 d after bull removal, ultrasonography was again used to determine seasonal pregnancy rate and confirm conception date, based on fetal crown-rump length. Estrous-detection-patch data were compared with actual pregnancy data, as determined by ultrasonography.

Statistical analysis. Statistical analysis was performed using SAS (SAS Inst. Inc., Cary, NC). Chi-square analysis was used to determine differences between ultrasound and PS data collected during the fourth wk of each evaluation period to determine the accuracy of predicting prebreeding cycling status in heifers, and AI and seasonal pregnancy rates in both heifers and cows. As a practical consideration, PS taken during the fourth wk were used for statistical

analysis because retention of patches over a 4-wk period would be adequate time for all animals to exhibit at least one complete estrous cycle. The null hypothesis was that patch scores (observed) and ultrasound (expected) data were independent, meaning no relationship existed between the two variables. However, rejection of the null hypothesis ($P \leq 0.05$) demonstrates that the 2 variables are related. An ANOVA was performed using the mixed procedure of SAS to determine effects of estrous-synchronization treatment of cows on follicle size at GnRH administration.

Results and discussion

Reproductive management can have a significant impact on the economic sustainability and viability of beef production but is often underused by beef producers. National Animal Health Monitoring System (NAHMS) survey data shows that only 1.2% of small-scale beef producers (i.e., fewer than 50 head of cows) evaluate the reproductive (cyclic) status of breeding-age heifers before start of the breeding season (USDA NAHMS, 1994). Less than 6% of small-scale beef producers have ever used estrous synchronization or AI, and less than 12% of producers check their cows or heifers for pregnancy (USDA NAHMS, 2011). A simple, cost-effective reproductive-management tool that beef producers might use would allow them to make better management decisions.

Evaluation of the reproductive status before the breeding season allows producers to make culling decisions and select estrous-synchronization protocols that have been shown to be effective in inducing cyclicity. Measure of progesterone in blood samples collected 10 d apart is often used by researchers to identify cyclic animals. However, the stress of handling and restraining animals can result in release of adrenal progesterone along with cortisol, resulting in

elevation of plasma progesterone above 1 ng/ml, and misidentification of prepubertal animals as cyclic (Cooke and Arthington, 2009). Ultrasonography has been shown to be accurate in identifying animals with a corpus luteum and in determining the diameter of dominant follicles (Pierson and Ginther, 1987).

The method of RTS (via ultrasonography) used in this study was first developed by Anderson et al. (1991; Table 1) and was found to be correlated with reproductive factors such as age of puberty, responsiveness to estrous synchronization, and pregnancy rates achieved via estrous synchronization. Reproductive-tract scores have also been found to be an accurate and repeatable method of distinguishing between pubertal and prepubertal beef heifers prior to start of the breeding season (Rosenkrans and Hardin, 2003).

As an alternative to reproductive-tract scoring, this study evaluated the use of estrous-detection patches for identifying cyclic and non-cyclic heifers. In a preliminary study, it was noted that if Estrotect patches were placed on the rump of a heifer, about midpoint between the tail head and hip bones, using only the self-adhesive back, the patches were often torn lose and lost after a few days. By clipping the hair, using spray adhesive, and placing the patches with the front edge aligned with the hipbones, the patches were retained for a period of weeks. In the current study, the patch retention rate on heifers was 98.8% during the prebreeding evaluation period.

After a preliminary assessment, it was decided to compare the accuracy of the fourth wk of patch score data to ultrasound data. As a practical consideration, retention of patches over a 4-wk period would be adequate time for all heifers to exhibit at least one complete estrous cycle. Also, it was decided to categorize PS of 1 or 2 as nonactivated and 3 or 4 as activated patches. Of the 81 heifers used in this study, RTS determined by ultrasonography identified 53 heifers as

cyclic (RTS of 4 or 5) and 28 heifers to be noncyclic (RTS of 1, 2, or 3) before the breeding season. The Yes or No patch-scoring method correctly ($P < 0.01$; Table 2) identified 42 of 53 (79.3%) heifers as cyclic and 24 of 28 (85.7%) heifers as noncyclic. The PS of Yes (activated patches) misidentified 4 heifers as cyclic when they were not (false positive). The PS of No (nonactivated patches) misidentified 11 heifers as noncyclic, but ultrasonography confirmed the heifers were cyclic (false negative).

The numerical PS method indicated that PS of 1 and 2 (assumed non-cyclic) also correctly identified 24of 28 (85.7%) of noncyclic heifers but incorrectly identified 11 cyclic heifers as noncyclic (false negative). Only 11 of 81 heifers received a wk-4 PS of 3, with 7 of 11 (63.6%) correctly identified as cyclic. All 35 heifers receiving a PS of 4 were correctly identified as cyclic. In comparison to ultrasound data, PS of 3 and 4 combined correctly identified 42 of 53 (79.2%) cyclic heifers ($P < 0.01$). This accuracy (~79%) compares favorably to other methods of determining cyclic status, such as estrous detection. In beef cattle, the efficiency of estrus detection (i.e., the percentage of animals in estrus that are actually detected) has been reported to range of about 50 to 75% (Stevenson et al., 1996; Rae et al., 1999). Therefore, visual observation has a failure rate of 25 to 50% in detecting cyclic animals. Patch placement may have contributed to the high incidence of false negatives (~ 31%). To improve patch retention during this study, patches were placed on the rump with the front edge of the patch in line with the hip bones, rather than about midpoint between the tail head and hip bones as is recommended. Placement of patches in this forward position may have prevented activation of patches on some cyclic heifers.

Synchronized heifers were visually observed for estrus but also received an Estrotect estrous-detection patch at the time of PGF treatment. All but 1 (PS 3) of the heifers observed in

estrus over a 72-h period also were noted to have fully activated patches at the time of insemination. Forty-eight heifers were detected in estrus and artificially inseminated. The lower-than-expected (48/81) estrus response was likely due to ~35% of the heifers being noncyclic at the start of estrous-synchronization treatment. Ultrasonography later confirmed that 24 (50%) heifers were pregnant by AI. The Yes or No method of patch scoring correctly identified 23 of 24 (95.8%) of the heifers pregnant to AI, but was only 58.3% (14/24) accurate in identifying open heifers (Table 3; $P < 0.01$). Patch scores 1 and 2 combined correctly identified 22 of 24 (91.7%) heifers pregnant after AI but misidentified another 10 heifers as pregnant when they were open (Table 4; $P < 0.01$). Only 1 heifer received a PS of 3 but was incorrectly identified as pregnant. Of 15 heifers scored as PS 4, 14 (93.3%) were correctly identified as open.

Estrotect estrous-detection patches were placed on heifers at the end of the breeding season and monitored for 4 wk to determine seasonal pregnancy rates. Ultrasonography was then used to determine pregnancy status, for comparison to patch data. Ultrasonography confirmed that 72.8% (59/81) of the heifers to be pregnant. At the 4-wk evaluation, 3 heifers had lost their estrous-detection patches; 2 of 3 of these heifers were confirmed pregnant. The Yes or No PS method correctly identified 45 of 59 (76.3%) pregnant heifers but correctly identified only 9 of 22 (40.9%) open heifers (Table 3; $P = 0.02$). The combination of PS 1 and 2 correctly identified 39 of 59 (66.1%) of pregnant heifers (Table 4; $P < 0.01$). Patch scores of 3 and 4 correctly identified only 10 of 22 (45.5%) open heifers. Accuracy of using estrous-detection patches to determine pregnancy status of heifers is dependent on the heifers being cyclic. Estrous-detection patches cannot differentiate between pregnant and noncyclic animals, because neither would be expected to have activated patches. Fully activated patches appear accurate in

identifying cyclic or open heifers. In the current study, ~35% of the heifers were not cyclic at the start of the study. During the final ultrasonography to determine seasonal pregnancy rate, it was noted that 5 of 22 open heifers were not cyclic based on absence of corpus luteum or any follicles greater than 10 mm in diameter on either ovary. It was concluded that noncyclic heifers contributed to the error rate noted in the ability of estrous-detection patches to correctly identify reproductive status.

Pregnancy rates increase through the use of estrous-synchronization protocols, such as long-term progestin treatment, that synchronize estrus in cycling cows and induce estrus in prepubertal heifers and anestrous postpartum cows (Patterson et al., 2011). Long-term treatment with progestins in the presence of subluteal progesterone concentrations results in development of large persistent follicles (Siriois and Fortune, 1990). Good estrous response and AI pregnancy rates (> 75%) has been achieved when lactating cows were synchronized with 14-d progestin treatment followed by GnRH on d 16 and PGF on d 23 (Powell et al., 2011). Injection of GnRH on d 16 was expected to induce ovulation and synchronize follicle growth so that cows express estrus more consistently after PGF treatment on d 23. However, effectiveness of GnRH is dependent on the presence of a dominant follicle (≥ 9 mm) at the time of treatment (Martinez et al., 1999).

A preliminary study, where follicles present on the ovaries of cows were measured at withdrawal of a 14-d progestin treatment, indicated more than 90% of cows had at least 1 follicle measuring 9 mm or larger. Hence, treatment with GnRH at progestin removal would likely be effective, while reducing labor costs and processing of cows through a working facility. Therefore, the current study investigated the effects of timing of GnRH administration, when given either at CIDR removal (GnRH+0) or 24 h later (GnRH+1). Approximately 93% of cows

(138/149) were cyclic before estrous synchronization; however, only 63.1% (94/149) of cows were observed in estrus after synchronization. Estrus was visually observed for all (n = 76) cows with estrous-detection PS of 3 or 4, and another 13 cows with missing patches at the time of insemination. An additional 5 cows were observed in estrus, but only had patch scores of 1 or 2 at insemination. Chi-square analysis indicated that estrous response was similar ($P = 0.99$) between both treatments, at 63.0% (46/73) in the GnRH+0 versus 63.2% (48/76) in the GnRH+1 group. The poor estrous response observed, compared to the number of animals cycling prior to synchronization, may have been due to severe winter weather conditions that occurred during estrous synchronization. The mean temperature at time of CIDR removal was 10.1˚C, but conditions declined over the next week, during which time the mean temperature plunged to -9.3˚C, with a low of -13.3 and high of -6.1˚C. Weather conditions continued to worsen with an accumulation of approximately 15.2 cm of sleet and snow (The Old Farmer's Almanac, 2014).

Of the 94 cows exhibiting estrus and inseminated, pregnancy rates were similar ($P = 0.91$) at 76.1% for GnRH+0 and 77.1% for GnRH+1. Cows failing to exhibit estrus within 84 h of PGF treatment received GnRH treatment in conjunction with insemination at 96 h post PGF. The timed insemination resulted in an 11% AI pregnancy rate. Administration of GnRH triggers massive release of luteinizing hormone (LH), and follicle stimulating hormone, resulting in synchronization of follicular waves and ovulation (Pursley et al., 1995). Ovulatory capability has been reported to occur once follicles have reached approximately 10 mm in diameter under massive stimulation of luteinizing hormone (Martinez et al., 1999; Sartori et al., 2001). An ANOVA indicated that follicular diameter was similar for both GnRH+0 and GnRH+1 treatments (15.21 vs. 15.75 mm respectively, $P = 0.63$). Treatment with GnRH at the time of

CIDR removal reduced labor and the number of times animals have to be processed through working facilities during synchronization.

Estrotect estrous-detection patches were also used to determine AI and seasonal pregnancy rates of cows. Ultrasonography confirmed 81 cows to be pregnant by AI and a total of 125 cows to be pregnant at the end of the breeding season. The Yes or No patch scoring method correctly identified 79 of 81 (97.5%) cows pregnant by AI but only 39 of 68 (57.4%) open cows following AI ($P < 0.01$; Table 3). The Yes or No scoring incorrectly identified 28 cows as pregnant when they were open. Patch scores of 1 and 2 correctly identified 77 of 81 (95.1%) cows to be pregnant by AI but misidentified 23 cows as pregnant when they were not (Table 4; $P < 0.01$). A total of 44 of 68 (64.7%) open cows were correctly identified as open by PS of 3 or 4. The PS of 3 or 4 incorrectly identified 3 cows as open, but were determined to be pregnant. Two cows lost their patch during the 4-wk post-AI evaluation period; one of these cows was found to be pregnant while the other was open. Any animal that lost an estrous detection patch was considered a failure to correctly predict pregnancy status and was considered as such in the analysis.

Pregnancy data (from ultrasonography) was compared to patch score data collected 4 wk after bull removal. Of the 125 cows confirmed to be pregnant at the end of the breeding season, the Yes or No PS correctly identified ($P < 0.01$; Table 3) 109 pregnant (87.2%) but only 5 (20.8%) open cows. The numerical PS method correctly identified 108 of 125 (86.4%) cows as pregnant but correctly detected only 5 of 24 (20.8%) as open ($P < 0.01$; Table 4). Both patch-scoring methods misidentified approximately 11% of open cows (PS of 1 or 2, or "No") as pregnant. The cows used in this study lost condition (initial BCS 5.5 vs. final BCS 4.6) from synchronization until final pregnancy check at the end of the breeding season. As a result, 12 of

24 open cows were confirmed by ultrasonography to be noncyclic after the breeding season. As was observed with heifers, estrous-detection patches cannot differentiate between pregnant and open, noncyclic cows, because neither would be expected to have activated patches.

The heifers and cows used in this study were synchronized to start the breeding season in late November and early December, respectively. Clipping the winter hair coat where the patch was to be applied, using spray adhesive, and placing the patch further up the rump than usual resulted in good long-term patch retention. It was noted that the majority of patches that were lost were those applied on very cold days, where the spray adhesive never got tacky. Similar difficulties in patch retention of HeatWatch mount detector patches have been observed in dairy heifers during cold weather (Ambrose et al., 2005). Although it is commonly assumed that missing patches are the result of increased mount activity during estrus (Stevenson et al., 2008), data from this study indicates otherwise, at least when patches are worn for an extended period of time. One of the two cows that lost their estrous-detection patch during the post-AI evaluation period was confirmed pregnant, as well as, 14 of 19 cows that lost patches during the seasonal pregnancy-evaluation period. It should be noted that the loss of patches contributed to the error rate in predicting open or pregnant animals in this study because a lost patch was considered a prediction failure.

Overall, the results of this study indicate that estrous-detection patches can be used to incorporate reproductive management into cow-calf operations at minimal cost. Estrous-detection-patch scoring was more accurate in identifying pregnant than open animals and dependent on the animals being cyclic. To wit, neither pregnant nor non-cyclic animals would be expected to have activated patches, so both groups might be assumed to be pregnant. Although estrous detection patches can be used to provide some information to producers for

making reproductive-management decisions, either palpation or ultrasound approximately 45 to 60 d after the end of the breeding season is still the preferred and most accurate method for pregnancy determination.

Implications

Data from this study indicate Estrotect estrous-detection patches can be used to provide producers with useful information regarding cyclicity and pregnancy rate after insemination or natural service. However, the predictive accuracy of estrous-detection patches is dependent upon the cyclity of the herd and retention of patches on cows or heifers over a 4-wk period. Data also suggest that acceptable AI pregnancy rates can be achieved in lactating beef cows synchronized with a modified progesterone-Select Synch protocol where GnRH administration occurs at CIDR removal.

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Tables

Table 1: Heifer reproductive tract score[a] (RTS) criteria

		Ovary			
RTS	**Uterine Horne**	**Length, mm**	**Height, mm**	**Width, mm**	**Ovarian structures**
1	Immature <20 mm diameter, no tone	15	10	8	No palpable structures
2	20 to 25 mm diameter, no tone	18	12	10	8-mm follicles
3	25 to 30 mm diameter, slight tone	22	15	10	8 to 10 mm follicles
4	30 mm diameter, good tone	30	16	12	>10 mm follicles, CL possible
5	>30 mm diameter, good tone, erect	>32	20	15	>10 mm follicles, CL present

[a]Table reproduced from Anderson et al., 1991. Heifers with a RTS of 4 or 5 are identified as cyclic.

Table 2: Use of Estrotect estrous detection patches to predict pre-breeding cyclicity in beef heifers

	Prediction of patches confirmed via ultrasonography			
Method	**Cyclic**	**FP[1] (%)**	**Non-cyclic**	**FN[2] (%)**
Patch activated[3,5]				
Yes/No	42/53 (79.3)	4/46 (8.7)	24/28 (85.7)	11/35 (31.4)
Patch score[4,5]				
1 & 2	-	-	24/28 (85.7)	11/35 (31.4)
3 & 4	42/53 (79.2)	4/46 (8.7)	-	-

[1]FP = False positive. Heifer identified as cyclic but confirmed non-cyclic.

[2]FN = False negative. Heifer identified as non-cyclic but confirmed cyclic.

[3]Patch activated. Yes = patch was activated due to mount activity suggesting an animal is open and has returned to estrus. No = patch was not activated suggesting an animal is pregnant.

[4]Patch score. 1 = 25% or less of the patch had been activated, including minor scratches, 2 = up to 50% of the patch had been activated, 3 = up to 75% of the patch has been activated, and 4 = fully activated patch.

[5]Fisher's exact test. $P < 0.01$

Table 3: Yes/No scoring of Estrotect estrous detection patches to predict AI and seasonal pregnancy rates in beef heifers and cows.

Confirmed by ultrasonography			Predicted by Yes/No scoring			
Preg. rate	**Preg.**	**Open**	**Preg. (%)**	**FP[1] (%)**	**Open (%)**	**FN[2] (%)**
Heifers						
AI[3]	24	24	23 (95.8)	10/33 (30.3)	14 (58.3)	1/15 (6.7)
Seasonal[4]	59	22	45 (76.3)	12/57 (21.1)	9 (40.9)	12/21 (57.1)
Cows						
AI[3]	81	68	79 (97.5)	28/107 (26.2)	39 (57.4)	1/40 (2.5)
Seasonal[3]	125	24	109 (87.2)	14/123 (11.4)	5 (20.8)	2/7 (28.6)

[1]FP = False positive. Animals identified as pregnant but confirmed open.

[2]FN = False negative. Animals identified as open but confirmed pregnant.

[3] Fisher's exact test. $P \leq 0.01$

[4] Fisher's exact test. $P \leq 0.05$

Table 4: Scoring of Estrotect estrous detection patches as 1 to 4 to predict AI and seasonal pregnancy rates in beef heifers and cows.

	Confirmed by ultrasound		Predicted by PS[1] 1 to 4			
			Pregnant		Open	
Preg. Rate	**Preg.**	**Open**	**PS 1 & 2 (%)**	**FP[2] (%)**	**PS 3 & 4 (%)**	**FN[3] (%)**
Heifers						
AI[4]	24	24	22 (91.7)	10/32 (31.3)	14 (58.3)	2/16 (12.5)
Seasonal[4]	59	22	39 (66.1)	11/50 (22.0)	10 (45.5)	18/28 (64.3)
Cows						
AI[4]	81	68	77 (95.1)	23/100 (23.0)	44 (64.7)	3/47 (6.4)
Seasonal[4]	125	24	108 (86.4)	14/122 (11.5)	5 (20.8)	3/8 (37.5)

[1]PS = Patch score. 1 = 25% or less of the patch had been activated, including minor scratches, 2 = up to 50% of the patch had been activated, 3 = up to 75% of the patch has been activated, and 4 = fully activated patch.

[2]FP = False positive. Animals identified as pregnant but confirmed open.

[3]FN = False negative. Animals identified as open but confirmed pregnant.

[4]Fisher's exact test. $P \leq 0.01$

Chapter 3: Comparison of two estrous synchronization protocols for use with X sorted semen in lactating beef cows

Abstract

A study investigated whether prostaglandin injection on d 7 of a modified 14-d progesterone protocol improved estrous response in beef cows, and the effect of insemination timing on conception rate when using X-sorted semen. Treatment 1 (Control; n = 132) cows received a CIDR progesterone insert from d 0 to d 14, gonadorelin (GnRH) treatment on d 16, and prostaglandin F2alpha (PGF) treatment on d 23. Treatment 2 (D7PGF; n = 132) cows received the same synchronization treatment, except an additional dose of PGF was given on d 7 of CIDR treatment. Cows were observed for estrus over an 84-h period and inseminated with X-sorted semen either 9 to 15, or 16 to 24 h after detected estrus, followed 10 d later by exposure to fertile bulls for 45 d. Percentage of cows exhibiting estrus did not differ ($P = 0.33$) at 76.5 and 71.2% for treatments 1 and 2, respectively. Conception rates after AI with X-sorted semen were similar ($P = 0.64$) at 63.3 and 66.7% for treatments 1 and 2, respectively. Time of insemination had no effect ($P = 0.72$) on conception rate. At the end of the breeding season, overall pregnancy rates were also similar ($P = 0.74$) at 83.3 and 84.9% for cows in treatments 1 and 2, respectively. Results demonstrated no benefit to addition of PGF on d 7 to the estrous synchronization protocol, and that acceptable conception rates can be achieved in lactating beef cows when using X-sorted semen over a range of insemination times.

Keywords: estrous synchronization, insemination timing, X-sorted semen

Introduction

Good estrous response and AI conception rates (> 75%) have been reported for cows synchronized with a 14-d controlled internal drug release (**CIDR**) progesterone treatment, followed by gonadorelin (**GnRH)** on d 16 and prostaglandin F2alpha (**PGF**) on d 23 (Powell et al., 2011). Martinez et al. (1999) reported that the effectiveness of GnRH is dependent on the presence of a dominant follicle (≥ 9 mm) at the time of treatment. When progesterone concentrations are low (sub-luteal), long-term treatment with progestins results in the development of persistent dominant follicles (Siriois and Fortune, 1990). In the estrous synchronization described above, GnRH injection on d 16 should induce ovulation of any persistent follicles forming during progestin treatment and synchronize follicle growth so that cows express estrus more consistently after PGF treatment on d 23. However, if cows are cyclic at the start of synchronization treatment, a functional corpus luteum could be present, resulting in elevated circulating progesterone that would prevent development of a persistent follicle. Thus, an objective of this study was to determine if the estrous synchronization protocol reported by Powell et al. (2011) might be improved by the addition of PGF on d 7 of the CIDR treatment, to regress any corpus luteum present, and insure a persistent follicle will develop that should be responsive to GnRH.

In preliminary study (Rorie et al., 2012), a trend for greater conception rates was noted when AI with X-sorted semen in beef cows was delayed until about 16 to 18 h after detected estrus. A study comparing the effects of timing of insemination with X-sorted semen in Jersey heifers reported higher conception rates for heifers inseminated at 16 to 24 h versus 12 to 16 h after onset of estrus (Fihlo et al., 2010). Compared with conventional unsorted semen, sex-sorted semen is processed to contain a lower insemination dose (≤ 2 x 10^6) and may have

reduced viability due to potential damage during the sorting process (Frijters et al., 2009). Therefore, insemination with X-sorted semen closer to the time of ovulation might compensate for reduced viability, and improve conception rates. A second objective of this study was to further evaluate the effect of time of insemination after onset of estrus on conception rate when using X-sorted semen in beef cows.

Materials and methods

The University of Arkansas Animal Care and Use Committee approved all animal procedures utilized in this study (protocol # 12010). The study utilized Angus-based, multiparous (n = 264) and primiparous (n = 74) lactating beef cows located at the University of Arkansas Beef Cattle Research Unit near Fayetteville, Arkansas, that were bred during the fall of 2011 and 2012. All cows were maintained on pasture and supplemented (ad libitum) with mixed grass hay. Prior to synchronization, transrectal ultrasonography (Ibex Pro, E.I. Medical Imaging, Loveland, Co) was performed using a L6.2 (8-5 MHz linear array) transducer to determine cyclic status of all cows. Cows with a corpus luteum and/or at least one follicle >10 mm in diameter were classified as cyclic. Cows were stratified across treatment groups based on cyclic status, body condition, days postpartum, parity and weight (Table 1). Treatment 1 (Control) cows received a CIDR progesterone insert (Eazi-Breed CIDR; 1.38 g progesterone, Zoetis, Florham Park, NJ) on d 0. The CIDR was removed on d 14, followed by treatment with GnRH (100 µg i.m., Factrel, Zoetis) on d 16, and PGF (25 mg i.m., Lutalyse, Zoetis) on d 23. Treatment 2 (**D7PGF**) cows received the same synchronization treatment, except an additional dose of PGF was given on d 7 of the CIDR treatment.

An estrous detection patch (Estrotect; Rockway Inc., Spring Valley, WI) was placed on all cows at the time of PGF treatment on d 23. Cows were visually observed for estrus continuously from 0800 until 2000 h, then at least every 4 h overnight, over the 84-h period following PGF. Cows exhibiting estrus were inseminated with X-sorted semen between 9 and 24 h after detected estrus. Conception rates for cows inseminated either 9 to 15, or 16 to 24 h after detected estrus were compared retrospectively. A single, experienced technician performed all inseminations. Ten days after the estrus detection period, all cows were exposed to fertile bulls for 45 d. Transrectal ultrasonography was used to determine pregnancy status of cows at approximately 45 d of gestation, and again 45 to 55 d after bull removal for overall pregnancy rate. Differences in fetal crown-rump length were used to determine if pregnancies resulted from artificial insemination or subsequent matings.

Data were analyzed using SAS statistical software (8.3, SAS Inst., Inc., Cary, NC) with animal as the experimental unit. Estrous response was defined as the percentage of all treated cows that were detected in estrus within the 84-h period following PGF dosing. The AI conception rate was defined as the number of cows that were determined to be pregnant to AI service divided by the number of cows exhibiting estrus and inseminated during the 84-h period following PGF dosing. Overall pregnancy rate was defined as the percentage of all cows that were pregnant at the end of the breeding season. Estrous response, AI conception rate, and overall pregnancy rate were evaluated using the Chi-square analysis (Proc Logistic). The conception rates for cows inseminated 9 to 15 h versus 16 to 24 h after detected estrus were compared retrospectively to determine any effect of insemination timing on conception rate. Effects of synchronization treatment on interval from PGF treatment to detected estrus were evaluated by general linear model (Proc GLM) of SAS. Initial models for reproductive

responses contained fixed effects of year, treatment, BCS, days postpartum, parity and their interactions. Effects not found significant were removed from the model. No significant year or treatment x year interactions was detected ($P \geq 0.35$), so data for both years were combined for analysis. The reduced model evaluated the effects of synchronization treatment on estrous response, interval from PGF to estrus, interval from onset of estrus to AI on AI conception rate, and overall pregnancy rate. Also evaluated, were the effects of cyclic status within synchronization treatments on these parameters.

Results and discussion

An estrous synchronization protocol consisting of a 14 d CIDR treatment, followed by GnRH on d 16 and PGF on d 23 has resulted in good estrous response (> 80%) and AI conception rates (> 75%) synchronization in beef cows (Powell et al., 2011). The protocol was based on the assumption that the long-term CIDR (progesterone) treatment would result in development of a large persistent follicle capable of ovulating in response to GnRH when given within 2 d of CIDR removal. However, if a cow has a functional corpus luteum during the CIDR treatment period, the additional progesterone from the corpus luteum could prevent a persistent follicle from developing and the GnRH treatment will be ineffective. This potential problem might be avoided if PGF treatment were given on d 7 of CIDR treatment to regress any corpus luteum present and insure a persistent follicle develops that can respond to GnRH. Thus, this study was conducted to determine if such a PGF treatment would improve the estrous response to the synchronization protocol.

Percentage of cows exhibiting estrus did not differ ($P = 0.33$) at 76.5 and 71.2% for the control and D7PGF treatments, respectively (Table 2). The estrus response was good,

considering that at the start of estrous synchronization about 30% of the cows were acyclic (Table 1). It is well established that exogenous progestogens can be used to induce cyclicity in postpartum, anestrous cows (Yavas and Walton, 2000). Over 50% of the anestrous cows in each treatment exhibited estrus (Table 3). If cows are not cyclic, they would not have had a functional corpus luteum on d 7 of CIDR treatment, so could not respond to PGF. This might explain at least in part, why no treatment differences were detected in estrus response to synchronization. The mean interval from PGF treatment on d 23 until detected estrus was 3 h longer ($P = 0.03$) for cows in the D7PGF than the control treatment. This delay in onset of estrus was due to an effect on cows identified as cyclic at the start of synchronization, rather than acyclic cows (Table 3). The delay in onset of estrus resulted in a more synchronous estrus in the D7PGF treatment group. Within 48 h of PGF treatment, 25% of the control cows were observed in estrus compared to 6% in the D7PGF treatment. During a 24-h period (from 48 to 72 h after PGF) 89% of the cows detected in estrus in the D7PGF group had expressed estrus compared with 69% of the cows in the control group. Select Synch (GnRH followed by PGF 7 d later) is known to reduce variability in the time of estrus in cows and heifers (Pursley et al., 1995). In the current study, the D7PGF treatment may have increased the number of cows with persistent dominant follicles capable of responding to GnRH and resulted in more synchronous estrus.

Conception rates after AI with X-sorted semen were similar ($P = 0.64$) at 63.3 and 66.7% for the control and D7PGF treatments, respectively (Table 2). The AI conception rate tended ($P = 0.08$) to be greater for cows classified as acyclic (at the start of synchronization) in the D7PGF group as compared with acyclic cows in the control group (70 versus 42%, respectively; Table 3). Synchronization treatment had no effect ($P = 0.74$) on overall pregnancy rate (Table 2), or on the pregnancy rate of cyclic cows ($P = 0.37$; Table 3). However, cows that were acyclic at the

start of synchronization in the D7PGF treatment tended (P = 0.09) to have a greater overall pregnancy rate than similar cows in the control group (85 versus 69%, respectively; Table 3). The majority of the cows that were acyclic at the start of the study were those with the shortest postpartum interval. In dairy cows, treatment with PGF on d 14 to 16 postpartum tended to reduce days open, and reduced mean services per conception (McClary et al., 1989). In another study, Salasel and Mokhtari (2011) reported that 2 injections of PGF given 8 h apart to dairy cows on d 20 postpartum increased first service conception rate, while reducing mean services per conception and mean days open. A plausible mechanism by which PGF treatment given early postpartum improves fertility parameters is through enhancement of uterine involution.

Rorie et al. (2002) compared conception rates in beef cows that were inseminated with conventional frozen-thawed semen, at 4-h intervals, ranging from 8 to 24 h after the onset of estrus. Time of insemination had no effect on AI conception rates, indicating there is flexibility in time of insemination in beef cows when using high quality, conventional semen. However, preliminary data (Rorie et al., 2012) suggested conception rates might be improved by delaying insemination a few h later than the usual 12 h after onset of estrus, when using sex-sorted semen. Conception rates from sex-sorted semen are often reported to be lower than that achieved with conventional, unsorted semen, due to the reduced number of sperm per insemination dose and potential damage to sperm during the sorting process (Frijters et al., 2009). Funston and Meyer (2012) directly compared single service conception rates in in beef heifers inseminated with either conventional or sex-sorted semen from the same sires. All inseminations occurred approximately 18 to 24 h after detected estrus. Conception rates resulting from insemination with conventional and sex-sorted semen were 58.4 and 41%, respectively. In Jersey dairy heifers, conception rate is highest when insemination with X sorted semen occurs from 16 to 24

h after onset of estrus (Filho et al., 2010). Inseminating earlier at 12 to 16 h, or later than 24 h after onset of estrus, both reduced conception rates when compared to the 16 to 24 h time frame.

In the current study, a similar (P = 0.72) number of cows (45/72; 62.5%) inseminated between 9 and 15 h after estrus conceived, as compared with cows (80/123; 65.0%) inseminated between 16 to 24 h after estrus. The overall conception rate of approximately 64% is higher than often reported in other studies (Funston and Meyer, 2012; Filho et al., 2012; Schenk et al., 2009). All cows were inseminated, using X-sorted semen from 2 sires with very good fertility, as evidenced by high conception rates when used for timed insemination (Stan Lock, Genex Coop. Inc., personnel communication). Although our data did not show an effect of time of insemination with X-sorted semen on conception rate, there is no evidence to suggest that delaying insemination until 16 to 24 h after onset of estrus would be detrimental to fertility.

Implications

An estrous synchronization protocol consisting of CIDR for 14 d, GnRH on d 16 and PG on d 23 was effective in synchronizing over 70% of lactating beef cows within an 84-h period. Addition of PGF on d 7 of the synchronization protocol did not increase estrous response or conception rate, but did result in tighter synchrony of estrus. Results demonstrate that acceptable conception rates can be achieved in lactating beef cows when using high quality, X-sorted semen over a range of insemination times.

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Tables

Table 1. Distribution of beef cows across synchronization treatments

	Synchronization treatment		
Parameter	**Control**	**D7PGF**	***P* value**
Weight (kg)	527.3 ± 6.6	524.2 ± 6.6	0.74
Body condition (BCS)	5.23 ± 0.1	5.22 ± 0.1	0.97
Post partum interval (d)	57.6 ± 1.4	58.8 ± 1.4	0.53
Cows cyclic at synchronization	93/132 (70.5%)	92/132 (70.1%)	0.89

Table 2. Effect of synchronization treatment on estrus response and pregnancy rates

Parameter	Control[1]	D7PGF[2]	*P* value
Expressed estrus	101/132 (76.5%)	94/132 (71.2%)	0.33
Interval, PGF to estrus (h)	54.3 ± 1.0	57.4 ± 1.0	0.03
AI conception rate[3]	57/90 (63.3%)	58/87 (66.7%)	0.64
Overall pregnancy rate	110/132 (83.3%)	112/132 (84.9%)	0.74

[1]Control - Synchronization protocol consisting of 14 d CIDR, GnRH on d 16, PGF on d 23.

[2]D7PGF - Same as Control treatment, except a dose of PGF was given on d 7 of CIDR treatment.

[3]AI conception rate - Excludes 7 cows in the control group and 3 cows in the D7PGF group that were inseminated with conventional, unsorted semen.

Table 3. Effect of cyclic status on estrus response and pregnancy rates by treatment

Parameter	Control[1]	D7PGF[2]	*P* value
Expressed estrus			
Cyclic cows	80/93 (86.0%)	73/92 (79.4%)	0.23
Acyclic cows	21/39 (53.9%)	21/40 (52.5%)	0.90
Interval, PGF to estrus (h)			
Cyclic cows	53.6 ± 1.1	57.1 ± 1.1	0.03
Acyclic cows	57.0 ± 2.5	58.5 ± 2.5	0.65
AI conception rate[3]			
Cyclic cows	49/71 (69.0%)	44/67 (65.7%)	0.68
Acyclic cows	8/19 (42.1%)	14/20 (70.0%)	0.08
Overall pregnancy rate			
Cyclic cows	83/93 (89.3%)	78/92 (84.8%)	0.37
Acyclic cows	27/39 (69.2%)	34/40 (85.0%)	0.09

[1]Control – 14 d CIDR, GnRH on d 16, PGF on d 23

[2]D7PGF – same as Control treatment, except an additional dose of PGF was given on d 7 of CIDR treatment.

[3]AI conception rate - Excludes 7 cows in the control group and 3 cows in the D7PGF group that were inseminated with conventional, unsorted semen.

Chapter 4: Prostaglandin F2alpha treatment 24 hours before CIDR progesterone insert removal improves synchrony of estrus in lactating beef cows

Abstract

An estrus synchronization protocol, where CIDR removal is delayed until 24 h after prostaglandin F2α (PGF) administration might prevent early expression of estrus and improve synchrony. Therefore, a study compared estrus response and conception rates of Angus and Angus x Hereford cows (n = 61) that received PGF on d 6, with CIDR removal occurring concurrent with PGF or one day later on d 7. Cows were stratified across treatments based on BW, BCS, cyclicity, and postpartum interval. After PGF administration, all cows received estrous detection patches and were observed for estrus for 4 d. Estrous response was similar (P = 0.61) at 76.7% and 71% for the 6 and 7 d CIDR treatments, respectively. The mean interval from PGF administration to onset of estrus was greater (69.1 vs. 52.3 h; P < 0.01) for the 7 d CIDR cows than the 6 d CIDR cows. All cows detected in estrus in the 7 d CIDR group expressed estrus within a 10-h period (68 to 77 h post PGF), whereas cows detected in estrus in the 6 d CIDR group expressed estrus over a 26-h period (44 to 70 h post PGF). Conception rate after AI was similar (P = 0.46) at 65 and 54.6% for the 6 and 7 d CIDR treatment cows, respectively. After the breeding season, the overall pregnancy rate was 93.3% for 6 d CIDR cows and 95.1% for the 7 d CIDR cows (P = 0.53). Overall, the results indicate that delaying CIDR removal until 24 h after PGF administration delayed expression of estrus, which in turn resulted in better estrous synchrony. Although delaying CIDR removal until 24 h after PGF requires additional labor, the improvement in synchrony could improve the success of timed inseminations.

Key words: artificial insemination, bovine, CIDR, estrous synchronization

Introduction

Reproductive management has been identified as the single most important factor contributing the economic success of cow-calf producers (Dziuk and Bellows, 1983). Among reproductive biotechnologies, estrous synchronization and artificial insemination (AI) have been referred to as the most important and applicable to producers (Seidel, 1995). A variety of estrous synchronization products and protocols have been available for well over 30 years (Lamb et al., 2010). However, beef producers have been slow to adopt these technologies. Currently, only about 7% of beef cows in the United States are artificially inseminated.

A commonly used estrous synchronization protocol is the use of a controlled internal drug release (CIDR) progesterone insert for 6 or 7 d, with PGF administered at CIDR removal. The mean interval from prostaglandin F2 alpha (PGF) administration to onset of estrus in beef cows is about 60 h (Rorie et al., 2002). However, HeatWatch data shows that individual beef cows may express estrus as early as 12 h or as late as 96 h after PGF treatment (Rorie, unpublished data). Producers would be more likely to utilize AI if synchronization protocols resulted in a more synchronous expression of estrus, allowing for an acceptable pregnancy rate from the use of timed inseminations.

Although it would require more labor, delaying CIDR removal until 24 h after PGF administration might delay estrus in some cows resulting in a more synchronous estrus compared to protocols where CIDRs are removed at time of PGF injection. Therefore the objective of this study was to compare estrous response and conception rates of lactating beef cows, where PGF is given on d 6 after CIDR insertion with the CIDR removed concurrent with or 1 d after PGF.

Materials and methods

All animal procedures used in this study were approved by the University of Arkansas Animal Care and Use Committee (IACUC protocol # 12010). Sixty-one Angus and Angus X Hereford cows from the University of Arkansas Savoy Beef Research Unit were used in this study. Cows had a mean BW of 581 ± 8.5 kg with an average BCS of 5.5 and a post-partum interval of approximately 59 d. Body condition was scored from 1 to 9 with a score of 1 being emaciated and 9 being extremely fat (Richards et al., 1986). The cows were maintained on mixed grass pastures containing entophyte-infected tall fescue, and supplemented with hay from calving until the initiation of the study.

Immediately prior to start of estrous synchronization, reproductive ultrasonography (8-5MHz 66-mm linear array transducer, Ibex Pro, E.I. Medical Imaging, Loveland, Co) was performed on all cows to determine cyclicity. Cows with a corpus luteum or a large (> 10 mm diameter) pre-ovulatory follicle on either ovary were identified as cyclic. Body weight and BCS were recorded at the time of ultrasonography. After ultrasonography, the cows were stratified cross synchronization treatments based on weight, body condition, post-partum interval and cyclic status (Table 1). All cows received a CIDR progesterone insert (EAZI-Breed CIDR; 1.38g progesterone, Zoetis, Florham Park, NJ) on d 0.

On d 6, all cows were administered PGF (25 mg, i.m., Lutalyse, Zoetis). The CIDR was removed at the time of PGF administration on d 6 in the treatment 1 (6 d CIDR) cows (n = 30) and 24 h post PGF administration in treatment 2 (d 7 CIDR) cows (n = 31). At PGF administration, all cows were relocated to dry lot pens and received ad libitum grass hay and water. An Estrotect estrous detection patch (Rockway Inc., Spring Valley, WI) was placed on

the rump of each cow, approximately mid-point between the tail head and hipbones, to aid in estrous detection.

All cows were observed for estrus behavior over a 96-h period post PGF. The cows were observed continuously from 0700 to 1830 h and at 4-h intervals overnight. Cows were inseminated by an experienced technician between 8 and 24 h after detected estrus, using conventional, frozen-thawed semen from the same Angus sire. Any cows that failed to display estrus within 96 h of CIDR removal were time inseminated and given an injection of gonadorelin (GnRH; 100 µg i.m., Factrel, Zoetis). Approximately 10 d after insemination, cows were returned to pasture and exposed to fertile bulls for a 45 d breeding season. Ultrasonography was used to determine AI conception rates approximately 45 d following the last insemination, and again 30 to 45 d following bull removal to determine overall pregnancy rate. Fetal crown-rump length was measured to confirm conception date.

Estrous response was based on the percentage of cows in each synchronization treatment that were detected in estrus within the 84 h of CIDR removal. Artificial insemination conception rates were calculated by dividing the number of cows detected in estrus by the number of cows confirmed to be pregnant by AI via ultrasonography. Overall pregnancy rates represent the total percentage of cows determined to be pregnancy at the end of the breeding season. Cows inseminated by timed AI at 96 h post PGF were not included in calculation of AI conception rates, but were included in the calculation of overall pregnancy rates.

Statistical analysis was performed using JMP Pro 10.0 software (SAS Inst. Inc., Cary, NC). Analysis of variance was used to determine differences between the intervals from CIDR removal to onset of estrus between synchronization treatments. The model included cow as the experimental unit with synchronization treatment, BCS, and cyclic status as fixed effects with

PPI and BW as random effects. Interactions that were not found to be significant were removed from the model and the model refitted until the final model included only fixed and random effects. Chi-square analysis was used to determine the relationship between estrous synchronization treatment and estrous response, AI conception rate and overall pregnancy rates.

Results and discussion

Individuals providing breeding services to cattle producers typically use timed insemination following estrous synchronization. Conception rates achieved from various estrous synchronization protocols utilizing fixed time AI are often reduced when compared with cows inseminated after detected estrus (Patterson et al., 2011). With timed inseminations, AI is scheduled to occur at a specific interval after PGF administration, without regard to individual cow variation in the onset of estrus. Cows expressing estrus early or late will fail to conceive. Timed insemination is not recommended when using sorted semen because timing of insemination is critical, therefore conception rate will be low (Schenk et al., 2009). The conception rate resulting from timed inseminations might be improved if the variation in expression of estrus of individual cows could be reduced.

An estrous synchronization protocol such as OvSynch with CIDR, where GnRH is given at CIDR insertion, PGF at CIDR removal and GnRH again at insemination, improves the conception rate achieved with timed inseminations (Kawate et al., 2004; Stevenson et al., 2006), but may be too expensive for producers to consider using. The current study was conducted to determine if delaying CIDR removal in a simple CIDR-PGF synchronization protocol might improve the synchrony of estrus, and be an alternate to more expensive synchronization protocols for potential use with timed inseminations. Administration of PGF 24 h before CIDR

removal should allow the corpus luteum more time to regress before progesterone supplementation is withdrawn, possibly resulting in a more synchronized expression of estrus.

In the current study, estrus response to synchronization was similar (P = 0.61) at 76.7 and 71% for 6 and 7 d CIDR treatments, respectively (Table 2). However, the mean interval from PGF administration to onset of estrus was greater (69.1 vs. 52.3 h; P = 0.001) for the d 7 CIDR cows than the d 6 CIDR cows. The delay in estrus for cows in the 7 d CIDR treatment resulted in a more synchronous expression of estrus (Figure 1). All cows detected in estrus in the d 7 CIDR group expressed estrus within a 10-h period (68 to 77 h post PGF), whereas cows detected in estrus in the d 6 CIDR group expressed estrus over a 26-h period (44 to 70 h post PGF). The synchrony of estrus in the 7 d CIDR treatment would likely work well with inseminations timed to occur at 80 hours post PGF. Such timing in the current study would have resulted in insemination of cows between 3 and 12 h after onset of estrus. Although insemination at 3 h after onset of estrus might seem too early, studies have shown that insemination once daily (resulting in a range of insemination times from at or near the onset of estrus, up to 24 h after onset) results in acceptable conception rates. Studies in dairy cattle have shown that twice-daily estrus detection, but once-daily insemination, results in conception rates similar to that of inseminations based on the a.m.-p.m. rule (Nebel et al., 1994; Graves et al., 1997). In beef cows, Rorie et al. (2002) showed that good pregnancy rates could be achieved when cows are inseminated over a broad range of insemination times, ranging from 7 to 25 h after onset of estrus.

Conception rates after AI were similar (P = 0.46) at 65 and 54.6% for the 6 and 7 d CIDR treatment cows, respectively. Of the 16 cows receiving “clean up" AI at 96 h post PGF, 4 (57.1%) in the 6 d CIDR group and 4 (44.4%) in the 7 d CIDR group, respectively, conceived to

the timed AI. A study reported by Lucy et al. (2001) compared estrous response, first service conception rates and overall pregnancy rates in beef cows receiving one of three synchronization treatments: Control (CON) - no treatment, single injection of PGF (PGF), or 7 d CIDR with PGF administration occurring on d 6 (CIDR+PGF). Across locations, the CIDR+PGF treatment resulted in improved synchronization of anestrous (45% versus 19 and 11%) and cyclic (72% versus 49 and 19%) as compared with PGF and CON cows, respectively. Although no differences were observed in first-insemination conception rates (average of 63% across all locations), data indicated a higher percentage of cows became pregnant within the first 3 d of the breeding period in the CIDR+PGF treatment group (36%) as compared with the PGF (22%) and CON group (7%), regardless of cyclic status prior to synchronization (Lucy et al., 2001).

For cows in the 7 d CIDR group in the current study, the overall pregnancy rate for all inseminations, (including the TAI at 96 h) was 51.6% (16/31). These results are in agreement with a large multistate study (Larson et al., 2006), where one of the synchronization treatments consisted of a 7 d CIDR treatment, where PGF was administered at CIDR removal, then cows were observed for estrus and inseminated, followed by TAI and GnRH to non-responders at 84 h. In that study, the overall AI pregnancy rate for 506 cows was 53%. Larson et al. (2006) reported an overall (seasonal) pregnancy rate of 92.2%, which was similar to the overall pregnancy rates of 93.3 and 95.1% for the 6 d and 7 d CIDR treatment cows, respectively.

Although beef cattle producers have been slow to adapt to estrous synchronization and AI, improvements to protocols which reduce time and labor associated with estrus detection may make estrous synchronization and AI more attractive options for producers (Larson et al., 2006). Development of fixed-time AI protocols reduces labor associated with AI because they eliminate the need for estrus detection (Larson et al., 2006). In the current study, estrous synchronization

using a 7 d CIDR in which PGF administration occurred 24 h before CIDR removal reduced the variation in expression of estrus to a 10-h period in lactating beef cows. Additional studies are needed to determine the suitability of this estrous synchronization protocol for use with timed inseminations.

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Tables

Table 1. Distribution of beef cows across synchronization treatments.

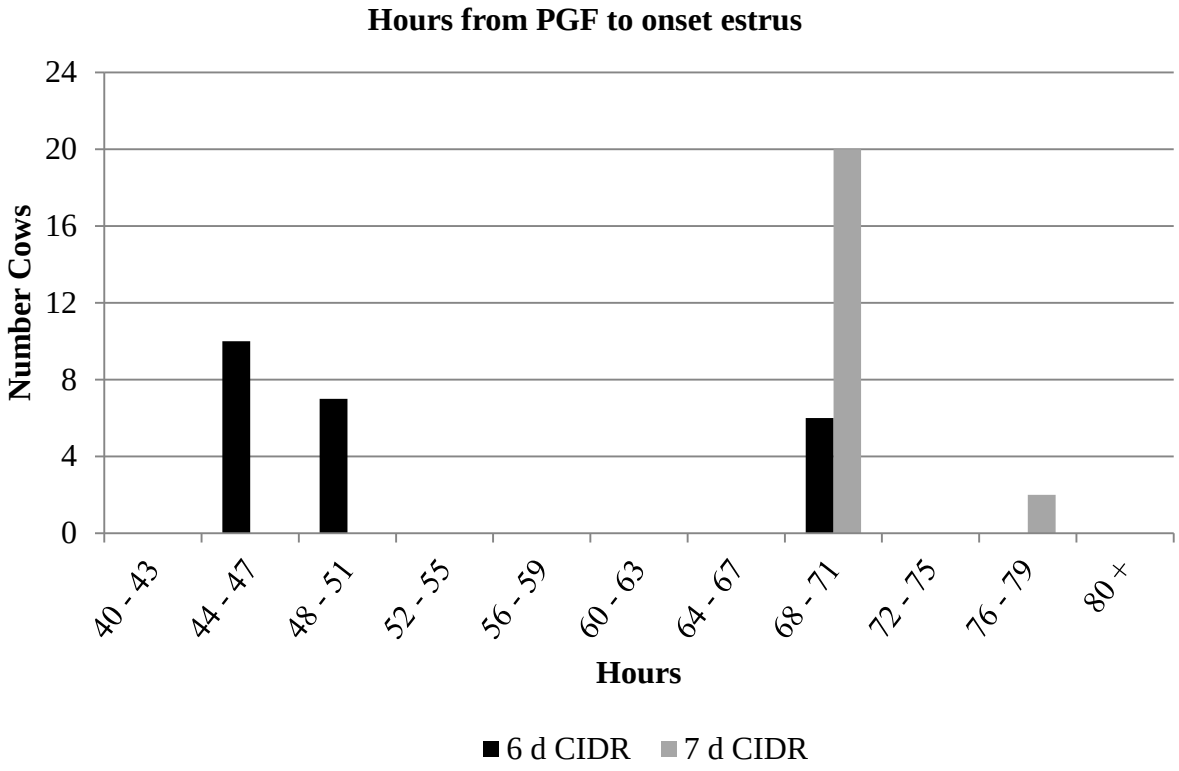
Parameter	6D CIDR	7D CIDR	*P* value
Weight (kg)	584.5 ± 12.6	577.3 ± 11.5	0.67
BCS	5.6 ± 0.2	5.4 ± 0.2	0.64
PPI (d)	58.4 ± 3.3	60.0 ± 3.2	0.73
Cyclic %	22/30 (73.3%)	23/31 (74.2%)	0.94

Table 2. Effect of synchronization treatment on estrus response and pregnancy rates

Parameter	6D CIDR	7D CIDR	*P* value
Estrus response	23/30 (76.7%)	22/31 (71.0%)	0.61
PGF to estrus (h) ± SE	52.3 ± 1.6	69.1 ± 1.6	< 0.01
AI conception rate	15/23 (65.0%)	12/22 (54.6%)	0.46
Overall pregnancy rate	28/30 (93.3%)	30/31 (95.1%)	0.53

Figures

Figure1: Occurrence of estrus after PGF administration in lactating beef cows.



Conclusion

Many factors contribute to the economic success of cow-calf operations but none more important than reproductive efficiency. However, basic reproductive management practices are underutilized by the majority of beef producers because these technologies are often viewed as time and labor intensive or difficult to use (USDA NAHMS, 2011). Thus three studies were conducted to improve and incorporate reproductive management practices into beef cow-calf production.

Estrotect estrous-detection patches proved to be a valuable tool for providing producers information regarding the cyclic status of breeding age heifers and determining AI and seasonal pregnancy rates in beef heifers and cows. However, accuracy of estrous-detection patches at predicting cyclic and pregnancy status is dependent upon the cyclic status of the herd because patches cannot differentiate between pregnant versus noncyclic animals. Accuracy is also dependent upon retention of patches over a 4 wk period. Although placing of patches further up on the rump of heifers may have improved patch retention, it may have resulted in a reduced number of activated patches.

Good estrus response and AI pregnancy rates have been reported using a modified progesterone-Select Synch protocol using a 14 d CIDR with GnRH on d 16 and PGF on d 23 (Powell et al., 2011). Data presented herein demonstrated that this protocol could be simplified and associated labor cost reduced, by administering GnRH at CIDR removal without compromising protocol effectiveness. It was also proposed that addition of PGF on d 7 of the protocol developed by Powell et al. (2011) may improve estrus response through regression of corpus luteum present, thus ensuring development of a persistent follicle capable of responding

to GnRH. Although addition of PGF on d 7 did not improve estrous response or AI conception rate, it did result in tighter synchrony of estrus.

Artificial insemination is the primary means for rapidly improving the genetic merit of a herd (Foote, 2002). Nevertheless successful use of AI is dependent upon proper timing of insemination, particularly when utilizing sex-sorted semen due to the reduced number of sperm per insemination dose and damages incurred during the sorting process (Frijters et al., 2009). However, data presented herein suggests that acceptable AI conception rates can be achieved in lactating beef cows when using high quality, X-sorted semen over a range of insemination times between 9 to 24 h after onset of estrus.

Fixed-time AI protocols (FTAI) have become an attractive option for producers because these protocols reduce labor associated with animal handling and the need for estrus detection (Lamb et al., 2010); however, FTAI often results in lower pregnancy rates compared to insemination based on detected estrus (Beef Reproduction Task Force, 2006). Thus development of synchronization protocols resulting in more uniform expression of estrus, while achieving acceptable pregnancy rates, should promote the use of FTAI among beef producers. Although it resulted in additional labor and animal handling, delaying CIDR removal until 24 h after PGF administration reduced the variation in expression of estrus to a 10-h period in lactating beef cows. However, further research is needed to determine the suitability of this estrous synchronization protocol for use with timed inseminations. Overall, results presented herein indicate that basic reproductive management can be incorporated into beef cow-calf operations at minimal cost. Improvement in synchronization protocol's effectiveness and successful use of sex-sorted semen over a range of insemination times may encourage beef producers to incorporate artificial insemination into their operations.

Literature cited

Beef Reproduction Task Force: Protocols for synchronization for estrus and ovulation. 2006. http://www.ansci.wisc.edu/jjp1/ansci_repro/lab/cow_project/syncprotocols.pdf.

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

Frijters, A. C. J., E. Mullaart, R. M. G. Roelofs, R. P. van Hoorne, J. F. Moreno, O. Moreno, and J. S. Merton. 2009. What affects fertility of sexed bull semen more, low sperm dosage or the sorting process? Theriogenology 71:64-67.

Lamb, G. C., C. R. Dahlen, J. E. Larson, G. Marquezini, and J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: A review. J. Anim. Sci. 88:E181-E192.

Powell, J. G., T. D. Lester, M. P. Rowe, C. L. Williams, and R. W. Rorie. 2011. Evaluation of a modified progestin-select synch estrous synchronization protocol in beef cows and heifers. Prof. Anim. Sci. 27:535-539.

USDA NAHMS: Small-scale U.S. Cow-Calf Operations. 2011. http://www.aphis.usda.gov/animal_health/nahms/smallscale/downloads/Small_scale_beef.pdf

Appendices

Appendix A: University of Arkansas Animal Use Protocol (IACUC 12010)

Animal Use Protocol
University of Arkansas, Fayetteville
Coversheet

IACUC use only:
Protocol number: 12010
Date Received: _____
Approval Date: _____
Start Date: _____
End Date: _____

Category(s) of animal use:
☒ Agricultural
☐ Biomedical
☐ Field
LATA Training Verified ☐ Yes ☐ No

Instructions:

- *This is a MicroSoft Word (MSWord) "form". Use MSWord to fill in the information asked for in either the blanks ("_____"), or the box ("☐") provided. You can put as much information in the blanks or boxes as you need to. (Note -- It may cause minor complications to use the "Tab" key to move from box to box since the boxes are a cell in a table (consisting of one cell). Therefore, it should cause less problems to avoid using the tab key. However, if you need to use the Tab key in the cell, you will need to use the Ctrl-Tab combination.)*
- *Submit an electronic copy of your completed protocol to crodlun@uark.edu and be sure to sign (with a scanned signature) the appropriate form(s). If you cannot send a signed electronic copy, then also send a signed paper copy of the completed protocol to Carol Rodlun; CLAF, A-42 ANSC.*
- *Failure to follow these instructions and adequately fill out the required information may result in the protocol being returned.*
- *The deadline for getting this form to Carol Rodlun, is 12:00 Noon on Monday of the week of the IACUC meeting when it will be acted upon.*

Project Title: Evaluation of estrous synchronization protocols in beef cattle

Project length (3 years maximum): 3 years

Start date: Nov. 14, 2011 End date: Dec. 30, 2014

	Principal Investigator:	Co-Investigator(s) (if applicable):	
Name:	Rick Rorie	Jeremy Powell	_____
Department/Division:	ANSC	ANSC	_____
Campus Mail Address:	AFLS B103	AFLS B110	_____
Telephone:	(479) 575-6398	(479) 575-5136	_____
Fax:	_____	_____	_____
E-mail:	rrorie@uark.edu	jerpow@uark.edu	_____

Individual(s) responsible for animal care:

Name:	Jeremy Powell	_____	_____
Office address:	AFLS B110	_____	_____
Office City, State, Zip:	Fayetteville, AR 72701	_____	_____
Office phone:	575-5136	_____	_____
Home address:	13650 Lakepoint Dr	_____	_____
Home City, State, Zip:	Lowell, AR 72745	_____	_____
Home phone:	927-2227	_____	_____

Individual(s) responsible for euthanasia:

Name:	_____	_____	_____
Office address:	_____	_____	_____
Office City, State, Zip:	_____	_____	_____
Office phone:	_____	_____	_____
Home address:	_____	_____	_____
Home City, State, Zip:	_____	_____	_____
Home phone:	_____	_____	_____

Animal Use Protocol
University of Arkansas, Fayetteville
Coversheet

Animals used

Species: Bos taurus
Common name: Cattle
Calculated number to be used (by species; not a combined number): 275
-- Note: This number (or these numbers) must agree with those listed in Section 2B (Experimental Design) of the Narrative.
Supplier (all purchases must be from a licensed supplier)
Name: N/A
Address: _____

Locations (building and room)

Animal housing: Savoy Beef Farm - Beef Cow Facility
Surgical facility: N/A
Data collection: At the Savoy Beef farm

Animal Use Protocol
University of Arkansas, Fayetteville
Narrative

Title of Project: Evaluation of estrous synchronization protocols in beef cattle

Principal Investigator: Rick Rorie

Type of Project:

☒ Research ☐ Teaching → Course Number(s) _____

Category of research and teaching for which this protocol was written:

☐ Biomedical ☒ Agricultural ☐ Field

Funding Source (check all that apply):

☐ NIH ☐ NSF ☐ USDA ☐ private industry ☒ U of A ☐ State of Arkansas

☐ other (identify): _____

Level of pain or stress: Check only one level, which should be the most severe level the animals will be subjected to during the course of the study

	Level	Examples and Comments
☒ Level 1	Level 1 Experiments on vertebrate animals that are expected to produce little or no discomfort.	Simple procedures such as injections and blood sampling; observational field behavior; physical examinations; experiments on anesthetized animals that do not regain consciousness; food/water deprivation for short periods and methods of euthanasia that induce rapid unconsciousness.
☐ Level 2	Level 2 Experiments that involve some minor stress or short-duration pain to vertebrate animals.	With anesthesia, "cut downs" or implantation of catheters; behavioral experiments on conscious animals that involve restraint; immunization employing Freund's adjuvant; noxious stimuli from which escape is possible; surgical procedures under anesthesia that may result in postsurgical discomfort.
☐ Level 3	Level 3 Experiments that involve significant unavoidable stress or pain to vertebrate animals.	Deliberate induction of behavioral stress; major surgical procedures under anesthesia that result in significant postoperative discomfort or an anatomic or physiologic deficit that will result in pain or distress; noxious stimuli from which escape is impossible; prolonged periods of physical restraint; procedures that produce pain in which anesthetics are not used (toxicity testing, radiation sickness, certain injections, and stress and shock research, experimental infection producing systemic disease or death). Level 3 mandates responsibility on the part of the investigator to explore alternative designs.
☐ Level 4	Level 4 Procedures that involve inflicting severe pain on unanesthetized, conscious animals.	Use of muscle relaxants or paralytic drugs without the use of anesthetics; surgery, severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior or severe stress or terminal stress. Many of these procedures are specifically prohibited and therefore may result in withdrawal of federal funds and/or institutional USDA registration.

Note: The preceding levels correspond to the following animal use categories on the APHIS annual report form: Level 1 = Category C or D; Level 2 = Category D; Levels 3 and 4 = Category E.

Animal Use Protocol
University of Arkansas, Fayetteville
Narrative

Surgical Procedures:
If any of the methods/techniques listed below will be used, check the appropriate space and *provide the requested details in Section 2D of the Narrative (Surgical Procedures)*:

- ☒ none
- ☐ non-survival surgery (euthanasia will be administered before recovery from anesthesia)
- ☐ survival surgery (animal will be allowed to recover from anesthesia)
- ☐ multiple survival surgeries *(requires explicit justification in Narrative)*

Non-Surgical Procedures:
If any of the methods/techniques listed below will be used, check the appropriate space and *provide the requested details in Section 2C of the Narrative (Non-Surgical Procedures)*:

- ☒ Non-surgical invasive procedures (blood collection, catheterization, intubation, etc.).
  Provide appropriate details (volume, site, frequency, etc.)
- ☐ Exposure of a living animal to a hazardous, toxic, and/or radioactive substance.
  Provide substance name, route of administration, dose, volume, frequency.
- ☐ Exposure of a living animal to an infectious agent.
  Provide name of agent, means of exposure, and amount and frequency of exposure. Specify in "Method of Euthanasia" section, the criterion you will use to determine if euthanasia is necessary to relieve suffering.
- ☐ Immunization protocol.
  Provide name of adjuvant(s) used; injection site; volume per site; frequency of injection; method, frequency, and volume of blood withdrawn (including anesthetic, if used). **Note: this does NOT apply to standard prophylactic vaccinations.**
- ☐ Prolonged restraint.
  Provide method, duration, frequency, procedure by which animal is adapted to restraint device.
- ☐ Food/water deprivation.
  Provide duration, frequency, extent (total/partial), methods used to assess and monitor distress. **Note: removal of food and/or water for 24 hours in preparation for surgery or some other procedure is NOT considered to be food/water deprivation.**
- ☐ Abnormal environment.
  Provide information on departure from normal conditions (temperature, humidity, light, duration, etc.).
- ☐ Aversive stimuli.
  Provide type and intensity of stimulus, duration, justification for use.
- ☐ Hybridoma protocol.
  Provide priming agent, cells injected, schedule for collection of ascites, number of abdominal taps, size of needle used. **Important: Provide justification for use of the *in vivo* mouse ascites method versus the various *in vitro* methods currently available, providing adequate documentation.**
- ☐ Use of neuromuscular blocking agents (muscle paralytics) during surgery.
  Provide a rationale for their use and explain how you will determine that adequate anesthesia is maintained.
- ☐ Use of death (without euthanasia) as an endpoint of the study.
  Provide justification why an earlier endpoint is not acceptable.

Method of Euthanasia -- Identify the method(s) of euthanasia to be used; it (they) must comply with the most recent AVMA Guidelines on Euthanasia

- ☒ none needed
- ☐ overdose of anesthetic (specify agent, dose, and route of administration)
- ☐ inhalation of carbon dioxide
- ☐ physical means under general anesthesia (identify the specific means that will be used; cervical dislocation, etc.)
- ☐ physical means without anesthesia (the use of captive bolt pistol on large farm animals, cervical dislocation on chickens, and some other physical methods [such as gunshot] are permitted, if done properly by trained personnel; otherwise physical means without anesthesia can be used only when scientifically justified and requires specific written justification). If to be used, write justification here: _____
- ☐ other (identify here and describe): _____

Disposal of remains:

- ☐ Incineration at University Farm (this is the disposal site for dead animals that are placed in the freezer at CLAF)
- ☐ Other (describe): _____

Animal Use Protocol
University of Arkansas, Fayetteville
Narrative

1. ABSTRACT (approximately 100-300 words)

Please provide, in lay language, a concise but specific statement of the scientific objective for the proposed research, the rationale behind this objective, the species of animal to be used, and an overview of the procedures to be followed. This statement should stand alone and be comprehensible to a non-scientist. This is NOT the place for a lengthy introduction.

Abstract here ↓

The objective of the proposed studies is to optimize estrous synchronization protocols for artificial insemination using X- and Y-chromosome sorted semen, as well as unsorted semen. Currently, the expected pregnancy rate after insemination with sorted semen is about 80% of that typically achieved using unsorted semen. If sorted semen is used for fixed-time insemination, the pregnancy rate is further reduced. Estrous synchronization protocols and time of insemination in relation to estrus (or in relation to synchronization treatments in the case of fixed-time insemination) need to be optimized for artificial insemination with sorted semen. The proposed studies will utilize ~ 275 beef cows and heifers in the fall calving herd at Savoy. During the first year, variations of a 14-day progestin-Select Synch protocol will be evaluated for artificial insemination after estrous detection. Another study will compare variations of a 5-day Co-Synch plus CIDR estrous synchronization protocol suitable for fixed-time artificial insemination. Estrous synchronization protocols utilized in subsequent years will depend on results from previous years.

2. METHODS

Using the headings listed below, describe the methods to be used in your project. The level of detail for procedures involving animals should be comparable to that in the Methods section of a journal article (i.e., sufficient to enable another researcher competent in your field to replicate your study). Please, do not cut & paste from your grant proposal, it usually includes information that the IACUC does not need to review. Also, do not repeat information in the different sections below any more than is absolutely necessary to communicate clearly what you plan to do. (In other words, do not repeat descriptions in B, C, and/or D.)

A. Housing

(Note: Cage size, amount of room per animal, etc. must conform to the dimensions listed in one of the following; *ILAR Guide for the Care and Use of Laboratory Animals*; *Animal Welfare Act (USDA) Regulations*; *PHS Policy on Humane Care and Use of Laboratory Animals*; or *FASS GUIDE For the Care and Use of Agricultural Animals in Agricultural Research and Teaching*. The only exceptions are those protocols that are to be done under "commercial conditions", these must be appropriately documented and approved.)

Describe how the animals will be housed, including cage or pen size (indicate dimensions),

- number per cage where applicable (indicating area of floor space allotted to each animal), and
- a concise description of routine husbandry practices

Describe here ↓

The beef cows and heifers will be maintained on pasture at the Savoy Beef Farm. All animals will have ab libitum access to water and mineral, and will be fed mixed grass hay.

B. Experimental design

Provide an overview of the experimental design, including:

- numbers of groups (include some sort of table, list, chart, etc., indicating treatment groups, etc.)
- numbers of animals in each group (Note: These numbers must agree with the number listed on the Coverpage)
- a schedule or timetable of the treatments animals will be exposed to
- duration of treatments
- the terminal fate of the animals (sent to processing, subjected to a terminal procedure under anesthesia, euthanized for tissue collection, etc.)

Describe here ↓

The cows will be randomly and equally distributed across treatments based on BCS, age and days postpartum and reproductive status. Likewise, heifers will be assigned to treatment based on BCS, weight, age and reproductive status. Reproductive status will be determined based on ultrasonography of ovarian structures at the initiation of synchronization treatment.

Synchronization Experiment 1 - Cows .(n=70) assigned to Trt. 1 will receive a CIDR progesterone insert from D 0 to 14, GnRH on D 16 and PGF on D 23. Cows (n=70) assigned to Trt. 2 will receive the same treatment as those in Trt. 1, but with the addition of a PGF injection on D 7 of the 14-d CIDR treatment. Ultrasonography will be used to determine the presence or absence of a corpus luteum and to measure the diameter of the dominant follicle at GnRH treatment on D 16. Starting at PGF treatment on D 23, all cows will be observed at least twice daily for onset of estrus. All cows will be inseminated by an experienced technician ~12 h after the onset of estrus. On D 27 (96 h

post PGF), any cows or heifers not previously detected in estrus will receive a timed insemination in conjunction with GnRH treatment. On D 37, cleanup bulls will be placed with the herd for a 50-d breeding season. Ultrasonography will be used 45 d after initial AI to determine the AI conception rate. A final ultrasonography will be performed 45 d after bulls are removed from the herd to determine the overall pregnancy rate. Treatment effectiveness will be based on expression and synchrony of estrus, AI and overall pregnancy rates. Data analysis will take into consideration, BCS, weight, age, and reproductive status in determining treatment effectiveness.

Synchronization Experiment 2 – The cows (n=70) and heifers (n=65) will be randomly and equally distributed across treatments based on BCS, age and days postpartum and reproductive status. Animals assigned to Trt. 1 will be synchronized using the 5-d CO-Synch protocol where GnRH is given in conjunction with a CIDR on D 0. On D 5 the CIDR will be removed and 2 PGF treatments will be given 12 h apart. On D 8 all cows and heifers will be given GnRH again and inseminated. In Trt. 2, cows and heifers will receive a CIDR for D 0 to 14 and GnRH on D 16. On D 21, two injections of PGF will be given at 12-hour intervals, followed by GnRH and insemination on D 24. All animals will be monitored with a HeatWatch system for onset of estrus. Timing of insemination may be adjusted, based on when the majority of heifers in each treatment group express estrus. Animals in both treatments will be exposed to bulls for a 50-d breeding season, starting 10 d after the timed insemination. Ultrasonography will be used 45 d after initial AI to determine the AI conception rate. A final ultrasonography will be performed 45 d after bulls are removed from the herd to determine the overall pregnancy rate. Treatment effectiveness will be based on AI and overall pregnancy rates. The AI pregnancy rate will be evaluated for any effect of time of insemination in relation to onset of estrus. Data analysis will take into consideration, BCS, weight, age, and reproductive status in determining treatment effectiveness.

C. Non-surgical procedures involving animals

Be particularly detailed regarding any procedures that are invasive, involve stress, or cause tissue damage. ***Be sure this section explains what is indicated on the Checklist.***

Describe here ↓

Animal Handling Procedures: Cows with calves will be gathered from pasture and placed into sorting pens. Calves will be separated from cows to insure calves are not injured while processing cows. All procedures below will be performed after restraining cows and/or heifers in a cattle catch chute to insure both animals and personnel are not injured.

An Aloka 500 ultrasound with either a 5 or 7.5 Mhz Transrectal transducer will be used to evaluate ovarian structures and to determine pregnancy status.

HeatWatch transmitters will be placed into patches designed for the transmitters, and glued to the rump of cows and heifers using a spray adhesive. The patches/transmitters will be removed after ~ 4 days of estrus detection.

All necessary injections will be given as directed by product labels, using sterile syringes and 20 gauge needles.

CIDR progesterone inserts will be inserted into and removed from the vagina of cows and heifers as directed by the product label.

The insemination procedure will be the same as standard practice in the cattle industry.

D. Surgical procedures

Note: Written records of surgery and anesthesia must be kept for each animal. Animals must be observed daily following surgery and observations must be recorded from the time surgery is completed until incisions are healed. These records must be made available for semi-annual inspection by the IACUC. ***Be sure this section explains what is indicated on the Checklist.***

1. **Surgeon(s)** (list qualifications for the procedures to be carried out)
   List here ↓

N/A

2. **Procedure** (must use aseptic techniques)

Describe here ↓

N/A

3. **Medication**

For all medications, specify:

- the agent,
- the route of administration (e.g., IM),
- the dose (mg/kg), and,
- when appropriate, the frequency of administration.

A. Pre-operative medication and preparation

Describe here ↓

N/A

B. Anesthesia and other medication during surgery

Describe here ↓

N/A

C. Post-operative medication and observation

Describe here ↓

N/A

E. Euthanasia

If animals to become seriously ill or injured (even if this is not an expected occurrence), specify the criterion you will use to determine if, and when euthanasia will be used to relieve suffering. If euthanasia is not included as part of the protocol, indicate what will happen to the animals at the end of the study. *Again, information included should conform to what is indicated on the Checklist.*

Describe here ↓

All animals will be observed daily for signs of illness. If any animal exhibits signs of illness then proper medical therapy will be applied. If an animal becomes seriously injured or ill and requires euthanasia, then pentobarbital will be administered intravenously at an overdosed rate. Otherwise, all animals will return to the production herd at the conclusion of the study.

3. QUALIFICATIONS OF INDIVIDUALS PERFORMING WORK WITH ANIMALS

Please list all individuals who will be carrying out procedures involving animals during this project. Please indicate who will be performing each procedure and their qualifications for that procedure. If individuals are to be trained in a procedure during this project, please indicate who will provide the training and supervision and their qualifications.

A. Principal Investigator (a current vita should be on file with the IACUC)

Any Additional Information here ↓

Rick Rorie

B. Students (attach resume or provide a brief description of qualifications)

Any Additional Information here ↓

None currently.

C. **Lab Technicians** (attach resume or provide a brief description of qualifications)
Any Additional Information here ↓

Toby Lester

D. **Individuals Providing Training or Supervision** (attach resume or provide a brief description of qualifications)
Any Additional Information here ↓

Rick Rorie and Jeremy Powell

All personnel listed on this protocol must complete the 2 base modules; (1) *The Humane Care and Handling of Laboratory Animals* and (2) *Policy and Procedures*, of the Laboratory Animal Training Association (LATA) online training program. Any questions regarding this training should be directed to Carol Rodlun (575-2994 or crodlun@uark.edu). Please fill out the table on the following page regarding the completion of this training.

Animal Use Protocol
University of Arkansas, Fayetteville
Narrative

LATA Training Documentation

Person: / Responsibility - PI, Tech, Student, or Other	List Names →	Jeremy Powell	Rick Rorie	Toby Lester				
		☒ PI ☐ T ☐ S ☐ O	☒ PI ☐ T ☐ S ☐ O	☐ PI ☒ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O
Required Modules*								
Base Modules								
The Humane Care and Use of Laboratory Animals	Date Completed →	1/23/08	11/28/07					
Policy and Procedures	Date Completed →	1/23/08	11/28/07					
Optional Modules:								
Species Modules								
The Humane Care and Use of the Laboratory Rat	Date Completed →							
The Humane Care and Use of the Laboratory Mouse	Date Completed →							
The Humane Care and Use of the Laboratory Hamster	Date Completed →							
The Humane Care and Use of the Laboratory Guinea Pig	Date Completed →							
The Humane Care and Use of the Laboratory Rabbit	Date Completed →							
The Humane Care and Use of the Laboratory Goat	Date Completed →							
The Humane Care and Use of Laboratory Swine	Date Completed →							
The Humane Care and Use of Laboratory Fish	Date Completed →							
Techniques Modules								
Aseptic Surgery of Rodents	Date Completed →							
Anesthesia and Analgesia of Rodents	Date Completed →							
Safety Module								
Occupational Health and Safety	Date Completed →							

Person: / Responsibility - PI, Tech, Student, or Other	List Names →							
		☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O	☐ PI ☐ T ☐ S ☐ O
Required Modules*								
Base Modules								
The Humane Care and Use of Laboratory Animals	Date Completed →							
Policy and Procedures	Date Completed →							
Optional Modules:								
Species Modules								
The Humane Care and Use of the Laboratory Rat	Date Completed →							
The Humane Care and Use of the Laboratory Mouse	Date Completed →							
The Humane Care and Use of the Laboratory Hamster	Date Completed →							
The Humane Care and Use of the Laboratory Guinea Pig	Date Completed →							
The Humane Care and Use of the Laboratory Rabbit	Date Completed →							
The Humane Care and Use of the Laboratory Goat	Date Completed →							
The Humane Care and Use of Laboratory Swine	Date Completed →							
The Humane Care and Use of Laboratory Fish	Date Completed →							
Techniques Modules								
Aseptic Surgery of Rodents	Date Completed →							
Anesthesia and Analgesia of Rodents	Date Completed →							
Safety Module								
Occupational Health and Safety	Date Completed →							

4. STATEMENT OF COMPLIANCE:

As the individual responsible for this research or teaching project,

I confirm that the information contained herein is accurate and, to the best of my knowledge, conforms with all applicable University, PHS, and USDA policies on the use of animals in research and teaching.

I confirm that I have completed the following online LATA training modules: 1) The Humane Care and Use of Laboratory Animals, 2) Policy and Procedures.

I confirm that all individuals involved with the animals used in this project will complete the above online LATA training modules and will be instructed in the humane care, handling, and use of animals, prior to participation in the project, and I will have reviewed their qualifications.

I agree not to proceed with any portion of this project or purchase animals until I receive written approval from the University of Arkansas Institutional Animal Care and Use Committee (IACUC).

I agree that no substantive change will be made in the procedures contained in this proposal without prior written notification to and approval by the IACUC.

I agree to allow inspection of my research facilities by members of the IACUC and the Animal Welfare Veterinarian and to comply promptly if informed of any violations of the University of Arkansas, Fayetteville's Policy on Animal Care and Use.

I understand that failure to comply with the University of Arkansas, Fayetteville's Policy on Animal Care and Use will jeopardize the University's Animal Welfare Assurance on file with the PHS (and with it all federal funding for the University), and may ultimately lead to revocation of my privileges to conduct animal research at the University of Arkansas.

(place a scanned signature in the box ↑)
Signature of Principal Investigator:
Date:
Assurance Statement of Compliance

Animal Use Protocol
University of Arkansas, Fayetteville
Assurance Statements for Biomedical Research and Teaching

DO NOT COMPLETE THIS SECTION IF PROTOCOL IS SPECIFIED AS
AGRICULTURAL or FIELD RESEARCH

The regulations for the Animal Welfare Act, the United States Department of Agriculture, and the Public Health Service **require** that in protocols for biomedical research and teaching involving animals the **following concerns be specifically addressed in writing by the Principal Investigator**. Items in brackets [] identify the source of the requirement [AWA = Animal Welfare Act regulations; NIH = NIH Guide for Care and Use of Laboratory Animals, 1996 edition].

A. Animals should not be used if other methods exist that would provide substantially the same information. Indicate why the use of live animals is required in this research. [AWA 2.31 (e) (2); NIH p. 8] : *Indicate here ↓*

B. Justify your choice of species by listing some of the important characteristics of the species that make it suitable for use in the proposed research. Cost alone is not sufficient rationale. [AWA 2.31 (e) (2); NIH p. 8] : *Justify here ↓*

C. The number of animals used should be the minimum number that can be expected to provide valid results. Describe how the number of animals to be used was determined. [AWA 2.31 (e) (2); NIH p. 8] : *Describe here ↓*

D. The principal investigator should not unnecessarily duplicate previous experiments, and must consider less invasive alternatives to procedures that may cause more than momentary or slight pain or distress to animals (i.e., Level 3 or higher). **Provide a statement** that a literature review has been carried out demonstrating that this research does not unnecessarily duplicate previous experiments, and that appropriate alternative research methods are not available for any proposed procedures that are Level 3 or higher. The database used must be identified (check below). [AWA 2.31 (d) (1) (i, ii, and iii); NIH p. 8] : *Provide statement here ↓*

(Be sure to list date(s) of search for each database used and the keywords that were used.)

	Database	Date(s) of Search	Key Words Used
☐	Medline	____	____
☐	Agricola	____	____
☐	Index Medicus	____	____
☐	Biol. Abstracts	____	____
☐	Animal Welfare Information Center (National Agricultural Library)	____	____
☐	Other (please specify below): ____	____	____

(place a scanned signature in the box ↑)
Signature of Principal Investigator
Date: ____

Assurance Statement for Biomedical Research