Utilization of sex-sorted semen in beef cattle

by

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Abstract

Reproduction is one of the most important traits for cattle producers. The continued and consistent production of calves for market and replacement purposes is the backbone of many cattle operations' economic success. The value of male and female calves can vary greatly depending on the purpose that animal is meant to serve. This difference in value has made the ability to control the gender of a calf crop a topic of great interest. The development of sex-sorted semen has given producers the ability to shift the ratio of male and female offspring within their operation. The relative novelty of sex-sorted semen means there are still improvements being made to the technology and room for further adoption to occur. In the first chapter, a review of the literature encompasses the development of sex-sorted semen, its uses in different industries and operations, and its differences from unsorted semen. Three studies were conducted to evaluate the use of sex-sorted semen in beef cows and heifers. The second chapter describes research evaluating the use of X- and -Y- sorted semen in beef cows and heifers using various estrous synchronization and timing of insemination. The finding of this study further supports existing evidence of the importance of detection of estrus when utilizing sex-sorted semen and also showed the potential for use of Y-sorted semen in terminal mating systems. The third chapter evaluates the use of sex-sorted semen in beef heifers following synchronization using MGA-PG and insemination following heat detection. Results indicate that inseminating at a later time following observation of estrus behavior is advantageous when utilizing sex-sorted semen. The last chapter evaluated the difference between the novel 7&7 Synch and the classic 7-Day CO-Synch+CIDR estrous synchronization protocols following fixed-time AI (FTAI) with Y-sorted semen. Results of this study showed no difference between the 7&7 Synch and the 7-Day CO-Synch+CIDR protocols in the mature cows, but that the 7-Day CO-Synch+CIDR was

more effective on two-year-old cows. Results of these studies indicate that sex-sorted semen can be utilized in beef cattle operations and acceptable pregnancy rates can be achieved. Further research is needed to improve the technology.

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List of Abbreviations

ADG-Average daily gain

AI-Artificial insemination

BCS-Body Condition Score

OPR-Overall pregnancy rates

BW-birth weight

CIDR-Controlled Internal Drug Release

CL-Corpus Luteum

cm-centimeters

cwt-hundredweight

d-Days

DNA-Deoxyribonucleic acid

DPP-Days postpartum

ET-Embryo transfer

FTAI-Fixed-timed artificial insemination

g-grams

GnRH-Gonadotropin releasing hormone

hr-hours

IVF-In Vitro fertilization

kg-kilograms

LH-Luteinizing hormone

LLNL-Lawrence Livermore National Laboratory

MGA-Melengestrol Acetate

mg-milligram

MHz-megahertz

ml-milliliter

mm-millimeters

NAAB-National Association of Animal Breeders

NNR-non return rate

P/AI-AI Pregnancy rate or Pregnant AI

P_4-Progesterone

PG-Prostaglandin $F_{2\alpha}$

PMT-Photomultiplier tube

SD-standard deviation

STAI-Split-timed artificial insemination

USDA-United States Department of Agriculture

APHIS-Animal and Plant Health Inspection Service

ERS-Economic Research Service

vs- versus

wt-weight

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Chapter 1 - Review of Literature

Introduction

Reproduction is arguably the most important trait for a beef cattle producer, and artificial insemination (AI) has been used to improve reproductive efficiency and assist in genetic progress. (Foote, 2002). It has been less than 40 years since one of the most important advancements in reproductive technologies was developed, sex-sorted semen. Sex-sorted semen is semen that has been further processed after collection to have a high percentage of sperm cells that bear either the X- or Y- gender-determining chromosome. With unsorted semen, the normal distribution of gender amongst offspring (in cattle) is 51% male and 49% female (Seidel, 2011). Sex-sorted semen gives producers the ability to determine gender with a >90% accuracy. The control of the sex ratio allows producers to take advantage of sex-specific traits, improve genetic progress and productivity, improve animal welfare (through reduced dystocia or calving difficulties), and reduce environmental impact by eliminating the resources used for the undesired gender (Rath and Johnson, 2008). Due to these advantages and others, the dairy industry has widely adopted the technology; however, there is limited use in the beef industry.

History of Artificial Insemination

Development of AI

The field of reproductive physiology has a long history of groundbreaking discoveries. These discoveries include an improved understanding of the anatomy and physiology of the male and female reproductive tract; understanding and control of the endocrine system; technological advancements such as AI, embryo transfer (ET), and in-vitro fertilization (IVF).

The history of the study of AI can be traced back to the 1600s when Leeuwenhoek and Hamm were the first to observe sperm cells in 1678 (Leeuwenhoek, 1678). In 1784, Spallanzani

reported the first successful insemination in a dog resulting in the birth of three pups (Spallanzani, 1784). Heape (1897) reported on the isolated uses of AI in rabbits, dogs, and horses in multiple countries. However, the first published literature on the practical use of AI came out of Russia in 1899 from Professor Ivanov (Ivanoff, 1922). Professor Ivanoff evaluated AI of multiple species including dogs, foxes, rabbits, poultry, cattle, and sheep, but especially in horses (Ivanow, 1907 and Ivanoff, 1922).

One of the next greatest advancements was the development of the artificial vagina in 1914 which made the collection of ejaculates simpler (Perry, 1968; Smith et al., 2018). Though using rectal massage to stimulate ejaculation was still the primary method of semen collection until the 1920s (Perry, 1968; Smith et al., 2018). Successful AI in dairy cattle was performed in Russia by 1931 and in the United States in the early 1930s (Walton, 1933; Foote, 2005). The first AI cooperative was formed in Denmark in 1936 with cooperatives in New York and New Jersey being the first formed in the United States in 1938 (Perry, 1968; Sipher, 1991; and Smith et al., 2018).

Cryopreservation

The biggest challenge that early AI faced was the storage and shipment of semen from AI cooperatives to the farm (Foote, 2002). Early methods of shipping semen used for AI involved transporting the liquid semen protected by media called “extender” (Smith et al., 2018). Philips and Lardy (1940) developed an extender consisting of a phosphate-buffered egg yolk solution that became a standard extender. In 1945, glucose was added to the egg yolk extender (Salisbury and VanDemark, 1945). Foote and Bratton (1949) reported that egg yolks could be used to protect sperm cells cooled to 5 degrees Celsius. Antibiotics were added to the extender to increase fertility (Almquist et al., 1949; Foote and Bratton, 1950).

The next major discovery came out of England by Polge et al. (1949). Polge reported the first cryopreservation of bull semen by adding glycerol to the semen extender. Following this discovery, the first offspring born from cryopreserved semen was reported in 1952 (Polge and Rowson, 1952). The egg yolk plus glycerol extender became the most commonly used media for cryopreserved semen for bulls and other species (Iritani, 1980).

Until the late 1950s both liquid and frozen semen were packaged in capped glass tubes and later in glass ampules (Smith et al., 2018). This packaging system proved to be problematic because the glass ampules would often break during the freezing or thawing process (Foote, 2002). This problem was solved by packaging semen in plastic straws. In the 1940s, Sorensen, a Danish scientist, invented the first semen straw, but straws would not become popular until Cassou (1964) modified Sorensen's original design into the French straw that is commercially used worldwide (Sorensen, 1940; Cassou, 1964; Foote, 2002). Original French straws were 0.5 mL in capacity, but 0.25 mL straws would later be developed because they required less storage space (Foote, 2002).

Cryopreserved semen storage methods have also changed. Originally frozen semen was stored using solid carbon dioxide (dry ice) at a temperature of -79 degrees Celsius (Foote, 2002). Research revealed that storage in liquid nitrogen at -196 degrees Celsius would provide a storage system with a significant advantage over dry ice because metabolic activity within sperm cells still occurred in dry ice storage (Foote, 2002). While liquid nitrogen is the gold standard in terms of semen storage Today, that was not always the case. In the beginning, the inefficiency of the insulation used in liquid nitrogen tanks required frequent refilling to maintain the appropriate temperature of -196 degrees Celsius and thus limited its use as a storage method (Foote, 2002). It would not be until J. Rockefeller Prentice, the owner of the American Breeders Service, invested

in the development of improved liquid nitrogen tanks that liquid nitrogen would become the standard storage system (Foote, 2002). While cryopreservation has been critical in the development and adoption of AI, it is important to note that frozen semen is less fertile than fresh semen (Shannon and Vishwana, 1995).

Sex-Sorted Semen

While the ability to determine sex at conception was a long-sought-after technology, it would not be until the 1980s that any major research or breakthroughs would occur. Garner and Seidel (2008) describe the history of commercializing sex-sorted semen. In 1981, Colorado State University (CSU) was approached by Mr. William Goddard of Warwick Land Co., Providence, RI, USA, regarding researching the sexing of sperm cells. While the university would refuse the funding, Drs. Rupert Amann and George Seidel of CSU agreed to host a symposium on the basics of sperm biology Thought to be relevant to sperm sexing. In the same year, Oklahoma State University (OSU) and the Lawrence Livermore National Laboratory (LLNL) demonstrated the use of flow cytometry to identify X- and Y-sperm based on the DNA content of the sperm cells. The problem with this early sorting by DNA content was that the dyes used to assist in determining the DNA content of the sperm cells killed the sperm cells. Johnson et al. (1987 and 1988) demonstrated that removal of the sperm membranes was not necessary for staining. This was done using the bisbenzimidazole fluorescent dye, Hoechst 33342, which was able to penetrate the membrane of the sperm cells without killing the cells. Morrell et al. (1988 and 1989) demonstrated the first uses of Hoechst 3342 on live sperm cells for insemination from the bull and rabbit. The next breakthrough and arguably most important one came in 1989 when the USDA Beltsville Research Group reported the first live offspring from a rabbit inseminated with sex-sorted semen (Johnson et al., 1989). Initially, the use of sex-sorted semen was not practical

due to the time it took to sort an adequate number of sperm cells for a single dose (Garner and Seidel, 2008). Cran et al. (1993) tried to overcome the problem of the slow sorting process using IVF due to the lower numbers of sperm cells required when using IVF. And while there was some success in further studies, IVF did not prove to be financially feasible (Cran et al., 1995; Garner and Seidel, 2008). The early limitations on the speed of sorting originally excluded AI as a tool for use of sex-sorted semen. In 1995, Dr. George Seidel showed that relatively low doses of sex-sorted sperm could result in conception rates near 50% when insemination occurred within 12 hours post-sort (Seidel et al., 1996). Seidel found that the fertility of sex-sorted semen used 17 hours or more after sorting was problematic (Seidel et al., 1997). These initial results were enough for further research to be funded and eventually led to the commercialization of sex-sorted semen in 2003-2005 (Garner and Seidel, 2008; Seidel and DeJarnette, 2021). Further improvements in the fertility of sex-sorted semen would be seen when ST Genetic released their SexedULTRA 4M product due to increasing the dosage of sex-sorted semen from $2.1x10^6$ sperm cells per dose to $4x10^6$ sperm cells (Castellani, 2017).

Sexing Technology

Sorting Process

Today, the standard for sorting semen includes using the process of flow-cytometry. Johnson et. al. (1999) and Garner and Seidel (2008) describe in detail the process of flowcytometry regarding sorting sperm cells by sex. This process is described below:

- After collection sperm are dyed with a fluorescent dye, Hoechst 33342. The stained sperm cells emit a bright blue fluorescence.
- A crystal vibrator then breaks the semen into individual droplets that contain one sperm cell per droplet.

- A photomultiplier tube (PMT) is used to measure the fluorescence of the sperm cells.
    - The level of fluorescence is proportionate to the DNA content of the sperm cells. Because sperm bearing an X chromosome has roughly 4% more DNA than sperm bearing a Y chromosome, the blue fluorescence will be brighter for sperm which will result in female offspring upon fertilization.
    - A computer analysis of the fluorescence of the stream is collected via two separate detectors.
        - The first detector positioned at zero degrees determines the DNA content of the sperm cells from the flattened face of the sperm head
        - The second detector positioned at 90 degrees determines the orientation of the sperm cells
- After the computer determines the DNA content of the sperm cells, the individual droplets are assigned an electrical charge
- The stream of sperm cells then passes through opposing electrical fields
    - X- and Y-bearing sperm are attracted to opposing electrical fields with:
        - X-sorted sperm being attracted to the negative electrical field, and the
        - Y-sorted sperm being attracted to the positive electrical field
- Validation of both the X and the Y samples is conducted post sort to verify that the correct gender has been sorted in each sample

During the sorting process, another stream of droplets goes unsorted into a third sample. This third sample consists of uncharged droplets that are unable to be sorted through the applied electrical fields. There are several reasons that the droplets are unable to be sorted including droplets containing no sperm cells, rare cases of two sperm cells per droplet, as well as dead

sperm cells. This third sample is discarded and comprises over half of the volume of the semen sorted. Though this third stream represents a loss of the total volume of the semen sample, the removal of dead sperm cells improves the overall quality of the semen sample.

The entire process of sorting sperm is done very rapidly via flow cytometry. The process is known as the Beltsville Sperm Sexing Technology patented by the USDA with Dr. Lawrence Johnson attributed as the inventor (see Figure 1.1).

Figure 1.1. Diagram of sperm sorting process using flow cytometry

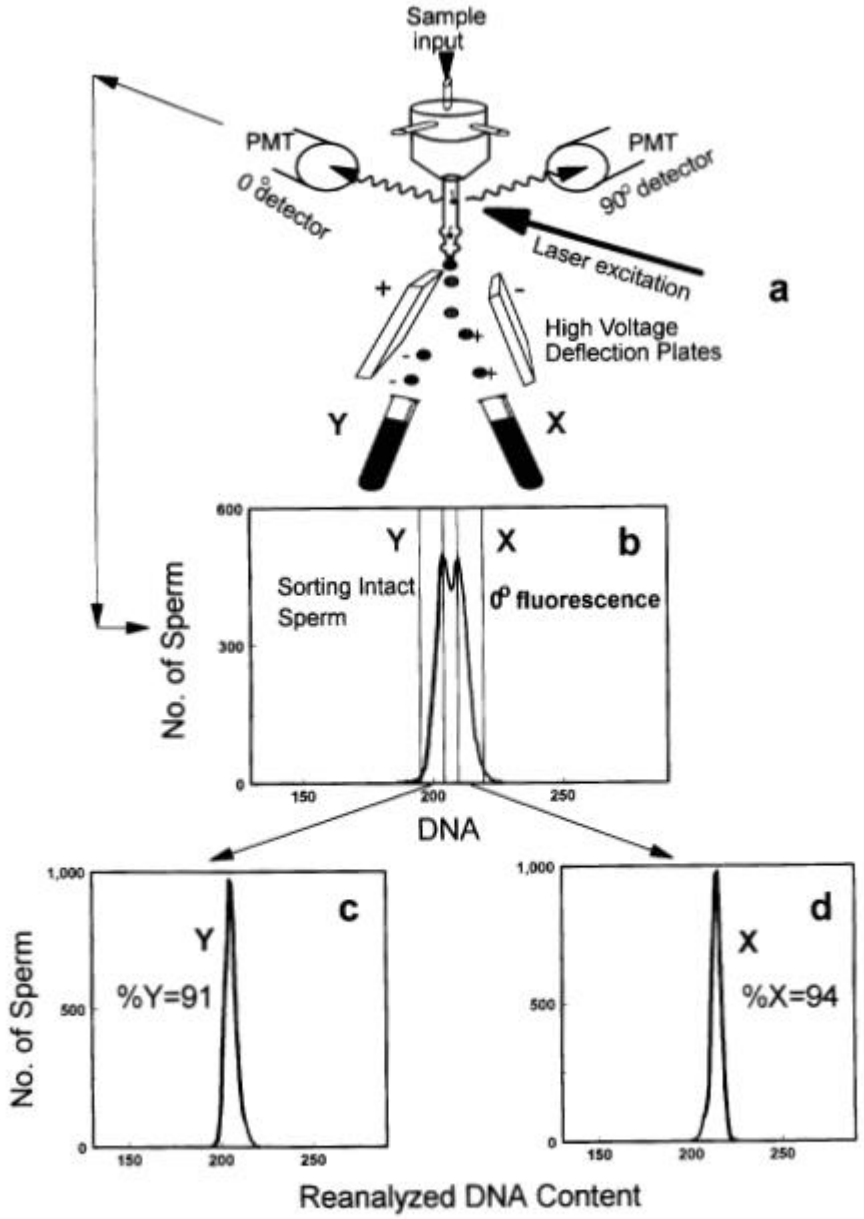

Figure 1. Sperm are flow cytometrically analyzed (a) for their DNA content by collecting fluorescence information that is proportionate to the DNA content from the flattened face of the sperm head. This information is collected in the 0° (a modification) fluorescence detector. The 90° fluorescence detector is used to determine how the sperm is oriented. Sort windows (b) around the dimmer Y chromosome-bearing sperm and the brighter X chromosome-bearing sperm are used to determine which sperm are collected. Validation of the sorted sperm is done by reanalyzing some of the sperm from both collected fractions, Y (c) and X (d).

Figure 1.1 Borrowed from Johnson et al., 1999

While flow cytometry is the most popular method of sex-sorting semen there is another method. ABS Global launched its sex-sorted product in 2017 under the name of Sexcel (ABS Australia News, 2017). While little literature exists describing the specific process ABS uses in their Sexcel product, what is known is that the sorting is done through the process of ablation (Perry et al, 2020). This process of ablation kills the undesired gender of sperm cells (Perry et al, 2020).

It is also possible to sort unsorted semen that has already been frozen through the process of reverse sorting. Reverse sorting is taking frozen semen, thawing the semen, sorting the sperm, and refreezing the now sorted sperm cells (Kasimanickam, 2015). Reverse sorting uses the same process of flow cytometry as semen sorted immediately after collection. This is a useful technique when bulls are located far from sorting machines or when the bull is deceased (Kasimanickam, 2015).

Sperm Concentrations

When comparing the dosage of unsorted semen to sex-sorted semen there are major differences in the concentrations of sperm cells per unit between the two semen types. Early research found that 50 percent non-return rates (NRR) could be achieved with as little as $0.38x10^6$ sperm cells post-thaw in cows (Jondet, 1972). Today, common concentrations for unsorted bovine semen are around 20-25 million sperm cells per unit before freezing (Thomas et al., 2017; Thomas et al., 2018; Perry et al., 2020). In comparison, sex-sorted semen is packaged at much lower concentrations. Sexing Technologies, the major supplier of sex-sorted semen, offers its SexedULTRA 4M semen product at $4x10^6$ sperm cells per 0.25 ml dose before freezing. Before the production of SexedULTRA 4M, sex-sorted semen was packaged at around

$2.1x10^6$ sperm per dose. ABS Global's Sexcel gender ablated product is packaged at $1.25x10^6$ sperm cells per dose post-thaw (Perry et al., 2020).

The first trial in which a dosage effect on NRR with sex-sorted semen was observed was conducted by Lenz et al. (2016) and German Genetics International. This study compared the 56-Day NRR of cows bred to semen sorted with the old sorting technology, XY Legacy; the new SexedULTRA technology; and unsorted processed semen. They evaluated three different dosages with the SexedULTRA (SU) technology, $2.1x10^6$ $3x10^6$, and $4x10^6$ sperm cells per straw. Alternatively, the old XY Legacy technology was packaged at the standard dosage of $2.1x10^6$ sperm cells per straw, and unsorted was packaged at $15x10^6$ sperm cells per straw. It was reported that the SexedULTRA technology produced superior NRR as compared with the XY Legacy Technology and they also found that SexedULTRA packaged at $4x10^6$ sperm cells had NRR comparable to unsorted semen (XY $2.1x10^6$=55.9%, SU $2.1x10^6$=59.9%, SU $3x10^6$=60.0%, SU $4x10^6$=66.7%, and unsorted $15x10^6$=66.5%). The results from this study led to the offering of SexedULTRA 4M which was released in June 2017 (Castellani, 2017).

Current Utilization of Sexed Semen

Currently, the most common use of sex-sorted semen is in dairy heifers to produce replacement dairy heifers (Seidel and DeJarnette, 2021). From National Association of Animal Breeders (NAAB) data Seidel and DeJarnette (2021) reported, in North America, that roughly one-third of total beef and dairy semen sales are attributed to sex-sorted semen. Despite this, sex-sorted semen only accounts for roughly 1-2% of total beef semen sales in North America (Seidel and DeJarnette, 2021). Despite the NAAB data, accurate numbers of sex-sorted semen used are difficult to determine due to incomplete data and the number of doses produced exceeding the number sold and used (Seidel and DeJarnette, 2021).

Dairy Industry use of Sex-Sorted Semen

The dairy industry has more readily adopted sex-sorted semen than the beef industry. Two key factors of the dairy industry's structure have influenced the widespread adoption of sex-sorted semen. The first and most obvious reason is the importance of female calves for dairy producers and the lack of value in male dairy calves (Vishwanath and Moreno, 2018). The second reason is less obvious, but the dairy industry's wider use of AI technologies has put them at an advantage in the adoption of the technology. As of 2007, over 70 percent of dairy operations, regardless of size, utilize AI (USDA APHIS, 2009). In comparison, only about six percent of beef cattle are inseminated using AI (Karisch, 2020). This massive difference in the use of AI is seen, even though, as of 2019, beef cow numbers were over three times higher than the number of dairy cows, 31.69 vs 9.35 million animals respectively, (Shahbandeh, 2021).

The most common use of sex-sorted semen is in dairy heifers for the production of replacement dairy heifers (Seidel and DeJarnette, 2021). In large dairies, the standard practice for sex-sorted semen has been for use largely on young, genetically superior females while older females are limited to unsorted semen (Seidel and DeJarnette, 2021). The use of sex-sorted semen in dairies is also typically recommended only for first services because the lower fertility of the semen can result in extended breeding seasons (Seidel, 2011). DeJarnette et al. (2009) reported that most inseminations with sex-sorted semen were done in first and second-service virgin heifers. Norman et al. (2010) reported that in 2008, 80.5% of heifers and 68.6% of cows inseminated with sex-sorted semen were for the first service and that 63.1% of inseminations with sex-sorted semen were during the first parity.

The use of sex-sorted semen in dairy cattle has grown. A 2009 report from the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS)

stated that sex-sorted semen was used in 11.4% of heifers and 3.5% of cows (USDA APHIS, 2009). In the years 2006, 2007, and 2008 the growth in the use of sex-sorted semen was 1.4%, 9.5%, and 17.8% in heifers and 0.1%, 0.2%, and 0.4% in cows (Norman et al., 2010). Hutchison and Bickhart (2016) reported that sex-sorted semen use from the years 2007 to 2015 increased from 9.4% to 30.7% in heifers, and from 0.2% to 1% in cows. Seidel and DeJarnette (2021) reported over 20% of dairy heifers were inseminated with sex-sorted semen while roughly 2% of dairy cows were.

De Vries et al. (2008) estimated that sex-sorted semen would change the structure of the dairy industry over the next decade. This prediction appears to have been accurate. The use of sex-sorted semen to advance genetic progress within the dairy industry has been one of the primary applications of the technology (Seidel and DeJarnette, 2021). Yet, there are downsides to the widespread use of sex-sorted semen in the dairy industry. The use of sex-sorted semen has led to the production of more replacement heifers than there is a demand for, and this has led to diminishing markets for dairy replacement heifers (Seidel and DeJarnette, 2021).

This use of sex-sorted semen and diminishing market for replacement heifers has led more dairies to utilize beef semen in their females not being selected for replacement females (McWhorter et al. 2020). McWhorter et al. (2020) state that the use of beef semen in dairy cows has doubled in the past four years. This beef-on-dairy mating strategy could help with the issue of surplus replacement heifers and eliminate the $10 dairy bull meant for slaughter (Seidel, 2011). The use of Y-chromosome-bearing sex-sorted semen seems to be a natural next step for this system, but this is limited by the increased semen costs and reduced fertility (Seidel and DeJarnette, 2021).

Comparison of Unsorted and Sex-Sorted Semen

Fertility

The importance of reproduction to beef producers makes fertility, or the ability to produce offspring, an important trait that producers will want to measure and track. Sex-sorted semen is well known for its decreased fertility compared to unsorted semen resulting in fewer pregnancies to AI, with the reasons for this reduction not completely understood (Steele et al., 2020). AI pregnancy rates with sex-sorted semen are typically around 70-80% of those observed with unsorted semen (Siedel and DeJarnette, 2021). Studies evaluating AI pregnancy rates in cows comparing unsorted semen to sex-sorted semen have reported the following results: 72% vs 52%, 60% vs 44%; 67% vs 52%; 65% vs 48%, 65% vs 50%, respectively (Andersen et al., 2021; Perry et al., 2020; Thomas et al., 2018). Similar studies conducted with heifers have reported the following results in AI pregnancy rates: 60% vs 52%; 59% vs 48% with unsorted vs sex-sorted semen, respectively (Thomas et al., 2017; Ketchum et al., 2021). Based on the results from the studies above it appears that heifers inseminated with sex-sorted semen are more likely to have AI pregnancy rates closer to those seen with unsorted semen. Alternatively, cows appear to have a greater difference in AI pregnancy rates when comparing sex-sorted semen to unsorted semen. There appears to be minimal difference in the AI pregnancy rates to sex-sorted semen when comparing traditional flow cytometry and gender ablation (Perry et al., 2020; Thomas et al., 2018).

Gender Accuracy

The technology of sex-sorted semen has been shown to shift the number of offspring toward one gender, but what is the accuracy, or likelihood of getting offspring of the correct gender, in the sorting process? The speed of sorting affects the accuracy of sex-sorted semen.

Sorting sperm more rapidly can potentially result in lower concentrations of the desired gender. Reducing the concentrations of sperm cells of the desired gender could result in a cheaper product that is more similar in cost to unsorted semen, however, the proportion of offspring that are the correct gender is going to be reduced (Seidel and DeJarnette, 2021). The initial report by Johnson et al. (1989) indicated the accuracy of the sorting process to be 81% male for the Y-chromosome-bearing sperm cells, and 94% female for the X-chromosome-bearing sperm cells. Sexing Technologies (Navasota, TX), the major US producer of sex-sorted semen, advertises >90% sperm cells for the desired gender per 0.25 ml straw with their SexedULTRA 4M product. However, does this accuracy in the sorting process results in >90% of the desired gender following AI? In other words, does the use of sex-sorted semen result in >90% offspring of the desired gender? Perry et al. (2020) reported an 81.3% gender accuracy from animals that conceived to AI with X-skewed sex-sorted semen. The Perry et al. (2020) study was not conducted using SexedULTRA 4M (Sexing Technologies; Navasota, TX) but was conducted using Sexcel ABS Global's (DeForest, WI) gender ablated product. Thomas et al. (2017) reported a 96% gender accuracy for X-skewed sex-sorted semen in heifers based on fetal sexing with transrectal ultrasound. Kasimanickam et al. (2021) reported an accuracy of 88% of the desired gender in offspring of cows bred to Y-skewed sex-sorted semen. From these observations, it could be inferred that the gender accuracy with sex-sorted semen can vary from the 90% accuracy target, but, on average, 90% accuracy is achieved. These results show that sex-sorted semen can shift the gender in a producer's herd from roughly 50/50 with unsorted semen to a high percentage of the desired gender.

Sperm abnormalities and calf normalcy following sex-sorting

With the additional processing that sex-sorted semen undergoes, the question arises about whether the sorting process compromises sperm cell quality, viability, and normalcy of calves born from sex-sorted semen following the sorting process. At one point, it was thought that the further processing of the sperm cells followed by cryopreservation interacted to affect sperm physiology. Studies have shown that the fertility of fresh sex-sorted semen is only marginally lower than the fertility of fresh unsorted semen (Seidel, 2012; Vishwanath and Moreno, 2018). This result suggests that the sorting process may not be as damaging to the sperm cells as originally thought (Seidel, 2012; Vishwanath and Moreno, 2018). Xu (2014) conducted a similar study in New Zealand where cows were inseminated with fresh sex-sorted sperm (at $1.0x10^6$ sperm cells /mL) resulting in a Non-return rate (NRR) of 93% to 97% as compared with fresh unsorted sperm (at $2.0x10^6$ sperm cells/mL). When evaluating the physiology of the sorted sperm cells some differences can be observed. It has been found that due to a more advanced membrane state, sex-sorted sperm resembled unsorted sperm that have undergone in vitro capacitation (Bucci et al., 2012). This may lead to sex-sorted sperm cells not needing to be in the female tract as long to capacitate. Earlier sperm cell functionality supports the concept of inseminating closer to the time of ovulation when using sex-sorted semen (Winters et al., 2017; Vishwanath and Moreno, 2018). Suh et al. (2005) and de Graf et al., (2006) showed that sorted sperm differed from unsorted sperm in motility, velocity, the amplitude for lateral head displacement, and the ability to penetrate the cervical mucus. Winters et al. (2017) reported that the location of binding in the oviduct was similar when comparing unsorted and sex-sorted sperm showing potential differences in the rate of capacitation.

Steele et al. (2020) investigated the morphokinetics of sex-sorted sperm cells to determine if there were observable differences following the sorting process that affected early embryonic development. This study showed that sex-sorted semen had a significant reduction in the proportions of fast and slow progressively motile sperm, and hyperactive sperm cells. Additionally, there was an increased chance for embryos to arrest at the 4-cell stage; along with an increased chance (1.71 times greater) of shrinkage or fusion of blastomeres resulting in significantly reduced blastocyst rates. There was 2.36 times greater chance of cleavage and decreased embryo survival time when sex-sorted semen was used. The percentage of apoptotic cells and the timing of reaching developmental stages with sex-sorted semen were similar to those seen with unsorted semen. Overall, this research suggests that sex-sorting sperm results in altered morphokinetics which results in a decreased chance of sperm reaching and fertilizing an oocyte and resulting in altered early embryonic development.

Norman et al. (2010) investigated the incidences of stillbirths and dystocia when using sex-sorted semen in dairy cows and heifers. When X-chromosome-bearing sex-sorted semen was used, a greater percentage of female calves were born resulting in reduced incidences of dystocia. Proportions of stillbirths were reduced for cows carrying twins when sex-sorted semen was used. The stillbirth rate was increased in heifers bearing bull calves after being inseminated with sex-sorted semen; however, only 10% of calves born were bulls (Kasimanickam, 2015). Other studies have found no differences in the rates of abortion, length of gestation, neonatal death, calving difficulty, birth weight, weaning weight, or live births when comparing unsorted semen to sex-sorted semen (Tubman et al., 2004; DeJarnette et al., 2007).

Breeding Considerations

When utilizing AI, the standard practice for beef cattle is to breed females approximately 12 hours after observation of estrus. This practice is referred to as the A.M.-P.M. rule and was first established by Trimberger (1948). When using the A.M.-P.M. rule, if a female is in estrus in the morning a producer would breed her in the evening and vice versa allowing producers to only need to check for estrus and breed twice daily. But does this rule apply to sex-sorted semen? The most common recommendation when considering using sex-sorted semen is to limit the use to estrual females only (Seidel, 2011; Thomas et al., 2018; Perry et al., 2020, Ketchum et al., 2021). The importance of positive signs of estrus has been shown with unsorted semen as well but due to the decreased fertility of sex-sorted semen, the expression of estrus seems to be even more critical (Thomas et al., 2018; Perry et al., 2020, Ketchum et al., 2021). With this in mind, a breeding strategy to consider when using sex-sorted semen is to breed all females that show positive signs of estrus with sex-sorted semen and breed the remaining females with unsorted semen (Ketchum et al., 2021).

The optimal timing of insemination using sex-sorted semen is not well established. Multiple studies have been conducted to determine the optimal timing of insemination when using AI, but many variations in the timing of insemination were observed between these studies. The exact timing of ovulation is very rarely known (Diskin, 2018). Dransfield et al. (1998) concluded that the optimal time of insemination to AI with unsorted semen is 4 to 16 hours after the onset of estrus. Seidel (2011) concluded that the optimum time of insemination is around 18 hours after the onset of estrus, or recommended breeding 6 hours later than the time recommended for unsorted semen when using fixed-time AI. Oosthuizen et al. (2021) evaluated AI pregnancy rates following pre-synchronization and delayed fixed-timed AI at either 54 or 72

hours after CIDR removal using unsorted and sex-sorted semen. This study found no difference in AI pregnancy rates whether heifers were inseminated at 54 hours or 72 hours after CIDR removal regardless of semen type (Oosthuizen et al., 2021). This study found that increased pregnancy rates with sex-sorted semen were observed after heifers were exposed to presynchronization and delayed timing of insemination. Another study conducted by Ketchum et al. (2021) evaluated the use of split-timed AI when using unsorted and sex-sorted semen. No difference in AI pregnancy rate was observed when STAI was done at 66 hours or 72 hours with either semen type. This study also found that delaying insemination by 6 hours to 72 hours resulted in an increased number of cows having displayed estrus before insemination. While no difference in AI pregnancy rate was reported at the sample size used, the increased estrous response at 72 hours may warrant further research due to the importance of estrous response when using sex-sorted semen. With these two studies in mind, utilization of either presynchronization, split-timed AI, or both may be breeding strategies to consider when using sex-sorted semen. Overall, more research needs to be done to find the optimal timing of insemination when using sex-sorted semen.

Factors Influencing the Use of Sex-Sorted Semen

Cost

Cost is one of the major factors that influence whether producers choose to use AI over natural service. This is an even more crucial factor to consider when using sex-sorted semen because the semen itself is more expensive per unit than unsorted semen.

Little literature exists outlining the specific costs of using sex-sorted semen in a beef cattle operation. A comparison of the cost of unsorted and sex-sorted semen sold by STgenetics, the current leader in sex-sorted semen, shows the cost of sex-sorted semen is $15-30 more per

unit than unsorted semen from the same sire (STgenetics sire directory, 2022). Rhinehart (2016) showed that using AI on mature beef cows using the CO-Synch estrous synchronization protocol (injection of GnRH on Day 0, followed by injection of PG on Day 7, and injection of GnRH followed by AI on Day 9) with a 50% pregnancy rate averages around $35.50 per insemination when accounting for drug/hormone costs, semen costs, and cost of a technician. The same study showed that using AI on beef heifers with the same assumed pregnancy rate of 50% and accounting for the same cost factors averaged $37.50 per insemination. Overton (2005) analyzed the cost of AI on a dairy operation and determined that the average net cost per insemination was $37.21. With these figures in mind, it can be assumed that the cost of using sex-sorted semen would be anywhere from $15-$25 more due solely to the additional cost of the semen itself, likely averaging from $45-$50 per insemination (Seidel, 2011). It is important to remember that the cost per insemination will vary greatly depending on the situation. For example, an estrous synchronization protocol requiring more injections such as the 7&7 Synch would have additional drug costs involved compared to an MGA-PG protocol.

Labor

While labor is one of the most critical factors in determining whether producers use AI or natural service, the use of sex-sorted semen alone does not add to the labor of AI. However, if specific estrous synchronization protocols, like the STAI or 7&7 Synch, are used with sex-sorted semen, labor needs increase (Andersen et al, 2021; Ketchum et al, 2021; Thomas et al, 2018). These protocols may be chosen to be used due to the increased AI pregnancy rates that have been observed when utilizing them with sex-sorted semen (Andersen et al, 2021; Ketchum et al, 2021; Thomas et al, 2018). However, these protocols often require additional days of work and

additional animal handling. This will increase the time and labor involved with AI regardless of the semen type used.

Semen Handling

Another factor to consider when using sex-sorted semen rather than unsorted semen is differences in semen handling. Traditionally, unsorted semen has been packaged in 0.5 ml straws, while in comparison, sex-sorted semen has been packaged in 0.25 ml straws. The smaller straws used for sex-sorted semen can be more difficult to handle compared to the larger 0.5 ml straws. Due to the larger surface-to-volume ratio, 0.25 ml straws are more susceptible to temperature stress when handling (Diskin, 2018; Miller et al, 2011). Handling sex-sorted semen or any semen packaged in 0.25 ml straws requires extra care to minimize temperature shock. Semen packaged in 0.25 ml straws also require different AI guns than traditional 0.5 ml straws, adding another potential expense (Seidel, 2011). It is important to note that some bull studs are beginning to package conventional unsorted semen in 0.25 ml straws as well. With this change, the necessary precautions that need to be taken when handling sex-sorted semen due to straw size will apply to unsorted semen as well.

Applications

Current literature detailing the specific applications of sex-sorted semen in beef cattle is limited. Seidel (2011) reported numerous profitable methods of utilizing sex-sorted semen depending on whether the production of females or males is the goal. For the production of females, Seidel (2011) outlines four major applications for the use of sex-sorted semen:

- Increasing the percentage of heifer calves born to either grow herd size or for the sale of replacements,

- Increase selection intensity for replacement females by selecting only the genetically superior dams,
    - With this system, only 25% of dams would be needed to produce replacement females,
    - The remaining 75% could be used in terminal matings (potentially with Y-chromosome bearing sex-sorted semen) to increase the value of feeder calves sold,
- Utilizing X- bearing sex-sorted semen on first-calf heifers to decrease the incidence of dystocia, and
- Utilizing sex-sorted semen for in vitro fertilization and embryo transfer
    - This system may be more profitable because if female (or male) offspring are more desired, any recipients receiving an embryo are >90% likely to have offspring of the desired gender.

For the production of males, Seidel (2011) outlines two major applications for the use of sex-sorted semen:

- Increasing number of male calves for meat production,
    - This has value, as steers are typically $100-$150 greater in value than their heifer counterparts,
    - The added value that steers bring is needed to compensate for the reduced fertility seen when using sex-sorted semen,
- Increasing the number of bull calves born for sale by seedstock producers
    - This application is similar to the application of choosing superior dams to create replacements.

Seedstock producers will be much more likely to utilize sex-sorted semen than commercial producers because they typically focus breeding programs on genetic advancement which leads them to utilize AI more often than commercial producers (Parish et al., 2014). Of the roughly six percent of US beef cattle bred using AI, a considerable proportion are seedstock producers (Karisch, 2020).

Two novel approaches to the use of sex-sorted semen are described by Seidel and DeJarnette (2021). The first approach is the all heifer/no cows system. This system developed by Seidel and Whittier (2015) is meant to have each female replace herself by giving birth to a heifer calf following insemination with sex-sorted semen. The basics of this system are to have heifers calve at 22-24 months of age, wean the calves at 3-4 months of age, and slaughter all the dams by 28-30 months of age. This is meant to eliminate the inefficiency of the nutritional maintenance cost of a cow throughout the year to produce a 6-8-month-old weaned calf. This can potentially avoid market discounts for cattle slaughtered over 30 months of age. This system does not account for any open females, calf loss, and the 10% of calves that are born male (though these calves still have value).

The second approach is to select for increased sexual dimorphism. This system is largely theoretical, but sexual dimorphism is the sexual and physiological differences found between males and females (such as size, growth efficiency, and behavioral differences). This application is meant to take advantage of these differences to create specialized animals. The problem with this is that any males born from a maternal specialized mating or females born from a terminal specialized mating are not optimal animals in terms of beef production (Seidel and DeJarnette, 2021).

Conclusion

Sex-sorted semen has been an amazing advancement in technology with many potential benefits to the cattle industry. Though the technology is amazing, there are challenges with the technology that limit adoption in the beef industry. Further research and improvements in sex-sorted semen are needed to increase adoption, along with further adoption of AI itself by more beef producers. Looking to the future, the increasing challenges cattle producers face with decreasing land availability and resource availability, sex-sorted semen may become a powerful tool for beef and dairy producers.

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Chapter 2 - Description of a field study using X-and Y-bearing sex-sorted semen in commercial beef cows and heifers; Summer 2019 through Fall 2020

Abstract

Sex-sorted semen holds the potential to create a high percentage of either bull or heifer calves but comes with a reduction in fertility. Our objective was to evaluate the use of sex-sorted semen (both X-and Y-sorted semen) in commercial beef cows and heifers. For this trial 320 Angus, SimAngus cows, and heifers were utilized. The groups were: yearling heifers (Group 1, n=101) and young cows (Group 2, n=51) which were bred to an Angus sire sorted for >90% X-bearing sperm, 168 mature cows (Group 3, n=80; and Group 4, n=88) which were bred to a Charolais sire sorted to have >90% Y-bearing sperm. Heifers were synchronized using melengestrol acetate plus prostaglandin $F_{2\alpha}$ (MGA-PG) and were bred based on visual estrous detection. The three cow groups were synchronized using the 7-Day CO-Synch+CIDR protocol. The young cow group and mature cows Group 3 were inseminated using Split-Time AI (STAI)., while mature cows in Group 4 were inseminated using Fixed-Time AI (FTAI). The estrous responses were: 95.1% (Group 1), 88.2% (Group 2), 75.0% (Group 3), and 69.3% (Group 4). The AI pregnancy rates were: 63.4% (Group 1), 47.1% (Group 2), 46.3% (Group 3), and 40.2% (Group 4). In summary, the heifers had a high estrous response and AI pregnancy rate likely due to the greater fertility of heifers, and the intensive estrous detection used. The mature cows had lower estrous responses and lower overall AI pregnancy rates that were consistent with or lower than the literature. The two groups inseminated using STAI had higher estrous responses and AI pregnancy rates than the group inseminated using only FTAI. This is because STAI allows

further time for cows to display estrus, and higher proportions of cows showing estrus can result in greater AI pregnancy rates. Finally, the Charolais-sired calves averaged around 100 lbs. heavier than their Angus-sired counterparts. This result shows the commercial potential of using Y-chromosome-sorted semen in terminal sire programs.

Introduction

Sex-sorted semen is a relatively recent technology, with commercially availability for beef cattle since 2003 (Garner and Seidel, 2008; Seidel and DeJarnette, 2021). The ability to shift the distribution of a calf crop from roughly 50/50 male to female to a majority of the desired gender has made the use of sex-sorted semen increasingly popular in the dairy industry. However, the beef industry has been much slower to adopt the technology. A major hurdle that sex-sorted semen faces are the reduction in fertility in comparison to unsorted semen (Seidel, 2011). The objective of this field study was to evaluate the use of sex-sorted semen on commercial beef cows and heifers using a variety of estrous synchronization protocols and both X- and Y-sorted semen.

Materials & Methods

The design of the project and descriptive information is shown in Table 2.1.

Table 2.1. Descriptions of animal age, estrous synchronization method, and breeding method by sire and group

Group/Sire	N	Average Age (yr)	Estrous Synchronization Method	Breeding Method
Sire A[5]	152	1.4±0.6		
1-Yearling Heifers	101		MGA-PG	Bred by Estrus[1]
2-Young Cows	51	2.2±0.5	7-Day CO-Synch+CIDR	STAI [2]
Sire B[6]	168	6.4±2.4		
3-Mature Cows A	80	6.0±2.2	7-Day CO-Synch+CIDR	STAI [3]
4-Mature Cows B	88	6.8±2.6	7-Day CO-Synch+CIDR	FTAI [4]

[1] Heifers were inseminated 15 to 21 hours after the observation of estrus
[2]Split-Time AI- Young cows that did not display estrus by 70 hours after PG received an injection of GnRH and were inseminated 24 hours later
[3]Split-Time AI- Cows that did not display estrus at 70 hours after PG received an injection of GnRH and were inseminated 12 hours later
[4] Fixed-Time AI-All cows were inseminated at 70 hours after PG, and cows that did not show signs of estrus by 70 hours received an injection of GnRH
[5] Angus sire was sorted to contain >90% X-chromosome bearing sperm cells at a concentration of $4x10^6$ per 0.25 ml straw
[6] Charolais sire was sorted to contain >90% Y-Chromosome bearing sperm cells at a concentration of $4x10^6$ per 0.25 ml straw

Animals

This trial was conducted in north-central South Dakota and south-central North Dakota from the Summer of 2019 through the Fall of 2020. There were a total of 320 Angus and SimAngus females ranging in age from yearling heifers to 13-year-old mature cows enrolled in this field trial in four groups. Group 1 consisted of yearling heifers (n=101) that were housed in a dry lot setting. The remaining three groups consisted of suckled cows on pasture. Group 2 was made up of young cows (n=51, aged 2-4 years old). Groups 3 and 4 consisted of mature suckled beef cows (Mature cows A, n=80; Mature cows B, n=88; aged 5-12 years old).

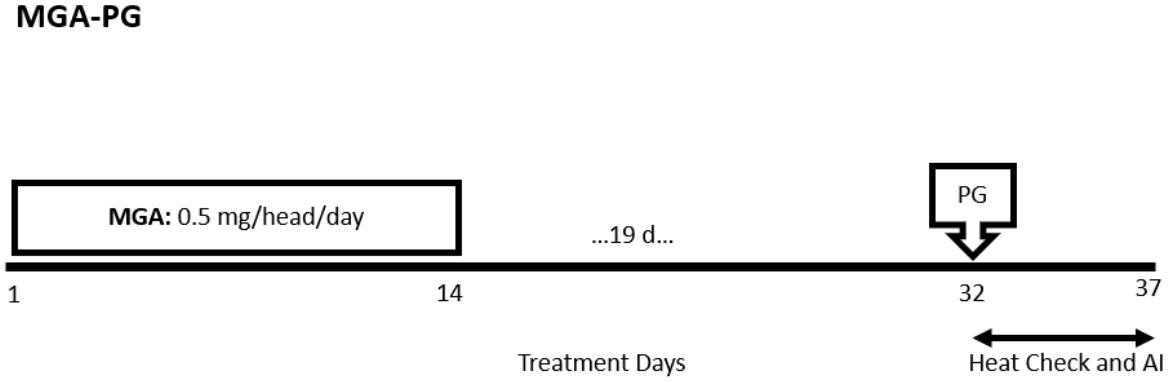
Sire Selection

Two commercially available sires were selected by Sexing Technologies (Navasota, TX) to be used in this field trial. Sire A was an Angus sire selected to be used for the yearling heifers and the young cows (n=152); while Sire B was a Charolais sire selected to be used on the two mature cow groups (n=168). Sire A was sorted to contain x chromosome-bearing sperm cells, while Sire B was sorted to contain y chromosome-bearing sperm cells. Semen was packaged at 4.0 $x10^6$ live cells per 0.25 ml straw before freezing with >90% sorting accuracy typical of SexedULTRA 4M™.

Estrous Synchronization

Estrous cycles of yearling heifers were synchronized using the MGA-PG protocol. Heifers were fed melengestrol acetate for 14 days at a concentration of 0.5 mg/head/day. After feeding MGA for 14 days, MGA was removed from the ration. On Day 32 of the protocol, heifers received a 5 ml injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) (refer to Figure 2.1).

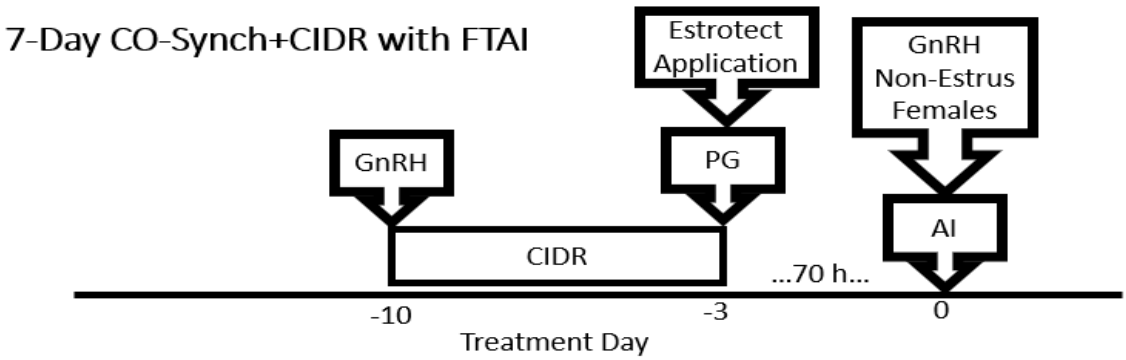
Figure 2.1. MGA-PG



Heifers (Group 1) were fed 0.5 mg/head/day of melengestrol acetate (MGA) for 14 days starting on Day 1 of the protocol. On Day 14 MGA was removed from the ration and 19 days later, a 5 ml injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) was administered. Estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied at the time of PG injection. Following injection of PG, heifers were visually observed for estrus every 4-5 hours for the next 5 days. Heifers were inseminated approximately15-21 hours after observation of estrus.

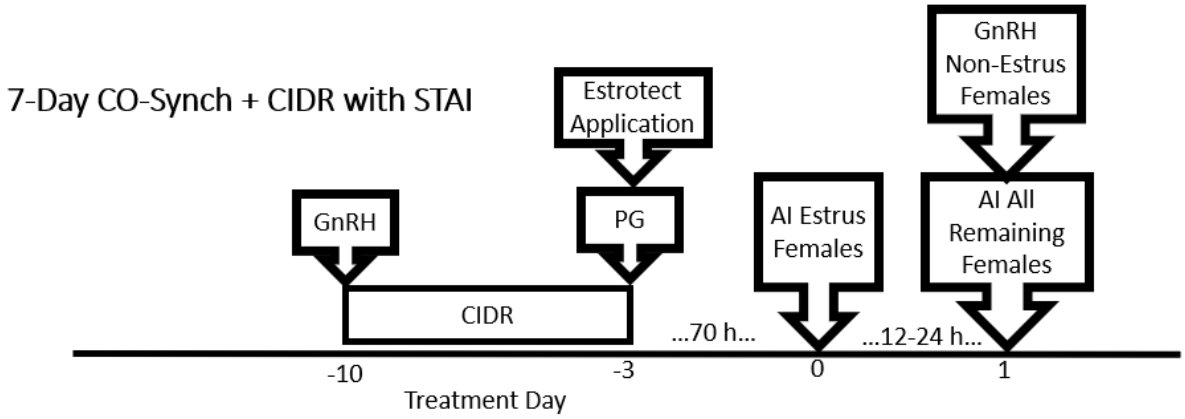
Estrus was synchronized for the young and mature cow groups using the 7-Day CO-Synch+CIDR protocol. Cows received a progesterone insert (CIDR; Zoetis, Madison, NJ) along with a 2 ml injection of a GnRH analog (Gonadorelin; Cystorelin; Merial, Athens, GA) at the start of the protocol. After seven days CIDRs were removed and cows received a 5 ml injection of PG (Lutalyse; Zoetis, Madison, NJ) (refer to figures 2.2 and 2.3).

Figure 2.2. 7-Day CO-Synch+CIDR with FTAI



Eighty-eight cows (Group 4, Mature cows B) were synchronized with the 7-Day CO-Synch+CIDR protocol and inseminated using fixed-timed AI (FTAI). On Day -10 of the protocol, cows received an intravaginal insert (CIDR) containing 1.38 g of progesterone alongside an injection of 2 ml of a GnRH analog (Gonadorelin; Cystorelin; Merial, Athens, GA). Seven days later on Day -3, CIDRs were removed, estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied to the front of the tail head, and an injection of 5 ml injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) was given. Approximately 70 hours post CIDR removal all cows were inseminated with all non-estrual (patches with less than 50% coating removal) cows receiving an injection of GnRH.

Figure 2.3. 7-Day CO-Synch+CIDR with STAI



One hundred thirty-one cows (Group 2, Young cows, n=51; Group 3, Mature cows A, n=80) were synchronized with the 7-Day CO-Synch+CIDR protocol and inseminated using Split-timed AI (STAI). On Day -10 of the protocol, cows received an intravaginal insert (CIDR) containing 1.38 g of progesterone alongside an injection of 2 ml of a GnRH analog (Gonadorelin; Cystorelin; Merial, Athens, GA). Seven days later on Day -3, CIDRs were removed, estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied to the front of the tail head, and an injection of 5 ml injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) was given. Approximately 70 hours post CIDR removal, cows that had displayed estrus before 70 hours were inseminated. The remaining females were given an additional 12-24 hours to display estrus. All cows after the additional 12-24 hours were inseminated with the non-estrual cows receiving a GnRH injection.

Estrous Detection

Estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied at the time of PG injection at all locations. Estrus was defined as standing to be ridden and (or) as >50% of the patch coating removed, or the absence of the patch at the time of insemination (n=25; Group 2, n=2; Group 3, n=14; Group 4, n=9). Yearling heifers were visually observed for signs of estrus every four hours for five days following injection of PG.

Artificial Insemination

Heifers were inseminated to Sire A at 15 to 21 hours after the onset of estrus (Figure 2.1). The young cows and mature cows A were inseminated using a split timed AI system (Figure 2.2) while mature cows B were inseminated using a fixed timed AI system (Figure 2.3). The young

cows in group 2 that displayed estrus were bred to Sire A approximately 70 hours post-PG injection; non-estrual females were injected with GnRH at 70 hours and were inseminated approximately 24 hours later. All estrual females in the mature cows A were bred to Sire B approximately 70 hours post-PG injection; non-estrual females at 70 hours received an injection of GnRH and were inseminated approximately 12 hours later. All of the cows in the mature cow B group were inseminated to Sire B approximately 70 hours post-PG injection; with the non-estrual females receiving an injection of GnRH at the time of insemination. Females were exposed to bulls approximately 5-7 days after AI for the remainder of the breeding season.

All semen was thawed by a single individual at all locations. All inseminations were conducted by a single technician for the yearling heifers, young cows, and mature cows B; with two technicians conducting the inseminations for the mature cows A.

Pregnancy Diagnosis

Pregnancy diagnosis was conducted approximately 65-90 days post-insemination via transrectal ultrasonography (ReproScan XTC equipped with a 4.0 MHz 60mm convex rectal probe; ReproScan, Winterset, IA). Fetal size was used to differentiate AI pregnancies from natural service pregnancies. Gender was determined at birth.

Calf Data

Gender accuracy and gender skew to sex-sorted semen for each sire and group were calculated at the end of the calving season. Gender accuracy, or how many of the desired gender resulted from using sex-sorted semen, was calculated by taking the number of calves of the desired gender born divided by the number of AI calves born. Gender skew was calculated to determine how much sex-sorted semen could shift or skew the ratio of male to female calves in a

calf crop. Gender skew was calculated by taking the number of calves of the desired gender born divided by the total number of calves born by the end of the calving season.

All calves were weighed before weaning in the fall. An adjusted 200-Day calf weight was calculated using the fall weight and average birth weight of 36.3 kg using the equations below.

(Fall Wt -Standard BW (36.3 kg))/Days of age=Average Daily Gain (ADG)

(ADG*200 Days) + Standard BW (36.3 kg) = Adjusted 200-Day Wt

Results

Estrous Response

Refer to table 2.2. The overall estrous response for all four groups was 78.8%. The yearling heifers in group 1 showed an estrous response of 95.1%. The young cows in group 2 showed an estrous response of 88.2%. The mature cows A in group 3 showed an estrous response of 75.0%. Group 4 mature cows A showed an estrous response of 69.3%.

Pregnancy Data

Refer to table 2.2. The overall AI pregnancy rate (P/AI) was 51.3%, with an overall pregnancy rate (total number of pregnancies resulting from AI plus natural service at the time of pregnancy detection) of 90.6%. Sire A had an overall P/AI of 58.6%, and an overall pregnancy rate of 87.5%. The yearling heifers had a P/AI of 63.4%, and a pregnancy rate of 86.1%. Group 2, the young cows, had a P/AI rate of 47.1%, and an overall pregnancy rate of 92.3%. Sire B had an overall P/AI of 44.0%, and an overall pregnancy rate of 92.9%. Mature cows A, group 3, had a P/AI rate of 46.3% with an overall pregnancy rate of 92.2%. The mature cows B, group 4, had a P/AI rate of 40.2%, and an overall pregnancy rate of 96.6%.

Table 2.2. Estrous Response, AI Pregnancy Rate (P/AI), and Overall Pregnancy Rates by Sire and Group

Group/Sire	N	Estrous Response[1]	AI Pregnancy Rate (P/AI)	Overall Pregnancy Rate[2]
Overall	320	78.8%	51.3%	90.6%
Sire A[3]	152	91.4%	58.6%	87.5%
1-Yearling Heifers	101	95.1% [5]	63.4%	87.0%
2-Young Cows	51	88.2% [6]	47.1%	92.3%
Sire B[4]	168	66.9%	44.0%	92.9%
3-Mature Cows A	80	75.0%	46.3%	92.2%
4-Mature Cows B	88	69.3%	40.2%	96.6%

[1] Estrous response was determined using visual observation of estrus, Estrotect patch status, or a combination of the two. Calculated using the following equation: Estrous response=number of animals in estrus/number of animals treated

[2]Overall pregnancy rate was the combined total number of pregnancies accounting for both AI and natural service pregnancies at the time of pregnancy detection. Calculated using the following equation: Overall pregnancy rate=AI pregnancies + natural service pregnancies

[3]Angus sire was sorted to contain >90% X-chromosome bearing sperm cells at a concentration of $4x10^6$ per 0.25 ml straw

[4] Charolais sire was sorted to contain >90% Y-Chromosome bearing sperm cells at a concentration of $4x10^6$ per 0.25 ml straw

[5]This response rate is the number of cows showing estrus by 70 hours post-injection plus those showing estrus by 94 hours post-injection

[6]This response rate is the number of cows showing estrus by 70 hours post-injection plus those showing estrus by 82 hours post-injection

Calving Data

Table 2.3 outlines the following results. Sire A, which was sorted to contain >90% X-chromosome-bearing sperm cells, had a gender accuracy of 92.8% heifers (77 heifers/83 AI calves) and a gender skew of 77.9% heifer calves (102 heifers/131 total calves). The yearling heifers had a gender accuracy of 94.9% heifer calves (56 heifers/59 AI calves) and an overall skew of 77.7% heifer calves (66 heifers/85 total calves) in the calf crop. The young cows had a gender accuracy of 87.0% heifers (20 heifers/23 AI calves) and 78.3% heifer calves (36 heifers/46 total calves) born. Sire B, which was sorted to contain >90% Y-chromosome-bearing sperm cells, had a gender accuracy of 87.7% bulls (57 bulls/65 AI calves). Mature cows A, group 3, had a gender accuracy of 90.9% bull calves (30 bulls/33 AI calves) and 76.4% bulls calves born (55 bulls/72 total calves). Finally, group 4, mature cows B, had a gender accuracy of 84.4% bulls (27 bulls/32 AI calves) with a final gender skew of 63.3% bull calves born (50 bulls/79 total calves). Overall, the gender accuracy of sex-sorted semen was 90.5% (134 calves of the correct gender/148 total AI calves).

The overall average 200-Day adjusted calf weight for all four groups was 248.3 kg. Sire A's calves averaged 226.1 kg for a 200-Day adjusted calf weight. The yearling heifers' calves averaged 225.3 kg while the young cows' calves averaged 229.3 kg for their respective 200-Day adjusted calf weights. Sire B's calves averaged 272.8 kg for their adjusted 200-Day calf weight. Mature cows A and B had 200-Day adjusted calf weights of 275.1 kg and 270.2 kg, respectively.

Table 2.3. Gender Accuracy, Gender Skew, and 200-Day Adjusted Calf Weight by Sire and Group

Group/Sire	N	Gender Accuracy [1]	Gender Skew [2]	200-Day Adjusted Calf Weight [3] (kg)
Overall	320	90.5%	Heifers:47.5%, Bulls: 52.5%	248.3
Sire A [4]	152	92.8%	77.9%	226.1
1-Yearling Heifers	101	95%	77.7%	225.3
2-Young Cows	51	87%	78.3%	229.3
Sire B [5]	168	87.7%	69.5%	272.8
3-Mature Cows A	80	91%	76.4%	275.1
4-Mature Cows B	88	84%	63.3%	270.2

[1] Gender Accuracy is the total number of AI calves of the desired gender divided by the total number of AI calves born
[2] Gender Skew is the total number of calves born of the desired gender divided by the total number of calves born
[3] (Fall Wt -Standard BW (of 36.3 kg))/Days of age=Average Daily Gain (ADG)
(ADG*200 Days) + Standard BW (of 36.3 kg) = Adjusted 200-Day Wt
[4] Angus sire was sorted to contain >90% X-chromosome bearing sperm cells at a concentration of $4x10^6$ per 0.25 ml straw
[5] Charolais sire was sorted to hold >90% Y-Chromosome bearing sperm cells at a concentration of $4x10^6$ per 0.25 ml straw

Discussion

In this study, the AI pregnancy rates varied amongst the four groups of cattle likely due to a combination of differences in female age, estrous synchronization method, and time of insemination. The heifers (group 1) had an exceptionally high AI pregnancy rate with sex-sorted semen (63.4%; 64/101) when compared to the existing literature. One study similar to the present study evaluated the use of sex-sorted semen after synchronizing heifers using MGA and inseminating after detection of estrus and reported overall AI pregnancy rates of 41% (Funston and Meyer, 2012). Thomas et al. (2017) reported an AI pregnancy rate in heifers of 52% while Ketchum et al. (2021) reported an AI pregnancy rate in heifers of 48% when using sex-sorted semen following synchronization with a 14-Day CIDR protocol and insemination using STAI. It is important to note that the latter two studies were studies evaluating the use of sex-sorted semen on heifers using a CIDR protocol and a timed breeding protocol (FTAI or STAI) rather than the MGA-PG protocol and estrous detection used in our experiment. Our results were higher than the AI pregnancy rates achieved with unsorted semen from the previously mentioned studies. The best explanation for our result was the intensive estrous detection that was conducted, resulting in an overall estrous response of 95.1% (96/101).

The AI pregnancy rates for our 3 groups of cows were comparable to existing literature (Andersen et al., 2021; Perry et al., 2020; Thomas et al., 2018). The young cows (Group 2) and the mature cows A (Group 3) had AI pregnancy rates of 47.1% (24/51) and 46.3% (37/80) respectively. Andersen et al. (2021) reported an AI pregnancy rate of 52% and 44%. Perry et al. (2020) reported 52%, and Thomas et al. (2018) reported 48% and 50% AI pregnancy rates. The Andersen et al. (2021) and Thomas et al. (2018) studies reported two AI pregnancy rates using sex-sorted semen because those studies were evaluating different estrous synchronization

protocols (7-Day CO-Synch+CIDR vs 7&7 Synch) or time of insemination (FTAI vs STAI). The mature cows B (Group 4) had the lowest AI pregnancy rate at 40.2% (35/87) which would be comparable to Funston and Meyer's (2012) results though that study was done in heifers. The reasoning behind why the mature cows B had a lower AI pregnancy rate compared with the other two groups may be because that group was inseminated using the FTAI approach where all females were inseminated at one time regardless of estrous status. The young cows and mature cows A were inseminated using an STAI approach giving any non-estrual females at the first breeding time more time to express estrus. This may be supported by the estrous responses of these three groups: the young cows and mature cows A had overall estrous responses of 88.2% (48/51) and 75.0% (60/80) respectively, while mature cows B had an estrous response of 69.3%. These results support the recommendation of only using sex-sorted semen on females that have displayed estrus (Seidel, 2011; Thomas et al., 2018; Perry et al., 2020, Ketchum et al., 2021).

There was a major difference in AI pregnancy rates between the two sires used (Sire A 58.6% [89/152] vs Sire B 44.0% [74/168]). This result is likely due to the exceptionally high AI pregnancy rates seen in the heifers bred to Sire A and the lower AI pregnancy rate of the mature cows B bred to sire B. Overall pregnancy rates were similar across the four groups and between the two sires (87.0%, 92.3%, 92.9%, 96.6% respectively) (87.5% and 92.9% respectively) being slightly higher in the three mature cow groups.

Gender accuracy of the four groups (Heifers 95.0%, young cows 87.0%, mature cows A 91.0%, and mature cows B 84%) was comparable to the literature. Other studies have reported gender accuracies of 81% accuracy for male offspring and 94% accuracy for female offspring (Johnson et al., 1989), 81.3% (Perry et al., 2020), 96% (Thomas et al., 2017), and 88% (Kasimanickam et al., 2021). The resulting gender skew from using sex-sorted semen was:

77.7% and 78.3% heifer calves (Heifers and young cows), and 76.4% and 63.3% bull calves (mature cows A and B). The gender skew between the two sires was: Sire A with 77.9% heifer calves and Sire B with 69.5% bull calves. These results show that sex-sorted semen can be used to shift the ratio of gender in a calf crop away from the roughly 50/50 ratio of males to females that are seen with unsorted semen and natural mating. The lower percentage gender skew seen in the mature cows B may be a result of the reduced AI pregnancy rate resulting in fewer total AI calves born. This would have in turn affected the gender skew seen for Sire B.

When evaluating the weight of the calves from each sire, we opted to use a 200-d adjusted calf weight that accounted for the calves' weight in the fall, calf age in days, and a standard birthweight of 36.3 kg Sire B's (Charolais sire sorted for Y-chromosome bearing sperm cells) calves had 200-Day adjusted calf weights 45 to 50 kg heavier than those recorded from Sire A (Angus sire sorted for X-chromosome bearing sperm cells). This can likely be attributed to several factors such as a greater percentage of male calves born into groups inseminated with Sire B, Charolais sire, steers received an implant while heifer did not, a greater degree of heterosis, and these calves were born and raised by mature cows. This result could be important in figuring out if using Y-chromosome-bearing sex-sorted semen from a terminal sire could be a relevant breeding strategy for commercial producers. Using a high-growth sire breed such as Charolais on commercial cows in a terminal cross is not a new system; however, traditionally one of the challenges with this cross is that with unsorted semen 50% of the offspring would be heifers.

It is well known that steers finish at a heavier weight, and have higher average daily gains, and lower feed-to-gain ratios than their heifer counterparts (Stehle et al., 2018). There is also a sale price difference between steers and heifers. In December 2021 227-250 kg steers were

worth $187.26/cwt, 272-295 kg steers were worth $165.51/cwt, and 340-363 kg steers were worth $165.51/cwt (UDSA, ERS; 2022). During the same time 204-227 kg heifers were worth $160.13/cwt and 317.5-340 kg heifers were worth $155.14/cwt (UDSA, ERS; 2022). Steers are typically worth more than heifers of similar weights. Another factor that is important to this equation is the industry trend of increasing carcass weights. In January 1986, the average slaughter cattle carcass weight was 294.4 kg while in December 2021, it was 381.5 kg which is an 87.1 kg difference. With this trend of increasing carcass weights, the price difference between steers and heifers, and that steers physiologically have an advantage in growth rate over heifers the price advantage for steers may continue to grow.

Conclusion

The results from this field trial show that sex-sorted semen can be used within a commercial cow/calf operation using various synchronization methods and times of breeding. The increased cost of sex-sorted semen in combination with the reduction in fertility is a barrier to adoption. Our results are consistent with existing literature on the importance of only inseminating cows that have been in estrus when using sex-sorted semen. Finally, the results from this study supply insight into the potential of using Y-chromosome-bearing sex-sorted semen in a terminal mating strategy and the potential increased revenue. Further research may be warranted on the topic of the economics of using sex-sorted semen in a terminal mating system.

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Chapter 3 - Effect of timing of insemination using sex-sorted semen on estrus synchronized beef heifers

Abstract

The objective of the study was to evaluate the effect of timing of insemination relative to the onset of estrus when using sex-sorted semen in beef heifers. Heifers (n=150) were synchronized using the MGA-PG protocol and were inseminated following visual estrous detection with semen from either an Angus or Simmental sire. Ninety-eight heifers were visually observed in standing estrus and were used to investigate the three-timing intervals. These were: 1) 12.5-15.9 hr post-onset of estrus; 2) 16.5-21.0 hr post-onset of estrus; and 3) 21.4-27.5 hr post-onset of estrus. The late-bred group had a higher ($P<0.05$) AI pregnancy rate than the early-bred group. The middle group did not differ ($P>0.05$) from either of the other two groups. It appears advantageous to inseminate heifers later than 21 hours post-onset of estrus when using sex-sorted semen.

Introduction

The ability to determine gender at conception has been a widely sought-after technology that has only been available commercially for less than 20 years (Garner and Seidel, 2008; Seidel and DeJarnette, 2021). While sex-sorted semen has been widely used in the dairy industry, the adoption of this technology has been limited in the beef industry due to increased cost and reduced fertility (Seidel, 2011). Due to the reduced fertility of sex-sorted semen, heifers are the most likely candidates for sex-sorted semen in the dairy industry (Seidel and DeJarnette, 2021). The objective of this study was to evaluate the effect of timing of insemination relative to the onset of estrus when using sex-sorted semen in beef heifers.

Materials & Methods

Animals

This trial was conducted in north-central South Dakota during the Summer and Fall of 2021. There were 150 Angus and SimAngus yearling heifers enrolled in this field trial housed in a dry lot setting.

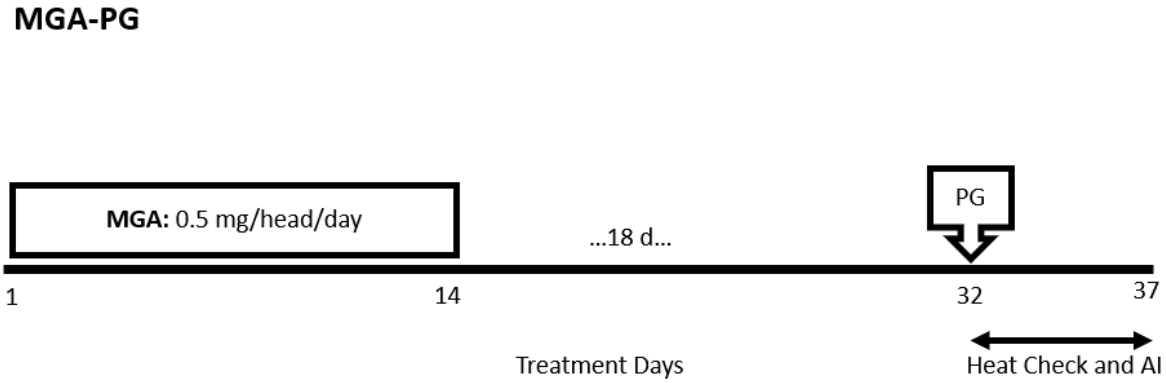
Sire Selection

Two commercially available sires, an Angus sire, and a Simmental sire were selected to be used in this field trial. Semen from each sire was sorted to contain X chromosome-bearing sperm cells. Equal numbers of heifers were assigned to be inseminated with either Angus or Simmental semen (n=75/sire). Semen was packaged at 4.0 $x10^6$ live cells per 0.25 ml straw before freezing with expected >90% sorting accuracy of SexedULTRA 4M™.

Estrous Synchronization

Estrus was synchronized using the MGA-PG protocol. Heifers were fed melengestrol acetate (MGA) for 14 Days at a concentration of 0.5 mg/head/day. Eighteen days after the last feeding of MGA, heifers were injected with 5 ml of PG. (PG; Lutalyse; Zoetis, Madison, NJ). At the time of PG injection, a weight, body condition score (BCS) using a 1 to 9 scale (1=emaciated and 9 =obese), and a hip height that was used to calculate a frame score were collected (Olsen and Walker, 2017). See figure 3.1.

Figure 3.1. MGA-PG



Heifers were fed 0.5 mg/head/day of melengestrol acetate (MGA) for 14 days starting on day 1 of the protocol. Eighteen days after the last feeding of MGA, a 5 ml injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) was administered. Estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were also applied at the time of PG injection. Following injection of PG heifers, were visually observed for estrus every 4 hours for the next 5 days.

Estrus Detection and Artificial Insemination

Heifers were visually observed for signs of estrus every four hours for five days following injection of PG. Estrous detection aids (ESTROTECT, Rockway Inc., Spring Valley, WI) were applied at the time of PG injection. Heifers were determined to have exhibited estrus when they were seen standing or >50% of the patch coating was removed or her patch was missing (n=1). After the earliest observation of estrus, heifers were inseminated 12.5-27.5 hours later.

Of the 150 heifers in the original group, eight were not detected in estrus in the five days and were excluded. Of the remaining heifers, 98 were visually observed in standing estrus. These 98 head were retrospectively divided into three groups for analysis. The first interval was 12.5-15.9 hr (n=37) between the onset of estrus and insemination, the second was 16.5-21.0 hr (n=33), and the final interval was 21.4-27.5 hr (n=28).

Pregnancy Diagnosis

Pregnancy diagnosis was conducted 65-70 days post-insemination via transrectal ultrasonography (ReproScan XTC equipped with a 4.0 MHz 60mm convex rectal probe; ReproScan, Winterset, IA). Fetal size was used to differentiate AI pregnancies from natural service pregnancies.

Statistical Analysis

The statistical analysis for this experiment was done using the GLIMMIX procedure in SAS (SAS Inst. Inc., Cary, NC) using the binomial distribution, and link logit function. The dependent variable was AI pregnancy rate. Interval from the onset of estrus to time of insemination, sire, and sire by interval from onset of estrus to time of insemination were included as fixed effects. Sire and the interaction of sire and interval from the onset of estrus to the time of insemination were not significant ($P>0.1$), so were removed from the final model. Significance was determined when $P\leq0.05$.

Results

Description of the mean weight, BCS, and frame score of all the heifers enrolled in the study are shown in Table 3.1.

Table 3.2 shows the AI pregnancy rates by the onset of estrus to time of insemination interval. The range in hours from onset of estrus to insemination in the first interval was 12.5-15.9 hr, with an average time of 14.7 hr The range for interval two was 16.5-21.0 hr, with an average of 17.9 hr The final interval ranged from 21.4-27.5 hr, averaging 24.3 hr. The late bred group had a higher ($P<0.05$) AI pregnancy rate than the early bred group. The middle group did not differ ($P>0.05$) from either of the other two groups.

Table 3.1. Description of heifer weight (kg), body condition score (BCS), and frame score

N	WT (kg)	BCS[1]	Frame Score
150	424.4±32.8	5.6±0.6	4.5±0.5

[1] Body condition score (mean±SD) was collected on the day of PG (1-9 scale, where 1=emaciated and 9=obese)

Table 3.2. The interval from observed estrus to insemination and the overall corresponding AI pregnancy rates (P/AI)

Estrus to Insemination Interval [1]	N	AI Pregnancy Rate (P/AI)
Overall	98	60.2%
12.5-15.9 hr[2]	37	51.4%[a]
16.5-21.0 hr[3]	33	56.3%[ab]
21.4-27.5 hr[4]	28	75.9%[b]

[1] Interval of time from when heifers were detected in estrus to the time of insemination; Average interval from estrus to insemination was 18.5 hr with a range of 12.5-27.5 hr
[2]Average interval from estrus to insemination was 14.7 hr with a range of 12.5-15.9 hr
[3]Average interval from estrus to insemination was 17.9 hr with a range of 16.5-21.0 hr
[4]Average interval from estrus to insemination was 24.3 hr with a range of 21.4-27.5 hr
[ab] Values within a column without a common superscript are different ($P \leq 0.05$)

Discussion

The results of this study appear to support the theory that breeding later when using sex-sorted semen may be advantageous for increasing AI pregnancy rates. This study overall showed a difference in AI pregnancy rates when breeding was conducted at interval 3 (21.4-27.5 hr after observed estrus). The timing from the onset of estrus to ovulation in cattle has been found to range from 24 to 32 hours (Senger, 2012). Research done by White et al., (2002) has shown that

the average time of ovulation relative to the onset of estrus was 31.1 hr. It is possible that it is advantageous to delay insemination relative to the onset of estrus because of differences in capacitation rate of sex-sorted semen as compared with unsorted sperm. It has been demonstrated that sex-sorted sperm capacitates in the female reproductive tract at a different rate than unsorted semen (Winters et al., 2017). Bucci et al, (2012) found that sex-sorted bull and boar sperm had a more advanced membrane state where sex-sorted sperm resemble unsorted sperm that have undergone in vitro capacitation. Additionally, Bucci et al, (2012) observed that around 40% of bull spermatozoa, "had a staining pattern indicative of capacitation." If sex-sorted semen is more capacitated than unsorted semen, then in theory the sperm cells would need less time in the female tract to undergo capacitation. Thus sex-sorted semen would need less time in the tract prior to ovulation to be viable for proper fertilization. Overall, further research may be warranted to determine the optimal timing of insemination following estrus when using sex-sorted semen. Furthermore, determining the optimal timing of insemination following estrus when using sex-sorted semen would be beneficial to develop timed AI protocols specific to the use of sex-sorted semen.

Conclusion

Based on the results of this study it appears advantageous to inseminate heifers later than 21 hours post onset of estrus when using sex-sorted semen.

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Chapter 4 - Comparison of 7&7 Synch and 7-Day CO-Synch+CIDR in beef cows using a fixed-time AI and Y-bearing sex-sorted semen

Abstract

The objective of this study was to compare pregnancy rates for beef cows that were synchronized using either the 7-Day CO-Synch+CIDR or 7&7 Synch estrous synchronization protocols using sex-sorted semen. This study used 204 Angus and SimAngus beef cows. Two groups of mature cows (n=130) and one group of 2-year-old cows (n=74) were assigned to the either the 7&7 Synch or the 7-Day CO-Synch+CIDR protocols. Following estrous synchronization, cows were inseminated using Y-chromosome-bearing sex-sorted semen from two Charolais bulls at a fixed time. No differences were observed among mature cows in estrous response (79.4% vs 76.9%; P=0.74), FTAI pregnancy rates (52.4% vs 44.6%; P=0.39), or overall pregnancy rates (88.9% vs 92.3%; P=0.51). No difference was observed in the estrous response of two-year-old cows (58.3% vs 73.0%; P=0.19). A greater ($P\leq0.05$) proportion of two-year-old cows were pregnant to the AI breeding following the 7-Day CO-Synch+CIDR (56.8%) compared with the 7&7 Synch (33.3%). A greater ($P\leq0.05$) overall pregnancy rate was observed among the two-year-old cows following the 7-Day CO-Synch+CIDR (91.9%) compared with the 7&7 Synch (69.4%) protocol. Results of the study saw no differences between synchronization protocols in mature cows. However, 7-Day CO-Synch+CIDR treatment resulted in a higher AI pregnancy rate and overall pregnancy rate in two-year old cows.

Introduction

The adoption of sex-sorted semen has been limited within the beef cattle industry due to the added cost and reduced fertility associated with the product (Seidel 2011; Seidel and

DeJarnette, 2021; STgenetics, Sire Directory, 2022). The limited use of AI by beef producers has also limited the adoption of the technology. The development of the SexedULTRA technology and later the release of the SexedULTRA 4M product have increased the fertility of sex-sorted semen, but AI pregnancy rates with sex-sorted semen are still only achieving 70-80% of those seen with unsorted semen (Seidel and DeJarnette, 2021; Thomas et al., 2018). Developing an estrous synchronization protocol to increase success with sex-sorted semen is an important objective. Traditionally, sex-sorted semen was only recommended for use after visual observation of estrus; however, multiple studies have shown that the use of sex-sorted semen with timed-AI protocols can achieve acceptable results (Perry et al., 2020; Thomas et al., 2018). The dairy industry has seen widespread use of sex-sorted semen due to the greater value of female calves. Within the dairy industry, the use of presynchronization protocols has proven effective in increasing AI pregnancy rates, but these presynchronization protocols are not commonly used on beef cattle due to the additional time and handlings required (El-Zarkouny et al., 2004).

The most common and popular estrous synchronization protocol used in beef cows is the 7-Day CO-Synch+CIDR protocol. A concern with this protocol is that due to differences in follicular maturity at the time of synchronization, some cows do not respond to the injection of GnRH. Failure to respond to GnRH causes various subgroups of cows to form which can result in variation in the timing of ovulation. To answer this challenge, Thomas et al., (2021) at the University of Missouri developed a new protocol termed 7&7 Synch. The 7&7 Synch estrous synchronization protocol is a presynchronization type protocol that is meant to tighten the window of ovulation within a group of synchronized cows while only requiring one additional handling event (Andersen et al., 2021a; Bonacker et al., 2020a; Thomas et al., 2021). Figures 4.1,

4.2, and 4.3 highlights the basic physiology of the 7-Day CO-Synch+CIDR and the 7&7 Synch (Bonacker et al., 2020a) protocols. Multiple studies comparing this protocol to the classic 7-Day CO-Synch and Bee Synch II protocols have shown promising results with unsorted and sex-sorted semen along with embryo transfer (Andersen et al., 2021a and b; Bonacker et al., 2020a and b). The goal of the current study was to evaluate the use of Y-chromosome-bearing sex-sorted semen in a fixed-time AI system when using the 7-Day CO-Synch+CIDR and the 7&7 Synch estrous synchronization protocols in mature and 2-year-old cows.

Figure 4.1. Physiology of the 7-Day CO-Synch+CIDR protocol with successful induction of ovulation following injection of GnRH

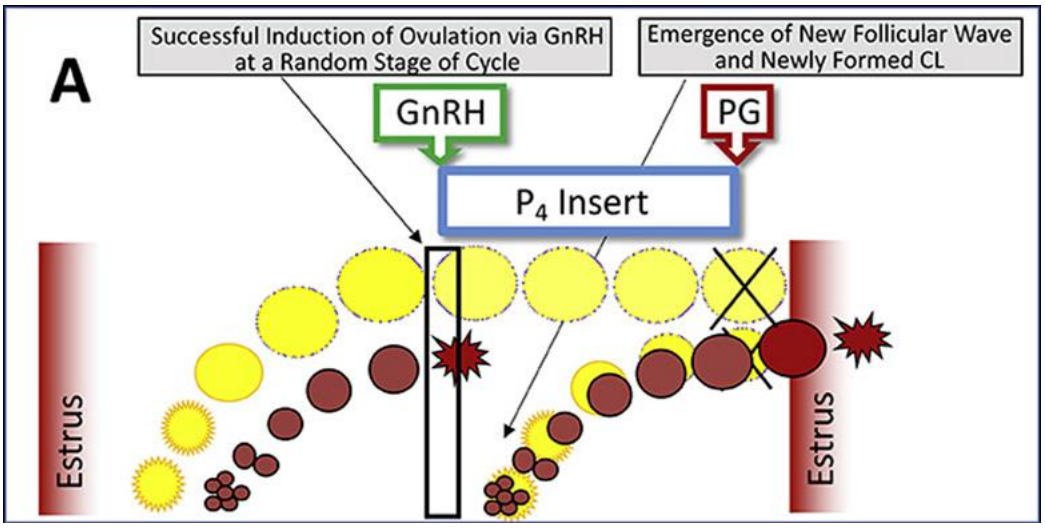


Figure 4.1 A: shows the basic physiology of the 7-Day CO-Synch+CIDR protocol when injection of GnRH is successful in inducing ovulation causing the emergence of a new follicular wave and new CL. Modified from Bonacker et al., 2020a

Figure 4.2. Physiology of the 7-Day CO-Synch+CIDR protocol with unsuccessful induction of ovulation following injection of GnRH

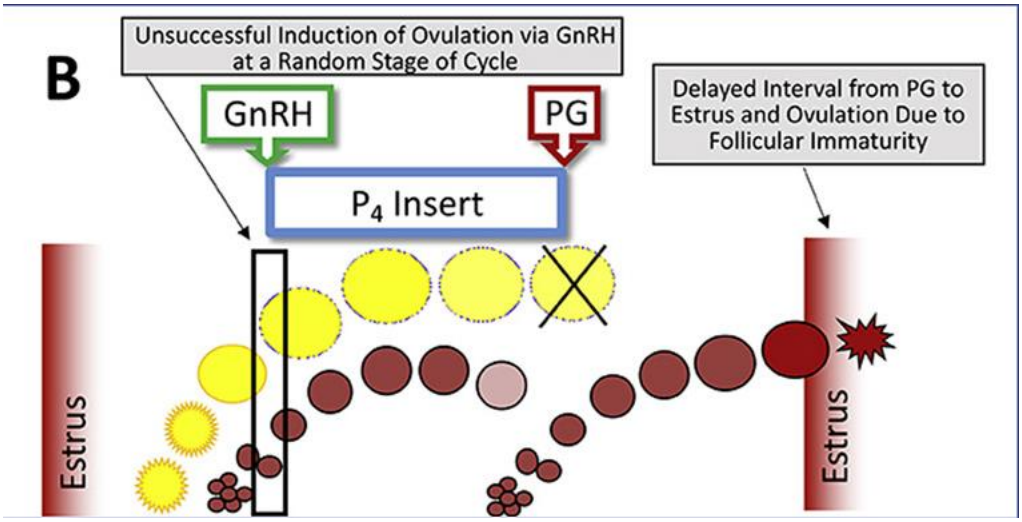


Figure 4.1 B: shows the basic physiology of the 7-Day CO-Synch+CIDR protocol when injection of GnRH is unsuccessful in inducing ovulation thus delaying the onset of a new follicular wave and formation of a new CL. Modified from Bonacker et al., 2020a

Figure 4.3. Physiology of the 7&7 Synch protocol

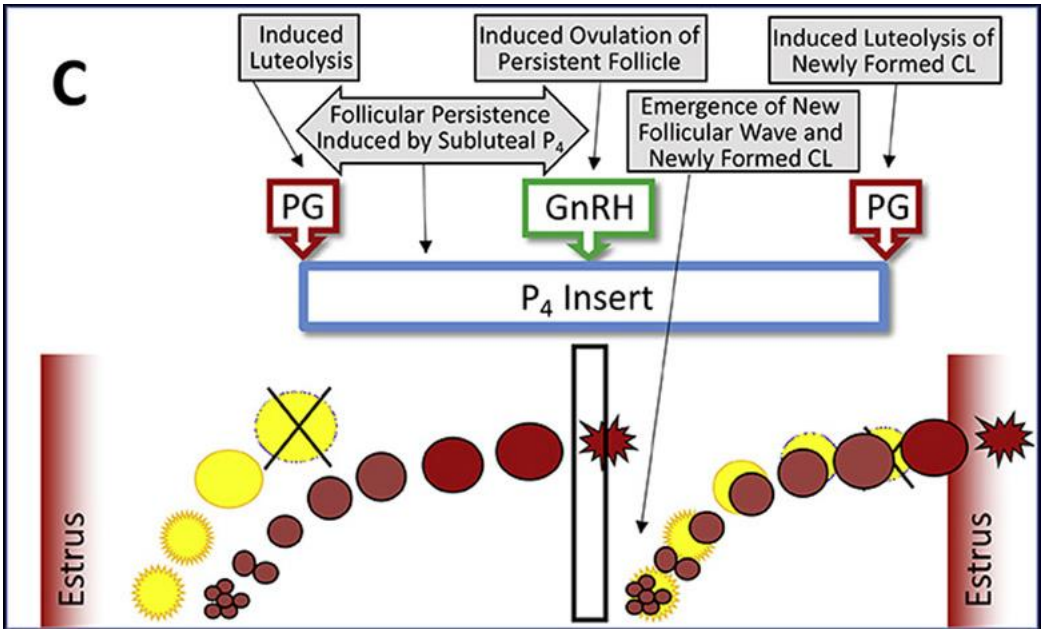


Figure 4.1 C: shows the basic physiology of the 7&7 Synch protocol. Injecting prostaglandin at the beginning of the protocol causes luteolysis of any existing CL and induces follicular persistence which results in an increased likelihood of any existing follicles ovulating due to GnRH. This then will result in a new follicular wave and formation of a new CL which will then undergo induced luteolysis at the time of prostaglandin injection at the end of the protocol. This protocol allows for more follicular and luteal control during synchronization on cows that are at random stages of follicular development. Modified from Bonacker et al., 2020a

Materials & Methods

Animals

This trial was conducted in north-central South Dakota in the Summer and Fall of 2021. A total of 204 Angus and SimAngus beef cows ranging in age from 2 to 12-year-olds and managed in three separate locations were enrolled in this trial. All three groups were kept in pasture settings before breeding through post-pregnancy detection. Mature cows were managed in two different pasture locations (Location 1: n=50 and Location 2: n=80) while two-year-old cows (n=74) were managed separately.

Sire Selection

Cows were randomly assigned to one of two sires at the start of the experiment. Two commercially available Charolais sires were selected to be used for this experiment. Sex-sorted semen containing Y-chromosome-bearing sperm cells from each sire was used in equal proportions across groups and treatments. Semen was packaged at 4.0 $x10^6$ live cells per 0.25 ml straw before freezing with expected >90% sorting accuracy.

Estrous Synchronization

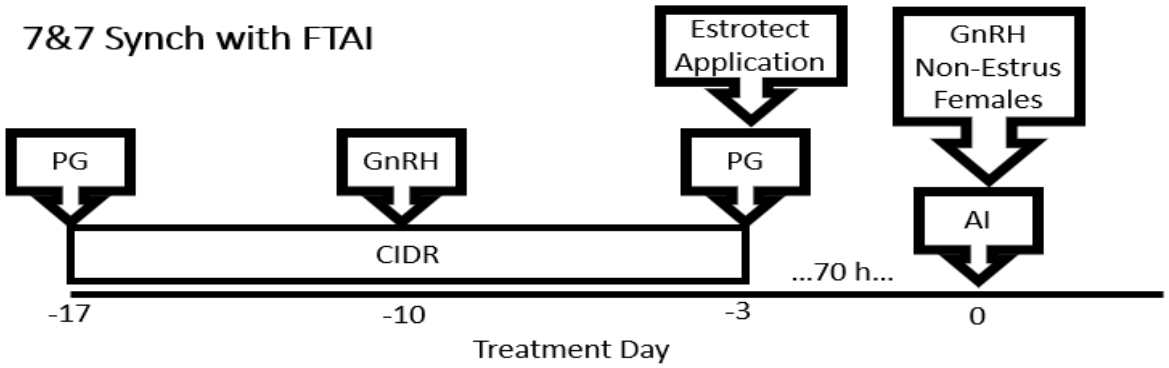
Day 0 was established as the day of insemination for both treatment groups. On Day -17, a weight, a body condition score (BCS) using a 1 to 9 scale (1=emaciated and 9 =obese), and a hip height were collected. The hip height measurement was used to calculate a frame score (Olsen and Walker, 2017).

The 7&7 Synch treatment group (see Figure 4.4 for details) received a CIDR (CIDR; Zoetis, Madison, NJ) and a 5 ml injection of prostaglandin $F_{2\alpha}$ (PG; Lutalyse; Zoetis, Madison, NJ) on Day -17. On Day -10, cows received a 2 ml injection of a GnRH analog (Gonadorelin; Cystorelin; Merial, Athens, GA). On Day -3 (CIDR removal), cows received a 5 ml injection of PG.

For the 7-Day CO-Synch+CIDR treatment group (see Figure 4.5 for details), cows received a CIDR and an injection of 2 ml of GnRH on Day -7 and an injection of 5 ml of PG at the time of CIDR removal (Day -3). Estrous detection aids (ESTROTECT, Rockway Inc., Spring Valley, WI) were applied to all animals at time of CIDR removal (Day -3). Cows were determined to have expressed estrus if at the time of insemination >50% of the patch coating was removed or if patches were missing. Cows in both groups were inseminated at approximately 70 hr post-CIDR removal. Cows that were not in estrus were injected with 2 ml of GnRH at the time

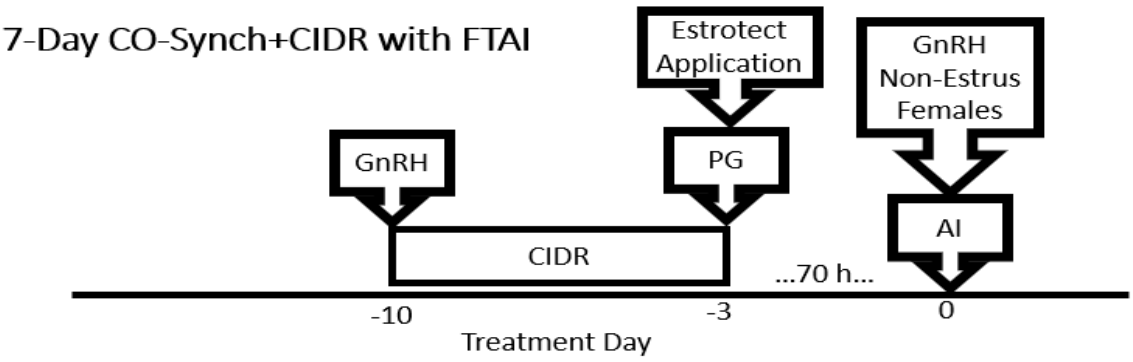
of insemination. Cows were exposed to bulls approximately five days after insemination for the remainder of the breeding season.

Figure 4.4. 7&7 Synch with FTAI



Ninety-nine cows were synchronized using the 7&7 Synch protocol. On Day -17 cows received an intravaginal insert (CIDR) containing 1.38 g of progesterone alongside an injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ). On Day -10 of the protocol cows an injection of 2 ml of a GnRH analog (Gonadorelin; Cystorelin; Merial, Athens, GA). Seven days later on Day -3 CIDRs were removed, estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied to the front of the tail head, and an injection of 5 ml of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) was given. Approximately 70 hours post CIDR removal all cows were inseminated, with all non-estrual (patches with less than 50% coating removal) cows receiving an injection of GnRH

Figure 4.5. 7-Day CO-Synch+CIDR with FTAI



One hundred and two cows were synchronized with the 7-Day CO-Synch+CIDR protocol and inseminated using a fixed-timed AI (FTAI) strategy. On Day -10 of the protocol cows received an intravaginal insert (CIDR) containing 1.38 g of progesterone alongside an injection of 2 ml of a GnRH analog (Gonadorelin; Cystorelin; Merial, Athens, GA). Seven days later on Day -3 CIDRs were removed, estrous detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied to the front of the tail head, and an injection of 5 ml injection of prostaglandin $F_{2\alpha}$ (PG) (Lutalyse; Zoetis, Madison, NJ) was given. Approximately 70 hours post CIDR removal all cows were inseminated, with all non-estrual (patches with less than 50% coating removal) cows receiving an injection of GnRH

Pregnancy Diagnosis

Pregnancy diagnosis was conducted 77 to 83 days post insemination via transrectal ultrasonography (ReproScan XTC equipped with a 4.0 MHz 60mm convex rectal probe; ReproScan, Winterset, IA). Fetal size was used to differentiate AI pregnancies from natural service pregnancies. Overall pregnancy rates (total number of pregnancies resulting from AI plus natural service at the time of pregnancy detection) were calculated following pregnancy diagnosis. Weights and body condition scores (BCS) using a 1 to 9 scale (1=emaciated and 9 =obese) were also collected at this time.

Statistical Analysis

The statistical analysis for this experiment was done using the GLIMMIX procedure in SAS (SAS Inst. Inc., Cary, NC) using the binomial distribution, and link logit function. The mature cows and young cows were analyzed using separate models.

The model to evaluate the effect of treatment on AI pregnancy rate included sire and estrous synchronization protocol as fixed effects, but sire was not a significant source of variation, so it was not included in the final model. Location was only included as a random variable in the mature cow model.

Significance was determined when a P-value of $P \leq 0.05$ was detected.

Results

Three cows (Group 1, n=0; Group 2, n= 2; Group 3, n=1) were excluded from the analysis due to missing CIDRs on either Day -10 or -3 of the protocol.

See Tables 4.1 and 4.2 for mean ages, days postpartum, frame scores, weights at breeding and pregnancy detection, and BCS of the mature cows.

See Tables 4.3 and 4.4 for mean ages, days postpartum, frame scores, weights at breeding and pregnancy detection, and BCS of the two-year-old cows.

Table 4.5 shows the results in the mature cows by treatment. No difference was observed between the two treatments (7&7 vs 7-Day CO-Synch+CIDR) in estrous response (79.4% vs 76.2%; P=0.74), AI pregnancy rates (52.4% vs 44.6%; P=0.39) or overall pregnancy rates (88.9% vs 92.3%; P=0.51).

Table 4.6 shows the treatment effects (7&7 vs 7-Day CO-Synch+CIDR) for the two-year old cows. No difference was observed in estrous response between the two treatments (58.3% vs 73.0%; P=0.19). A greater ($P \leq 0.05$) proportion of two-year-olds were pregnant to FTAI

following the 7-Day CO-Synch+CIDR protocol (56.8%) compared with the 7&7 Synch (33.3%). A significant ($P \leq 0.05$) difference in overall pregnancy rates was observed between the two treatments with an advantage for the 7-Day CO-Synch+CIDR group (91.9%) compared with the 7&7 Synch group (69.4%).

Table 4.1. Average animal age, days postpartum, weight, BCS, and frame score during breeding and pregnancy detection for the mature cows

			Breeding		Pregnancy Detection		
N	Age (yr)	Days Postpartum	WT (kg)[1]	BCS[2]	WT (kg)[1]	BCS[2]	Frames Score[3]
128	5.1±2.1	58.4±6.6	580.0±69.1	5.0±0.7	568.5±82.4	4.8±0.5	5.6±0.8

[1] Bodyweight was collected on Day -17 of the protocol and 77-83 days post-AI
[2] Body condition score was collected on Day -17of the protocol and 77-83 days post-AI (1-9 scale, where 1=emaciated and 9=obese)
[3]Hip heights were collected on Day -17 of the protocol (Olsen and Walker, 2017)

Table 4.2. Average animal age, days postpartum, weight, BCS, and frame during breeding and pregnancy detection by treatment for the mature cows

				Breeding		Pregnancy Detection		
Treatment	N	Age (yr)	Days Postpartum	WT (kg)[1]	BCS[2]	WT (kg)[1]	BCS[2]	Frames Score[3]
7&7 Synch	63	5.1±2.0	59.7±7.0	578.0±61.0	5.0±0.6	566.9±93.5	4.9±0.5	5.6±0.9
7-Day CO-Synch+CIDR	65	5.1±2.2	59.1±6.2	581.5±76.5	5.0±0.7	571.6±70.7	4.8±0.6	5.6±0.8

[1] Bodyweight was collected on Day -17 of the protocol and 77-83 days post-AI
[2] Body condition score was collected on Day -17of the protocol and 77-83 days post-AI (1-9 scale, where 1=emaciated and 9=obese)
[3]Hip heights were collected on Day -17 of the protocol (Olsen and Walker, 2017)

Table 4.3. Average animal age, days postpartum, weight, BCS, and frame score during breeding and pregnancy detection for the two-year-old cows

			Breeding		Pregnancy Detection		
N	Age (yr)	Days Postpartum	WT (kg)[1]	BCS[2]	WT (kg)[1]	BCS[2]	Frames Score[3]
73	2±0.0	77.4±11.5	467.3±40.5	5.2±0.5	449.4±37.3	4.5±0.6	5.5±0.6

[1] Bodyweight was collected on Day -17 of the protocol and 77-83 days post-AI
[2] Body condition score was collected on Day -17of the protocol and 77-83 days post-AI (1-9 scale, where 1=emaciated and 9=obese)
[3]Hip heights were collected on Day -17 of the protocol (Olsen and Walker, 2017)

Table 4.4. Average animal age, days postpartum, weight, BCS, and frame during breeding and pregnancy detection by treatment for the two-year-old cows

				Breeding		Pregnancy Detection		
Treatment	N	Age (yr)	Days Postpartum	WT (kg)[1]	BCS[2]	WT (kg)[1]	BCS[2]	Frames Score[3]
7&7 Synch	36	2.0±0.0	77.9±10.2	467.0±39.8	5.2±0.5	440.5±38.8	4.5±0.6	5.6±0.7
7-Day CO-Synch+CIDR	38	2.0±0.0	76.5±12.7	471.5±41.7	5.2±0.5	462.2±34.1	4.5±0.5	5.5±0.5

[1] Bodyweight was collected on Day -17 of the protocol and 77-83 days post-AI
[2] Body condition score was collected on Day -17of the protocol and 77-83 days post-AI (1-9 scale, where 1=emaciated and 9=obese)
[3]Hip heights were collected on Day -17 of the protocol (Olsen and Walker, 2017)

Table 4.5. Estrous response, AI pregnancy rate (P/AI), and overall pregnancy rate by treatment for the mature cows

Group	N	Estrous Response[1]	AI Pregnancy Rate (PAI)[2]	Overall Pregnancy Rate[3]
7&7 Synch	63	79.4%[a]	52.4%[a]	88.9%[a]
7-Day CO-Synch+CIDR	65	76.2%[a]	44.6%[a]	92.3%[a]

[1] Estrous response was calculated from Estrotect patch status. Cows with lost or activated patches (>50% of patch coating removed) were considered in estrus

[2] FTAI pregnancy rates were determined following transrectal ultrasonography 77-83 days after AI with fetal size being used to differentiate AI pregnancies from natural service pregnancies.

[3] Overall pregnancy rate was the combined total number of pregnancies accounting for both AI and natural service pregnancies at the time of pregnancy detection. Calculated using the following equation: Overall pregnancy rate=AI pregnancies + natural service pregnancies

[ab] Values within a column without a common superscript are different ($P \leq 0.05$)

Table 4.6. Estrous response, AI pregnancy rate (P/AI), and overall pregnancy rate by treatment for the two-year-old cows

Group	N	Estrous Response[1]	AI Pregnancy Rate (PAI)[2]	Overall Pregnancy Rate[3]
7&7 Synch	36	58.3%[a]	33.3%[a]	69.4%[a]
7-Day CO-Synch+CIDR	37	73.0%[a]	56.8%[b]	91.9%[b]

[1] Estrous response was calculated from Estrotect patch status. Cows with lost or activated patches (>50% of patch coating removed) were considered in estrus

[2] FTAI pregnancy rates were determined following transrectal ultrasonography 77-83 days after AI with fetal size being used to differentiate AI pregnancies from natural service pregnancies.

[3] Overall pregnancy rate was the combined total number of pregnancies accounting for both AI and natural service pregnancies at the time of pregnancy detection. Calculated using the following equation: Overall pregnancy rate=AI pregnancies + natural service pregnancies

[ab] Values within a column without a common superscript are different ($P \leq 0.05$)

Discussion

While there was no significant difference in AI pregnancy rate among the mature cows, the eight-percentage point greater AI pregnancy rate for 7&7 Synch is similar to results in other studies (Andersen et al., 2021a). Andersen et al. (2021a) reported a difference in AI pregnancy rate in favor of the 7&7 Synch (52% vs 44%) as compared with the 7-Day CO-Synch+CIDR. The power of test used in this study (n=201 total, 128 mature cows and 73 young cows) may explain the lack of significance in AI pregnancy rate observed in this study with the mature cows. A study conducted by Bonacker et al. (2020a) resulted in a numerical advantage to 7&7 Synch compared to the 7-Day CO-Synch+CIDR (63% vs 51%).

The young cows in this study had the opposite results of what we had hypothesized based on the literature. It is likely that the two-year old cows had a lower percentage having estrous cycles at the beginning of treatment. The 7&7 Synch program may not work as well if there is a significant portion of the herd that do not have corpora lutea at the time of the initial PG injection. The young cows had a greater AI pregnancy rate with the 7-Day CO-Synch+CIDR protocol compared to the 7&7 Synch (56.7% vs 33.4%). Overall pregnancy rate was also higher for the 7-Day CO-Synch+CIDR group. The numerically higher estrous response in the 7-Day CO-Synch+CIDR group may help explain the differences observed in treatment effects on AI pregnancy rate and overall pregnancy rate. The difference in AI pregnancy rate in the two-year old cows can also likely be attributed to several environmental and basic physiological factors. Two-year old cows have several physiological factors that provide additional challenges to them in regard to reproduction. They have just undergone their first parturition, are going through their first lactation, alongside the fact that they are still a growing animal themselves. All of these factors provide challenges that she needs to overcome before reproduction becomes a priority. In

addition, the two-year old cows also faced some environmental challenges. At the time of this study drought conditions existed in north-central South Dakota and the pasture these females were grazing had been overgrazed in previous years resulting in relatively poor forage availability. The effect these factors likely had on the two-year old cows can be seen in the change in their weight and BCS from breeding to pregnancy detection. Referring to Table 4.3 the two-year old cows overall lost 17.9 kg of body weight and 0.7 of a body condition score from breeding to pregnancy detection. Referring to Table 4.4 it was also observed that the heifers assigned to the 7&7 Synch lost more weight than those assigned to the 7-Day CO-Synch+CIDR protocol (26.5 kg lost vs 9.3 kg lost, respectively), though no difference in the loss of body condition was observed between treatments.

Conclusion

While no significant differences in treatment for AI pregnancy rate were observed, there was a numerical advantage (eight percent) for 7&7 Synch estrous synchronization protocol in the mature cow group. More research is needed to determine the effectiveness of the 7&7 Synch treatment in two-year old cows and the effectiveness of the treatment in anestrous beef cows.

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