CHARACTERISTICS OF ESTRUS AND MANIPULATION OF THE ESTROUS CYCLE TO IMPACT FERTILITY OF BEEF FEMALES

A Dissertation

by

NICOLA OOSTHUIZEN

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Graham C. Lamb
Committee Members,	David P. Anderson
	Rodolfo C. Cardoso
	Reinaldo F. Cooke
Head of Department,	Graham C. Lamb

May 2020

Major Subject: Physiology of Reproduction

ABSTRACT

Reproductive efficiency of beef females directly impacts the overall efficiency and profitability of cow-calf operations. By making use of reproductive management technologies, such as estrus synchronization, presynchronization, and sex-sorted semen, reproductive efficiency, and thus profitability of beef cattle production systems may be improved. Three experiments were performed to evaluate the effects of estrus intensity, and presynchronization and delayed fixed-time artificial insemination (**TAI**) on fertility in beef females. In experiment one, the impacts of estrus expression and intensity, assessed via physical activity, were evaluated on parameters associated with fertility in beef cows. Lactating, multiparous cows were classified as not expressing estrus, or expressing estrus with net physical activity greater or below the median. Expression of estrus during the 7-d CO-Synch + controlled internal drug release (**CIDR**) protocol increased ($P < 0.01$) pregnancy rates to TAI (**PR/AI**), whereas estrus intensity increased ($P < 0.01$) dominant follicle and corpus luteum dimensions but not PR/AI ($P = 0.46$). Experiment two was performed to determine the effects of presynchronization with prostaglandin $F_{2\alpha}$ (**PGF**) and a CIDR insert in conjunction with delayed TAI on PR/AI in beef heifers. Heifers presynchronized with PGF and a CIDR insert had greater ($P = 0.03$) PR/AI than control heifers. Moreover, there was a tendency ($P = 0.10$) for greater PR/AI when heifers were presynchronized with PGF and had delayed TAI when compared to control heifers. Finally, experiment three was performed to determine whether delayed timing of TAI after presynchronization with PGF enhances PR/AI with sex-sorted semen. Pregnancy rates to TAI were greater ($P < 0.04$) when conventional semen was utilized as opposed to sex-sorted

semen; however, the combination of PGF administration 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol and TAI at 72 h after CIDR removal succeeded in enhancing (P = 0.02) PR/AI with sex-sorted semen. Outcomes of these experiments will be utilized to develop strategies to improve reproductive efficiency in beef females. Furthermore, these results will be utilized to develop a decision aid tool that will assist beef cattle producers in their decision on whether or not to incorporate sex-sorted semen in their operations.

DEDICATION

This dissertation is dedicated to my mother, Glenda Oosthuizen; sister, Gaby Oosthuizen; grandparents, Louis Snr and Pat Thomas; uncle, Louis Thomas Jnr; and cousins, Michaela and Jean-Luke Thomas.

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my committee chair, Dr. G. Cliff Lamb, for providing me with the opportunity to be a part of his lab and to attend Texas A&M University. I have thoroughly enjoyed working for him over the past few years and am thankful for his support, guidance, and great sense of humor. I appreciate how Dr. Lamb's door was always open when I had questions or needed advice, and how willing he was to make time to meet. I learnt a great deal from him and it was truly a privilege to have had him as my mentor and friend.

I would also like to thank Dr. Reinaldo F. Cooke, Dr. Rodolfo C. Cardoso, and Dr. David P. Anderson for agreeing to be a part of my committee; their guidance and support throughout the course of this research was greatly appreciated. I would also like to thank Dr. Tryon Wickersham for his friendly demeanor, great sense of humor, and his kind heart; Dr. Tom Welsh for being such a great supporter and advocate for graduate students; Dr. Katie Dunlap for her support and listening ear, and for being willing to serve as my teaching mentor; and Dr. David Forrest for his support of the Animal Science Graduate Student Association; my time at Texas A&M University would not have been the same without them. Furthermore, I would like to thank Dr. Bruce Carpenter for his friendship over the years and for many happy memories of teaching rectal palpation and artificial insemination at the monthly Bovine Elite artificial insemination school.

I appreciate the Animal Science Department staff members, Maggie Berger, Amy Carwile, Rebecca Binder, Jennifer Houston, Sarah Salas, Robbie Lukeman, and Linda Rice.

They are a wonderful group of people and I am thankful for their friendship, willingness to provide assistance, and for all of the laughs we have shared over the years.

I am thankful for the great friendships that I made during my time in College Station. I would like to acknowledge the graduate students, interns, and professors from the Cooke and Pohler labs for all of the fun happy hours, barbecues, and parties we enjoyed together as part of a joint lab group. More specifically, I would like to thank Kitty Halloran, Claire Stenhouse, Genevieve D'Souza, and Kelsey Schubach for their support during the tough times and for all of the laughs during the good; John Long, Lacey Quail, and Fernando Dias for their friendship; my roommate, Ramiro Oliveira, for all of the jokes and good memories; and to my South African friend, Rosie Olson, for teaching me all about the Aggie Traditions. I am particularly grateful to Pedro Levy Piza Fontes for the large role that he played throughout the course of my degree. His passion for science and research is unmatched, and I have learnt a significant amount from him throughout my graduate career. I am so thankful for his friendship and support; graduate school would not have been the same without him.

Finally, I would like to express my profound appreciation to my family in South Africa for their unfailing support and love, and for their continuous encouragement. Thank you to my mom, Glenda, for her incomparable strength and unconditional love; to my sister, Gaby, for her kind heart and incredible sense of humor; to my grandparents, Tappy and Pa, for always being tremendously proud of me; to my uncle, Louis, for getting me started on my journey in the United States and for all the laughs along the way; to my cousins, Michaela and Jean-Luke, for their love; and lastly to my dad, Jimmy, who was truly my biggest fan, and who was always in my thoughts throughout this journey - you are sorely missed. This accomplishment would not have been possible without them.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Cliff Lamb, Dr. Reinaldo Cooke, and Dr. Rodolfo Cardoso of the Department of Animal Science, and Dr. David Anderson of the Department of Agricultural Economics.

Collaborators included Dr. Ky Pohler of the Department of Animal Science at Texas A&M University; Drs. Charles Looney and Jeremy Powell of the Department of Animal Science at the University of Arkansas; Dr. Vitor Mercadante of the Department of Animal & Poultry Science at Virginia Tech; Dr. George Perry of the Animal Science Department at South Dakota State University; Dr. George Seidel of the Animal Science Department at Colorado State University; Dr. David Grieger of the Department of Animal Sciences and Industry at Kansas State University; Dr. John Hall of the Department of Animal and Veterinary Science at the University of Idaho; Dr. Carl Dahlen of the Department of Animal Sciences at North Dakota State University; Dr. Ligia Prezotto of the Department of Animal and Range Sciences at Montana State University; Dr. Ryon Walker of the Nobel Research Institute; and Drs. Vish Vishwanath and Aaron Arnett of ST Genetics. In addition, sincere appreciation is expressed to Kristina Porter of Kleinsasser-Porter Veterinary Service, as well as the graduate students and staff members of Texas A&M University that contributed to these projects.

Funding Sources

A portion of this work was supported by an ST Genetics research grant (M1900768) and by the Oregon Beef Council.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Over the course of the next 30 years the world's population is expected to reach 9.1 billion, and as a result, food production will need to increase by 70% (FAO, 2009). Of the total increase in food production required, meat production will need to rise by approximately 200 million tonnes and accordingly, there will be a greater demand for beef products. Urbanization will continue to rise and approximately 70% of the world's population will reside in urban areas by 2050. Consequently, urban areas will expand dramatically and agricultural land availability may be reduced. Therefore, increasing the amount of land utilized by food animal production systems may not be a feasible solution to improve food production but instead efforts should be focused on increasing the efficiency of the animals that are already a part of these systems.

The United States (**US**) is the largest producer of beef in the world, and as of January 1, 2020, the US had a cattle inventory of 94.4 million head, of which 31.3 million head were beef cows and 5.8 million head were beef replacement heifers (USDA, 2020). The reproductive performance of these beef females will not only determine the overall efficiency of cow-calf operations and the US beef industry but will significantly impact the world's food supply. Beef production has become a more efficient process over the past few decades, which is largely due to the development and adoption of new technologies, as well as an overall improvement in herd genetics. However, additional advancements in both management and technologies are required to reach a level of animal production that can provide for the population by 2050 (FAO, 2009).

Reproductive management technologies can be utilized to increase the reproductive efficiency and profitability of beef cattle production systems. Estrus synchronization is a reproductive management tool that may be utilized to increase the proportion of beef females becoming pregnant earlier in the breeding season, and as a result, is able to reduce the duration of the calving season and improve calf crop uniformity (Rodgers et al., 2012). When combined with fixed-time artificial insemination (**TAI**), estrus synchronization protocols have achieved pregnancy rates to TAI (**PR/AI**) similar to protocols that make use of estrus detection; therefore, estrus detection and its associated labor can be minimized or removed completely (Lamb et al., 2006; Larson et al., 2006). Numerous estrus synchronization protocols are currently available for use in beef heifers; however, in order to improve current PR/AI, enhancements to these protocols are necessary. Through presynchronization it is possible to increase the proportion of females at a certain stage of the estrous cycle prior to the initiation of an estrus synchronization protocol. Consequently, the synchrony of subsequent follicular waves and estrus expression can be improved (Kojima et al., 2000; Busch et al., 2007; Atkins et al., 2008), which may potentially lead to an increase in PR/AI. Estrus synchronization allows reproductive biotechnologies, such as sex-sorted semen, to be easily incorporated into a herd. By using sex-sorted semen, more progeny of the desired sex can be generated and genetic progress can be hastened; however, PR/AI are significantly lower than with that of conventional semen, and there are currently no TAI protocols developed specifically for the use of sex-sorted semen. Therefore, research into the development of estrus synchronization protocols specifically designed to increase PR/AI with sex-sorted semen is warranted.

Through the incorporation of reproductive management strategies and biotechnologies, there is potential to improve reproductive efficiency, genetic quality, and animal performance, which are required to support the ever-expanding world population.

1.1. References

Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. Lucy, and M. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: effect of day of the cycle. J. Anim. Sci. 86:83–93.

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933–1939. doi:10.2527/jas.2006-845.

FAO. 2009. How to feed the world in 2050. Insights from an Expert Meet. FAO. 2050:1–35. doi:10.1111/j.1728-4457.2009.00312.x.

Kojima, F. N., B. E. Salfen, J. F. Bader, W. A. Ricke, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 synch. J. Anim. Sci. 78:2186–2191.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2α, and progesterone. J. Anim. Sci. 84:3000–3009. doi:10.2527/jas.2006-220.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. Dejarnette, F. N. Schrick, A. Dicostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J. Anim. Sci. 84:332–342.

Rodgers, J. C., S. L. Bird, J. E. Larson, N. Dilorenzo, C. R. Dahlen, A. Dicostanzo, and G. C. Lamb. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. J. Anim. Sci. 90:4055–4062. doi:10.2527/jas.2011-4836.

USDA, NASS, and Agricultural Statistics Board. 2020. Cattle. Available from: https://usda.library.cornell.edu/concern/publications/h702q636h

2. LITERATURE REVIEW

2.1. The Bovine Estrous Cycle

Once a female attains puberty, she transitions into a period of reproductive cyclicity that continues throughout her lifetime; however, cyclicity may be interrupted by pregnancy and periods of postpartum anestrus. Bovine females are polyestrous, therefore, have uniform and regular estrous cycles throughout the year. The bovine estrous cycle typically ranges from 17 to 24 d, with a mean length of 21 d (Amstalden and Williams, 2015), and consists of two primary phases known as the follicular and luteal phases. These two phases may be further characterized into four stages which include metestrus and diestrus (during the luteal phase), and proestrus and estrus (during the follicular phase; Senger, 2011). Each stage is characterized by different hormonal concentrations, changes in follicular dynamics, and presence or absence of a corpus luteum (**CL**). Estrous cycle regulation is carried out by hormones that are secreted from the hypothalamus, pituitary gland, ovaries, and the uterus, which circulate throughout the circulatory system to their respective receptors in various tissues (Smith et al., 2005).

2.1.1. The Luteal Phase

Metestrus occurs between 1 to 5 d after ovulation and is characterized by a shift in hormonal dominance from estradiol (**E_2**) to progesterone (**P4**). The ovulated follicle briefly forms into a corpus hemorrhagicum before transforming into a CL (Senger, 2011). This transformation is complete when the granulosa and thecal cells of the follicle, which normally secrete E_2, undergo morphological and physiological changes, as a result of luteinizing hormone (**LH**) stimulation, to become P4-secreting luteal cells (Hansel and

Convey, 1983). Granulosa cells differentiate into large luteal cells, whereas the thecal cells differentiate into small luteal cells. This transformation is referred to as luteinization, and results in concentrations of E_2 declining to basal concentrations (Adams et al., 2008). In turn, luteal cells undergo hypertrophy, and the CL increases in size until it becomes a fully functioning P4-producing tissue (Yoshioka et al., 2013).

Diestrus is associated with high concentrations of P4, which are the result of a fully functioning CL, and occurs for a period of 10 to 14 d. The length of diestrus is directly related to the amount of time that the CL remains functional while secreting P4. Progesterone has a negative feedback on gonadotropin-releasing hormone (**GnRH**) secretion by the hypothalamus, which prevents preovulatory follicles from ovulating and inhibits behavioral estrus by inhibiting production of E_2 (Hansel and Convey, 1983). Towards the end of diestrus in a female that is not pregnant, prostaglandin $F_{2\alpha}$ (**PGF**) is released in pulses from the endometrium of the uterus and initiates regression of the CL, known as luteolysis. Luteolysis induces both functional and structural regression of the CL, and as a result, the CL decreases in size (Louis et al., 1974).

2.1.2. The Follicular Phase

Proestrus varies between 1 and 5 d (Bridges et al., 2010) and is characterized by a rapid decline in concentrations of P4, as a result of luteal cell apoptosis and consequent CL regression. The length of proestrus is determined by the size of the preovulatory follicle at the beginning of this stage (Sirois and Fortune, 1988). A decrease in P4 results in a decreased negative feedback to the hypothalamus and allows for an increase in GnRH and LH secretion (Rahe et al.,1980). Luteinizing hormone binds to receptors on thecal cells of the follicle to produce androgens that diffuse into the granulosa cells. Similarly, follicle-stimulating

hormone (**FSH**) binds to receptors on the granulosa cells and stimulates the synthesis of the enzyme, aromatase, which is responsible for the conversion of androgens into E_2 (Smith et al., 2005). As a result of this conversion, concentrations of E_2 rapidly increase. High concentrations of E_2 stimulate kisspeptin neurons in the hypothalamus to release kisspeptin, which stimulates GnRH neurons in the preoptic area to release GnRH (Pinilla et al., 2012). Gonadotrophin-releasing hormone is transported to the anterior pituitary via the hypothalamic-hypophysial portal system where it stimulates gonadotropes to release LH, and thereby increases LH pulse frequency (Rahe et al., 1980).

Estrus is characterized by high concentrations of E_2 and estrus behavior, and culminates with ovulation. The mean duration of estrus is ≈15 h but ranges from 6 to 24 h (Senger, 2011). High concentrations of E_2 result in large quantities of GnRH being released from the hypothalamus, and as a result, an LH surge occurs (Hansel and Convey, 1983). The LH surge triggers a series of biochemical events that lead to ovulation. Blood flow to the dominant follicle is elevated, which leads to an increase in follicular pressure. Prostaglandin $F_{2\alpha}$ is secreted by the endothelium of the uterus and induces smooth muscle contractions of the ovary. These contractions lead to the rupture of granulosa cell lysosomes which then release their enzymes. The release of these enzymes aids in additional follicle deterioration (Senger, 2011), and as a result, the dominant follicle ruptures and the oocyte is released.

2.1.3 Follicular Dynamics

A heifer calf is born with ≈156,000 primordial follicles, which is reduced to ≈117,000 primordial follicles by one year of age (Erickson, 1966). Throughout the duration of the bovine estrous cycle, follicles undergo a number of growth waves and degeneration periods before the final dominant follicle is ovulated. Based on transrectal ultrasonography,

most bovine estrous cycles consist of either two or three follicular waves (Sirois and Fortune, 1988). Cows typically have two follicular waves, where the second wave culminates in ovulation, and heifers generally have three follicular waves with the third wave terminating in ovulation (Fortune et al., 1988; Adams et al., 1992). Follicular development can be classified into four phases; recruitment, selection, dominance, and atresia.

Folliculogenesis is the process of follicular growth characterized by the physical and functional development of follicles. Follicular growth is stimulated by FSH (Adams et al., 2008), which binds to its receptors on granulosa cells of antral follicles present on the ovary, and stimulates E_2 production (Hansel and Convey, 1983). The first follicular growth wave following ovulation is stimulated around the onset of metestrus. Initially, the process of recruitment occurs where a cohort of antral follicles from the follicular pool will begin to grow and produce E_2. The majority of these follicles will reach a point at which their development will cease and they will undergo atresia (Smith et al., 2005). A cluster of follicles that did not undergo atresia will enter a selection process where one follicle will be selected to become dominant, and as a result, the other subordinate follicles will undergo atresia. The dominant follicle will continue to grow and will suppress the growth of subordinate follicles as well as suppress the emergence of a new follicular wave (Adams et al., 2008). If dominance occurs during diestrus, the high concentrations of P4 from the CL will suppress LH pulse frequency, and the dominant follicle may regress (Adams et al., 2008). If regression occurs, a new follicular wave will be initiated and the new dominant follicle may then either undergo atresia in the presence of P4, or may be destined to ovulate. After PGF induces luteolysis of the CL, the dominant follicle will produce increasing quantities of E_2 and will begin to secrete inhibin from granulosa cells, which inhibits the

release of FSH from the anterior pituitary. Therefore, the dominant follicle is destined to ovulate as a result of the positive feedback between E_2 and LH that results in an LH surge (Louis et al., 1974).

2.2. Manipulation of the Estrous Cycle Using Exogenous Hormones

Estrus synchronization has been utilized by cattle producers for over five decades to incorporate artificial insemination (**AI**) in their operations. Previously, the major deterrent for AI use was the time and labor associated with estrus detection and AI over a period of 60 to 90 d or more (Lauderdale, 2005). Subsequently, exogenous hormone analogues and estrus synchronization protocols were developed, and can be utilized to manipulate the estrous cycle and synchronize estrus in both cows and heifers. Estrus synchronization protocols differ in the combination of hormones used, method of hormone administration, number of injections, number of cattle handlings, timing of injections, and heat detection requirements (Parish et al., 2012). A number of protocols are specifically adapted for estrus synchronization in heifers, and make use of heat detection, heat detection in combination with fixed-time artificial insemination (**TAI**), and TAI only (BRTF, 2020). These protocols facilitate an improvement in reproductive rates and accelerate genetic progress when applied to estrous-cycling heifers, and can potentially induce an ovulatory estrus in prepubertal heifers (Patterson et al., 2013). The use of exogenous hormones in estrus synchronization enables AI of a group heifers at a similar, pre-determined time. Advantages of estrus synchronization include easier facilitation of AI and embryo transfer, reduced time and labor associated with estrus detection, earlier conception during the breeding season, and earlier calving resulting in older, heavier calves at the time of market (Rodgers et al., 2012).

Understanding the roles of these hormones in the estrous cycle is essential in order to improve current estrus synchronization protocols.

Hormones used in synchronization protocols typically include progestins, prostaglandins, and GnRH analogues. These hormones can be used to stimulate the estrous cycle at certain points and to mimic certain phases of the estrous cycle, such as supplemental progesterone to mimic diestrus (Senger, 2011). Estrous cycle control methods used to develop estrus synchronization protocols include: inhibition of ovulation with the use of a progestin, induced CL regression with PGF, induced ovulation with the use of GnRH (Smith et al., 2005), induction of follicle atresia with E_2 and a progestin, induction of a new follicular wave through follicle aspiration, or a combination of these strategies.

The first exogenous hormone to be found suitable for the control of the estrous cycle in a beef heifer was PGF in the 1970s (Lauderdale, 1972; Rowson et al., 1972; Tervit et al., 1973; Louis et al., 1974). Most of the early estrus synchronization protocols utilized PGF to induce luteolysis of the CL followed by detection of estrus. Concurrently, progestins were being researched for their ability to inhibit estrus from occurring in bovine females. Once protocols involving a single PGF injection and heat detection were successfully developed, double PGF injection protocols were investigated. The subsequent advance was to combine the administration of PGF with the estrus delaying capabilities of exogenous progestins, such as norgestomet (Heersche et al., 1979). Gonadotropin-releasing hormone was first reported to induce a release of LH in beef heifers in 1974 (Kaltenbach et al., 1974) and was shown to be an effective means of synchronizing the preovulatory LH surge 64 h after an injection of PGF (Fernandez-Limia et al., 1977). After the discovery of follicular growth waves (Sirois

and Fortune, 1988), GnRH was utilized for more accurate control of the bovine estrous cycle by synchronizing the selection of a new large growing follicle (Twagiramungu et al., 1995).

2.2.1. Prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ is a fatty acid hormone that is synthesized in, and primarily released from the uterine endometrium after stimulation by oxytocin (Lafrance and Goff, 1988). In cattle, PGF diffuses into the ovarian artery from the utero-ovarian vein, via a counter current transport mechanism, in order to bind to receptors on the CL and avoid being metabolized in the lungs (Lauderdale, 1972; Hansel et al., 1973; Lamond et al., 1973). Endogenous PGF specifically induces luteolysis of the CL (Lamond et al.,1973) at the end of diestrus in non-pregnant females (Louis et al., 1974), promotes uterine myometrium contractions during parturition, and aids in ovulation (Senger, 2011). Similarly, PGF analogues are used in estrus synchronization protocols to induce CL regression but are only effective at regressing a functional CL between d 5 and 16 of the estrous cycle (Rowson et al., 1972). Administration of PGF during this period will initiate premature regression of the CL where responding females will return to estrus within approximately 3 d after administration (Tervit et al., 1973; Lauderdale et al., 1974). Regression of the CL by PGF analogues has been shown to cause a 50% decrease in serum P4 concentrations approximately 12 h after administration (Louis et al., 1974).

Synthetic PGF analogues such as dinoprost tromethamine and cloprostenol sodium, are the active ingredients in common luteolytic exogenous hormones commercially marketed in the US as estroPLAN®, Estrumate®, In-Synch®, Lutalyse®, Lutalyse HighCon®, Prostamate®, and SYNCHSURE™. Numerous studies on the efficacy of different PGF analogues have been performed. Collectively, these studies reported no

differences among PGF products in their ability to decrease concentrations of P4 (Guay, Rieger, & Roberge, 1988; Schams & Karg, 1982; Stevenson & Phatak, 2010), to induce an estrus response (Plata et al., 1990; R. R. Salverson et al., 2002; Oosthuizen et al., 2018a), and have shown no significant differences in pregnancy rates (Plata et al., 1990; Hiers et al., 2003; Stevenson and Phatak, 2010; Oosthuizen et al., 2018a). The comparison of Lutalyse and Estrumate has been extensively studied, with no differences between products for estrus detection, conception, and pregnancy rates reported (Salverson et al., 2002; Martineau, 2003; Lauderdale, 2005). More recently, Lutalyse was compared to Lutalyse HighCon and no differences in estrus expression, time to estrus expression, or pregnancy rates to TAI (**PR/AI**) were determined (Oosthuizen et al., 2018a).

Use of a single injection of PGF was the foundation for current estrus synchronization protocols in beef cattle. The first protocol included 5 d of estrus detection and AI, administration of PGF, followed by estrus detection and AI for an additional 7 d. This single PGF injection protocol involves labor intensive estrus detection periods but is regarded as a cost effective estrus synchronization method. Studies have shown that the length of the estrous cycle is significantly reduced in heifers that have been treated with a single injection of PGF when compared to controls (Roche, 1973). Beef heifers that were exposed to a single PGF injection protocol had similar estrus detection rates in the first 5 d of the protocol when compared to controls (25%, 24%; Lauderdale, 2002). However, the number of heifers detected in estrus between d 1 and 9 was significantly greater in the treated group when compared to controls (64%, 38%). Conception rates did not differ between treated or control heifers on d 1 to 5 (62%, 62%), 1 to 9 (53%, 56%), and 1 to 24 (57%, 59%) of the breeding season. Conversely, pregnancy rates from d 1 to 9 of the breeding season

were significantly greater in treated heifers (45%) compared with the controls (24%; Lauderdale, 2002).

A two-injection PGF protocol was developed to account for females that had a non-responsive CL at the time of the first injection of PGF and those where a new CL formed after the first injection (Lauderdale, 2005). The two-injection PGF protocol involves two PGF injections administered 11 to 14 d apart to all females, followed by AI after estrus detection. This protocol aims to maximize the percentage of a herd that will respond to the treatment. Beef heifers synchronized with the two-injection PGF protocol had significantly greater estrus expression during the first 5 d of the AI season (64%) when compared with controls (17%; Lauderdale, 2002). Conception rates were similar between treated heifers and controls for the first 5 d (52%, 49%), and d 1 to 24 (53%, 56%) of AI. Pregnancy rates in the first 5 d of the breeding season were greater for treated heifers (34%) compared to controls (9%; Lauderdale, 2002).

2.2.2 Progestins

Endogenous P4 is secreted by the luteal cells of a functioning CL during diestrus, and is responsible for the maintenance of pregnancy. Progestins are a synthetic form of P4 that when used in estrus synchronization protocols, serve as an 'artificial' CL. The primary objective of administering progestins is to inhibit estrus activity and ovulation, which is associated with the negative feedback of P4 on the hypothalamus. Progesterone down-regulates E_2 receptors in the brain and therefore, inhibits many of E_2's effects (Brenner et al., 1974). When the progestin is removed, females initiate proestrus and estrus within 2 to 3 d of removal (Senger, 2011). Progestins are also able to induce estrous cyclicity in non-cycling, prepubertal heifers, and can improve their chances of establishing pregnancy during

a defined breeding period (Parish et al., 2012). Often, the luteal phase preceding the onset of puberty in heifers is short, which has been attributed to a premature release of PGF (Zollers et al., 1989). If conception is followed by a short luteal phase, the pregnancy will fail, as the embryo would not have had sufficient time to secrete interferon τ for maternal recognition of pregnancy (d 15 to 16 in cattle; Bazer et al., 2008). A period of P4 and a subsequent rise in preovulatory E_2 concentrations are necessary to establish a normal luteal lifespan and prevent the premature release of PGF (Kieborz-Loos et al., 2003). Therefore, the inclusion of a progestin in estrus synchronization protocols for heifers has the potential to prevent short-cycling and premature pregnancy loss (Atkins et al., 2013).

In the US, P4 is typically provided to cattle in the form of a controlled internal drug releasing (**CIDR**) vaginal insert, or is fed in the form of the orally active melengestrol actetate (**MGA**). When feeding MGA, heifers each need to consume a daily amount of 0.5 mg for it to be effective. Heifers that do not consume the required amount may return to estrus prematurely, which may reduce the estrus response prior to AI (Patterson et al., 2005). Melengestrol acetate is effective at synchronizing estrus, where the average interval to estrus following removal of MGA ranges from 3 to 7 d (Patterson et al., 1989). The initial MGA protocol involved feeding MGA for 14 d followed 10 d later by exposure to bulls. However, fertility has been shown to be decreased in cattle receiving only an oral progestin, as well as conception rates for the first estrus following removal of MGA from the feed, when compared to non-treated cattle (Zimbelman & Smith, 1966; Lamond et al., 1971). Therefore, it is generally recommended that females not be AI or exposed to bulls at the first estrus following MGA removal (Patterson et al., 2013).

2.2.3. Gonadotropin-Releasing Hormone

Gonadotropin-releasing hormone is secreted by GnRH neurons in the basal hypothalamus through kisspeptin stimulation and is responsible for the release of gonadotropins, FSH and LH, from the anterior pituitary (Amstalden and Williams, 2015). No GnRH receptors have been detected on the bovine ovary (Brown and Reeves, 1983); therefore, it has been deduced that GnRH acts indirectly on the ovary via induced FSH and LH release. After the discovery of follicle growth waves (Sirois and Fortune, 1988), GnRH analogs were used to increase precision of estrus synchronization protocols as the selection of a new large growing follicle could be synchronized (Twagiramungu et al., 1995). An injection of GnRH results in a significant release of FSH and LH 15 minutes later, with peak concentrations being reached at 120 minutes post-administration (Rodger & Stormshak, 1986; Chenault et al., 1990; Rettmer et al.,1992; Martínez, 2003). If a dominant follicle (≥ 10 mm) is present on the ovary at the time of GnRH administration, then the resulting LH surge (after 2 to 4 h) will induce ovulation (24 to 36 h later; Pursley et al., 1995), before the initiation of a new follicular wave approximately 1.6 d later (Roche et al., 1999). Administration of GnRH has no effect on the progression of a follicular growth wave if it is administered prior to dominant follicle selection (Roche et al., 1999). The stage of the estrous cycle at the time of GnRH administration also affects response, with cattle responding most consistently when GnRH is administered between d 5 and 12 of the estrous cycle (Vasconcelos et al., 1999; Atkins et al., 2008).

Different forms of native GnRH are used in estrus synchronization protocols in the US, such as gonadorelin diacetate tetrahydrate (sold as Cystorelin®, Fertagyl®, and OvaCyst®), gonadorelin acetate (sold as GONAbreed®), and gonadorelin hydrochloride

(sold as Factrel®). A study was conducted to test the different effects of Cystorelin, Fertagyl and Factrel on LH release, ovulation, and follicle wave emergence in cross-bred beef heifers. Heifers treated with Cystorelin had greater mean concentrations of LH and greater LH peaks; however, there were no differences in ovulation rate or the d of emergence of the next follicular wave between treatments (Martínez et al., 2003). Dairy cows treated with Factrel had reduced ovulation rates compared to those that received Cystorelin, Fertagyl, or Ovacyst (55.3 vs. 76.7, 73.6, and 85%; Souza et al., 2009); nonetheless, no differences in time to LH peak, or peak LH concentration were determined. Furthermore, no differences in PR/AI were noted in dairy cows treated with either Factrel or Cystorelin (35.7 vs. 38.4%; Poock et al., 2015).

2.2.4 Combining Progestins, PGF, and GnRH

When GnRH and PGF are both incorporated into an estrus synchronization protocol, it is possible to completely avoid estrus detection through the facilitation of TAI. By utilizing both hormones, the CL as well as the follicle can be controlled, and ovulation can be synchronized (Kasimanickam, 2015). In protocols that involve a combination of GnRH-PGF-GnRH followed by TAI, the first injection of GnRH is administered to all females, which are at various stages of the estrous cycle; therefore, if a dominant follicle is present on the ovary it may undergo ovulation, depending on its size, and a new follicular wave will be initiated. When a dominant follicle is not present, the GnRH injection has no effect. An injection of GnRH at random stages of the estrous cycle has resulted in different ovulation rates between cows and heifers. In cows, 75 to 90% (Pursley et al., 1995; Thompson., 1999) ovulated a follicle, whereas in heifers only 38 to 60% ovulated a follicle in response to GnRH administration at random stages of the estrous cycle (Macmillan & Thatcher, 1991; Pursley

et al., 1995; Dahlen et al., 2011a; Dahlen et al., 2011b). When ovulation occurs as an outcome of the first GnRH injection, luteolysis of the resulting CL is likely to be induced when PGF is administrated 7 d later. Once the inhibitory effect of P4 is removed, the dominant follicle is allowed to mature and may ovulate after the administration of the second GnRH injection (Whittier & Geary, 2000). The induction of ovulation through the use of GnRH without the expression of estrus may lead to decreased conception rates in TAI protocols (Bridges et al., 2012; Whittier et al., 2013).

The OvSynch protocol involves the administration of the second GnRH injection 2 d after PGF administration, followed by TAI approximately 16 h later (Kasimanickam, 2015). However, pregnancy rates to this protocol are reduced compared to heifers inseminated to a detected estrus (Schmitt et al., 1996 ; Pursley et al., 1997; Tenhagen et al. 2005), and are reduced when compared to cows (Martinez et al., 2002). Currently, there are two recommended OvSynch protocols in the dairy industry, the OvSynch 56 and OvSynch 48 TAI protocols (DCRC, 2020). The time between PGF administration and the second injection of GnRH differs between the protocols, which is either 56 or 48 h. However, both protocols have a 72 h period between PGF and TAI. In CO-Synch protocols, the second injection of GnRH is administered at the time of TAI instead of before TAI. Therefore, the CO-Synch protocol requires one less d of cattle handling compared to the Ovsynch protocol, and as a result, is favorable for estrus synchronization in beef cattle. Pregnancy rates to the CO-Synch protocol in cows have ranged from 31 to 61% (Geary et al. 1998; Martinez et al., 2002; Stevenson et al., 2003; Lamb et al., 2001). Pregnancy rates in heifers are generally low in GnRH-PGF based TAI protocols that do not include a progestin, and have ranged between 26 and 39% (Schmitt et al., 1996; Pursley et al., 1997; Martinez et al., 2002). The

lower pregnancy rates in heifers may be related to the lower response rate to the first injection of GnRH when compared to cows (Macmillan & Thatcher, 1991; Pursley et al., 1995; Martinez et al., 2002); therefore, it is more difficult to synchronize their follicular waves. Furthermore, lower pregnancy rates could be attributed to heifers expressing estrus greater than 24 h before TAI (Hall et al., 2009).

All recommended TAI protocols in beef heifers involve the use of GnRH, PGF, and a progestin (BRTF, 2020). By including a progestin in TAI protocols, greater pregnancy rates can be achieved, and the exposure to low levels of P4 for seven to ten d may induce puberty in heifers (Hall et al., 2009). Addition of a CIDR to the CO-synch protocol (7-d CO-Synch + CIDR and the 5-d CO-Synch + CIDR protocols) has resulted in greater PR/AI. The 5-d CO-Synch + CIDR protocol involves the insertion of a CIDR on Day 0 at the time of GnRH administration, CIDR removal on Day 5 along with administration of two injections of PGF given 8 ± 2 h apart, and followed 72 ± 2 h later by TAI and an injection of GnRH. This protocol has yielded pregnancy rates between 52 to 69% in heifers (Kasimanickam et al., 2009; Palomares et al., 2015; Say et al., 2016; White et al., 2016). The 7-d CO-Synch + CIDR protocol is one of the most commonly used estrus synchronization protocols in beef females and involves the addition of a CIDR insert between Day 0 and 7 of the CO-Synch protocol (Lamb et al., 2006). In a study where the CO-Synch and the 7-d CO-Synch + CIDR protocols were compared, pregnancy rates were greater in heifers that received the 7-d CO-Synch + CIDR over the CO-Synch protocol (39.1 vs. 68%; Martinez et al., 2002). Pregnancy rates in heifers exposed to the 7-d CO-Synch + CIDR protocol have been reported to range from 40 to 68% (Martinez et al., 2002; Busch et al., 2007; Dahlen et al., 2011b; Oosthuizen

et al., 2018a). These protocols are widely used to synchronize estrus in heifers but do require a moderate amount of labor, and can incur high pharmaceutical costs.

2.2.5. Presynchronization

More recently, research into the administration of exogenous hormones prior to the initiation of estrus synchronization protocols has been performed, and has been termed presynchronization. By utilizing hormones before the initiation of a TAI protocol, the percentage of females at a similar stage in their estrous cycle may be increased, which may lead to an increase in the response to the initial injection of GnRH, and may improve the synchronization of follicular waves (Kojima et al., 2000; Busch et al., 2007; Atkins et al., 2008). Furthermore, presynchronization may synchronize estrus more effectively, with resulting greater fertility (Patterson et al., 2003).

Presynchronization with a progestin before the administration of GnRH and PGF in beef heifers has led to improvements in PR/AI (Busch et al., 2007). The CIDR Select protocol involves presynchronization with a CIDR for 14 d, followed 9 d later by initiation of the Select Synch and CO-Synch TAI protocols. Heifers treated with the CIDR Select protocol had greater pregnancy rates (62%) compared to those receiving the 7-d CO-Synch + CIDR protocol (47%), and had a greater synchronized estrus response (87 vs. 69%; Busch et al., 2007). The CIDR Select protocol improved the synchrony of estrus and ovulation compared to the Select Synch + CIDR protocol (Leitman et al., 2008). Suckled beef cows presynchronized with a once-used CIDR insert for 15 d prior to the CO-Synch protocol had greater dominant follicle diameters at the first GnRH injection, had an increased ovulation rate to the first injection of GnRH, had a greater response rate to the PGF injection, and had

larger follicles at TAI (Small et al., 2009). However, no differences in PR/AI were determined.

Utilization of GnRH as a presynchronization strategy provides an additional opportunity to induce CL formation, and thereby an endogenous source of P4 prior to estrus synchronization (DeJarnette et al., 2001). In beef cows, presynchronization with GnRH increased conception rates to TAI compared to controls (26 vs. 13%) but did not alter the synchrony of estrus (DeJarnette et al., 2001). In beef heifers, presynchronization with GnRH 6 d before the initiation of a GnRH-PGF-GnRH TAI protocol produced similar synchronized pregnancy rates compared to the TAI protocol alone (25.4 vs. 22.1%; Dahlen et al., 2003).

Presynchronization with PGF is currently used in protocols, such as the PGF 6-d CIDR and TAI protocol, where an injection of PGF is administered 3 d prior to a GnRH injection and CIDR insertion, followed 6 d later by administration of PGF as well as CIDR removal. A GnRH injection is administered at the time of TAI 72 to 84 h later. This protocol makes use of estrus detection and AI 3 d prior to CIDR insertion and 3 d after CIDR removal. In beef cows, this protocol yielded similar pregnancy rates compared to cows that were synchronized with the 7-d CO-Synch + CIDR protocol (55.5 vs. 52.2%; Hill, 2013). Nevertheless, the presynchronized cows had larger follicles on Day -10 and more follicles ovulated after the first GnRH injection (60.6 vs. 36.5%). Similar results were achieved in another study where more beef cows ovulated after the initial GnRH injection in the PGF 6-d CIDR protocol, compared with the 5-d CO-Synch + CIDR (88 vs. 68%); however, in this study, pregnancy rates were greater in the presynchronized treatment group (64 vs. 55%; Perry et al., 2012). In beef heifers, the addition of an injection of PGF 3 d before the PGF 6-d CIDR protocol increased the percentage of heifers initiating a new follicular wave (88 vs.

60%), and increased the synchrony of estrus after CIDR removal compared to the Select Synch + CIDR protocol (Grant et al., 2011). Conversely, an injection of PGF 7 d prior to the initiation of a 7-d CO-Synch + CIDR protocol decreased estrus expression between CIDR removal and TAI but had no effect on PR/AI, indicating that presynchronized heifers may have ovulated later than those which were not presynchronized (Oosthuizen et al., 2018b).

The combination of PGF and a progestin as a presynchronization strategy has also been evaluated. Presynchronization with PGF and a once-used CIDR insert 7 d prior to the initiation of the 7-d CO-Synch protocol was able to increase the diameter of the largest follicle at the first GnRH injection (12 vs. 16 mm), increased the ovulation response to the first GnRH injection (55 vs. 77%), and increased the mean diameter of the dominant follicle at the second PGF from 13 to 14 mm (Small et al., 2009). In addition, presynchronization with an injection of PGF and a twice-used CIDR insert 5 d prior to the 7-d CO-Synch + CIDR protocol succeeded in increasing the response to the initial injection of GnRH (60 vs. 36%; Small et al., 2009).

2.3. The Use of Sex-Sorted Semen in the Beef Cattle Industry

One of the more recent biotechnologies incorporated into beef cattle operations is that of sex-sorted semen. Through flow cytometry, sperm cells carrying either an X (X-sperm) or Y chromosome (Y-sperm) are separated based on DNA content, as X-sperm contain approximately 4% more DNA than Y-sperm (Seidel, 2014). When flow cytometry was initially developed in the early 1980s, it produced de-membraned, unviable sperm. By 1989 the procedure had been refined and was able to sort sperm cells without killing or severely damaging them, and in 1991 the sorting procedure was patented by the US

Department of Agriculture (Seidel, 2014). In 1989, the first live birth from sex-sorted semen was achieved when rabbits were surgically inseminated with X-sperm (Johnson et al., 1989); however, it was only in 1997 that the first calves were produced using sex-sorted semen by nonsurgical AI (Seidel et al., 1997). Commercialization of sexed semen took off when Sexing Technologies Inc. (Navasota, TX) was granted a sorting license in 2003 (DeJarnette et al., 2009).

2.3.1. Semen Sorting Procedure

Since 2007, the commercialization of sex-sorted semen has dramatically increased due to enhancements in equipment and processing procedures. During the sorting procedure, sperm cells are stained using a fluorescent dye, Hoechst 33342, which penetrates the sperm membranes and is able to bind to the DNA. A laser provides a wavelength of light that causes sperm cells to fluoresce, and a computer will detect and analyze the amount of fluorescence given off. X-sperm give off approximately 4% more fluorescence than Y-sperm because they have an additional 4% of DNA. A different electrical charge is placed on X- and Y-sperm, which allows them to be sorted into different containers when passing between two oppositely charged electrical fields. If a sperm droplet contains an X-sperm, a positive charge is added; if it contains a Y-sperm, a negative charge is added; if the droplet does not contain a sperm cell, if the sperm cell is damaged, or if the fluorescence is indistinguishable, then no charge is added and the droplet will be collected separately (Seidel, 2007).

2.3.2. Application of Sex-Sorted Semen

By incorporating sex-sorted semen as a reproductive management strategy, producers are able to select calf gender with greater than 90% accuracy and can achieve faster genetic progress (Seidel, 2014). In addition, it is easy to incorporate the use of sex-

sorted semen into a management system if AI is already being performed, as it will not change the workflow. However, pregnancy rates with sex-sorted semen are significantly lower than those of conventional semen, and sex-sorted semen is more expensive, which limits its economic feasibility.

Sex-sorted semen is primarily utilized in the dairy industry in heifers to generate heifer calves that will eventually be used as replacements. Traditionally, the greater utilization in heifers was due to concerns over reduced pregnancy rates associated with sex-sorted semen in cows, where reductions of 13 to 18 % were reported in dairy cows (DeJarnette et al., 2009). However, more recent research suggests that sex-sorted semen can be used effectively in both dairy cows and heifers (Butler et al., 2014). Utilization of sex-sorted semen in the beef industry is significantly lower, where it is either used to produce replacement females or to produce males for beef production, as bulls and steers are more efficient at converting feed to muscle. In purebred operations, sex-sorted semen can be used to generate progeny of the desired sex, such as bulls from superior sires or daughters from elite cows. When used in conjunction with *in vitro* fertilization (**IVF**), sex-sorted semen can be used to produce embryos of a desired sex. However, there are currently no official TAI protocols established specifically for the use of sex-sorted semen, which limits its adoption in the beef industry

2.3.3. Fixed-Time Artificial Insemination and Embryo Transfer

Pregnancy rates after estrus detection and AI with sex-sorted semen are in the range of 80 to 90% of those from conventional semen (Deutscher et al., 2002; DeJarnette et al., 2009; Seidel, 2014). This reduction in fertility is one of the largest hindrances to the use of sex-sorted semen in beef cattle operations, and is largely due to a lower post-thaw motility,

a reduced number of sperm cells with intact membranes, and acrosomal alterations that can occur during the sorting process (Schenk et al., 2009; Carvalho et al., 2010). However, no differences in fertilization rate, cleavage rate, or blastocyst rate have been determined between sex-sorted and conventional sperm (Carvalho et al., 2010).

Fixed-time AI with sex-sorted semen has resulted in PR/AI between 32 to 70% of those from conventional semen (Sales et al., 2011; Thomas et al., 2014). Numerous studies have attempted to improve PR/AI with sex-sorted semen, such as through deep uterine horn AI and by delaying AI in TAI protocols. In dairy heifers, the site of insemination and dominant follicle size at TAI did not affect PR/AI with either sex-sorted or conventional semen (Ingenhoff et al., 2017). Furthermore, delaying TAI in beef cows from 72 to 80 h was unsuccessful at increasing PR/AI (35.4 vs. 34.8%; Hall et al., 2017). Conversely, delaying TAI from 54 to 60 h after progestin removal in dairy heifers increased PR/AI (16.2 vs. 31.4%); however, PR/AI were still significantly lower than those of conventional semen (31.4 vs. 51.8%; Sales et al., 2011). Recently, improvements to sperm sexing technology led to the production of SexedULTRA™ semen (ST Genetics, Navasota, TX) that is sold at a concentration of 4 x 10^6 spermatozoa per straw as opposed to 2 x 10^6 in the conventional sex-sorted semen straws (Thomas et al., 2017). However, when SexedULTRA™ was incorporated into a split-time AI protocol, there was still a tendency for PR/AI to differ between conventional and sex-sorted semen (60 vs. 52%; Thomas et al., 2017).

In embryo production, there are a number of differences between the use of sex-sorted and conventional semen. In superovulated dairy heifers and cows, the number of viable embryos collected was significantly reduced in the females that received sex-sorted vs. conventional semen (Mikkola and Taponen, 2017). Furthermore, there were greater

numbers of unfertilized ova and degenerate embryos produced in the sexed treatment group, and embryo quality grades were significantly lower for cows and heifers receiving sex-sorted semen. However, conception rates to sex-sorted semen through IVF and fixed-time embryo transfer were greater than that of TAI in crossbred beef cows (42 vs. 30%; Pellegrino et al., 2016).

2.4. Economic Considerations

Economic viability of new technologies plays a large role in their adoption. If a new technology is going to increase initial costs, the associated advantage or profit needs to be perceived as great enough to overcome the additional costs for a producer to want to incorporate it into their operation. A partial budget analysis is a method utilized to analyze variations in revenue and expenses, and profit potential after minor changes are made to an initial budget (Kay et al., 2016). By creating a partial budget analysis, a possible outcome for a beef enterprise, based on the differences in management decisions and expected results, can be fine-tuned based on a default budget. Economic decisions regarding the incorporation of reproductive management technologies may be made using a partial budget analysis as a tool to calculate the expected change in profit.

2.4.1. Estrus Synchronization and Fixed-Time Artificial Insemination

By using estrus synchronization, more females will become pregnant closer to the beginning of the breeding season, and as a result, will give birth closer to the beginning of the calving season. A more concentrated early calving season leads to an older, more uniform calf crop; therefore, calves have more time to gain weight and may have greater weaning weights at market, which may result in a net increase in profit. When beef cows were exposed

to estrus synchronization and TAI, their calving distribution was shifted earlier than those cows that were not exposed (Rodgers et al., 2012). Furthermore, weaning weights were significantly greater for cows in the TAI treatment (193.4 ± 4.3 kg) compared to cows in the control treatment (175.9 ± 4.3 kg). According to a partial budget analysis, by increasing weaning weights of the calves, net profit was increased by $49.14 per cow exposed to TAI compared to untreated controls (Rodgers et al., 2012). Similarly, it was reported than calves born as a result of estrus synchronization and TAI were 10 d older and 72 pounds heavier at weaning (Anderson and Deaton, 2003). When savings on bull purchases were included, return on investment was calculated at $129 per cow that was synchronized when compared to those that were only exposed to natural service (Anderson and Deaton, 2003).

There are also opportunities to increase profitability of beef cattle operations by marketing targeted genetics from the use of superior AI sires. A stochastic model was developed to compare costs of various estrus synchronization and AI systems with natural service and to identify factors that play large roles in the differences between the costs of breeding systems (Johnson and Jones, 2008). According to the model, semen cost and the premium for genetic value were consistently in the top three factors that determined expected economic differences between natural service and AI systems. The net present value (**NPV**) of semen from a particular sire is the difference between the value of the discounted net income earnings from genetic improvement and the cost of the semen. When AI service sires were ranked according to their NPV, the average NPV of the 20 most profitable sires was $22.51, which was substantially greater than the average NPV of all 552 sires in the analysis at $3.23 (Baker et al., 2004). Furthermore, the Angus Association reported that carcass value was $206 greater per head when Angus sires from the top 10% for carcass value were utilized

when compared to the bottom 10%, based on an average choice-select spread (Suther, 2000). In some cases, it may be difficult to quantify an exact increase in profit as a result of genetic gain from incorporation of AI. However, a survey reported that 17% of beef producers estimate that they receive a premium of $50 to $100 per calf that is born of AI, whereas 31% estimate that they receive a premium greater than $100 per calf that is born of AI vs. natural service (Johnson et al., 2011).

When creating a partial budget analysis for estrus synchronization and TAI, increased returns may be generated as a result of heavier weaned calves, improved genetics through the use of an AI sire, and increased uniformity of the calf crop; whereas decreased returns may be a result of having fewer cull bulls to sell. Decreased costs may be a result of fewer bulls needing to be purchased and maintained, less labor required in a more concentrated calving season, and more predictable calving ease; whereas increased costs may be incurred by the purchase of estrus synchronization products and semen, additional labor, and facilities that need to be improved (Johnson and Jones, 2005).

2.4.2 Sex-Sorted Semen

As a result of reduced PR/AI and the greater costs associated with purchasing sex-sorted semen, it may not be a viable reproductive management option in every beef cattle operation. The financial feasibility will rely on the achieved pregnancy success, the purchase cost of the sex-sorted semen, and the increased value of the calf crop.

A majority of the economic research on sex-sorted semen has been conducted in dairy cattle enterprises. An economic evaluation of the use of sex-sorted semen in the dairy industry reported that the primary value of sex-sorted semen is a result of the increased probability of having heifer calves born, as male dairy calves may sell for $50 whereas

female calves may be worth $450 (DeVries, 2008). This analysis determined that the value of sex-sorted semen breedings under default assumptions was $10.35 per heifer. Furthermore, it was concluded that the value of sex-sorted semen depends greatly on heifer price, sex-sorted semen price, and the relative decrease in conception rates. In the same study, a sensitivity analysis reported that when heifer calves were worth $300 then few scenarios made sense economically; however, when heifer calves were worth $500 then most scenarios made sex-sorted semen a profitable choice (DeVries, 2008). According to a simple economic analysis on dairy heifers AI with sex-sorted sperm, the calves from the more valuable sex should be worth at least $200 more to make sense economically based on their assumptions (Seidel, 2003). A case study conducted in dairy cattle revealed that production costs were influenced by the pregnancy rate, the resulting number of offspring produced of the desired sex, and the semen cost (Osada et al., 2019). In the same study, although pregnancy rates were lower when sex-sorted semen was utilized, birth rate of heifers and milk quality were improved, which suggests that utilizing sex-sorted semen may be beneficial economically. Consideration also needs to be placed on the reduced incidence of dystocia when X-sorted semen is utilized. A simple economic model showed that the use of sex-sorted semen in nulliparous heifers reduced the dystocia rate by 3.7%, of which the estimated savings were calculated as $5.38 per calving (Fetrow et al., 2007).

More research into the economics of incorporating sex-sorted semen in the beef industry is required. An accurate economic analysis needs to take the following into account: the sex-ratio with sex-sorted semen is approximately 90%, sex-sorted semen achieves reduced PR/AI when compared to conventional semen, there are advantages of reduced dystocia in heifers when X-sperm is utilized, there is genetic gain from sex-sorted sperm,

and more pounds of beef can be produced when Y-sperm is used. Until PR/AI can be increased, the sorting procedure is improved, or sex-sorted semen becomes more affordable, the incorporation of sex-sorted semen in the beef industry may be limited primarily to niche markets.

2.5. References

Adams, G. P., R. Jaiswal, J. Singh, and P. Malhi. 2008. Progress in understanding ovarian follicular dynamics in cattle. Theriogenology. 69:72–80. doi:10.1016/j.theriogenology.2007.09.026.

Adams, G. P., R. L. Matteri, J. P. Kastelic, J. C. Ko, and O. J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fertil. 94:177–188. doi:10.1530/jrf.0.0940177.

Amstalden, M., and G. L. Williams. 2015. Neuroendocrine control of estrus and ovulation. In: R. M. Hopper, editor. Bovine Reproduction. Wiley Blackwell. p. 203–218.

Anderson, L., and P. Deaton. 2003. Economics of estrus synchronization and artificial insemination. In: Beef Improvement Federation Research Symposium & Convention.

Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. Lucy, and M. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: effect of day of the cycle. J. Anim. Sci. 86:83–93.

Atkins, J. A., K. G. Pohler, and M. F. Smith. 2013. Physiology and endocrinology of puberty in heifers. Vet. Clin. North Am. - Food Anim. Pract. 29:479–492. doi:10.1016/j.cvfa.2013.07.008.

Baker, D. A., C. R. Shumway, and C. T. Gaskins. 2004. Selecting AI beef sires for maximum profit. Prof. Anim. Sci. 20:336–343.

Bazer, F. W., R. C. Burghardt, G. A. Johnson, T. E. Spencer, and G. Wu. 2008. Interferons and progesterone for establishment and maintenance of pregnancy: interactions among novel cell signaling pathways. Reprod. Biol. 8:179–211. doi:10.1016/S1642-431X(12)60012-6.

Brenner, R. M., J. A. Resko, and N. B. West. 1974. Cyclic changes in oviductal morphology and residual cytoplasmic estradiol binding capacity induced by sequential estradiol progesterone treatment of spayed rhesus monkeys. Endocrinology. 95:1094–1104.

Bridges, G. A., J. K. Ahola, C. Brauner, L. H. Cruppe, J. C. Currin, M. L. Day, P. J. Gunn, J. R. Jaeger, S. L. Lake, G. C. Lamb, G. H. L. Marquezini, R. K. Peel, A. E. Radunz, J. S. Stevenson, and W. D. Whittier. 2012. Determination of the appropriate delivery of prostaglandin f2a in the five-day co-synch + controlled intravaginal drug release protocol in suckled beef cows. J. Anim. Sci. 90:4814–4822. doi:10.2527/jas.2011-4880.

Bridges, G. A., M. L. Mussard, C. R. Burke, and M. L. Day. 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. Anim. Reprod. Sci. 117:208–215. doi:10.1016/j.anireprosci.2009.05.002.

Brown, J., and J. Reeves. 1983. Absence of specific luteinizing hormone releasing hormone receptors in ovine, bovine and porcine ovaries. Biol. Reprod. 29:1179–1182.

BRTF. 2020. Applied reproductive strategies in beef cattle. Available from: http://beefrepro.unl.edu/

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933–1939. doi:10.2527/jas.2006-845.

Butler, S. T., I. A. Hutchinson, A. R. Cromie, and L. Shalloo. 2014. Applications and cost benefits of sexed semen in pasture-based dairy production systems. Animal. 8:165–172. doi:10.1017/S1751731114000664.

Carvalho, J. O., R. Sartori, G. M. Machado, G. B. Mourão, and M. A. N. Dode. 2010. Quality assessment of bovine cryopreserved sperm after sexing by flow cytometry and their use in in vitro embryo production. Theriogenology. 74:1521–1530. doi:10.1016/j.theriogenology.2010.06.030.

Chenault, J. R., D. D. Kratzer, R. A. Rzepkowski, and M. C. Goodwin. 1990. LH and FSH response of Holstein heifers to fertirelin acetate, gonadorelin and buserelin. Theriogenology. 34:81–98. doi:10.1016/0093-691X(90)90579-I.

Dahlen, C. R., G. C. Lamb, C. M. Zehnder, L. R. Miller, and A. DiCostanzo. 2003. Fixed-time insemination in peripuberal, lightweight replacement beef heifers after estrus synchronization with PGF2alpha and GnRH. Theriogenology. 59:1827–1837. doi:10.1016/S0093-691X(02)01255-4.

Dahlen, C. R., G. H. L. Marquezini, J. E. Larson, and G. C. Lamb. 2011a. Human chorionic gonadotropin influences ovarian function and concentrations of progesterone in prepubertal Angus heifers. J. Anim. Sci. 89:2739–2749. doi:10.2527/jas.2010-3712.

Dahlen, C. R., G. H. L. Marquezini, J. E. Larson, and G. C. Lamb. 2011b. Fixed-time artificial insemination in replacement beef heifers after estrus synchronization with human chorionic gonadotropin or gonadotropin-releasing hormone. J. Anim. Sci. 89:2750–2758. doi:10.2527/jas.2010-3824.

DCRC. 2020. Reproductive Management Strategies for Dairy Cows. Available from: www.dcrcouncil.org

DeJarnette, J. M., M. L. Day, R. B. House, R. A. Wallace, and C. E. Marshall. 2001. Effect of GnRH pretreatment on reproductive performance of postpartum suckled beef cows following synchronization of estrus using GnRH and PGF2a. J. Anim. Sci. 79:1675–1682.

DeJarnette, J. M., R. L. Nebel, and C. E. Marshall. 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology. 71:49–58. doi:10.1016/j.theriogenology.2008.09.042.

Deutscher, G. H., R. Davis, G. Seidel, and J. Schenk. 2002. Use of sexed (female) sperm is successful in yearling heifers. In: 2002 Nebraska Beef Cattle Report. Rep. No. Univ. of Nebraska, Lincoln, NE. p. 12-14.

DeVries, A. 2008. Sexed semen economics. 45th Florida Dairy Prod. Conf. 67–83.

Erickson, B. H. 1966. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. 25:800–805.

Fernandez-Limia, O., B. D. Murphy, and J. G. Manns. 1977. Synchronization of preovulatory LH surges after PGF2a in beef cattle: the effects of D-Ala-GnRH and testosterone benzoate. Theriogenology. 7:73–81.

Fetrow, J., M. Overton, and S. Eicker. 2007. Sexed Semen: economics of a new technology. In: Western Dairy Management Conference.

Fortune, J.E., Sirois, J., Quirck, S. M. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology. 29:95–109.

Geary, T. W., and J. C. Whittier. 1998. Effects of a Timed Insemination Following Synchronization of Ovulation Using the Ovsynch or CO-Synch Protocol in Beef Cows. Prof. Anim. Sci. 14:217–220.

Grant, J. K., F. M. Abreu, N. L. Hojer, S. D. Fields, B. L. Perry, and G. A. Perry. 2011. Influence of inducing luteal regression before a modified controlled internal drug-releasing device treatment on control of follicular development. J. Anim. Sci. 89:3531–3541. doi:10.2527/jas.2011-3852.

Guay, P., D. Rieger, and S. Roberge. 1988. Superovulatory and endocrine responses in Holstein heifers treated with either prostaglandin F2a, cloprostenol or fenprostalene. Theriogenology. 29:1193–1199.

Hall, J. B., R. K. Kasimanickam, J. B. Glaze, and M. C. Roberts-Lew. 2017. Impact of delayed insemination on pregnancy rates to gender selected semen in a fixed-time AI system. Theriogenology. 102:154–161. doi:10.1016/j.theriogenology.2017.07.014.

Hall, J. B., A. Liles, and W. D. Whittier. 2009. Estrus Synchronization for Heifers. Virginia Cooperative Extension. Pub. No. 400-302. Virginia Tech Univ., Blacksburg, VA. p. 1-7.

Hansel, W., P. W. Concannon, and J. H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222–245.

Hansel, W., and E. M. Convey. 1983. Physiology of the estrous cycle. J. Anim. Sci. 57:404–424.

Heersche, G., G. H. Kiracofe, R. C. DeBenedetti, S. Wen, and R. M. McKee. 1979. Synchronization of estrus in beef heifers with a norgestomet implant and prostaglandin F2α. Theriogenology. 11:197–208.

Hiers, E. A., C. R. Barthle, M. V. Dahms, G. E. Portillo, G. A. Bridges, D. O. Rae, W. W. Thatcher, and J. V. Yelich. 2003. Synchronization of Bos indicus x Bos taurus cows for timed artificial insemination using gonadotropin-releasing hormone plus prostaglandin F2a in combination with melengestrol acetate. J. Anim. Sci. 81:830–835.

Hill, S. L. 2013. Presynchronizing $PGF_{2\alpha}$ or $PGF_{2\alpha}$ + GnRH before a Fixed Time Artificial Insemination (TAI) CO-Synch + CIDR Program in Suckled Beef Cows. PhD Diss. Kansas State Univ., Manhattan, KS.

Ingenhoff, L., E. Hall, S. Ranjbar NI, and J. House. 2017. Effect of insemination site and diameter of the pre-ovulatory follicle on the odds of pregnancy in heifers using sexed or non-sexed semen. Aust. Vet. J. 95:317–324. doi:10.1111/avj.12621.

Johnson, L. A., J. P. Flook, and H. W. Hawk. 1989. Sex preselection in rabbits: Live births from X and Y sperm separated by DNA and cell sorting. Biol. Reprod. 41:199–203.

Johnson, S. K., R. N. Funston, J. B. Hall, D. J. Kesler, G. C. Lamb, J. W. Lauderdale, D. J. Patterson, G. A. Perry, and D. R. Strohbehn. 2011. Multi-state beef reproduction Task Force provides science-based recommendations for the application of reproductive technologies. J. Anim. Sci. 89:2950–2954. doi:10.2527/jas.2010-3719.

Johnson, S. K., and R. Jones. 2005. Costs and comparisons of estrus synchronization systems. In: Applied Reproductive Strategies in Beef Cattle. p. 1–15.

Johnson, S. K., and R. D. Jones. 2008. A Stochastic Model to Compare Breeding System Costs for Synchronization of Estrus and Artificial Insemination to Natural Service. Prof. Anim. Sci. 24:588–595. doi:10.15232/s1080-7446(15)30909-8.

Kaltenbach, C. C., T. G. Dunn, T. E. Kiser, L. R. Corah, A. M. Akbar, and G. D. Niswender. 1974. Release of FSH and LH in beef heifers by synthetic gonadotrophin releasing hormone. J. Anim. Sci. 38:357–362.

Kasimanickam, R., M. L. Day, J. S. Rudolph, J. B. Hall, and W. D. Whittier. 2009. Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone-based synchronization protocol in beef cows. Theriogenology. 71:762–767. doi:10.1016/j.theriogenology.2008.09.049.

Kasimanickam, R. K. 2015. Pharmacological Intervention of Estrous Cycles. In: R. M. Hopper, editor. Bovine Reproduction. p. 304–313.

Kay, R. D., W. M. Edwards, and P. A. Duffy. 2016. Partial budgeting. In: Farm Management. Eighth. McGraw-Hill Education, New York. p. 215–225.

Kieborz-Loos, K. R., H. A. Garverick, D. H. Keisler, S. A. Hamilton, B. E. Salfen, R. S. Youngquist, and M. F. Smith. 2003. Oxytocin-induced secretion of prostaglandin F2α in postpartum beef cows: Effects of progesterone and estradiol-17β treatment. J. Anim. Sci. 81:1830–1836. doi:10.2527/2003.8171830x.

Kojima, F. N., B. E. Salfen, J. F. Bader, W. A. Ricke, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 synch. J. Anim. Sci. 78:2186–2191.

Lafrance, M., and A. K. Goff. 1988. Effects of progesterone and oestradiol-17 beta on oxytocin-induced release of prostaglandin F-2 alpha in heifers. J. Reprod. Fertil. 82:429–36.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:3000–3009. doi:10.2527/jas.2006-220.

Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253–2259. doi:/2001.7992253x.

Lamond, D. R., J. F. Dickey, D. M. Hendricks, J. R. Hill, and T. M. Leland. 1971. Effect of a progestin on the bovine ovary. J. Anim. Sci. 33:77–82.

Lamond, D. R., R. V. Tomlinson, M. Dorst, D. M. Henricks, and W. Jöchle. 1973. Studies of prostaglandin F2 alpha in the cow. Prostaglandins. 4:269–284. doi:10.1017/CBO9781107415324.004.

Lauderdale, J. W. 1972. Effects of PGF2alpha on pregnancy and estrous cycles of cattle. J. Anim. Sci. 35:246. (Abstr.).

Lauderdale, J. W. 2002. Use of prostaglandin F2alpha (PGF2alpha) in cattle breeding. In: M. J. Fields, R. S. Sand, and J. V. Yelich, editors. Factors Affecting Calf Crop: Biotechnology of Reproduction. p. 23–28.

Lauderdale, J. W. 2005. History, efficacy and prostaglandin F2 alpha for estrous synchronization. In: Applied Reproductive Strategies in Beef Cattle. p. 24–38.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of cattle following PGF2a injection. J. Anim. Sci. 38:964–967. doi:10.2527/jas1974.385964x.

Leitman, N. R., D. C. Busch, J. F. Bader, D. A. Mallory, D. J. Wilson, M. C. Lucy, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2008. Comparison of protocols to synchronize estrus and ovulation in estrous-cycling and prepubertal beef heifers. J. Anim. Sci. 86:1808–1818. doi:10.2527/jas.2008-0970.

Louis, T. M., H. D. Hafs, and D. A. Morrow. 1974. Intrauterine administration of prostaglandin $F_{2\alpha}$ in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347–353.

Macmillan, K. L., and W. W. Thatcher. 1991. Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. Biol. Reprod. 45:883–889. doi:10.1095/biolreprod45.6.883.

Martineau, R. 2003. Dinoprost versus cloprostenol: Does route of injection modulate their efficacy in dairy cattle? Bov. Pract. 37:10–19.

Martinez, M. F., J. P. Kastelic, G. P. Adams, B. Cook, W. O. Olson, and R. J. Mapletoft. 2002. The use of progestins in regimens for fixed-time artifcial insemination in beef cattle. 57:1049–1059.

Martínez, M. F., R. J. Mapletoft, J. P. Kastelic, and T. Carruthers. 2003. The effects of 3 gonadorelin products on luteinizing hormone release, ovulation, and follicular wave emergence in cattle. Can. Vet. J. 44:125–131.

Mikkola, M., and J. Taponen. 2017. Quality and developmental rate of embryos produced with sex-sorted and conventional semen from superovulated dairy cattle. Theriogenology. 87:135–140. doi:10.1016/j.theriogenology.2016.08.013.

Oosthuizen, N., A. C. Lansford, L. B. Canal, P. L. P. Fontes, C. D. Sanford, C. R. Dahlen, N. Dilorenzo, R. N. Funston, and G. C. Lamb. 2018a. Comparison of two alternate PGF 2 α products in two estrus synchronization protocols in beef heifers. 1388–1395. doi:10.1093/jas/sky059.

Oosthuizen, N., L. B. Canal, P. L. P. Fontes, C. D. Sanford, N. DiLorenzo, C. R. Dahlen, G. E. Seidel, and G. C. Lamb. 2018b. Prostaglandin $F_{2\alpha}$ 7 d prior to initiation of the 7-d CO-synch + CIDR protocol failed to enhance estrus response and pregnancy rates in beef heifers. J. Anim. Sci. 96:1466–1473. doi:10.1093/jas/sky058.

Osada, M., H. Iwabuchi, T. Aoki, K. Sasaki, H. Ushijima, and T. Ozawa. 2019. Economic evaluation of artificial insemination of sex-sorted semen on a Brown Swiss dairy farm—A case study. Anim. Sci. J. 90:597–603. doi:10.1111/asj.13156.

Palomares, R. A., H. J. Fishman, A. L. Jones, M. S. Ferrer, M. Jenerette, and A. Vaughn. 2015. Comparison of 4- versus 5-day Co-Synch + controlled internal drug release (CIDR) + timed artificial insemination protocols in dairy heifers. Theriogenology. 84:868–874. doi:10.1016/j.theriogenology.2015.05.021.

Parish, J. A., J. E. Larson, and R. C. Vann. 2012. Estrous Synchronization in Cattle. Mississippi State University Extension. Pub. No. 2614. Mississippi State Univ., Starkville, MS. p. 1-12.

Patterson, D. J., G. H. Kiracofe, J. S. Stevenson, and L. R. Corah. 1989. Control of the bovine estrous cycle with melengestrol acetate (MGA): a review. J. Anim. Sci. 67:1895–1906.

Patterson, D. J., F. N. Kojima, and M. F. Smith. 2003. A review of methods to synchronize estrus in replacement beef heifers and postpartum cows. J. Anim. Sci. 81:E166–E177.

Patterson, D. J., D. J. Schafer, and M. F. Smith. 2005. Review of estrus synchronization systems: MGA. In: Applied Reproductive Strategies in Beef Cattle. p. 53–90.

Patterson, D. J., J. M. Thomas, N. T. Martin, J. M. Nash, and M. F. Smith. 2013. Control of estrus and ovulation in beef heifers. Vet. Clin. North Am. - Food Anim. Pract. 29:591–617. doi:10.1016/j.cvfa.2013.07.009.

Pellegrino, C. A. G., F. Morotti, R. M. Untura, J. H. F. Pontes, M. F. O. Pellegrino, J. P. Campolina, M. M. Seneda, F. A. Barbosa, and M. Henry. 2016. Use of sexed sorted semen for fixed-time artificial insemination or fixed-time embryo transfer of in vitro–produced embryos in cattle. Theriogenology. 86:888–893. doi:10.1016/j.theriogenology.2016.03.010.

Perry, G. A., B. L. Perry, J. H. Krantz, and J. Rodgers. 2012. Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success. J. Anim. Sci. 90:489–494. doi:10.2527/jas.2011-4319.

Pinilla, L., E. Aguilar, C. Dieguez, R. P. Millar, and M. Tena-Sempere. 2012. Kisspeptins and Reproduction: Physiological Roles and Regulatory Mechanisms. Physiol Rev. 92:1235–1316. doi:10.1152/physrev.00037.2010.

Plata, N. I., J. C. Spitzer, C. E. Thompson, D. M. Henricks, M. P. Reid, and T. J. Newby. 1990. Synchronization of estrus after treatment with luprostlol in beef cows and in beef and dairy heifers. Theriogenology. 33:943–952.

Poock, S. E., W. R. Lamberson, and M. C. Lucy. 2015. Effect of different gonadorelin (GnRH) products used for the first or resynchronized timed artificial insemination on pregnancy rates in postpartum dairy cows. Theriogenology. 84:504–508. doi:10.1016/j.theriogenology.2015.04.002.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology. 44:915–923. doi:10.1016/0093-691X(95)00279-H.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997. Pregnancy Rates Per Artificial Insemination for Cows and Heifers Inseminated at a Synchronized Ovulation or Synchronized Estrus. J. Dairy Sci. 80:295–300. doi:http://dx.doi.org/10.3168/jds.S0022-0302(97)75937-X. X

Rahe, C. H., R. E. Owens, J. L. Fleeger, H. J. Newton, and P. G. Harms. 1980. Pattern of plasma luteinizing hormone in the cyclic cow: Dependence upon the period of the cycle. Endocrinology. 107:498–503. doi:10.1210/endo-107-2-498.

Rettmer, I., J. S. Stevenson, and L. R. Corah. 1992. Endocrine responses and ovarian changes in inseminated dairy heifers after an injection of a GnRH agonist 11 to 13 days after estrus. J. Anim. Sci. 70:508–517.

Roche, J. F. 1973. Synchronisation of oestrus and fertility following artificial insemination in heifers given Prostaglandin F2 alpha. J Reprod Fertil. 37:135–138.

Roche, J. F., E. J. Austin, M. Ryan, M. O'Rourke, M. Mihm, and M. G. Diskin. 1999. Regulation of follicle waves to maximize fertility in cattle. J. Reprod. Fertil. 54:61–71.

Rodger, D., and F. Stormshak. 1986. Gonadotropin-Releasing Hormone Induced Alteration of Bovine Corpus Luteum Function. 156:149–156.

Rodgers, J. C., S. L. Bird, J. E. Larson, N. Dilorenzo, C. R. Dahlen, A. Dicostanzo, and G. C. Lamb. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. J. Anim. Sci. 90:4055–4062. doi:10.2527/jas.2011-4836.

Rowson, L., H. R. Tervit, and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J. Reprod. Fertil. 29:145. (Abstr.).

Sales, J. N. S., K. A. L. Neves, A. H. Souza, G. A. Crepaldi, R. V. Sala, M. Fosado, E. P. C. Filho, M. de Faria, M. F. S. Filho, and P. S. Baruselli. 2011. Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. Theriogenology. 76:427–435. doi:10.1016/j.theriogenology.2011.02.019.

Salverson, R. R., J. M. DeJarnette, C. E. Marshall, and R. A. Wallace. 2002. Synchronization of estrus in virgin beef heifers using melengestrol acetate and $PGF_{2\alpha}$: An efficacy comparison of cloprostenol and dinoprost tromethamine. Theriogenology. 57:853–858. doi:10.1016/S0093-691X(01)00692-6.

Say, E., S. Coban, Y. Nak, D. Nak, U. Kara, S. White, V. Kasimanickam, and R. Kasimanickam. 2016. Fertility of Holstein heifers after two doses of PGF2alpha in 5-day CO-Synch progesterone-based synchronization protocol. Theriogenology. 86:988–993. doi:10.1016/j.theriogenology.2016.03.026.

Schams, D., and H. Karg. 1982. Hormonal responses following treatment with different prostaglandin analogues for estrous cycle regulation in cattle. Theriogenology. 17:499–513.

Schenk, J. L., D. G. Cran, R. W. Everett, and G. E. Seidel. 2009. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. Theriogenology. 71:717–728. doi:10.1016/j.theriogenology.2008.08.016.

Schmitt, E. J., T. Diaz, M. Drost, W. W. Thatcher, and J. Schmitt. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle The online version of this article , along with updated information and services , is located on the World Wide Web at : Use of a Gonadotropin. J. Anim. Sci. 1084–1091.

Seidel, G. E. 2003. Economics of selecting for sex: The most important genetic trait. Theriogenology. 59:585–598. doi:10.1016/S0093-691X(02)01242-6.

Seidel, G. E. 2007. Overview of sexing sperm. Theriogenology. 68:443–446. doi:10.1016/j.theriogenology.2007.04.005.

Seidel, G. E. 2014. Update on sexed semen technology in cattle. Animal. 8:160–164. doi:10.1017/S1751731114000202.

Seidel, G. E., C. H. Allen, L. A. Johnson, M. D. Holland, Z. Brink, G. R. Welch, J. K. Graham, and M. B. Cattell. 1997. Uterine horn insemination of heifers with very low numbers of nonfrozen and sexed spermatozoa. Theriogenology. 48:1255–1264. doi:10.1016/S0093-691X(97)00368-3.

Senger, P. L. 2011. Pathways to pregnancy and parturition. 3rd ed. Current Conceptions, Pullman, WA.

Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol. Reprod. 39:308–3. doi:10.1095/biolreprod39.2.308.

Small, J. A., M. G. Colazo, J. P. Kastelic, and R. J. Mapletoft. 2009. Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. Theriogenology. 71:698–706. doi:10.1016/j.theriogenology.2008.09.045.

Smith, M. F., G. A. Perry, J. A. Atkins, E. M. Jinks, K. G. Pohler, and D. J. Patterson. 2005. Physiological principles underlying synchronization of estrus. In: Applied Reproductive Strategies in Beef Cattle. p. 1–22.

Souza, A. H., A. P. Cunha, E. P. B. Silva, A. Gümen, H. Ayres, J. N. Guenther, and M. C. Wiltbank. 2009. Comparison of gonadorelin products in lactating dairy cows: Efficacy based on induction of ovulation of an accessory follicle and circulating luteinizing hormone profiles. Theriogenology. 72:271–279. doi:10.1016/j.theriogenology.2009.02.016.

Stevenson, J. S., G. C. Lamb, S. K. Johnson, M. A. Medina-Britos, D. M. Grieger, K. R. Harmoney, J. A. Cartmill, S. Z. El-Zarkouny, C. R. Dahlen, and T. J. Marple. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. J. Anim. Sci. 81:571–586.

Stevenson, J. S., and A. P. Phatak. 2010. Rates of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost. Theriogenology. 73:1127–1138.

Suther, S. 2000. What's "high quality" worth? In: January 2000 Angus Beef Bulletin. p. 1-3.

Tenhagen, B. A., S. Kuchenbuch, and W. Heuwieser. 2005. Timing of ovulation and fertility of heifers after synchronization of oestrus with GnRH and prostaglandin $F_{2\alpha}$ Reprod. Domest. Anim. 40:62–67. doi:10.1111/j.1439-0531.2004.00557.x.

Tervit, H. R., L. E. A. Rowson, and A. Brand. 1973. Synchronization of oestrus in cattle using a prostaglandin F2α analogue. J. Reprod. Fertil. 179–181.

Thomas, J. M., S. L. Lock, S. E. Poock, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2014. Delayed insemination of nonestrous cows improves pregnancy rates when using sex-sorted semen in timed artificial insemination of suckled beef cows. J. Anim. Sci. 92:1747–1752. doi:10.2527/jas2013-7131.

Thomas, J. M., J. W. C. Locke, R. Vishwanath, J. B. Hall, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2017. Effective use of SexedULTRA^{TM} sex-sorted semen for timed artificial insemination of beef heifers. Theriogenology. 98:88–93. doi:10.1016/j.theriogenology.2017.03.018.

Thompson, K. E., J. S. Stevenson, G. C. Lamb, D. M. Grieger, and C. A. Löest. 1999. Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and prostaglandin F2α. J. Anim. Sci. 77:1823–1832.

Twagiramungu, H., L. A. Guilbault, and J. J. Dufour. 1995. Synchrinization of ovarian follicular waves with a gonadotropin- releasing hormone agponist to increase the precision of oestrus in cattle: a review. J.Anim.Sci. 73:3141–3151.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology. 52:1067–1078. doi:10.1016/S0093-691X(99)00195-8.

White, S. S., R. K. Kasimanickam, and V. R. Kasimanickam. 2016. Fertility after two doses of $PGF_{2\alpha}$ concurrently or at 6-hour interval on the day of CIDR removal in 5-day CO-Synch progesterone-based synchronization protocols in beef heifers. Theriogenology. 86:785–790. doi:10.1016/j.theriogenology.2016.02.032.

Whittier, J. C., and T. W. Geary. 2000. Frequently asked questions about synchronizing estrus and ovulation in beef cattle with GnRH. In: Iowa Cooperative Extension CHIPS Beef Breeding Management Seminar. p. 1–15.

Whittier, W. D., J. F. Currin, H. Schramm, S. Holland, and R. K. Kasimanickam. 2013. Fertility in Angus cross beef cows following 5-day CO-Synch + CIDR or 7-day CO-Synch + CIDR estrus synchronization and timed artificial insemination. Theriogenology. 80:963–969.

Yoshioka, S., H. Abe, R. Sakumoto, and K. Okuda. 2013. Proliferation of luteal steroidogenic cells in cattle. PLoS One. 8:1–11. doi:10.1371/journal.pone.0084186.

Zimbelman, R. G., and L. W. Smith. 1966. Control of ovulation in cattle with melengestrol acetate I. Effect of dosage and route of administration. J. Reprod. Fertil. 11:185–191.

Zollers, W. G. J., H. A. Garverick, and M. F. Smith. 1989. Oxytocin-induced release of prostaglandin F2 alpha in postpartum beef cows: comparison of short versus normal luteal phases. Biol. Reprod. 41:262–267.

3. IMPACTS OF ESTRUS EXPRESSION AND INTENSITY ON VARIABLES ASSOCIATED WITH FERTILITY IN BEEF COWS RECEIVING A GNRH-BASED FIXED-TIME ARTIFICIAL INSEMINATION PROTOCOL[1]

3.1. Introduction

Cow-calf operations are dependent on females producing one healthy calf per year to generate revenue, which is directly influenced by overall reproductive performance and efficiency of the herd (Diskin and Kenny, 2016). Therefore, technologies aimed at improving the quantity and quality of calves generated are required to enhance the profitability in these enterprises. Synchronization of estrus and fixed-time artificial insemination (**TAI**) are reproductive technologies that have been successfully utilized to improve productivity and profits in cow-calf systems (Dahlen et al. 2014). Yet, management practices that maximize pregnancy success to TAI and single ovulations are still warranted to promote its adoption by producers, and further enhance reproductive efficiency of beef herds (Lamb et al., 2010)

Heifers and cows that exhibit estrus prior to TAI have greater pregnancy rates (**PR/AI**) when compared to non-estrual females (Perry et al., 2007; Richardson et al., 2016). Cows that exhibit estrus prior to AI were shown to have altered gene expression profile in their endometrium during the preimplantation phase, potentially favoring early conceptus development (Davoodi et al., 2016). Research on the intensity of estrus expression has also reported its associations with fertility (Cerri et al., 2017; Ferraz et al., 2017). The use of

[1] Reprinted with permission from "Effects of estrous expression and intensity of behavioral estrous symptoms on variables associated with fertility in beef cows treated for fixed-time artificial insemination" by Oosthuizen et al., 2020. *Animal Reproduction Science*, 214, 106308, Copyright 2020 by Animal Reproduction Science.

pedometers to estimate cow physical activity (Schubach et al., 2017) has been incorporated in both beef (Rodrigues et al., 2018) and dairy (Silper et al., 2015) herds to assess estrus intensity. Dairy cows with a greater mean peak of activity at estrus have been reported to have greater PR/AI (Madureira et al., 2015, Madureira et al., 2019). Research from our group was the first to evaluate estrus intensity via physical activity, and its impacts on fertility responses of beef cows (Rodrigues et al., 2018). In that study, estrus intensity was positively associated with follicle diameter, corpus luteum (**CL**) volume, and post-AI plasma progesterone (**P4**) concentrations in cows exposed to an estradiol-based TAI protocol (Rodrigues et al., 2018).

The use of estradiol in estrus synchronization, however, is not legal in the US and increases the incidence of pharmacologically induced estrus (Vasconcelos et al., 2014). Research is warranted to determine the relationships between estrus intensity and fertility responses in beef females subjected to GnRH-based TAI protocols, in which expression of estrus is induced by endogenous estradiol. Accordingly, the objective of this study was to determine the effects of expression of estrus and estrus intensity, estimated by physical activity, on fertility responses in beef cows receiving gonadotropin-releasing hormone (**GnRH**). We hypothesized that expression of estrus and estrus intensity would be positively associated with reproductive parameters associated with fertility in beef cows receiving a GnRH-based TAI protocol.

3.2. Materials and Methods

This experiment was conducted from May to July 2018 at the Texas A&M – Beef Cattle Systems (College Station, TX, USA). All animals were cared for in accordance with

acceptable practices and experimental protocols reviewed and approved by the Texas A&M - Institute of Animal Care and Use Committee (#2018/0093). All cows were healthy and no incidence of clinical disorders was noted during the experimental period, including lameness and other locomotor disorders that would influence cow physical activity.

3.2.1. Animals and Estrus Assessment

Two hundred and seventy three non-pregnant, lactating, multiparous beef cows (average ¾ *Bos taurus* and ¼ *Bos indicus*; BW = 456.5 ± 51.0 kg; BCS = 4.9 ± 0.5; days postpartum [**DPP**] = 73.4 ± 17.0 d) were assigned to this experiment (d -10 to 28). Cows were enrolled in the 7-d CO-Synch + controlled internal drug release (**CIDR**) estrus synchronization protocol, where they received a 100-µg injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ, USA) and a CIDR insert (EAZI-BREED CIDR; 1.38 g of P4; Zoetis Animal Health) on Day -10, a 25-mg injection of prostaglandin $F_{2\alpha}$ (**PGF**; Lutalyse HighCon; 12.5 mg/mL of dinoprost tromethamine; Zoetis Animal Health) at CIDR removal on Day -3. Cows received a second injection of 100-µg GnRH concurrently with TAI at 60 - 66 h after CIDR removal (d 0). All cows were inseminated by the same technician, using semen from the same bull and batch. Cows were maintained in a single pasture dominated by bermudagrass (*Cynodon* spp.) throughout the experiment, with *ad libitum* access to water and a commercial mineral-vitamin mix (Producers Cooperative Association; College Station, TX, USA). Cow BW and BCS (Wagner et al., 1988) were recorded on Day -10.

On Day -10 cows were fitted with a pedometer (HJ-321; Omron Healthcare, Inc., Bannockburn, IL, USA), which was placed inside a polyester pouch (Heat Watch II; Cow Chips, LLC, Manalapan, NJ, USA) and fixed behind their right shoulder to assess physical

activity as described by Rodrigues et al. (2018). Pedometer results were recorded concurrently with handling for estrus synchronization and TAI on Day -3 and 0, respectively. An estrus detection patch (Estrotect; Rockway Inc., Spring Valley, WI, USA) was applied to the tail-head of all cows, and patches were examined for estrus activity at TAI. Estrus was defined as removal of > 50% of the rub-off coating on the estrus detection patch (Thomas et al., 2014; Rodrigues et al., 2018), and no other visual assessment of estrus behavior was performed.

Basal physical activity of each cow was considered the average number of steps taken daily between Day -10 and -3, as expression of estrus was not expected during this period due to the presence of a CIDR insert (Lamb et al., 2001). Average daily steps from Day -3 to TAI was considered to be associated with the expression of estrus, as a result of CIDR removal and PGF injection on Day -3 (Louis et al., 1975). Similar to Rodrigues et al. (2018), net physical activity during estrus was calculated by subtracting basal physical activity (d -10 to -3) from activity during the expected estrual period (d -3 to 0). According to the estrus detection patch, cows that did not express estrus from Day -3 to 0 were classified as **NOESTR** regardless of their net physical activity. Those that expressed estrus were ranked by net physical activity; cows above the median were classified as **HIESTR** and the remaining cows as **LWESTR**. Fig. 3.1. provides an outline of the experimental schedule. Cows that were missing pedometers either on Day -3 or at TAI were excluded from all analyses (n = 5).

3.2.2. Ultrasonography

Transrectal ultrasonography (5.0-MHz linear multi-frequency transducer, Ibex EVO, E.I. Medical Imaging, Loveland, CO, USA) was performed on Day 0 and 7 to record

dominant follicle diameter (**DFD**; Day 0), and to determine CL volume (d 7). Dominant follicle diameter was estimated by taking the average of the greatest width and height of the dominant follicle. Corpus luteum volume was estimated using the formula for the volume of a sphere: volume = $4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter (Cooke et al., 2009). The presence of a CL cavity was accounted for in the calculation by subtracting the cavity volume (calculated as a sphere) from the CL volume. On Day 28, transrectal ultrasonography was performed to determine PR/AI by detecting a viable conceptus (5.0-MHz linear multi-frequency transducer, Ibex EVO, E.I. Medical Imaging). As in Rodrigues et al. (2018), cows were classified as responsive to the estrus synchronization protocol when diagnosed without the presence of a CL on Day 0, but with a CL greater than 0.38 cm^3 in volume on Day 7. Only data from cows that responded to the synchronization protocol [n = 224; synchronization rate = 82.0% (224/273 total cows)] and that did not lose a pedometer were maintained in the experiment (NOESTR, n = 119; LWESTR, n = 50; HIESTR, n = 50). Cows responsive to estrus synchronization but without a dominant follicle on Day 0 were classified as having ovulated prior to TAI.

3.2.3. Blood Collection and Analyses

Blood samples were collected via jugular venipuncture on Day 0 and 7 into heparinized blood collection tubes (BD Vacutainer, 10 mL; Franklin Lakes, NJ, USA). Following collection, blood samples were immediately placed on ice until they were centrifuged at 2,500 × g for 20 min at -4 °C. After centrifugation, plasma was transferred into micro tubes (1.5 mL; VWR International LLC, Radnor, PA, USA) and stored at −20 °C. Plasma samples collected on Day 0 and 7 were analyzed for concentrations of P4 using a radioimmunoassay (**RIA**) as previously described (Pohler et al., 2016). Plasma samples from

Day 0 were analyzed for estradiol-17β using a RIA as previously described (Kirby et al., 1997). The intra- and inter-assay CV were, respectively, 3.3 and 3.4% for P4, and 5.5 and 6.0% for estradiol-17β. The minimum detectable concentration of each assay was 0.05 ng/mL for P4 and 0.5 pg/mL for estradiol-17β.

On Day 20, blood samples were collected from a subset of cows randomly selected from each estrus characteristic group (NOESTR, LWESTR, and HIESTR; *n* = 28 cows/group) into PAXgene tubes (BD Diagnostics, Sparks, MD, USA) for whole blood RNA extraction and mRNA expression analysis of interferon-stimulated genes (**ISGs**; *interferon-stimulated gene 15*, *20,50-oligoadenylate synthetase*, and *myxovirus resistance 2*). Unfortunately, none of the NOESTR cows sampled on Day 20 were diagnosed as pregnant on Day 28. Given that expression of ISGs was analyzed to assess conceptus development (Fricke et al. 2016), whole blood samples collected from NOESTR cows were not analyzed for mRNA expression of ISGs. Total RNA was extracted from whole blood samples using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA, USA). Isolated RNA was assessed for quantity and quality via UV absorbance (Synergy LX Multi-Mode Reader; BioTek Instruments Inc., Winooski, VT, USA) at 260 nm and a 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). All samples had a 260/280 nm ratio between 1.92 and 2.18, and were deemed appropriate for cDNA synthesis as previously described (Fleige and Pfaffl, 2006). Extracted RNA (400 ng) was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA, USA). Real-time PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems) and gene-specific primers for the ISGs (20 pM each; Table 3.1) with the QuantStudio 3 Real-time PCR system (Applied Biosystems), according to procedures

described by Cooke et al. (2008). At the end of each RT-PCR, amplified products were subjected to a dissociation gradient (95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. Responses were quantified based on the threshold cycle (**C_T**), which is the number of PCR cycles required for target amplification to reach a predetermined threshold. Responses from ISGs were quantified based on C_T and normalized to the geometrical mean of C_T values from *β-actin* and *ribosomal Protein L19*. The CV for the geometrical mean of *β-actin* and *ribosomal Protein L19* C_T values across all samples was 2.7%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) as described by Ocón-Grove et al. (2008).

3.2.4. Statistical Analyses

Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA), whereas binary data were analyzed using the GLIMMIX procedure of SAS with a binomial distribution and logit link function. Cow was considered the experimental unit. Denominator degrees of freedom were adjusted using the Kenward-Rogers adjustment for the tests of fixed effects. All quantitative data were tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure of SAS, and considered normally distributed ($W \geq 0.90$). Model statements used for cow DPP, BW, BCS on Day -10, as well as physical activity parameters contained the effects of estrus characteristics (NOESTR, LWESTR, and HIESTR). All other model statements contained the effects of estrus characteristics and cow BCS on Day -10 as independent covariate. All random statements contained the effects of cow(estrus characteristic group). Results are reported as least square means (**LSM**) or covariately-adjusted LSM to BCS. Means were separated using PDIFF when the *P*-value for the main effect was ≤ 0.10. The probability of cows becoming pregnant to TAI was evaluated

according to DFD and plasma estradiol-17β concentrations on Day 0, plasma P4 concentrations on Day 0 and 7, and CL volume on Day 7. The GLM procedure of SAS was initially used to determine if each individual measurement influenced pregnancy maintenance linearly, quadratically, or cubically. The LOGISTIC procedure was then used to generate a regression model and to determine the intercept and slope(s) values according to maximum likelihood estimates from each significant continuous order effect. The probability of pregnancy was determined according to the following equation: Probability = ($e^{\text{logistic equation}}$)/(1 + $e^{\text{logistic equation}}$). Logistic curves were constructed according to values detected for each variable. For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined when $P > 0.05$ and ≤ 0.10.

3.3. Results

No differences in DPP were detected ($P = 0.57$) among estrus characteristic groups on Day -10 of the experiment (Table 3.2.). Cow BW and BCS on Day -10 were greater ($P \leq 0.04$) in HIESTR and LWESTR compared with NOESTR, and similar ($P \geq 0.94$) between HIESTR and LWESTR cows (Table 3.2.). According to the estrus detection patch, 45.7% of the cows classified as responsive to the synchronization protocol expressed estrus between Day -3 and TAI (100/219 of HIESTR and LWESTR cows/total cows). Average daily steps between Day -10 and -3 did not differ ($P = 0.12$) among estrus characteristic groups (Table 3.2.). In turn, average daily steps between Day -3 to 0 were greater ($P < 0.01$) in HIESTR vs. LWESTR and NOESTR, and greater ($P < 0.01$) in LWESTR vs. NOESTR cows (Table 3.2.). Consequently, net physical activity was greater ($P < 0.01$) in HIESTR and LWESTR vs. NOESTR cows, and greater ($P < 0.01$) in HIESTR vs. LWESTR cows according to the experimental design (Table 3.2.).

The percentage of cows with no dominant follicle at TAI (d 0) was similar ($P = 0.85$) among estrus characteristic groups (Table 3.3.). At TAI, DFD was greater ($P \leq 0.03$) in HIESTR vs. LWESTR and NOESTR, and greater ($P < 0.01$) in LWESTR vs. NOESTR cows. Plasma P4 and estradiol-17β concentrations at TAI did not differ ($P \geq 0.39$) among estrus characteristic groups (Table 3.3.). On Day 7, CL volume was greater ($P \leq 0.05$) in HIESTR vs. LWESTR and NOESTR, and also greater ($P < 0.01$) in LWESTR vs. NOESTR cows. Plasma P4 concentrations on Day 7 were greater ($P < 0.01$) in HIESTR and LWESTR vs. NOESTR, and did not differ ($P = 0.76$) between LWESTR and HIESTR cows (Table 3.3.). No differences ($P = 0.19$) in CL volume to plasma P4 ratio were detected among estrus characteristic groups on Day 7 (Table 3.3.).

No differences between HIESTR and LWESTR cows were observed ($P \geq 0.53$) for mRNA expression of *interferon-stimulated gene 15*, *myxovirus resistance 2*, or *20,50-oligoadenylate synthetase* (Table 3.4.). Nonetheless, mRNA expression of these ISGs were greater ($P < 0.01$) in pregnant vs. non-pregnant cows across estrus characteristic groups (Table 3.4.).

On Day 28, PR/AI was greater ($P < 0.01$) in HIESTR and LWESTR vs. NOESTR cows, and did not differ ($P = 0.46$) between HIESTR and LWESTR cows (Table 3.3.). Across estrus characteristic groups, the probability of cows becoming pregnant to TAI increased linearly as DFD on Day 0 and CL on Day 7 increased ($P < 0.01$), was affected quadratically ($P < 0.01$) by increasing plasma P4 concentrations on Day 7 (Fig. 3.2.), and was not influenced ($P \geq 0.26$) by plasma estradiol-17β and P4 concentrations on Day 0 (data not shown).

3.4. Discussion

The aim of this experiment was to evaluate the effects of expression of estrus and estrus intensity, assessed via physical activity using pedometers, on reproductive parameters in beef cows subjected to a GnRH-based TAI protocol. Our research group investigated this relationship in beef cows exposed to an estradiol-based TAI protocol (Rodrigues et al., 2018), which is known to pharmacologically induce estrus in beef females (Vasconcelos et al., 2014). Accordingly, incidence of estrus expression was 45.7% in cows responsive to the GnRH-based protocol in the current study, whereas 77% of the cows analyzed by Rodrigues et al. (2018) expressed estrus behavior. Values reported for expression of estrus herein, even when the cows that did not respond to the estrus synchronization protocol are accounted for (36.6% estrus expression; 100 cows/273 total cows), fall within the range reported by Richardson et al. (2016) in a meta-analysis based on GnRH-based TAI protocols.

Expression of estrus is primarily associated with increased circulating concentrations of estradiol, which triggers the hypothalamus to initiate the cascade of events leading to estrus behavior (Allrich, 1994). Estrus behavior is traditionally detected by observing cows and heifers standing to be mounted, whereas secondary signs such as restlessness can be measured through activity monitoring devices (Roelofs et al., 2010; Silper et al., 2015; Schubach et al., 2017). By monitoring physical activity during diestrus or prolonged exposure to P4, such as through the use of a CIDR insert, a baseline for non-estrual activity can be established, and increased physical activity during proestrus and estrus can be associated with expression of estrus (Madureira et al., 2015; Rodrigues et al., 2018). In the present experiment, average daily steps between Day -10 and -3 did not differ between estrus characteristic groups, denoting a similar baseline of physical activity among cows. In turn,

differences in net physical activity between HIESTR, LWESTR, and NOESTR should be associated with behavioral changes during proestrus and estrus (d -3 to 0; Rodrigues et al., 2018). The greater average daily steps (d -3 to TAI) and net physical activity noted for HIESTR and LWESTR cows corroborate the increased physical activity triggered by estrus behavior (Kiddy, 1977). It should be noted that physical activity of cows that did not respond to the synchronization protocol were not analyzed herein, as our hypothesis and research objectives focused on cows responsive to estrus synchronization. Future research should also investigate reproductive parameters in beef cows according to physical activity, estrus expression, and estrus intensity when response to estrus synchronization in unknown.

Across estrus characteristic groups, DPP at the beginning of the estrus synchronization protocol were adequate for optimal pregnancy rates of *B. indicus*-influenced cattle to TAI (Vasconcelos et al., 2014). Cow BW and BCS on Day -10 was positively associated with expression of estrus, as previously noted by our and other research groups (Madureira et al., 2015; Richardson et al., 2016; Rodrigues et al., 2018). The specific mechanisms by which nutritional status modulates estrus behavior, particularly among cows that successfully respond to estrus synchronization, are still unknown and deserves investigation. Nonetheless, all reproductive variables evaluated herein were covariately-adjusted to cow BCS on Day -10 to account for the potential differences in nutritional status and reserves between estrus characteristic groups.

Rodrigues et al. (2018) reported a greater percentage of cows expressing high intensity estrus without a dominant follicle at TAI compared with cohorts with low intensity or no expression of estrus. These outcomes were attributed, at least partially, to anticipated ovulatory LH surge due to increased preovulatory estradiol concentrations in cows

expressing high intensity estrus. In the present study, a similar percentage of cows in each estrus characteristic group had no dominant follicle at TAI. The use of GnRH at TAI herein, instead of estradiol cypionate and eCG 48 h prior to TAI in Rodrigues et al. (2018), likely resulted in slower follicle development (Sá Filho and Vasconcelos, 2011). Moreover, no differences in plasma estradiol-17β concentrations were detected among HIESTR, LWESTR, and NOESTR cows at TAI, despite expression of estrus and intensity being mostly driven by circulating estradiol concentration (Perry et al., 2007). Rodrigues et al. (2018) also failed to report differences in serum estradiol-17β concentrations at TAI among cows with different intensities on estrus behavior, and attributed this outcome to the use of exogenous estradiol. Although this experiment did not make use of exogenous estradiol as an ovulatory stimulus, one single blood sample at TAI provides a single snapshot of circulating estradiol, whereas LWESTR and HIESTR cows likely experienced an estradiol surge that elicited expression of estrus prior to blood sampling (Larimore et al., 2015). Research is still needed to characterize circulating concentrations of estradiol-17β in beef cows according to estrus intensity, including serial blood sampling beginning at CIDR removal in protocols based on TAI or spontaneous ovulation. This latter approach was not used herein because it requires additional handling of cows, which would influence physical activity assessment during the expected period of estrus expression.

During proestrus and estrus, reduced concentrations of circulating P4 facilitates follicular maturation, estradiol secretion, and estrus behavior (Adams et al., 1992; Allrich, 1994). Dairy cows that expressed estrus had lesser serum concentrations of P4 near the time of AI and greater DFD than those which did not express estrus (Pereira et al., 2016). In beef cows, expression of estrus (Carvalho et al., 2016) and intensity of estrus expression

(Rodrigues et al., 2018) were associated with reduced serum P4 and greater DFD at TAI. In the present experiment, however, plasma P4 concentrations at TAI did not differ among estrus characteristic groups. Cyclicity was not assessed herein, but it is plausible that anestrus incidence was elevated at the beginning of the TAI protocol given the BCS levels and *B. indicus* influence of the herd (Randel, 1990; Bridges et al., 2012; Larson et al., 2006). Cows with no functional CL at the beginning of the TAI protocol experience a rapid decrease in circulating P4 concentrations after CIDR removal on Day -3, resulting in low serum P4 concentrations on Day 0 (Fontes et al. 2019). In contrast, DFD increased according to expression and intensity of estrus herein, despite similar plasma P4 and estradiol-17β at among estrus characteristic groups at TAI. Rodrigues et al. (2018) also noted greater DFD but similar serum P4 concentrations at TAI in cows expressing high intensity vs. low intensity estrus; perhaps circulating P4 during proestrus and estrus does not play a substantial role in the DFD differences according to estrus intensity. Nonetheless, results from this and our previous experiment (Rodrigues et al., 2018) are the first to report a positive relationship among estrus intensity and DFD, suggesting fertility benefits when cows express high-intensity estrus behavior in estradiol- or GnRH-based protocols (Perry et al., 2005).

Granulosa and theca cells of the dominant follicle are converted to P4-secreting luteal cells of the CL following ovulation (Donaldson and Hansel, 1965). Subsequently, ovulation of larger dominant follicles results in greater CL volumes (Vasconcelos et al., 2001). Therefore, it was unsurprising that CL volume on Day 7 increased according to expression of estrus and estrus intensity, as they differed in DFD on Day 0. Circulating concentrations of P4 have been reported to follow the same pattern, where larger CL volumes often result in greater circulating P4 concentrations (Vasconcelos et al., 2001). Previous studies in beef

and dairy cows also reported differences in serum P4 concentrations after TAI according to expression of estrus and estrus intensity (Rodrigues et al., 2018; Madureira et al., 2019). In the present experiment, differences in CL volume resulted in the expected plasma P4 differences on Day 7 between cows that expressed estrus and those which did not, but not between LWESTR and HIESTR cows. Alternatively, Rodrigues et al. (2018) reported similar CL volume but greater serum P4 concentratins in cows expressing high-inteinsity vs. low-intensity estrus. Positive correlations between CL volume and circulating P4 concentrations have previously been reported (Gómez-Seco et al., 2017), whereas CL volume to plasma P4 ratio on Day 7 did not differ between estrus characteristic groups herein or in Rodrigues et al. (2018). Collectively, these outcomes indicate that plasma P4 concentration on Day 7 increased according to expression of estrus due to increasing CL volume rather than CL efficiency in P4 synthesis (Cipriano et al., 2016), despite this relationship not being fully retained when comparing cows with differing estrus intensity.

Interferon-tau secretion by the preimplantation conceptus is vital for pregnancy establishment and for the inhibition of luteolysis in ruminant species (Bazer, 2013). Interferon-tau also stimulates the expression of ISGs in peripheral blood leukocytes, which can be used to determine conceptus development and viability from d 15 to 22 of gestation (Gifford et al., 2007; Stevenson et al., 2007; Fricke et al., 2016). Expression of estrus at the time of insemination has been shown to increase ISG expression in reproductive tissues, stimulating a more receptive state for conceptus elongation during the preimplantation period (Davoodi et al., 2016; Cooke et al., 2019). In the present study, the impacts of expression of estrus on mRNA expression of ISGs could not be completed as none of the NOESTR cows sampled for whole blood were pregnant on Day 28. Nonetheless, no

differences between LWESTR and HIESTR were detected for mRNA expression of *interferon-stimulated gene 15*, *myxovirus resistance 2*, or *20,50-oligoadenylate synthetase*. Rodrigues et al. (2018) also reported similar mRNA expression of *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* according to expression of estrus and estrus intensity, although mRNA expression of *myxovirus resistance 2* were greater in cows expressing high-intensity estrus. The reason for differing outcomes in mRNA expression of ISGs according to estrus intensity among these research efforts are unknown, but require further investigation given the novelty of the subject (Rodrigues et al., 2018; Cooke et al., 2019).

It has been well documented that beef cows that express estrus prior to TAI have greater PR/AI compared to those that do not express estrus (Perry et al., 2007; Perry and Perry, 2008; Richardson et al., 2016). These differences in PR/AI are often attributed to larger DFD and greater concentrations of circulating estradiol prior to ovulation, leading to physiological events that promote fertility and pregnancy establishment (Perry and Perry, 2008a; Perry and Perry, 2008b; Pohler et al., 2012). Accordingly, PR/AI herein were greater in HIESTR and LWESTR when compared to NOESTR cows, representing a 2.4-fold increase in pregnancy rates in cows that expressed estrus near TAI. Conversely, no differences in PR/AI were determined between HIESTR and LWESTR cows, whereas Rodrigues et al. (2018) also failed to report such outcomes. Research in dairy cattle reported positive association among estrus intensity with PR/AI and pregnancy maintenance, representing a nearly 35% improvement in fertility (Madureira et al., 2015; Madureira et al., 2019). The number cows classified as LWESTR and HIESTR in the current study (n = 50) was limited to properly investigate differences in PR/AI between these estrus characteristic

groups. Nonetheless, results from this experiment and our previous research (Rodrigues et al., 2018) report improved fertility parameters in cows expressing high-intensity estrus, such as increased DFD at TAI and CL volume on Day 7, which are associated with improved pregnancy rates in cattle (Vasconcelos et al., 2001; Perry et al., 2005). Additional research is warranted to capture the potential benefits of high-intensity expression of estrus on pregnancy rates of beef cows, and replicate the outcomes already documented in dairy cattle (Madureira et al., 2019).

To corroborate the prospective fertility improvements when beef females express high-intensity estrus, the probability of cows becoming pregnant to TAI was evaluated across estrus characteristic groups according to ovarian and physiological responses measured herein. Cows with larger follicles had a greater likelihood of becoming pregnant, which corroborates the outcomes reported by Sá Filho et al. (2009) but differs from the quadratic relationship reported by our and other research groups (Perry et al., 2005; Pereira et al., 2016; Rodrigues et al., 2018). A similar linear relationship was observed between CL volume on Day 7 and probability of pregnancy, given that ovulation of larger dominant follicles yields larger CL (Vasconcelos et al. 2011). In contrast, plasma concentrations of estradiol-17β on Day 0 did not influence pregnancy probability, despite the known association between circulating estradiol near TAI and DFD in beef cows (Perry et al., 2007), whereas such outcome should be attributed to the single sampling schedule adopted herein. A quadratic relationship was noted between plasma P4 concentrations on Day 7 and pregnancy probability, denoting an optimal range in which plasma P4 concentrations maximize pregnancy rates, as reported by others (McNeill et al., 2006; Pereira et al., 2016; Rodrigues et al., 2018). Plasma P4 concentrations on Day 0, however, did not impact

pregnancy probability as in Rodrigues et al. (2018), which further suggests that the majority of cows had no CL at the beginning of the TAI protocol (Fontes et al. 2019). Within the population evaluated herein, these outcomes indicate maximized pregnancy rates to a GnRH-based TAI protocol as DFD on Day 0 and CL volume on Day 7 increase, with plasma P4 concentration on Day 7 near 3.10 ng/mL. Within the estrous characteristic groups evaluated herein, HIESTR cows are those that better fit this physiological profile, denoting the potential fertility benefits of high-intensity expression of estrus. However, caution should be adopted when interpreting these results, which can vary according to the population of cows evaluated and laboratory procedures for plasma P4 analysis.

3.5. Conclusions

Cows that expressed estrus during a GnRH-based TAI protocol (HIESTR and LWESTR) had greater physical activity during the proestrus and estrus periods, DFD at TAI, CL volume and plasma P4 concentration 7 d after TAI, and PR/AI compared to cohorts that did not express estrus (NOESTR). Moreover, cows that expressed high-intensity estrus (HIESTR) based on physical activity had greater DFD on Day 0 and CL volume on Day 7 compared with cohorts that expressed low-intensity estrus (LWESTR). Pregnancy rates to timed-AI were not impacted by estrus intensity, although HIESTR had improved indicators of fertility such as DFD on Day 0 and CL volume on Day 7 compared with LWESTR. These outcomes corroborate our previous findings in beef cows receiving an estradiol-based TAI protocol (Rodrigues et al, 2018). Collectively, expression of estrus near the time of TAI improves reproductive function and pregnancy rates, whereas estrus intensity modulates key fertility markers in beef cows assigned to estradiol- or GnRH-based TAI protocols.

3.6. References

Adams, G. P., R. L. Matteri, and O. J. Ginther. 1992. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. J. Reprod. Fertil. 95:627–640.

Allrich, R. D. 1994. Endocrine and Neural Control of Estrus in Dairy Cows. J. Dairy Sci. 77:2738–2744. doi:10.3168/jds.s0022-0302(94)77216-7.

Bazer, F. W. 2013. Pregnancy recognition signaling mechanisms in ruminants and pigs. J. Anim. Sci. Biotechnol. 4:1. doi:10.1186/2049-1891-4-23.

Bridges, G. A., J. K. Ahola, C. Brauner, L. H. Cruppe, J. C. Currin, M. L. Day, P. J. Gunn, J. R. Jaeger, S. L. Lake, G. C. Lamb, G. H. L. Marquezini, R. K. Peel, A. E. Radunz, J. S. Stevenson, and W. D. Whittier. 2012. Determination of the appropriate delivery of prostaglandin f2a in the five-day co-synch + controlled intravaginal drug release protocol in suckled beef cows. J. Anim. Sci. 90:4814–4822. doi:10.2527/jas.2011-4880.

Cerri, R. L. A., B. F. Silper, T. A. Burnett, A. M. L. Madureira, L. B. Polsky, M. Kaur, D. Veira, and J. L. M. Vasconcelos. 2017. Estrus: Association with production parameters and implications on fertility. WCDS Adv. Dairy Technol. 29:201–216.

Cooke, R. F., J. D. Arthington, D. B. Araujo, and G. C. Lamb. 2009. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. J. Anim. Sci. 87:4125–4132. doi:10.2527/jas.2009-2021.

Cooke, R. F., J. D. Arthington, D. B. Araujo, G. C. Lamb, and A. D. Ealy. 2008. Effects of supplementation frequency on performance, reproductive, and metabolic responses of Brahman-crossbred females. J. Anim. Sci. 86:2296–2309. doi:10.2527/jas.2008-0978.

Davoodi, S., R. F. Cooke, A. C. C. Fernandes, B. I. Cappellozza, J. L. M. Vasconcelos, and R. L. A. Cerri. 2016. Expression of estrus modifies the gene expression profile in reproductive tissues on day 19 of gestation in beef cows. Theriogenology. 85:645–655. doi:10.1016/j.theriogenology.2015.10.002.

Diskin, M. G., and D. A. Kenny. 2016. Managing the reproductive performance of beef cows. Theriogenology. 86:379–387. doi:10.1016/j.theriogenology.2016.04.052.

Donaldson, L., and W. Hansel. 1965. Histological Study of Bovine Corpora Lutea. J. Dairy Sci. 48:905–909. doi:10.3168/jds.s0022-0302(65)88360-6.

Ferraz, P. A., M. V. G. Loiola, A. S. Rodrigues, M. C. C. Lima, T. C. B. dos S. C. de Bittencourt, and A. de L. Ribeiro Filho. 2017. The effect of the intensity of estrus expression on the follicular diameter and fertility of nellore cows managed under a FTAI program. Cienc. Anim. Bras. 18:1–9. doi:10.1590/1089-6891v18e-37643.

Fleige, S., and M. W. Pfaffl. 2006. RNA integrity and the effect on the real-time qRT-PCR performance. Mol. Aspects Med. 27:126–139. doi:10.1016/j.mam.2005.12.003.

Fricke, P. M., P. D. Carvalho, M. C. Lucy, F. Curran, M. M. Herlihy, S. M. Waters, and J. A. Larkin. 2016. Effect of manipulating progesterone before timed artificial insemination on reproductive and endocrine parameters in seasonal-calving, pasture-based Holstein-Friesian cows. J. Dairy Sci. 99:6780–6792. doi:10.3168/jds.2016-11229.

Gifford, C. A., K. Racicot, D. S. Clark, K. J. Austin, T. R. Hansen, M. C. Lucy, C. J. Davies, and T. L. Ott. 2007. Regulation of Interferon-Stimulated Genes in Peripheral Blood Leukocytes in Pregnant and Bred , Nonpregnant Dairy Cows. J. Dairy Sci. 90:274–280. doi:10.3168/jds.S0022-0302(07)72628-0.

Gómez-Seco, C., B. Alegre, F. Martínez-Pastor, J. G. Prieto, J. R. González-Montaña, M. E. Alonso, and J. C. Domínguez. 2017. Evolution of the corpus luteum volume determined ultrasonographically and its relation to the plasma progesterone concentration after artificial insemination in pregnant and non-pregnant dairy cows. Vet. Res. Commun. 41:183–188. doi:10.1007/s11259-017-9685-x.

Kirby, C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. J. Dairy Sci. 80:273–285.

Lamb, G. C., C. R. Dahlen, J. E. Larson, G. Marquezini, and J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. J. Anim. Sci. 88. doi:10.2527/jas.2009-2349.

Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253–2259. doi:/2001.7992253x.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. Dejarnette, F. N. Schrick, A. Dicostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J. Anim. Sci. 84:332–342.

Louis, T. M., H. D. Hafs, and J. N. Stellflug. 1975. Control of ovulation, fertility and endocrine response after prostaglandin F2alpha in cattle. Ann. Biol. anim. Bioch. Biophys. 15:407–417.

Madureira, A. M. L., L. B. Polsky, T. A. Burnett, B. F. Silper, S. Soriano, A. F. Sica, K. G. Pohler, J. L. M. Vasconcelos, and R. L. A. Cerri. 2019. Intensity of estrus following an estradiol-progesterone-based ovulation synchronization protocol influences fertility outcomes. J. Dairy Sci. 102:3598–3608. doi:10.3168/jds.2018-15129.

Madureira, A. M. L., B. F. Silper, T. A. Burnett, L. Polsky, L. H. Cruppe, D. M. Veira, J. L. M. Vasconcelos, and R. L. A. Cerri. 2015. Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. J. Dairy Sci. 98:7003–7014. doi:10.3168/jds.2015-9672.

McNeill, R. E., M. G. Diskin, J. M. Sreenan, and D. G. Morris. 2006. Associations between milk progesterone concentration on different days and with embryo survival during the early luteal phase in dairy cows. Theriogenology. 65:1435–1441. doi:10.1016/j.theriogenology.2005.08.015.

Ocon-Grove, O. M., F. N. T. Cooke, I. M. Alvarez, S. E. Johnson, T. L. Ott, and A. D. Ealy. 2008. Ovine endometrial expression of fibroblast growth factor (FGF) 2 and conceptus expression of FGF receptors during early pregnancy. Domest. Anim. Endocrinol. 34:135–145. doi:10.1016/j.domaniend.2006.12.002.

Pereira, M. H. C., M. C. Wiltbank, and J. L. M. Vasconcelos. 2016. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. J. Dairy Sci. 99:2237–2247. doi:10.3168/jds.2015-9903.

Perry, G. A., and B. L. Perry. 2008. Effects of standing estrus and supplemental estradiol on changes in uterine pH during a fixed-time artificial insemination protocol. J. Anim. Sci. 86:2928–2935. doi:10.2527/jas.2008-1181.

Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Anim. Sci. 85:684–689. doi:10.2527/jas.2006-519.

Pohler, K. G., M. H. C. Pereira, F. R. Lopes, J. C. Lawrence, D. H. Keisler, and M. F. Smith. 2016a. Circulating concentrations of bovine pregnancy-associated glycoproteins and late embryonic mortality in lactating dairy herds. J. Dairy Sci. 99:1584–1594. doi:10.3168/jds.2015-10192.

Pohler, K. G., R. F. G. Peres, J. A. Green, H. Graff, T. Martins, J. L. M. Vasconcelos, and M. F. Smith. 2016b. Use of bovine pregnancy-associated glycoproteins to predict late embryonic mortality in postpartum Nelore beef cows. Theriogenology. 85:1652–1659. doi:10.1016/j.theriogenology.2016.01.026.

Richardson, B. N., S. L. Hill, J. S. Stevenson, G. D. Djira, and G. A. Perry. 2016. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim. Reprod. Sci. 166:133–140. doi:10.1016/j.anireprosci.2016.01.013.

Rodrigues, A. D., R. F. Cooke, R. S. Cipriano, L. G. T. Silva, R. L. A. Cerri, and L. H. Cruppe. 2018. Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in Bos indicus-influenced beef cows 1. J. Anim. Sci. 96:236–249. doi:10.1093/jas/skx043.

Roelofs, J., F. López-Gatius, R. H. F. Hunter, F. J. C. M. van Eerdenburg, and C. Hanzen. 2010. When is a cow in estrus? Clinical and practical aspects. Theriogenology. 74:327–344. doi:10.1016/j.theriogenology.2010.02.016.

De Sá Filho, O. G., and J. L. M. Vasconcelos. 2011. Treatments to optimize the use of artificial insemination and reproductive efficiency in beef cattle under tropical environments. Vet. Med. Int. 2011. doi:10.4061/2011/923053.

Sá Filho, O. G., M. Meneghetti, R. F. G. Peres, G. C. Lamb, and J. L. M. Vasconcelos. 2009. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows II: Strategies and factors affecting fertility. Theriogenology. 72:210–218. doi:10.1016/j.theriogenology.2009.02.008.

Schubach, K. M., R. F. Cooke, A. P. Brandão, K. D. Lippolis, L. G. T. Silva, R. S. Marques, and D. W. Bohnert. 2017. Impacts of stocking density on development and puberty attainment of replacement beef heifers. Animal. 12:2260–2267. doi:10.1017/S1751731117001070.

Silper, B. F., A. M. L. Madureira, M. Kaur, T. A. Burnett, and R. L. A. Cerri. 2015. Short communication: Comparison of estrus characteristics in Holstein heifers by 2 activity monitoring systems. J. Dairy Sci. 98:3158–65. doi:10.3168/jds.2014-9185.

Stevenson, J. L., J. C. Dalton, T. L. Ott, K. E. Racicot, and R. C. Chebel. 2007. Correlation between reproductive status and steady-state messenger ribonucleic acid levels of the Myxovirus resistance gene, MX2, in peripheral blood leukocytes of dairy heifers. J. Anim. Sci. 85:2163–2172. doi:10.2527/jas.2007-0014.

Vasconcelos, J. L. M., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology. 56:307–314.

Table 3.1.
Primer sequence, accession number, and reference for all gene transcripts analyzed by real-time PCR

Gene name	Primer sequence	Accession number	Reference
ISG15	F: GGTATGAGCTGAAGCAGTT	NM_174366	Fricke et al. (2016)
	R: ACCTCCCTGCTGTCAAGGT		
20,50-OAS	F: ACCCTCTCCAGGAATCCAGT	NM_001040606	Fricke et al. (2016)
	R: GATTCTGGTCCCAGGTCTGA		
MX2	F: CTTCAGAGACGCCTCAGTCG	NM_173941	Fricke et al. (2016)
	R: TGAAGCAGCCAGGAATAGTG		
β-actin	F: CTGGACTTCGAGCAGGAGAT	AY141970	Gifford et al. (2007)
	R: GGATGTCGACGTCACACTTC		
RPL19	F: ATCGATCGCCACATGTATCA	NM_001040516	Monteiro et al. (2014)
	R: GCGTGCTTCCTTGGTCTTAG		

ISG15: Interferon-stimulated gene 15; *20,50-OAS:* 20,50-oligoadenylate synthetase; *MX2:* Myxovirus resistance 2; *RPL19:* Ribosomal Protein L19.

Table 3.2.
Physical activity, days postpartum, body weight (BW), and body condition score (BCS) of beef cows expressing or not expressing estrus and with differing intensities of behavioral estrous expression based on physical activity determinations[a]

Item	NOESTR	LWESTR	HIESTR	SEM	*P*-value
Activity variables					
Average daily steps, Day -10 to -3	2,146	2,501	2,221	240	0.12
Average daily steps, Day -3 to 0	2,528[c]	3,080[b]	4,220[a]	150	< 0.01
Net physical activity, daily steps	384[c]	621[b]	2,118[a]	195	< 0.01
Cow variables					
Days post-partum (d -10)	74	73	71	2	0.57
BW (d -10), kg	447[b]	465[a]	465[a]	9	0.02
BCS (d -10)[b]	4.77[b]	4.94[a]	4.93[a]	0.08	0.03

[a] Cows were assigned to an estrous synchronization + timed artificial insemination treatment regimen (Larson et al, 2007) from Day -10 to 0; Physical activity using pedometers as described by Rodrigues et al. (2018). An estrous detection patch (Estrotect; Rockway Inc., Spring Valley, WI, USA) was attached to the tail-head of each cow on Day -3, and expression of estrus defined as removal of >50% of the rub-off coating from the patch on Day 0 (Thomas et al., 2014). Only data from cows responsive to the estrous synchronization protocol and with physical activity recorded from Day -10 to 0 were utilized. Cows that did not express estrus were classified as NOESTR (n = 119). Cows that expressed estrus were ranked by net physical activity: those greater than the median were classified as HIESTR (n = 50) and the other cows as LWESTR (n = 50); Within rows, values with different superscripts (a,b,c) differ ($P \leq 0.05$).
[b] According to (Wagner et al., 1988).

Table 3.3.
Ovarian, physiological, and pregnancy variables in beef cows expressing or not expressing estrus and with differing intensities of behavioral estrous expression based on physical activity determinations[a]

Item	NOESTR	LWESTR	HIESTR	SEM	*P*-value
Ovarian variables[b]					
Cows with no dominant follicle at timed-AI (d 0), %	6.28	4.00	5.92	3.72	0.85
Dominant follicle at timed-AI (d 0), mm	11.6[c]	12.9[b]	13.8[a]	0.5	< 0.01
Corpus luteum volume (d 7), cm^3	3.34[c]	4.98[b]	5.70[a]	0.34	< 0.01
Physiological variables[b]					
Plasma progesterone, ng/mL					
d 0	0.168	0.181	0.170	0.018	0.75
d 7	1.95[b]	3.53[a]	3.61[a]	0.16	< 0.01
P4 to CL ratio (d 7)	0.672	0.805	0.766	0.067	0.19
Estradiol-17β (d 0), pg/mL	6.17	6.06	5.46	0.56	0.39
Pregnancy rates[c], %	19.6[b] (23/119)	50.3[a] (27/50)	43.9[a] (24/50)	8.4	< 0.01

[a] Cows were assigned to an estrous synchronization + timed artificial insemination treatment regimen (Larson et al, 2007) from Day -10 to 0. Physical activity using pedometers as described by Rodrigues et al. (2018). An estrous detection patch (Estrotect; Rockway Inc., Spring Valley, WI, USA) was attached to the tail-head of each cow on Day -3, and expression of estrus defined as removal of >50% of the rub-off coating from the patch on Day 0 (Thomas et al., 2014); Only data from cows responsive to the estrous synchronization protocol and with physical activity recorded from Day -10 to 0 were utilized. Cows that did not express estrus were classified as NOESTR (n = 119). Cows that expressed estrus were ranked by net physical activity: those greater than the median were classified as HIESTR (n = 50) and the other cows as LWESTR (n = 50). All values reported are covariately-adjusted to cow BCS (Wagner et al., 1988) recorded on Day -10; Within rows, values with different superscripts (a,b,c) differ ($P \leq 0.05$).

[b] Transrectal ultrasonography was performed concurrently with blood sampling on Day 0 and 7. Corpus luteum (CL) volume was estimated using the formula for the volume of a sphere (Cooke et al., 2009).

[c] Pregnancy status was verified by detecting a conceptus using transrectal ultrasonography on Day 28. Values reported within parenthesis correspond to number of pregnant cows/total cows responsive to the estrous synchronization treatment regimen and classified based on estrous characteristics.

Table 3.4.
Relative abundances of mRNA transcripts for genes associated with pregnancy establishment in whole blood of cows expressing or not expressing estrus and with differing intensities of behavioral estrous expression based on physical activity determinations[a]

Item	LWESTR	HIESTR	SEM	*P*-value
mRNA transcript abundance, fold effect[b]				
Interferon-stimulated gene 15				
Pregnant	15.00	14.30	3.20	0.83
Non-pregnant	9.80	4.45	2.30	0.26
20,50-oligoadenylate synthetase				
Pregnant	10.60	9.50	1.68	0.64
Non-pregnant	4.70	2.97	2.29	0.64
Myxovirus resistance 2				
Pregnant	9.87	11.30	1.55	0.53
Non-pregnant	5.02	3.27	2.11	0.56

[a] Cows were assigned to an estrous synchronization + timed artificial insemination protocol (Larson et al, 2007) from Day -10 to 0. Physical activity using pedometers as described by Rodrigues et al. (2018); An estrous detection patch (Estrotect; Rockway Inc., Spring Valley, WI, USA) was attached to the tail-head of each cow on Day -3, and expression of estrus defined as removal of >50% of the rub-off coating from the patch on Day 0 (Thomas et al., 2014). Only data from cows responsive to the estrous synchronization protocol and with physical activity recorded from Day -10 to 0 were utilized. Cows that did not express estrus were classified as NOESTR (n = 119); Cows that expressed estrus were ranked by net physical activity: those above the median were classified as HIESTR (n = 50) and the other cows as LWESTR (n = 50). All values reported are covariately-adjusted to cow BCS (Wagner et al., 1988) recorded on Day -10.

[b] On Day 20, blood samples were collected from 84 cows randomly selected from cows with each estrous characteristic (NOESTR, LWESTR, HIESTR) into PAXgene tubes (BD Diagnostics, Sparks, MD, USA) for whole blood RNA extraction. On Day 28, pregnancy status was verified by detecting a conceptus using transrectal ultrasonography. None of the cows in the NOESTR group sampled on Day 20 were diagnosed as pregnant, hence, samples from these cows were not included in the analysis. Values are expressed as relative fold change compared to threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

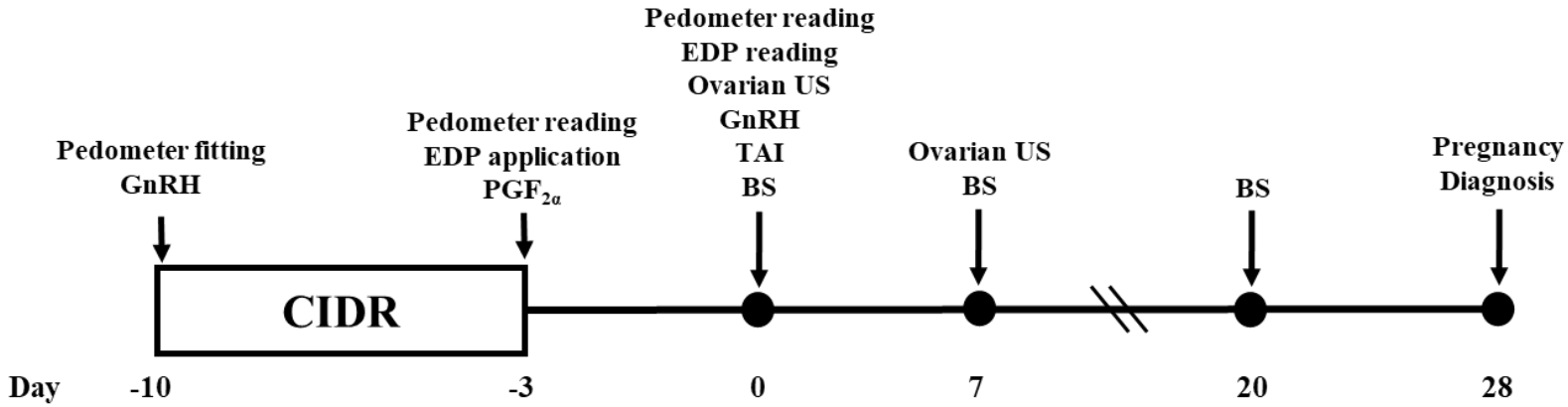

Figure 3.1. Outline of experimental protocol assigned to 273 multiparous suckled beef cows. GnRH = injection of gonadotropin releasing hormone; CIDR = intravaginal progesterone-releasing device; EDP = estrous detection patch; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$ injection; US = ultrasonography; TAI = fixed-time artificial insemination; and BS = blood sampling.

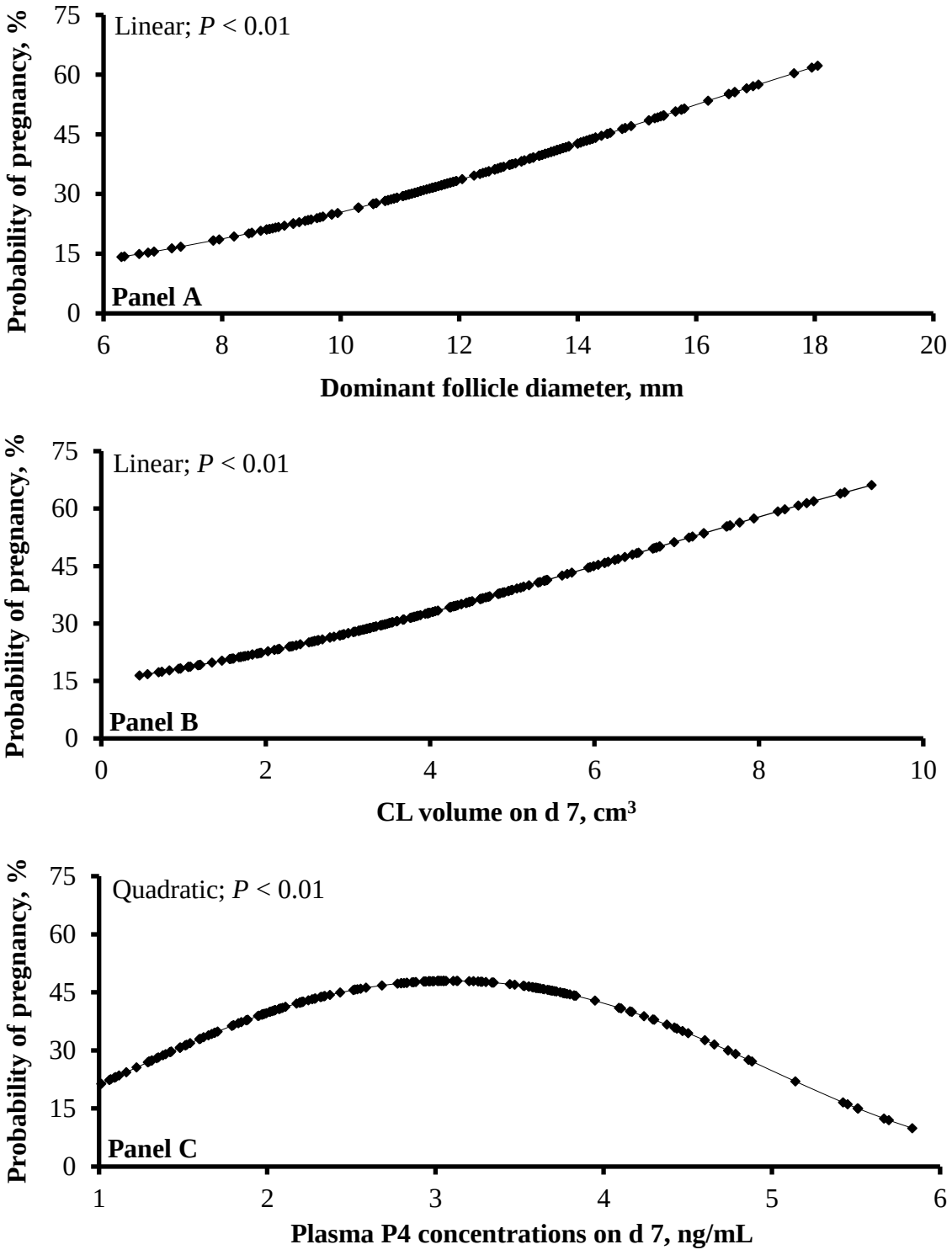

Figure 3.2. Probability of pregnancy to fixed-time artificial insemination (TAI; Day 0) in multiparous beef cows (n = 219) as associated with diameter of dominant follicle on Day 0 (Panel A), volume of the corpus luteum (CL) on Day 7 (Panel B), and plasma progesterone (P4) concentrations on Day 7 (Panel C). Pregnancy status was verified 28 d after TAI using transrectal ultrasonography.

4. PRESYNCHRONIZATION WITH PROSTAGLANDIN $F_{2\alpha}$ AND PROLONGED EXPOSURE TO EXOGENOUS PROGESTERONE IMPACTS ESTRUS EXPRESSION AND FERTILITY IN BEEF HEIFERS[2]

4.1. Introduction

Estrus synchronization is a tool that may be utilized to increase the proportion of beef females becoming pregnant earlier in the breeding season, and as a result, is able to reduce the duration of the calving season and improve calf crop uniformity (Rodgers et al., 2012). When combined with fixed-time artificial insemination (**TAI**), estrus synchronization protocols have achieved pregnancy rates to artificial insemination (**PR/AI**) similar to protocols that make use of estrus detection; therefore, estrus detection and its associated labor can be minimized or removed completely (Lamb et al., 2006; Larson et al., 2006). Numerous estrus synchronization protocols are currently available for use in beef heifers; however, in order to improve current PR/AI, enhancements to these protocols are necessary. By making use of presynchronization in addition to an estrus synchronization strategy in beef heifers, it may be possible to improve estrus expression prior to TAI, and as a result, improve PR/AI.

Through presynchronization, it is possible to increase the proportion of females at a certain stage of the estrous cycle prior to the initiation of an estrus synchronization protocol; consequently, the synchrony of subsequent follicular waves and estrus expression can be improved (Kojima et al., 2000; Busch et al., 2007; Atkins et al., 2008). An injection of

[2] Reprinted with permission from "Presynchronization with prostaglandin $F_{2\alpha}$ and prolonged exposure to exogenous progesterone impacts estrus expression and fertility in beef heifers" by Oosthuizen et al., 2020. *Theriogenology*, 146, 88-93, Copyright 2020 by Theriogenology.

prostaglandin $F_{2\alpha}$ (**PGF**) 3 d prior to estrus synchronization was reported to induce luteal regression before the initial GnRH injection (Grant et al., 2011; Perry et al., 2012), improve control of follicular turnover (Grant et al., 2011; Perry et al., 2012), and increase PR/AI from 55 to 64% (Perry et al., 2012). However, an injection of PGF 7 d prior to the initiation of a 7-d CO-Synch + controlled internal drug release (**CIDR**) protocol decreased estrus expression between CIDR removal and TAI from 55.6 to 39.7% (Oosthuizen et al., 2018). Nevertheless, PR/AI were similar between treatments (45.4 and 43.2%), indicating that presynchronized heifers may have ovulated later than those which were not presynchronized. Presynchronization with PGF and a once-used CIDR insert 7 d prior to the initiation of the 7-d CO-Synch protocol increased the diameter of the largest follicle at the first injection of GnRH (12 vs. 16 mm), increased the rate of ovulation to the first GnRH injection (55 vs. 77%), and increased the mean diameter of the dominant follicle at the second PGF from (13 to 14 mm; Small et al., 2009). Furthermore, presynchronization with an injection of PGF and a twice-used CIDR insert 5 d prior to the 7-d CO-Synch + CIDR protocol increased response to the initial GnRH (60 vs. 36%; Small et al., 2009). However, there is limited research associated with presynchronization with PGF in combination with a progestin on expression of estrus and PR/AI.

We hypothesized that delaying TAI to 72 ± 2 h, in heifers receiving an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol, would increase the proportion of heifers exhibiting estrus by TAI and, as a result, PR/AI would be increased. In addition, we hypothesized that presynchronizing heifers with a new CIDR insert in addition to the injection of PGF, would expose heifers to a reduced concentration of progesterone (**P4**) from the CIDR during the last 7 d of use (Chacher et al., 2017), thereby hastening

follicle development (Mercadante et al., 2015) and improving PR/AI when heifers are artificially inseminated 54 ± 2 h after CIDR removal.

4.2. Materials and Methods

All heifers were handled in accordance with procedures approved by Texas A&M University's Animal Care and Use Committee (IACUC #2018-0478).

4.2.1. Animals and Treatments

A total of 1,700 Angus beef heifers were enrolled in the study at three locations in South Dakota (**SD-1**, **SD-2**, and **SD-3**) over the course of 2018 and 2019 (Table 4.1.). Within location, heifers were randomly assigned to receive one of four treatments (Fig. 4.1.): 1) **PG54** (*n* = 434), heifers were administered with PGF (25 mg im; Lutalyse, dinoprost tromethamine; Zoetis Animal Health) 7 d prior [Day -14] to the initiation of the 7-d CO-Synch + CIDR protocol wherein they received gonadotropin-releasing hormone (**GnRH**; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a CIDR insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) on Day -7, PGF at CIDR removal on Day 0, and a second injection of GnRH concurrently with TAI 54 ± 2 h later; 2) **PG72** (*n* = 426), heifers were exposed to the same treatment as PG54, however, TAI was performed 72 ± 2 h after CIDR removal; 3) **PG-CIDR54** (*n* = 422), same as PG54 but heifers received a CIDR insert on Day -14 instead of Day -7, in conjunction with the injection of PGF; 4) **PG-CIDR72** (*n* = 418), same as PG-CIDR54, however, TAI was performed 72 ± 2 h after CIDR removal. Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to heifers in PG54 and PG72 treatments on Day -14 and were evaluated for activation on Day -7 (**EST1**). Additionally, estrus detection patches were applied to all

heifers on Day 0 and were evaluated for activation at TAI (**EST2**). Estrus patches were considered activated when at least 50% of the rub-off coating was removed from the patch or when the patch was missing. Estrus expression at EST1 was not recorded in PG-CIDR54 and PG-CIDR72 treatments, as P4 from the CIDR insert would inhibit estrus expression (Colazo et al., 2008). The time of CIDR removal and TAI were recorded for each heifer within a 15 min window; therefore, the time (± 30 min) between CIDR removal and TAI could be calculated. Time between CIDR removal and TAI was not recorded for 27 heifers. Heifer BW was recorded on Day -14 at SD-1 and SD-2, and body condition score (**BCS**) was recorded on Day 0 at SD-3 (Wagner et al., 1988). Two technicians performed the artificial insemination (**AI**) at all locations using conventional semen from two bulls. Bull A was used for all heifers at location SD-1, whereas Bull B was used for all heifers at SD-2 and SD-3. Transrectal ultrasonography (Ibex portable ultrasound, 5.0-MHz curved linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO) was performed by a veterinarian between 30 and 47 d after TAI to determine PR/AI.

4.2.2. Statistical Analyses

All data was analyzed as completely randomized design with a 2 by 2 factorial arrangement of treatments using the SAS statistical package (version 9.4; SAS/STAT, SAS Inst. Inc., Cary, NC, USA). The GLIMMIX procedure of SAS was used to analyze the binary response variables (EST1, EST2, and PR/AI), whereas the MIXED procedure of SAS was utilized to analyze the continuous response variables (BW and BCS). The models for both binary and continuous data included the fixed effects of CIDR treatment (yes or no), AI time (54 or 72 h), and the CIDR by AI Time interaction; as well as the random effect of location. Heifer was considered the experimental unit in all analyses. The effect of AI technician was

removed from the models due to non-significance ($P < 0.05$). Artificial insemination sire was confounded by location and, therefore, was not included in the models.

The probability of heifers in each treatment group of becoming pregnant to TAI was evaluated according to time between CIDR removal and TAI. The GLM procedure of SAS was initially used to determine if each individual measurement influenced PR/AI linearly, quadratically, or cubically. The LOGISTIC procedure was used to generate a regression model and to determine the intercept and slope(s) values according to maximum likelihood estimates from each significant continuous order effect. The probability of pregnancy was determined according to the following equation: Probability = $(e^{\text{logistic equation}})/(1 + e^{\text{logistic equation}})$. Logistic curves were constructed according to the values detected for each variable.

Statistical significance was declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered a tendency. Least square means ± SEM are reported.

4.3. Results

Body weight on Day -14 was similar ($P = 0.42$) among treatment groups at SD-1 and SD-2 and BCS at SD-3 was similar ($P = 0.61$) among treatments.

Estrus expression at EST1 was similar ($P = 0.35$) between PG54 and PG72 treatment groups (61.7 ± 0.02 and 63.0 ± 0.02%, respectively). The percentage of heifers exhibiting estrus at EST2 was greater ($P < 0.01$) in the PG72, PG-CIDR54, and PG-CIDR72 treatments compared to the PG54 treatment (Fig. 4.2.). Furthermore, estrus response at EST2 was greater ($P < 0.01$) in PG-CIDR54 and PG-CIDR72 heifers when compared to PG72. A tendency ($P = 0.09$) was observed on estrus expression at EST2 between PG-CIDR54 and PG-CIDR72 treatments.

Pregnancy rates to TAI were greater ($P < 0.05$) in the PG72 and PG-CIDR54 treatments when compared to PG-CIDR72 (Fig. 4.3.). In addition, PG-CIDR54 heifers had greater ($P = 0.03$) PR/AI than PG54. A tendency ($P = 0.10$) for an increase in PR/AI was observed between PG54 and PG72 heifers. No differences were determined between PG72 and PG-CIDR54 ($P = 0.64$) or between PG54 and PG-CIDR72 ($P = 0.16$).

The time between CIDR removal and TAI ranged from 49.2 to 56.3 h in PG54 and PG-CIDR54 heifers, and from 68.8 to 73.8 h in PG72 and PG-CIDR72 heifers. A linear relationship ($P = 0.04$) was determined between the probability of pregnancy in PG54 heifers and time between CIDR removal and TAI, where probability of pregnancy increased with increasing time (Fig. 4.4.). Furthermore, a tendency ($P = 0.08$) for a linear relationship was determined between the probability of pregnancy in PG-CIDR54 heifers and time between CIDR removal and TAI, where the probability of pregnancy increased as time increased. No relationship ($P \geq 0.72$) between the probability of pregnancy and time between CIDR removal and TAI was determined in either PG72 or PG-CIDR72 heifers.

Regardless of estrus expression, there was a tendency ($P = 0.08$) for a positive linear relationship between the probability of pregnancy and time between CIDR removal and TAI in PG54 heifers (Fig. 4.4.). However, no relationships ($P \geq 0.22$) between the probability of pregnancy and time between CIDR removal and TAI were determined in PG72, PG-CIDR54, or PG-CIDR72 heifers that either expressed or did not express estrus.

4.4. Discussion

We hypothesized that both estrus expression and PR/AI would be increased by delaying TAI to 72 h in heifers presynchronized with PGF. Our results indicate that delayed

TAI increased estrus expression in PG72 heifers; however, there was only a tendency for an increase in PR/AI. In addition, we hypothesized that heifers presynchronized with a CIDR insert in addition to an injection of PGF, would have greater PR/AI when TAI 54 h after CIDR removal. Our results indicate that the PG-CIDR54 treatment succeeded at increasing PR/AI.

Administration of PGF induces CL regression in the female bovine, which will return to estrus in approximately 3 d (Lauderdale et al., 1974; Louis et al., 1974). In the current study, no differences were determined in estrus expression at EST1, indicating that a similar percentage of heifers in the PG54 and PG72 treatments responded to the injection of PGF on Day -14, and underwent CL regression. When heifers at different stages of the estrous cycle receive an injection of PGF, ovulation occurs approximately 84 ± 4 h later (Nkuuhe and Manns, 1985) in a large proportion of those with a CL. Subsequently, a new follicular wave is initiated and a new CL is formed. In the present study, heifers likely responded to presynchronization with PGF in one of two ways. 1) The dominant follicles developing between Day -14 and -7 in PG54 and PG72 heifers were likely too small to respond to the injection of GnRH on Day -7, as smaller follicles (< 10.1 mm) are less likely to ovulate in response to a GnRH injection (Perry et al., 2007). Although concentrations of plasma P4 were not measured in this experiment, it is likely that concentrations of P4 were elevated between Day -7 and 0 in PG54 and PG72 heifers that responded to the PGF, due to the presence of both the CIDR insert and the newly formed CL. It is plausible that these greater circulating concentrations of P4 in PG54 and PG72 heifers resulted in the follicle growing at a reduced rate between Day -7 and 0 (Cerri et al., 2011; Mercadante et al., 2015); therefore, increasing the interval between CIDR removal and estrus expression. Heifers not responding

to the initial GnRH injection may have underwent follicular atresia between Day -7 and 0, and subsequently initiated a new follicular wave. The dominant follicle from this new follicular wave would likely have been induced to ovulate with the injection of GnRH at TAI. 2) A proportion of heifers may have had follicles sufficiently large enough to respond to the initial GnRH injection on Day -7, and would have initiated a new follicular wave between Day -7 and 0. This dominant follicle may have ovulated spontaneously or been induced to ovulate at TAI by the injection of GnRH. In our previous experiment, we noted that estrus expression was reduced between Day 0 and TAI at 54 h in heifers receiving an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol when compared to controls (Oosthuizen et al., 2018). In the present study, by delaying TAI to 72 h in the PG72 treatment, expression of estrus was increased compared to PG54 heifers. Expression of estrus prior to TAI has repeatedly been shown to result in greater PR/AI in beef females when compared to those that do not exhibit estrus (Perry and Perry, 2008; Richardson et al., 2016). However, in the present study, although estrus expression was increased by 47%, there was only a tendency for PR/AI to be increased by 5.7%. It is likely that heifers in the PG54 treatment group were beginning to come into estrus at TAI but were induced to ovulate by the injection of GnRH, resulting in acceptable PR/AI. Whereas it is likely that most PG72 heifers had spontaneously initiated ovulation prior to TAI and that the injection of GnRH at TAI had little effect on PR/AI. Therefore, the marginal increase in PR/AI when compared to estrus expression can likely be attributed to more heifers in the PG54 treatment group becoming pregnant as a result of induced ovulation.

High circulating concentrations of P4 are known to inhibit ovulation and subsequent CL formation (Colazo et al., 2008). Thus, we hypothesized that the addition of a CIDR insert

on Day -14 may prevent ovulation from occurring after the initial injection of PGF on Day -14, and that heifers would instead undergo follicular atresia prior to Day -7. It is, therefore, reasonable to assume that heifers receiving a CIDR on Day -14 responded in one of two ways. 1) A proportion of the heifers in PG-CIDR54 and PG-CIDR72 treatments may not have had a functional CL between Day -7 and 0. Hence, the only source of P4 during this period was derived from the CIDR insert, which results in reduced circulating concentrations of P4 after 7 d of use (Chacher et al., 2017). 2) A proportion of heifers may have responded to the injection of GnRH on Day -7 and would have initiated a new follicular wave shortly thereafter. The dominant follicle from this new follicular wave would have been destined to ovulate prior to or at TAI. Heifers in PG-CIDR54 and PG-CIDR72 treatments likely had lower circulating concentrations of P4 at CIDR removal when compared to PG54 and PG72 heifers. In the present experiment, estrus expression at EST2 did not differ between PG-CIDR54 and PG-CIDR72 treatments; however, was greater in PG-CIDR54 and PG-CIDR72 vs. the PG54 treatment. A rapid decrease in circulating concentrations of P4 after PGF administration and CIDR removal has the ability to enhance the proportion of beef heifers exhibiting estrus within 60 h (Fontes et al., 2019); therefore, decreased concentrations of P4 at CIDR removal likely reduced the interval between CIDR removal and estrus expression in PG-CIDR54 and PG-CIDR72 heifers. The increase in estrus expression led to greater PR/AI in PG-CIDR54 vs. PG54 heifers; however, PR/AI in PG-CIDR72 heifers were significantly lower than PG54, PG72, and PG-CIDR72 treatments. It is likely that the PG-CIDR54 and PG-CIDR72 treatments altered the distribution of estrus and ovulation, and that PG-CIDR72 heifers ovulated too early for TAI to be performed at 72 h, leading to a reduction

in PR/AI. Future research should include ovarian ultrasonography and hormonal analyses in order to determine the mechanism for the increase in PR/AI in the PG-CIDR54 treatment.

The positive linear relationship in PG54 heifers between the probability of pregnancy and the interval between CIDR removal and TAI indicates that heifers were more likely to become pregnant when this period was greater and, therefore, supports our initial hypothesis. Treatments were separated into estrus and non-estrus groups to determine if estrual status at EST2 affected the relationships between the probability of pregnancy and timing of TAI. In heifers that expressed estrus, a tendency for a positive linear relationship in the PG54 treatment indicates that heifers are more likely to become pregnant when the timing is extended. Similarly, PG54 heifers which did not exhibit estrus, tended to have greater PR/AI when the time between CIDR removal and TAI was extended, further supporting our hypothesis of delaying TAI to 72 h to improve PR/AI in presynchronized beef heifers.

In conclusion, the combination of PGF administration 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol and TAI at 72 h after CIDR removal resulted in a tendency for an increase in PR/AI in replacement beef heifers. Furthermore, presynchronization with a CIDR insert, in addition to PGF, succeeded in enhancing PR/AI. These treatments may potentially be utilized as alternatives to facilitate the use of TAI in beef heifers. Further research is required to determine the effectiveness of the PG72 and PG-CIDR54 treatments in comparison to the 7-d CO-Synch + CIDR protocol.

4.5. References

Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. Lucy, and M. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: effect of day of the cycle. J. Anim. Sci. 86:83–93.

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933–1939. doi:10.2527/jas.2006-845.

Cerri, R. L. A., R. C. Chebel, F. Rivera, C. D. Narciso, R. A. Oliveira, and W. W. Thatcher. 2011. Concentration of progesterone during the development of the ovulatory follicle : I . Ovarian and embryonic responses. J. Dairy Sci. 94:3342–3351. doi:10.3168/jds.2010-3734.

Chacher, M. F. ., A. Çolak, and A. Hayirli. 2017. Efficacy of repeatedly used CIDR device in cattle reproduction :a metaanalysis review of progesterone concentration and conception rate. Turkish J. Vet. Anim. Sci. 41:692–697. doi:10.3906/vet-1706-75.

Colazo, M. G., J. P. Kastelic, H. Davis, M. D. Rutledge, M. F. Martinez, J. A. Small, and R. J. Mapletoft. 2008. Effects of plasma progesterone concentrations on LH release and ovulation in beef cattle given GnRH. 34:109–117. doi:10.1016/j.domaniend.2006.11.004.

Fontes, P. L. P., R. F. Cooke, N. Oosthuizen, C. L. Timlin, N. W. Dias, J. F. Currin, S. Clark, K. G. Pohler, G. C. Lamb, and V. R. G. Mercadante. 2019. Impacts of administering prostaglandin F2α analogue 24 h prior to progesterone insert removal on expression of estrus in beef females. Livest. Sci. 226:82–86. doi:10.1016/j.livsci.2019.06.007.

Grant, J. K., F. M. Abreu, N. L. Hojer, S. D. Fields, B. L. Perry, and G. A. Perry. 2011. Influence of inducing luteal regression before a modified controlled internal drug-releasing device treatment on control of follicular development. J. Anim. Sci. 89:3531–3541. doi:10.2527/jas.2011-3852.

Kojima, F. N., B. E. Salfen, J. F. Bader, W. A. Ricke, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 synch. J. Anim. Sci. 78:2186–2191.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:3000–3009. doi:10.2527/jas.2006-220.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. Dejarnette, F. N. Schrick, A. Dicostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J. Anim. Sci. 84:332–342.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of cattle following PGF2a injection. J. Anim. Sci. 38:964–967. doi:10.2527/jas1974.385964x.

Louis, T. M., H. D. Hafs, and D. A. Morrow. 1974. Intrauterine administration of prostaglandin $F_{2\alpha}$ in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347–353.

Mercadante, V. R. G., L. E. Kozicki, F. M. Ciriaco, D. D. Henry, C. R. Dahlen, M. R. Crosswhite, J. E. Larson, B. E. Voelz, D. J. Patterson, G. A. Perry, R. N. Funston, T. L. Steckler, S. L. Hill, J. S. Stevenson, and G. C. Lamb. 2015. Effects of administration of prostaglandin F2alpha at initiation of the seven-day CO-Synch+controlled internal drug release ovulation synchronization protocol for suckled beef cows and replacement beef heifers. J. Anim. Sci. 93:5204–5213. doi:10.2527/jas.2015-8967.

Nkuuhe, J. R., and J. G. Manns. 1985. Relationship between time of prostaglandin injection and ovulation in beef cattle. Can. J. Anim. Sci. 65:405–409.

Oosthuizen, N., L. B. Canal, P. L. P. Fontes, C. D. Sanford, N. DiLorenzo, C. R. Dahlen, G. E. Seidel, and G. C. Lamb. 2018. Prostaglandin F2α 7 d prior to initiation of the 7-d CO-synch + CIDR protocol failed to enhance estrus response and pregnancy rates in beef heifers. J. Anim. Sci. 96:1466–1473. doi:10.1093/jas/sky058.

Perry, G. A., and B. L. Perry. 2008. Effects of standing estrus and supplemental estradiol on changes in uterine pH during a fixed-time artificial insemination protocol. J. Anim. Sci. 86:2928–2935. doi:10.2527/jas.2008-1181.

Perry, G. A., B. L. Perry, J. H. Krantz, and J. Rodgers. 2012. Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success. J. Anim. Sci. 90:489–494. doi:10.2527/jas.2011-4319.

Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Anim. Sci. 85:684–689. doi:10.2527/jas.2006-519.

Richardson, B. N., S. L. Hill, J. S. Stevenson, G. D. Djira, and G. A. Perry. 2016. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim. Reprod. Sci. 166:133–140. doi:10.1016/j.anireprosci.2016.01.013.

Rodgers, J. C., S. L. Bird, J. E. Larson, N. Dilorenzo, C. R. Dahlen, A. Dicostanzo, and G. C. Lamb. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. J. Anim. Sci. 90:4055–4062. doi:10.2527/jas.2011-4836.

Small, J. A., M. G. Colazo, J. P. Kastelic, and R. J. Mapletoft. 2009. Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. Theriogenology. 71:698–706. doi:10.1016/j.theriogenology.2008.09.045.

Wagner, J., K. Lusby, J. Oltjen, J. Rakestraw, R. Wettemann, and L. Walters. 1988. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603–612.

Table 4.1. Descriptive data by location[a]

Item	SD-1	SD-2	SD-3
No. of heifers	499	660	541
Breed	Angus	Angus	Angus
Breeding season	April to June 2018	June to July 2018	June to July 2019
Mean BW, kg[b]	397.8 ± 1.7	464.9 ± 1.5	-
Mean BCS[c]	-	-	6.0 ± 0.02
Mean time of TAI in 54 h groups, h[d]	51.9 ± 0.1	53.3 ± 0.1	53.7 ± 0.1
Mean time of TAI in 72 h groups, h[e]	71.3 ± 0.1	71.6 ± 0.1	71.2 ± 0.1

[a] Three locations in South Dakota (SD-1, SD-2, and SD-3).

[b] Body Weight was recorded on Day -14.

[c] Body Condition Score was recorded on Day 0.

[d] The mean interval between CIDR removal and TAI for heifers assigned to TAI at 54 ± 2 h.

[e] The mean interval between CIDR removal and TAI for heifers assigned to TAI at 72 ± 2 h.

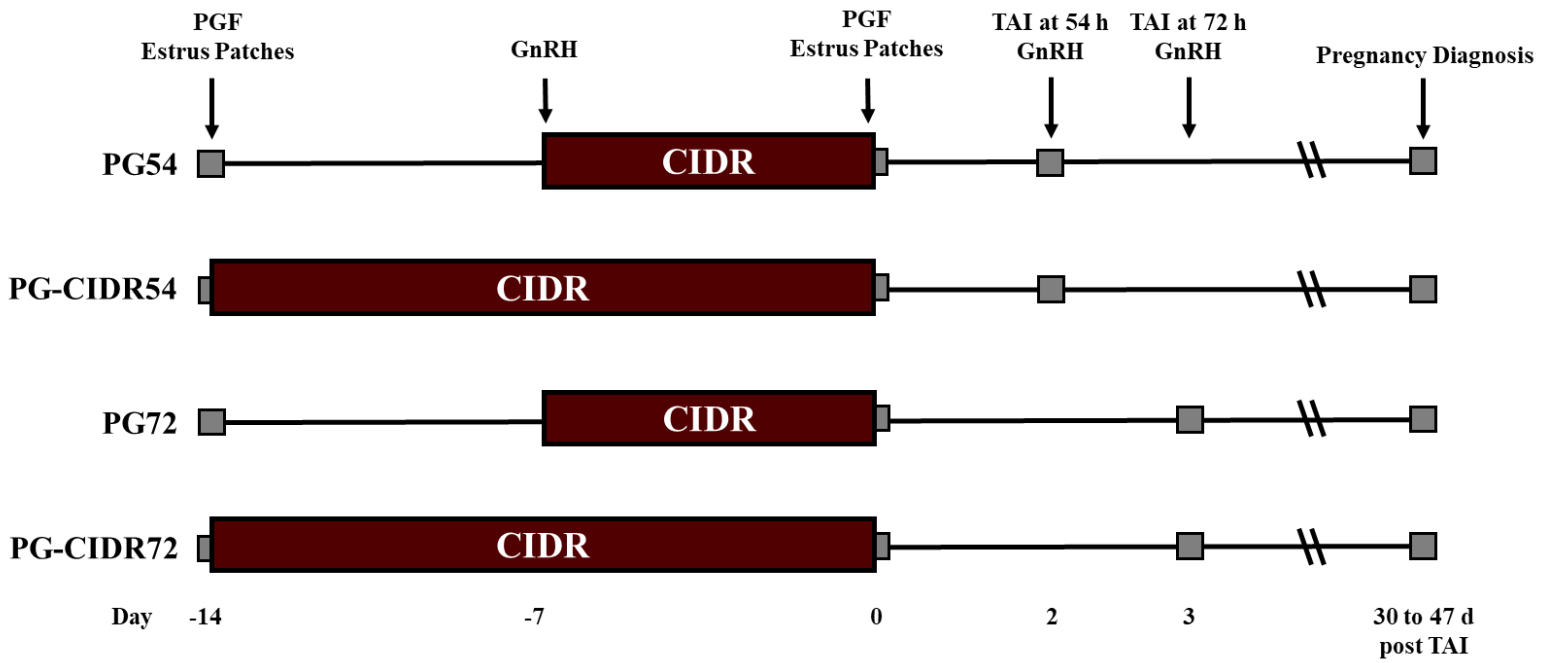

Figure 4.1. Schematic of treatments. PG54 (*n* = 434), heifers were administered prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse, dinoprost tromethamine; Zoetis Animal Health) 7 d prior [Day -14] to the initiation of the 7-d CO-Synch + CIDR protocol; PG72 (*n* = 426), heifers were exposed to the same treatment as PG54, however, TAI was performed 72 ± 2 h after CIDR removal; PG-CIDR54 (*n* = 422), same as PG54 but heifers received a CIDR insert (EAZI-BREED CIDR; 1.38 g progesterone; Zoetis Animal Health) on Day -14 in addition to the injection of PGF; PG-CIDR72 (*n* = 418), same as PG-CIDR54, however, TAI was performed 72 ± 2 h after CIDR removal. Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to heifers in PG54 and PG72 treatments on Day -14 and were evaluated for activation on Day -7. In addition, estrus detection patches were applied to all heifers on Day 0 and were evaluated for activation at TAI (54 ± 2 or 72 ± 2 h after CIDR removal). Pregnancy diagnosis was performed between 30 and 47 d after TAI.

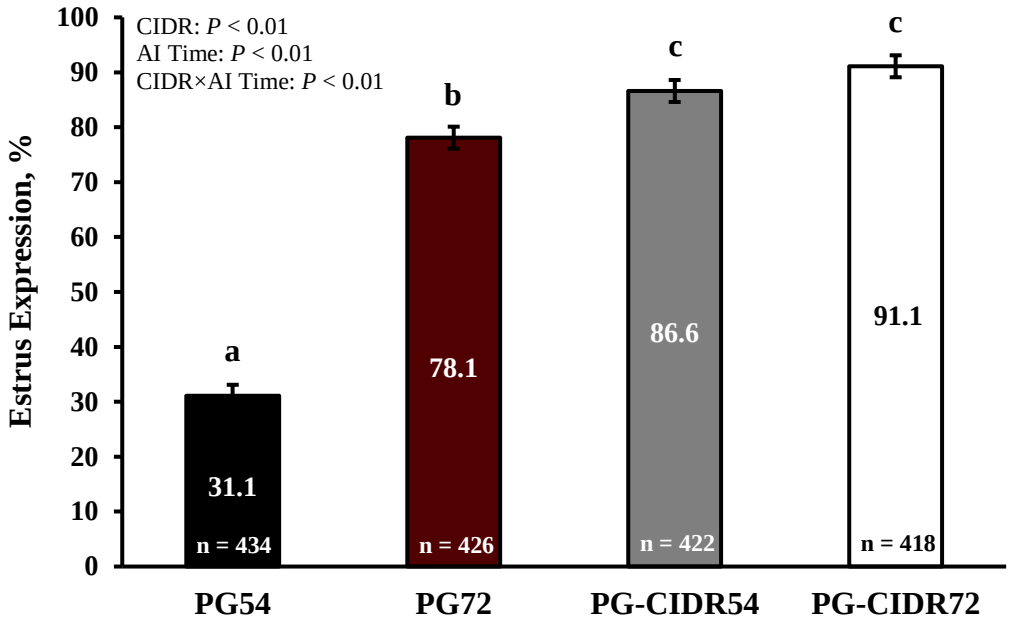

Figure 4.2. Estrus expression between CIDR removal and TAI among treatments. PG54 (*n* = 434), heifers were administered prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse, dinoprost tromethamine; Zoetis Animal Health) 7 d prior [Day -14] to the initiation of the 7-d CO-Synch + CIDR protocol; PG72 (*n* = 426), heifers were exposed to the same treatment as PG54, however, TAI was performed 72 ± 2 h after CIDR removal; PG-CIDR54 (*n* = 422), same as PG54 but heifers received a CIDR insert (EAZI-BREED CIDR; 1.38 g progesterone; Zoetis Animal Health) on Day -14 in addition to the injection of PGF; PG-CIDR72 (*n* = 418), same as PG-CIDR54, however, TAI was performed 72 ± 2 h after CIDR removal. Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to all heifers on Day 0 and were evaluated for activation at TAI (54 ± 2 or 72 ± 2 h after CIDR removal). [a,b,c]Bars with different superscripts differ ($P < 0.05$).

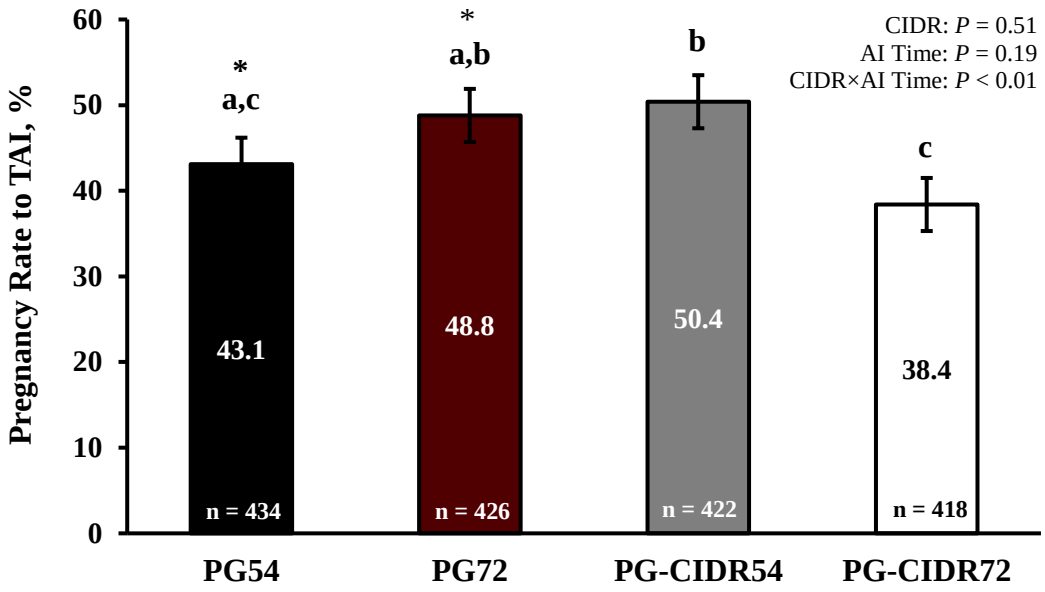

Figure 4.3. Pregnancy rates to fixed-time artificial insemination (TAI) among treatments. PG54 (n = 434), heifers were administered prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse, dinoprost tromethamine; Zoetis Animal Health) 7 d prior [Day -14] to the initiation of the 7-d CO-Synch + CIDR protocol; PG72 (n = 426), heifers were exposed to the same treatment as PG54, however, TAI was performed 72 ± 2 h after CIDR removal; PG-CIDR54 (n = 422), same as PG54 but heifers received a CIDR insert (EAZI-BREED CIDR; 1.38 g progesterone; Zoetis Animal Health) on Day -14 in addition to the injection of PGF; PG-CIDR72 (n = 418), same as PG-CIDR54, however, TAI was performed 72 ± 2 h after CIDR removal. Pregnancy to AI was diagnosed between 30 and 47 d after TAI (Ibex portable ultrasound, 5.0-MHz curved linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO). [a,b,c]Bars with different superscripts differ ($P < 0.05$). [*]Indicates a tendency ($0.05 < P \leq 0.10$) for treatment differences.

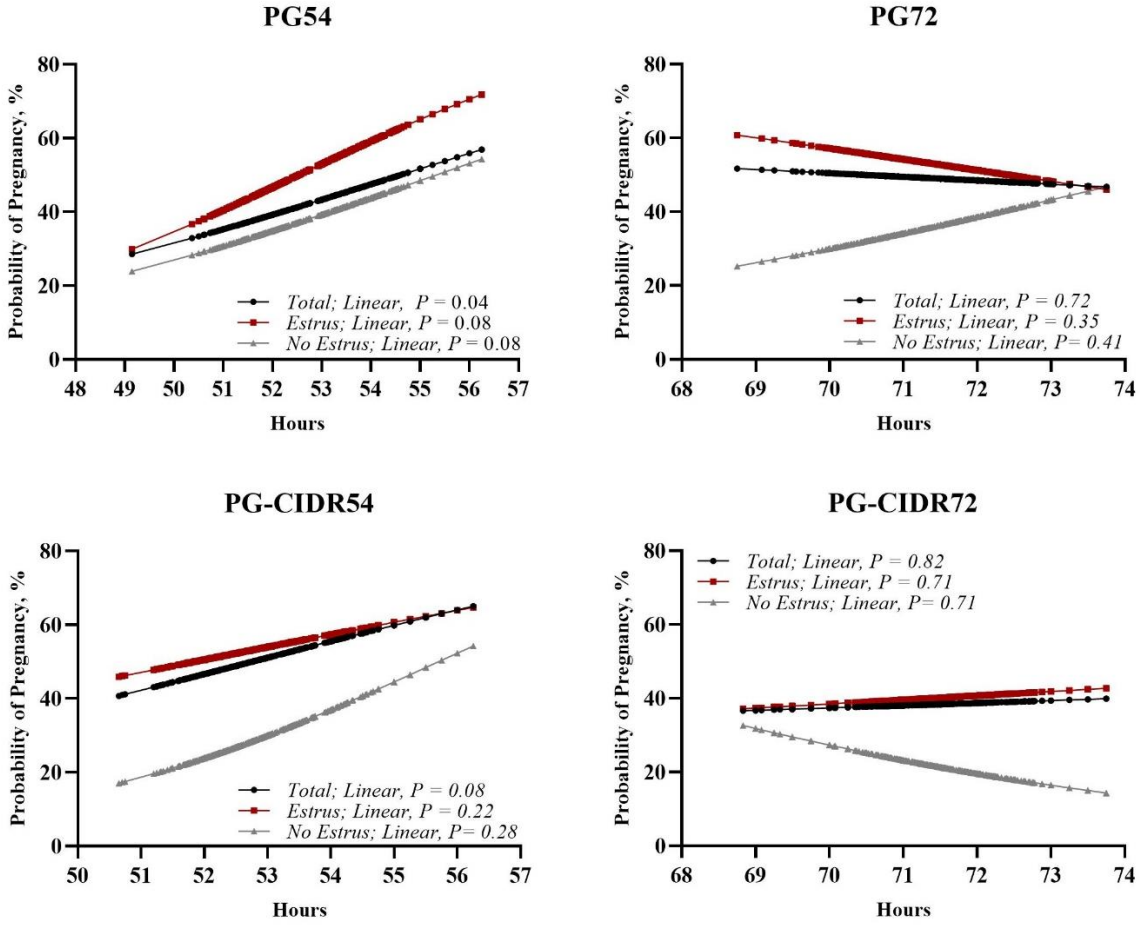

Figure 4.4. Probability of pregnancy to timed-AI (Day 0) according to time between CIDR removal and TAI in all heifers (*n* = 1,673), heifers which expressed estrus (*n* = 1,199), and heifers that did not express estrus (*n* = 474). PG54 (*n* = 426), heifers were administered prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse, dinoprost tromethamine; Zoetis Animal Health) 7 d prior [Day -14] to the initiation of the 7-d CO-Synch + CIDR protocol (Panel A); PG72 (*n* = 414), heifers were exposed to the same treatment as PG54, however, TAI was performed 72 ± 2 h after CIDR removal (Panel B); PG-CIDR54 (*n* = 416), same as PG54 but heifers received a CIDR insert (EAZI-BREED CIDR; 1.38 g progesterone; Zoetis Animal Health) on Day -14 in addition to the injection of PGF (Panel C); PG-CIDR72 (*n* = 417), same as PG-CIDR54, however, TAI was performed 72 ± 2 h after CIDR removal (Panel D). The time of CIDR removal and TAI were recorded for each heifer within a 15 min window; therefore, the time (± 30 min) between CIDR removal and TAI could be calculated. Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to all heifers on Day 0 and were evaluated for activation at TAI. Pregnancy to AI was diagnosed between 30 and 47 d after TAI (Ibex portable ultrasound, 5.0-MHz curved linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO).

5. EFFECTS OF PRESYNCHRONIZATION AND DELAYED FIXED-TIME ARTIFICIAL INSEMINATION WITH SEX-SORTED SEMEN ON FERTILITY IN BEEF HEIFERS

5.1. Introduction

Since 2007, the commercialization of sex-sorted semen has increased dramatically. Enhanced equipment and improvements in processing procedures, increased pregnancy rates to artificial insemination (**AI**), and reduction in costs have played large roles in this increase. There are a number of benefits associated with the utilization of sex-sorted semen, such as selecting calf gender with greater than 90% accuracy, faster genetic progress, and the removal of defective sperm through the sorting process (Seidel, 2014). In addition, it is easy to incorporate the use of sexed semen into a management system if AI is already being performed.

One of the primary challenges associated with the large-scale adoption of sex-sorted semen in the beef industry is the lower pregnancy rates to fixed-time AI (**PR/AI**) protocols typically achieved, which are usually in the range of 80 to 90% of PR/AI with conventional semen (DeJarnette et al., 2009; Seidel, 2014). Delayed fixed-time AI (**TAI**) has been suggested to improve PR/AI; however, delaying TAI by 8 h in beef cows did not improve PR/AI, despite an increase in estrus expression (Hall et al., 2017). Conversely, PR/AI were increased by 15% in dairy heifers when TAI with sex-sorted semen was delayed by 6 h (Sales et al., 2011). Currently, no official TAI protocols have been established specifically for the use of sex-sorted semen, which limits its adoption in the beef industry.

Presynchronization has the potential to increase the proportion of females at a certain stage of the estrous cycle prior to the initiation of an estrus synchronization protocol; thus, improving the synchrony of subsequent follicular waves and estrus expression (Kojima et al., 2000; Busch et al., 2007; Atkins et al., 2008). When prostaglandin $F_{2\alpha}$ (**PGF**) was administered 3 d prior to estrus synchronization, control of follicular turnover was improved (Grant et al., 2011; Perry et al., 2012) and PR/AI where increased from 55 to 64% (Perry et al., 2012). Moreover, when heifers received an injection of PGF 7 d prior to the 7-d CO-Synch + controlled internal drug release (**CIDR**) protocol, with TAI delayed to 72 h, there was a tendency for an increase in PR/AI (Oosthuizen et al., 2020).

Therefore, the objectives of this experiment were as follows: 1) to determine whether delayed timing of TAI after presynchronization with PGF 7 d prior to initiation of the 7-d CO-Synch + CIDR protocol in replacement beef heifers enhances PR/AI with sex-sorted semen, and 2) to compare PR/AI between conventional and sex-sorted semen. We hypothesized that heifers presynchronized with PGF and TAI at 72 h with sex-sorted semen would have greater PR/AI than heifers TAI with sex-sorted semen in the conventional 7-d CO-Synch + CIDR protocol. Furthermore, we hypothesized that PR/AI would be greater in conventional semen treatments when compared to sex-sorted semen treatments.

5.2. Materials and Methods

All heifers were handled in accordance with procedures approved by Texas A&M University's Animal Care and Use Committee (IACUC #2018-0478).

5.2.1. Animals and Treatments

A total of 2,855 *Bos taurus* beef heifers from 23 locations across 11 states were enrolled in a completely randomized design (Table 5.1.). Within location, heifers were randomly assigned to one of eight different treatment groups (Fig. 5.1.): 1 and 2), heifers were exposed to the 7-d CO-Synch + CIDR protocol wherein they received an injection of gonadotropin-releasing hormone (**GnRH**; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a CIDR insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) on Day 0, an injection of PGF (25 mg im; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health) upon CIDR removal on Day 7, and were TAI 54 ± 2 h later with either conventional (**CTRL-CNV**; *n* = 359) or sex-sorted semen (**CTRL-SEX**; *n* = 356); 3 and 4), heifers were treated the same as CTRL but were TAI at 72 ± 2 h with either conventional (**CTRL72-CNV**; *n* = 366) or sex-sorted semen (**CTRL72-SEX**; *n* = 360); 5 and 6), treated the same as CTRL but received an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol (Day -7) and were then TAI with either conventional (**PRE54-CNV**; *n* = 355) or sex-sorted semen (**PRE54-SEX**; *n* = 353); 7 and 8), treated the same as PRE54 treatments but had TAI delayed to 72 ± 2 h and were inseminated with conventional (**PRE72-CNV**; *n* = 351) or sex-sorted semen (**PRE72-SEX**; *n* = 355). All heifers were fitted with estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) at CIDR removal and were evaluated for activation at TAI to determine estrus expression. Estrus patches were considered activated when at least 50% of the rub-off coating was removed from the patch or when the patch was missing. Retrospectively, heifers were categorized into four estrus expression groups (**CTRL**, **CTRL72**, **PRE54**, and **PRE72**) according to their treatment up until but excluding TAI. On

Day -7, heifer body weight (**BW**) was recorded at 12 locations, and body condition score (**BCS**) was recorded at 19 locations (Wagner et al., 1988). Each location provided their own AI technician(s), selected semen from bulls based on their production system, selected X- or Y-sorted sperm, and received both conventional and sex-sorted semen (SexedULTRA™ 4M; ST Genetics, Navasota, TX) from their respective bull(s) of choice. In total, conventional (approximately 15 x 10^6 sperm cells per 0.5 ml straw pre-freezing) and sex-sorted (approximately 4 x 10^6 sperm cells per 0.25 ml straw pre-freezing) semen was used from 24 different bulls. Transrectal ultrasonography was performed by a veterinarian at each location between 30 and 45 d after TAI to determine PR/AI. Final pregnancy rates were determined at 18 locations at least 30 d after the end of the breeding season.

5.2.2. Statistical Analyses

All data was analyzed as completely randomized design using the SAS statistical package (version 9.4; SAS/STAT, SAS Inst. Inc., Cary, NC, USA). Heifer was considered the experimental unit in all analyses. The GLIMMIX procedure of SAS was used to analyze the binary response variables (Estrus expression, PR/AI, final pregnancy rate), as well as the continuous response variables (BW and BCS). The model for estrus expression included the fixed effect of Estrus Expression Group and the random effect of Location. The model for PR/AI included the fixed effects of Treatment, Estrus Expression, and the interaction; as well as the random effect of Location. All other binary and continuous models included the fixed effect of Treatment and the random effect of Location. To ensure that there was no effect of Sire on Treatments, the Sire × Treatment interaction was analyzed, revealing no significance. In addition, the effect of AI Tech was initially included in the model but was

removed due to non-significance. Statistical significance was declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered a tendency. Least square means ± SEM are reported.

5.3. Results

Body weight at 13 locations (371.3 ± 50.2 kg) and BCS at 19 locations (5.6 ± 0.6) on Day -7 were similar ($P \geq 0.86$) among treatment groups.

Percentage of heifers expressing estrus differed ($P < 0.01$) among estrus expression groups, where a greater ($P < 0.01$) percentage of CTRL72 heifers expressed estrus when compared to CTRL, PRE54, and PRE72 heifers (Fig. 5.2.). Furthermore, PRE72 heifers had greater ($P < 0.01$) expression of estrus than CTRL and PRE54 heifers. Finally, estrus expression was greater ($P < 0.001$) in CTRL heifers when compared to PRE54 heifers.

No treatment by estrus expression interaction ($P = 0.13$) was determined for PR/AI (Table 5.2.); however, PR/AI differed ($P < 0.01$) by estrus expression, where heifers that expressed estrus had greater PR/AI than those which did not express estrus (53.0 vs. 34.0%). Furthermore, PR/AI differed ($P < 0.01$) among treatment groups (Fig. 5.3.). CTRL-CNV heifers had greater ($P \leq 0.02$) PR/AI than CTRL-SEX, CTRL72-SEX, and PRE54-SEX heifers, but similar ($P \geq 0.20$) PR/AI to all other treatments. Heifers in the CTRL-SEX treatment group had similar ($P \geq 0.22$) PR/AI when compared to CTRL72-SEX and PRE54-SEX heifers; however, PR/AI were reduced ($P \leq 0.02$) in the CTRL-SEX treatment when compared to all other treatment groups. Pregnancy rates to TAI in CTRL72-CNV heifers were greater ($P < 0.01$) than CTRL72-SEX, and PRE54-SEX heifers but did not differ ($P \geq 0.57$) when compared to PRE72-CNV heifers. Furthermore, PR/AI of CTRL72-CNV treated heifers tended to be greater than those of PRE54-CNV ($P = 0.08$) and PRE72-SEX ($P =$

0.06) heifers. CTRL72-SEX heifers had greater ($P = 0.03$) PR/AI than PRE54-SEX heifers, lesser ($P \leq 0.03$) PR/AI compared to CTRL-CNV, CTRL72-CNV, and PRE72-CNV heifers, but had similar ($P \geq 0.22$) PR/AI compared to all other treatment groups. Heifers in the PRE54-CNV treatment had greater ($P \leq 0.02$) PR/AI that heifers in the PRE54-SEX treatment. Moreover, PR/AI of PRE54-CNV heifers were lesser ($P = 0.04$) than heifers in the PRE72-CNV treatment but did not differ ($P \geq 0.23$) from the PRE72-SEX treatment. Pregnancy rates to TAI in PRE54-SEX heifers were lesser ($P \leq 0.03$) than heifers in the PRE72-CNV, PRE72-SEX treatments but were greater than PRE72-SEX heifers. Lastly, PRE72-SEX heifers had greater ($P = 0.02$) PR/AI than CTRL-SEX heifers, lesser ($P = 0.04$) PR/AI than PRE72-CNV, and similar ($P \geq 0.20$) PR/AI when compared to CTRL-CNV, CTRL72-SEX, and PRE54-CNV heifers.

At the end of the breeding season no differences ($P = 0.86$) in final pregnancy rates from the 18 locations were determined among treatment groups. Furthermore, final pregnancy rates ranged from 79.5 to 83.6%.

5.4. Discussion

The goal of this experiment was to determine the influence of presynchronization with PGF, delayed TAI, and semen type on PR/AI in replacement beef heifers. We hypothesized that heifers in the PRE72-SEX treatment would have greater PR/AI than heifers in the CTRL-SEX treatment. Our results indicate that PR/AI were 8.3% greater in the PRE72-SEX treatment group compared to the CTRL-SEX treatment, which supports our initial hypothesis. In addition, we hypothesized that PR/AI would be greater in the treatments when conventional semen was used compared to sex-sorted semen, and PR/AI were 13%

greater in CTRL-CNV heifers when compared to CTRL-SEX heifers, 10.7% greater in CTRL72-CNV heifers compared to CTRL72-SEX, 12.1% greater in PRE54-CNV compared to PRE54-SEX, and 7.7% greater in PRE72-CNV when compared to PRE72-SEX heifers, supporting our second hypothesis.

5.4.1. Presynchronization

In heifers with a functional corpus luteum (**CL**), luteolysis can be induced through PGF administration, where responding heifers may return to estrus in approximately 3 d (Lauderdale et al., 1974; Louis et al., 1974). Estrus response after presynchronization with PGF was not determined in the current study; however, when beef heifers were administered PGF at random stages of their estrous cycle, 62 to 70% returned to estrus within 7 d (Oosthuizen et al., 2018; Oosthuizen et al., 2020). It is plausible that a large proportion of heifers in the PRE54 and PRE72 treatments responded to the initial injection of PGF on Day -7 and would have returned to estrus prior to Day 0. Responding heifers would likely have ovulated and initiated a new follicular wave between Day -7 and 0. The dominant follicle from this new follicular wave may have been too small to respond to the injection of GnRH on Day 0, as smaller follicles (<10.1 mm) are less likely to ovulate in response to an injection of GnRH (Perry et al., 2007). It is plausible that this follicle underwent atresia between Day 0 and 7 due to the high progesterone concentrations induced by a newly formed CL and the presence of the CIDR insert (Adams et al., 2008). The dominant follicle from the subsequent follicular wave would likely have grown at a reduced rate, as high circulating concentrations of progesterone have been reported to result in smaller follicles at CIDR removal (Cerri et al., 2011; Mercadante et al., 2015). This dominant follicle would have ovulated spontaneously or been induced to ovulate at TAI through GnRH administration.

Alternatively, the dominant follicle between Day -7 and 0 may have been large enough to respond to the injection of GnRH on Day 0 and a new follicular wave would have been initiated between Day 0 and 7, of which the dominant follicle would have been induced to ovulate or would have ovulated spontaneously at TAI.

Numerous studies have reported greater PR/AI when estrus is exhibited prior to TAI (Perry et al., 2007; Richardson et al., 2016). In the current study, presynchronization reduced the expression of estrus prior to TAI, where estrus expression in the PRE54 treatment was lesser than that of the CTRL treatment and expression of estrus in PRE72 heifers was lesser than that of CTRL72 heifers. However, PR/AI between CTRL-CNV and PRE54-CNV heifers, between CTRL72-CNV and PRE72-CNV, between CTRL-SEX and PRE54-SEX, and between CTRL72-SEX and PRE72-SEX heifers did not differ. Similarly, heifers synchronized with the 7-d CO-Synch + CIDR protocol had greater expression of estrus when compared to heifers presynchronized with PGF, yet PR/AI were not different (Oosthuizen et al., 2018). It is likely that presynchronized heifers were beginning to enter into estrus at TAI (54 h) as a result of smaller dominant follicles at CIDR removal; therefore, were induced to ovulate through the injection of GnRH, leading to acceptable PR/AI (Oosthuizen et al., 2020).

5.4.2. Timing of Fixed-Time Artificial Insemination

Beef heifers presynchronized with PGF 7 d prior to the 7-d CO-Synch + CIDR protocol likely require a greater amount of time between CIDR removal and TAI (54 h) in which to express estrus, as proestrus is likely to be extended (Oosthuizen et al., 2018). By delaying the timing of TAI, heifers are allowed more time to express estrus and a larger proportion may undergo spontaneous ovulation. When beef heifers were presynchronized

with PGF and had TAI delayed to 72 h, estrus expression before TAI was increased from 31 to 78% when compared to presynchronized heifers inseminated at 54 h (Oosthuizen et al., 2020). Furthermore, there was a tendency for heifers in the delayed TAI group to have greater PR/AI than those inseminated at 54 h. In the current study, delaying TAI to 72 h in CTRL72 and PRE72 treatments resulted in greater estrus expression; in addition, PR/AI were increased after delayed TAI in presynchronized heifers. This increase in PR/AI is likely the result of a greater number of spontaneous ovulations and fewer induced ovulations of smaller follicles, as follicle size has been associated with oocyte maturity (Driancourt and Thuel, 1998) and fertility in beef heifers (Perry et al., 2005). Furthermore, GnRH-induced ovulation of smaller follicles has led to reduced PR/AI and decreased embryonic survival in beef females (Perry et al., 2005; Perry et al., 2007; Atkins et al., 2008). Because no differences in PR/AI were determined between CTRL-CNV and CTRL72-CNV heifers, and between CTRL-SEX and CTRL72-SEX heifers, it is plausible that the time of insemination after synchronization with the 7-d CO-Synch + CIDR protocol may be more flexible than initially reported.

5.4.3. Semen Type

It has been well documented that pregnancy rates with sex-sorted semen are significantly lesser than those of conventional semen (Deutscher et al., 2002; DeJarnette et al., 2009; Seidel, 2014). When sex-sorted semen is used in TAI protocols for cows and heifers, PR/AI between 32 to 70% of conventional semen have been reported (Sales et al., 2011; Thomas et al., 2014). In the present study, PR/AI were significantly lower in each treatment when sex-sorted semen was utilized and ranged between 73.8 and 85.6% of PR/AI with conventional semen. This reduction in fertility is largely due to a lower post-thaw

motility, a reduced number of sperm cells with intact membranes, and acrosomal alterations that can occur during the sorting process (Schenk et al., 2009; Carvalho et al., 2010). In addition, it is plausible that the reduced number of sperm cells in straws of sex-sorted semen may limit the number of sperm cells capable of fertilization in the oviduct when the oocyte is present if females are inseminated too close to the onset of estrus (Bombardelli et al., 2016). Therefore, it is likely that sex-sorted sperm cells have a shorter capable lifespan in the female reproductive tract, and as a result, need to be introduced closer to the time of ovulation. In lactating dairy cows, insemination with sex-sorted semen closer to expected ovulation yielded greater PR/AI, where cows inseminated between 23 and 41 h after the onset of estrus had the greatest PR/AI (Bombardelli et al., 2016). In dairy heifers, PR/AI were 15.2% greater after insemination with sex-sorted semen when TAI was delayed from 54 to 60 h after progestin removal, yet PR/AI were still significantly lower than those of conventional semen (31.4 vs. 51.8%; Sales et al., 2011). In the current study, PR/AI with sex-sorted semen did not differ when TAI was delayed from 54 to 72 h; however, the combination of presynchronization and delayed TAI in the PRE72-SEX treatment succeeded in increasing PR/AI when compared to the standard TAI protocol utilized for sex-sorted semen (CTRL-SEX). Furthermore, PR/AI were increased to a great enough extent that they were similar to those of the standard TAI protocol for conventional semen (CTRL-CNV). The increase in PR/AI in the PRE72-SEX treatment is likely the result of a combination of greater estrus synchrony among heifers; prolonged proestrus, greater estrus expression, and a greater number of spontaneous ovulations; and insemination closer to expected ovulation. However, the exact mechanisms associated with this improvement are still unclear.

5.5. Economic Analysis

A partial budget analysis was performed to convert the results of this experiment into a decision aid tool for beef cattle producers. This tool will be used to determine the economic feasibility of incorporating sex-sorted semen or a combination of sex-sorted and conventional semen into a heifer production system when compared to conventional semen. There are a number of inputs that the producer would be required to enter, such as values that the producer could change if desired or that will remain as default values, and there are values that are unchangeable. Economic outcomes will be measured using increased returns and decreased costs compared with decreased returns and increased costs attributed to the use of conventional, sex-sorted, or a combination of the semen types. The gain/loss per heifer exposed to TAI and the gain/loss per herd will be calculated for three scenarios: 1) Conventional vs. sex-sorted semen, 2) conventional vs. combination, and 3) sex-sorted semen vs. combination. Conventional heifers are all TAI with conventional semen; sex-sorted heifers are all TAI with sex-sorted semen; and within the combination group, heifers that express estrus prior to TAI receive sex-sorted semen whereas heifers that do not express estrus before TAI receive conventional semen.

Producer inputs include the number of heifers in the herd, the number of clean-up bulls that will be utilized for the heifers, the desired sex of the semen, and the expected premium per head by utilizing sex-sorted semen (Table 5.3.). The changeable values include: expected PR/AI for conventional semen; mean calf weight gain per d; expected final pregnancy rates; clean-up bull purchase price, maintenance costs, useful life, salvage value, salvage weight, and the purchase cost interest rate; cost of labor and number of employees required; if an AI tech is required and the cost of AI tech per head; the cost of the estrus

synchronization drugs; the cost of the different types of semen; the amount borrowed to finance the costs and the interest rate on what was borrowed; the expected weaning weights of the male and female calves; and the expected price of male and female calves. The fixed values include the number of animal handlings for the different estrus synchronization protocols, the required hormonal doses, and the expected sex ratio per semen type.

Calculations included the cost per dose of each drug used in the estrus synchronization protocol; the labor cost per head; the total cost of the estrus synchronization drugs per head; the total semen cost for the combination scenario; the expected number of calves of each gender from conventional, sex-sorted, and combination semen; the expected number of calves of each gender from the clean-up bull(s) for each scenario; the weaning weights of calves born from the clean-up bull(s) for each gender; the total expense per heifer exposed based on the cost of TAI, costs of the clean-up bull(s), and the amount borrowed; and the total income based on the salvage value of clean-up bull(s), weaning weight of the calves, and the premium for calves of the desired sex (Table 5.4.). Final gain/loss per heifer was calculated based on increased/decreased returns and increased/decreased costs. Gain/loss per herd was calculated by multiplying the gain/loss per heifer exposed by the size of the herd.

There are a number of assumptions that have been taken into account: all heifers will be exposed to TAI; the PR/AI from sex-sorted semen is 4.7% lower than that of conventional semen, based on our data with the PRE72 protocol; the sex-ratio for conventional semen is 50%, for sex-sorted semen is 90%, and for the combination scenario is 79.2%; 65.8% of heifers will express estrus and 34.2% of heifers will not express estrus in the PRE72 protocol; and the PR/AI of heifers that express estrus and are TAI with sex-sorted semen are

54.2%, and 38.4% for heifer that did not express estrus and were TAI with conventional semen, based on our data.

A sensitivity analysis was performed to determine the differences in gain/loss per heifer exposed to TAI according to the three aforementioned scenarios: 1) Sex-sorted compared to conventional semen, 2) a combination of sex-sorted and conventional semen compared to conventional semen, and 3) a combination of sex-sorted and conventional semen compared to sex-sorted semen (Table 5.5.). When looking at the gain/loss per heifer exposed to sex-sorted semen compared to conventional semen, under our assumptions, positive returns are only achieved when Y-sorted sperm is used. Similarly, when a combination of sex-sorted and conventional semen is compared to conventional semen, positive returns per heifer exposed are mostly achieved when Y-sorted sperm is used. Lastly, when a combination of sex-sorted and conventional semen is compared to sex-sorted semen, the gain/loss per heifer exposed seems to depend more on herd size and the PR/AI with the CTRL-CNV treatment than the desired sex.

5.6. Conclusion

Pregnancy rates to TAI were greater when conventional semen was utilized as opposed to sex-sorted semen; however, the combination of PGF administration 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol and TAI at 72 h with sex-sorted semen succeeded in enhancing PR/AI. Therefore, the PRE72-SEX treatment may be utilized to facilitate the use of sex-sorted semen in replacement beef heifers.

Financially, the primary factors that influence the gain or loss per heifer exposed include the expected premium for the desired sex, the cost of sex-sorted semen, the size of

the herd, weaning weights, and the PR/AI of the CTRL-CNV treatment. According to our assumptions and values taken from each of the 23 herds included in this study, sex-sorted semen results in the greatest net returns when Y-sorted sperm is utilized. Furthermore, in order for X-sorted sperm to be more profitable, a perceived premium of greater than $154 per head is required.

5.7. References

Adams, G. P., R. Jaiswal, J. Singh, and P. Malhi. 2008. Progress in understanding ovarian follicular dynamics in cattle. Theriogenology. 69:72–80. doi:10.1016/j.theriogenology.2007.09.026.

Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. Lucy, and M. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: effect of day of the cycle. J. Anim. Sci. 86:83–93.

Bombardelli, G. D., H. F. Soares, and R. C. Chebel. 2016. Time of insemination relative to reaching activity threshold is associated with pregnancy risk when using sex-sorted semen for lactating Jersey cows. Theriogenology. 85:533–539. doi:10.1016/j.theriogenology.2015.09.042.

Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85:1933–1939. doi:10.2527/jas.2006-845.

Carvalho, J. O., R. Sartori, G. M. Machado, G. B. Mourão, and M. A. N. Dode. 2010. Quality assessment of bovine cryopreserved sperm after sexing by flow cytometry and their use in in vitro embryo production. Theriogenology. 74:1521–1530. doi:10.1016/j.theriogenology.2010.06.030.

Cerri, R. L. A., R. C. Chebel, F. Rivera, C. D. Narciso, R. A. Oliveira, and W. W. Thatcher. 2011. Concentration of progesterone during the development of the ovulatory follicle : I . Ovarian and embryonic responses. J. Dairy Sci. 94:3342–3351. doi:10.3168/jds.2010-3734.

DeJarnette, J. M., R. L. Nebel, and C. E. Marshall. 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology. 71:49–58. doi:10.1016/j.theriogenology.2008.09.042.

Deutscher, G. H., R. Davis, G. Seidel, and J. Schenk. 2002. Use of sexed (female) sperm is successful in yearling heifers. In: 2002 Nebraska Beef Cattle Report. Rep. No. Univ. of Nebraska, Lincoln, NE. p. 12-14.

Driancourt, M., and B. Thuel. 1998. Control of oocyte growth and maturation by follicular cells and molecules present in follicular fluid. A review. Reprod. Nutr. Dev. 38:345–362.

Grant, J. K., F. M. Abreu, N. L. Hojer, S. D. Fields, B. L. Perry, and G. A. Perry. 2011. Influence of inducing luteal regression before a modified controlled internal drug-releasing device treatment on control of follicular development. J. Anim. Sci. 89:3531–3541. doi:10.2527/jas.2011-3852.

Hall, J. B., R. K. Kasimanickam, J. B. Glaze, and M. C. Roberts-Lew. 2017. Impact of delayed insemination on pregnancy rates to gender selected semen in a fixed-time AI system. Theriogenology. 102:154–161. doi:10.1016/j.theriogenology.2017.07.014.

Klose, S. L., J. Smith, B. Thompson, M. Waller, S. Zapata, and W. Keeling. 2019. 2018 Texas Agricultural Custom Rates Survey.

Kojima, F. N., B. E. Salfen, J. F. Bader, W. A. Ricke, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 synch. J. Anim. Sci. 78:2186–2191.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. Dejarnette, F. N. Schrick, A. Dicostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J. Anim. Sci. 84:332–342.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of cattle following PGF2a injection. J. Anim. Sci. 38:964–967. doi:10.2527/jas1974.385964x.

Louis, T. M., H. D. Hafs, and D. A. Morrow. 1974. Intrauterine administration of prostaglandin F2α in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347–353.

Mercadante, V. R. G., L. E. Kozicki, F. M. Ciriaco, D. D. Henry, C. R. Dahlen, M. R. Crosswhite, J. E. Larson, B. E. Voelz, D. J. Patterson, G. A. Perry, R. N. Funston, T. L. Steckler, S. L. Hill, J. S. Stevenson, and G. C. Lamb. 2015. Effects of administration of prostaglandin F2alpha at initiation of the seven-day CO-Synch+controlled internal drug release ovulation synchronization protocol for suckled beef cows and replacement beef heifers. J. Anim. Sci. 93:5204–5213. doi:10.2527/jas.2015-8967.

Oosthuizen, N., L. B. Canal, P. L. P. Fontes, C. D. Sanford, N. DiLorenzo, C. R. Dahlen, G. E. Seidel, and G. C. Lamb. 2018. Prostaglandin $F_{2\alpha}$ 7 d prior to initiation of the 7-d CO-synch + CIDR protocol failed to enhance estrus response and pregnancy rates in beef heifers. J. Anim. Sci. 96:1466–1473. doi:10.1093/jas/sky058.

Oosthuizen, N., P. L. P. Fontes, K. Porter, and G. C. Lamb. 2020. Presynchronization with prostaglandin $F_{2\alpha}$ and prolonged exposure to exogenous progesterone impacts estrus expression and fertility in beef heifers. Theriogenology. 146:88–93. doi:10.1016/j.theriogenology.2020.02.010.

Paterson, J. 2015. What are the factors the influence weaning weights of calves. Natl. Cattlemen. 1. Available from: www.beefusa.org

Perry, G. A., B. L. Perry, J. H. Krantz, and J. Rodgers. 2012. Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success. J. Anim. Sci. 90:489–494. doi:10.2527/jas.2011-4319.

Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Natl. Acad. Sci. U. S. A. 102:5268–5273. doi:10.1073/pnas.0501700102.

Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Anim. Sci. 85:684–689. doi:10.2527/jas.2006-519.

Richardson, B. N., S. L. Hill, J. S. Stevenson, G. D. Djira, and G. A. Perry. 2016. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim. Reprod. Sci. 166:133–140. doi:10.1016/j.anireprosci.2016.01.013.

Sales, J. N. S., K. A. L. Neves, A. H. Souza, G. A. Crepaldi, R. V. Sala, M. Fosado, E. P. C. Filho, M. de Faria, M. F. S. Filho, and P. S. Baruselli. 2011. Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. Theriogenology. 76:427–435. doi:10.1016/j.theriogenology.2011.02.019.

Schenk, J. L., D. G. Cran, R. W. Everett, and G. E. Seidel. 2009. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. Theriogenology. 71:717–728. doi:10.1016/j.theriogenology.2008.08.016.

Seidel, G. E. 2014. Update on sexed semen technology in cattle. Animal. 8:160–164. doi:10.1017/S1751731114000202.

Thomas, J. M., S. L. Lock, S. E. Poock, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2014. Delayed insemination of nonestrous cows improves pregnancy rates when using sex-sorted semen in timed artificial insemination of suckled beef cows. J. Anim. Sci. 92:1747–1752. doi:10.2527/jas2013-7131.

USDA. 2020. Texas Weekly Summary. Available from: https://www.ams.usda.gov/mnreports/lswtxrecap.pdf

Wagner, J., K. Lusby, J. Oltjen, J. Rakestraw, R. Wettemann, and L. Walters. 1988. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603–612.

Table 5.1. Descriptive data of heifers by location[a]

Location	No. heifers	Breed	Mean BW, kg[b]	Mean BCS[c]
1	57	Angus	-	-
2	364	Angus	-	5.24 ± 0.02
3	238	Angus	378.25 ± 2.40	6.06 ± 0.03
4	170	Angus	353.19 ± 2.84	5.86 ± 0.03
5	145	Angus, Red Angus	400.60 ± 3.10	5.48 ± 0.03
6	141	Angus, Red Angus	406.48 ± 3.12	5.58 ± 0.03
7	149	Angus, Hereford, Simmental	376.91 ± 3.03	5.96 ± 0.03
8	61	Angus	-	-
9	51	Angus	-	5.85 ± 0.05
10	373	Angus	-	6.03 ± 0.02
11	56	Angus	367.88 ± 4.94	6.65 ± 0.05
12	50	Red Angus	483.48 ± 5.23	6.17 ± 0.05
13	81	Angus	343.65 ± 4.16	-
14	72	Angus	313.46 ± 4.36	5.15 ± 0.05
15	82	Angus	-	-
16	67	Angus	326.72 ± 4.51	5.50 ± 0.05
17	94	Angus, Hereford, Red Angus	-	5.05 ± 0.04
18	87	Angus x Simmental	331.43 ± 4.01	4.89 ± 0.04
19	100	Angus x Simmental	-	5.04 ± 0.04
20	96	Angus x Simmental	-	5.14 ± 0.04
21	145	Angus	-	5.48 ± 0.03
22	114	Angus, Charolais, Simmental	359.91 ± 3.46	4.87 ± 0.04
23	62	Angus, Hereford, Simmental	-	5.39 ± 0.05

[a] Twenty-three locations across 11 states.

[b] Body Weight was recorded on Day -7.

[c] Body Condition Score was recorded on Day -7.

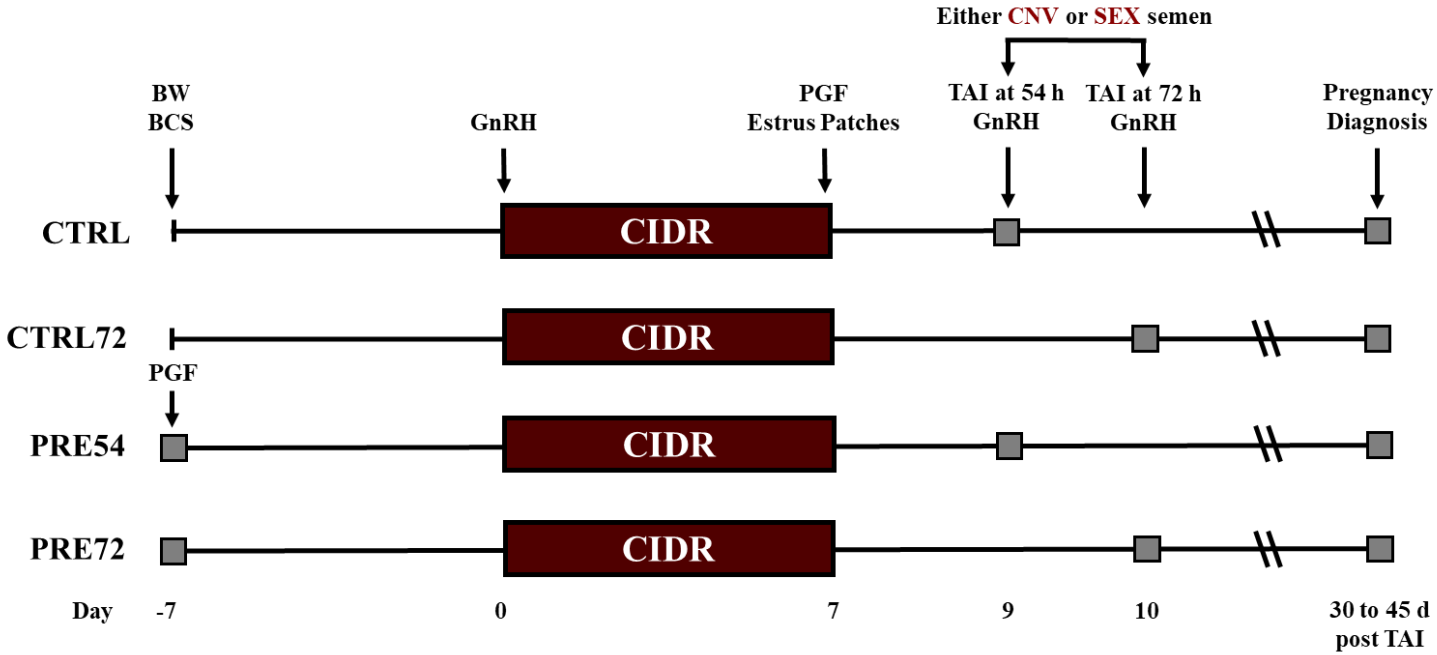

Figure 5.1. Diagram of treatments. CTRL, heifers were exposed to the 7-d CO-Synch + CIDR protocol wherein they received an injection of gonadotropin-releasing hormone (GnRH; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a CIDR insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) on Day 0, an injection of prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health) upon CIDR removal on Day 7, and were TAI 54 ± 2 h later with either conventional (CTRL-CNV; *n* = 359) or sex-sorted semen (CTRL-SEX; *n* = 356); CTRL72, heifers were treated the same as CTRL but were TAI at 72 ± 2 h with either conventional (CTRL72-CNV; *n* = 366) or sex-sorted semen (CTRL72-SEX; *n* = 360); PRE54, treated the same as CTRL but received an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol (Day -7) and were then TAI with either conventional (PRE54-CNV; *n* = 355) or sex-sorted semen (PRE54-SEX; *n* = 353); PRE72, treated the same as PRE54 treatments but had TAI delayed to 72 ± 2 h and were inseminated with conventional (PRE72-CNV; *n* = 351) or sex-sorted semen (PRE72-SEX; *n* = 355). Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to all heifers at CIDR removal and were evaluated for activation at their respective time of TAI.

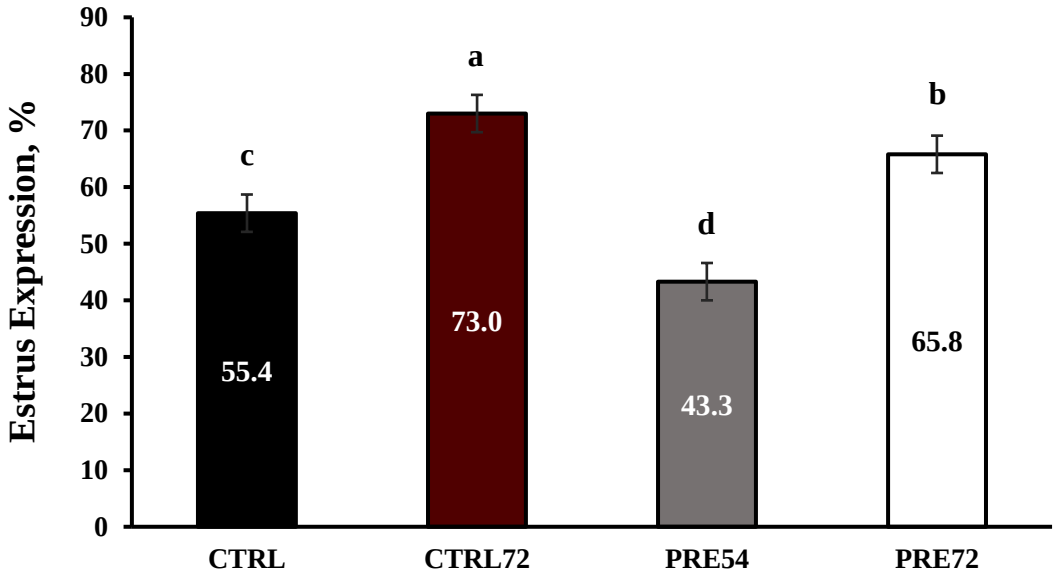

Figure 5.2. Estrus expression between CIDR removal and TAI (54 or 72 h). CTRL, heifers were exposed to the 7-d CO-Synch + CIDR protocol wherein they received an injection of gonadotropin-releasing hormone (GnRH; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a CIDR insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) on Day 0, an injection of prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health) upon CIDR removal on Day 7, and were TAI 54 ± 2 h later with either conventional (CTRL-CNV; *n* = 359) or sex-sorted semen (CTRL-SEX; *n* = 356); CTRL72, heifers were treated the same as CTRL but were TAI at 72 ± 2 h with either conventional (CTRL72-CNV; *n* = 366) or sex-sorted semen (CTRL72-SEX; *n* = 360); PRE54, treated the same as CTRL but received an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol (Day -7) and were then TAI with either conventional (PRE54-CNV; *n* = 355) or sex-sorted semen (PRE54-SEX; *n* = 353); PRE72, treated the same as PRE54 treatments but had TAI delayed to 72 ± 2 h and were inseminated with conventional (PRE72-CNV; *n* = 351) or sex-sorted semen (PRE72-SEX; *n* = 355). Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to all heifers at CIDR removal and were evaluated for activation at their respective time of TAI. [a,b,c]Bars with different superscripts differ ($P < 0.05$).

Table 5.2. Pregnancy rates to fixed-time artificial insemination (TAI) by estrus expression.

Treatment[a]	PR/AI, %[b]		*P*-value
	Estrus	Non-estrus	
CTRL-CNV	55.1 ± 4.1	44.3 ± 4.5	0.04
CTRL-SEX	43.3 ± 4.2	31.1 ± 4.4	0.02
CTRL72-CNV	57.6 ± 3.7	38.1 ± 5.7	< 0.01
CTRL72-SEX	50.4 ± 3.8	21.0 ± 5.3	< 0.01
PRE54-CNV	54.6 ± 4.5	39.7 ± 4.1	< 0.01
PRE54-SEX	42.0 ± 4.5	28.7 ± 4.1	0.01
PRE72-CNV	62.1 ± 3.9	38.4 ± 4.9	< 0.01
PRE72-SEX	54.2 ± 3.9	29.0 ± 5.0	< 0.01
Overall	53.0 ± 2.5	34.0 ± 2.7	< 0.01

[a] CTRL: heifers were exposed to the 7-d CO-Synch + CIDR protocol wherein they received an injection of gonadotropin-releasing hormone (GnRH; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a CIDR insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) on Day 0, an injection of prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health) upon CIDR removal on Day 7, and were TAI 54 ± 2 h later with either conventional (CTRL-CNV; *n* = 359) or sex-sorted semen (CTRL-SEX; *n* = 356); CTRL72: heifers were treated the same as CTRL but were TAI at 72 ± 2 h with either conventional (CTRL72-CNV; *n* = 366) or sex-sorted semen (CTRL72-SEX; *n* = 360); PRE54: treated the same as CTRL but received an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol (Day -7) and were then TAI with either conventional (PRE54-CNV; *n* = 355) or sex-sorted semen (PRE54-SEX; *n* = 353); PRE72: treated the same as PRE54 treatments but had TAI delayed to 72 ± 2 h and were inseminated with conventional (PRE72-CNV; *n* = 351) or sex-sorted semen (PRE72-SEX; *n* = 355). Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied to all heifers at CIDR removal and were evaluated for activation at their respective time of TAI.

[b] Pregnancy rates to TAI were determined via transrectal ultrasonography between 30 and 45 d after TAI.

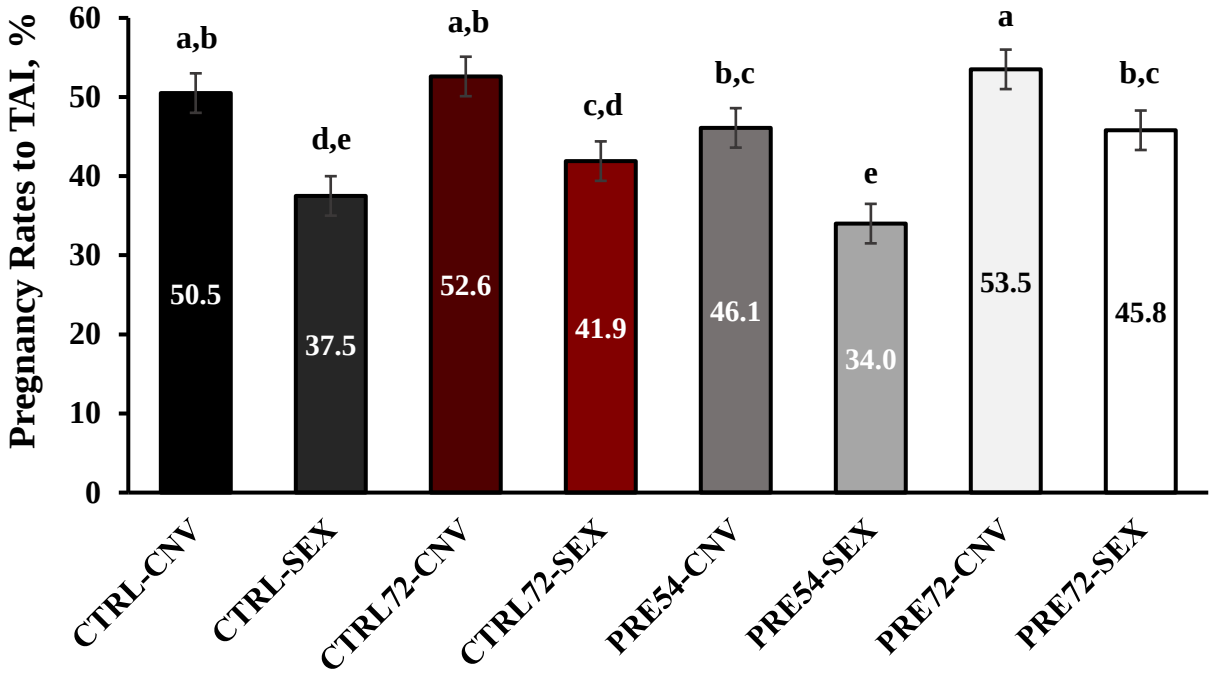

Figure 5.3. Pregnancy rates to fixed-time artificial insemination (TAI) among treatment groups. CTRL, heifers were exposed to the 7-d CO-Synch + CIDR protocol wherein they received an injection of gonadotropin-releasing hormone (GnRH; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a CIDR insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) on Day 0, an injection of prostaglandin $F_{2\alpha}$ (PGF; 25 mg im; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health) upon CIDR removal on Day 7, and were TAI 54 ± 2 h later with either conventional (CTRL-CNV; *n* = 359) or sex-sorted semen (CTRL-SEX; *n* = 356); CTRL72, heifers were treated the same as CTRL but were TAI at 72 ± 2 h with either conventional (CTRL72-CNV; *n* = 366) or sex-sorted semen (CTRL72-SEX; *n* = 360); PRE54, treated the same as CTRL but received an injection of PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol (Day -7) and were then TAI with either conventional (PRE54-CNV; *n* = 355) or sex-sorted semen (PRE54-SEX; *n* = 353); PRE72, treated the same as PRE54 treatments but had TAI delayed to 72 ± 2 h and were inseminated with conventional (PRE72-CNV; *n* = 351) or sex-sorted semen (PRE72-SEX; *n* = 355). Pregnancy rates were determined via transrectal ultrasonography between 30 and 45 d after TAI. ^{a,b,c}Bars with different superscripts differ ($P < 0.05$).

Table 5.3. Required inputs and default values for a partial budget analysis on the comparison of conventional, sex-sorted, and combination semen in a herd of heifers.

Inputs		Type of Value
Herd	No. of heifers	Required
	No. of clean-up bulls	Required
	Expected PR/AI with conventional semen	Default (50.5%)[a]
	Expected final pregnancy rate	Default (90.0%)[b]
	Mean calf weight gain per d, kg	Default (1.03 kg/d)[c]
Clean-up bulls		
	Bull maintenance costs	Default ($600.00)
	Mean purchase cost of bull	Default ($4,000.00)
	Useful life	Default (4)
	Salvage value, per 50.8 kg	Default ($70.00)
	Salvage weight, kg	Default (816.47 kg)
	Interest rate used	Default (6%)
Labor		
	Cost of labor per d	Default ($160.00)[d]
	No. of employees required	Default (3)
	AI technician required?	Default (Yes)
	Cost of AI technician per head	Default ($7.50)
Estrus synchronization		
	Cost of bottle (100 ml) prostaglandin $F_{2\alpha}$	Default ($57.89)[e]
	Doses of prostaglandin $F_{2\alpha}$ per bottle	Default (20)
	Cost of bottle (20 ml) of GnRH	Default ($24.29) [e]
	Doses of GnRH per bottle	Default (10)
	Cost per unit of CIDR inserts	Default ($127.79)[e]
	No. of CIDRs per unit	Default (10)
	Estrus detection patches per pack	Default ($62.95)[e]
	No. of patches	Default (50)
Sex-sorted semen		
	Cost of conventional semen	Default ($25.00)[f]
	Cost of sexed semen	Default ($45.00)[f]
	Desired sex	Required
	Desired sex premium per head	Default ($100.00)[g]
Financing		
	Percentage of costs borrowed	Default (100%)
	Interest on expenses per year	Default (4%)
Weaning weights		
	Mean expected weaning weight conventional males, kg	Default (254 kg)[h]
	Mean expected weaning weight conventional heifers, kg	Default (240 kg)[h]
	Mean expected weaning weight sexed males, kg	Default (254 kg)[h]

Table 5.3. Continued

Inputs		Type of Value
Weaning weights (Continued)		
	Mean expected weaning weight sexed heifers, kg	Default (240 kg)[h]
	Expected price of weaned conventional male calf, per 50.8 kg	Default ($155.00)[i]
	Expected price of weaned conventional heifer calf, per 50.8 kg	Default ($133.00)[i]
	Expected price of weaned sexed male calf, per 50.8 kg	Default ($155.00)[i]
	Expected price of weaned sexed heifer calf, per 50.8 kg	Default ($133.00)[i]

[a] Pregnancy rates to fixed-time artificial insemination (PR/AI) for the 7-d CO-Synch + CIDR protocol with conventional semen in the current study.
[b] An expected overall pregnancy rate for a 60 d breeding season.
[c] Calf average daily gain for a 247.2 kg calf weaned at 205 d.
[d] Labor cost taken from Klose et al., 2019.
[e] Drug costs taken from www.valleyvet.com.
[f] Semen cost of most commonly used bull in the current study (ST Genetics, Navasota, TX).
[g] Anticipated premium of desired sex when sex-sorted semen is utilized.
[h] Mean weaning weights taken from Paterson, 2015.
[i] Mean hundred weight taken from USDA, 2020 and converted to kg based on the average weaning weight for each sex.

Table 5.4. Calculations and values based on a herd of 100 heifers and two clean-up bulls, with input values from Table 5.3., and the desired calf sex of heifer.

Item	Calculation	Type of value
Estrus synchronization		
Products		
	Cost per dose of prostaglandin $F_{2\alpha}$	Default ($2.89)[a]
	Cost per dose of GnRH	Default ($2.43)[a]
	Cost per CIDR	Default ($12.78)[a]
	Cost per estrus detection patch	Default ($1.26)[a]
Conventional semen based on 7-d CO-Synch+CIDR protocol		
	No. of cattle working d	Fixed (3)
	Doses of prostaglandin $F_{2\alpha}$ required	Fixed (1)
	Doses of GnRH required	Fixed (1)
	No. of CIDRs required	Fixed (1)
	Labor Cost per head	$14.40[b]
	Total cost of estrus synchronization products	Default ($18.10)
	Semen cost	Default ($25.00)
	AI technician	Default ($7.50)
	Expected PR/AI	Default (50.5%)[c]
	Expected sex ratio	Fixed (50.0%)
Sexed semen based on PRE72 protocol		
	No. of cattle working d	Fixed (4)
	Doses of prostaglandin $F_{2\alpha}$ required	Fixed (2)
	Doses of GnRH required	Fixed (1)
	No. of CIDRs required	Fixed (1)
	Labor cost per head	$19.20[b]
	Total cost of estrus synchronization products	Default ($21.00)
	Semen cost	Default ($45.00)
	AI technician	Default ($7.50)
	Difference in PR/AI	Fixed (4.7%)[d]
	Expected PR/AI	Expected PR/AI for conventional minus 4.7% Default (45.8%)[e]
	Expected desired sex ratio	Fixed (90.0%)[f]
Combination based on PRE72 protocol		
	No. of cattle working d	Fixed (4)
	Doses of prostaglandin $F_{2\alpha}$ required	Fixed (2)
	Doses of GnRH required	Fixed (1)
	No. of CIDRs required	Fixed (1)

Table 5.4. Continued

Item	Calculation	Type of value
Combination based on PRE72 protocol (Continued)		
	No. of estrus detection patches required	Fixed (1)
	Labor cost per head	$19.20[b]
	Total cost of estrus synchronization products	Default ($22.26)
	Expected percentage of heifers in estrus	Fixed (65.8%)[g]
	Expected percentage of heifers non-estrus	Fixed (34.2%)[g]
	Expected PR/AI of heifers in estrus with sexed semen	Fixed (54.2%)[g]
	Expected PR/AI of heifers non-estrus with conventional semen	Fixed (38.4%)[g]
	Total expected PR/AI from sexed	Default (35.7%)[h]
	Total expected PR/AI from conventional	Default (13.1%)[h]
	Total PR/AI	Default (48.8%)[i]
	Conventional semen cost	Default ($855.00) [j]
	Sexed semen cost	Default ($2,961.00)[j]
	Total semen cost	Default ($3,816.00)
	Total semen cost per head	Default ($38.16)
	AI technician	Default ($7.50)
	Expected desired sex ratio	Fixed (79.2%)[k]
Calf crop		
Calves from TAI		
	Expected calf crop for conventional	50.5[l]
	Expected no. of male conventional	25.25[m]
	Expected no. of female conventional	25.25[m]
	Expected calf crop for sexed	45.8[l]
	Expected no. of male sex calves	4.6[m]
	Expected no. of female sex calves	41.2[m]
	Expected calf crop for combination	48.8[l]
	Expected no. of male combination	10.1[m]
	Expected no. of female combination	38.7[m]
Calves from clean-up bull		
	Conventional protocol: female calves from clean-up bull	19.75[n]
	Conventional protocol: male calves from clean-up bull	19.75[n]
	Sexed protocol: female calves from clean-up bull	22.1[n]

Table 5.4. Continued

Item	Calculation	Type of value
Calves from clean-up bull (Continued)		
	Sexed protocol: male calves from clean-up bull	22.1[n]
	Combination protocol: female calves from clean-up bull	20.6[n]
	Combination protocol: male calves from clean-up bull	20.6[n]
Clean-up bull weaning weights		
	Mean no. of d younger than TAI calves	29[o]
	Mean expected decrease in natural service weaning weights, kg	29.86[p]
	Mean expected weaning weight natural service males, kg	224.15[q]
	Mean expected weaning weight natural service heifers, kg	210.54[q]
Expenses		
Per heifer		
	Total cost per heifer for conventional	$65.00[r]
	Total cost per heifer for sexed	$92.70[r]
	Total cost per heifer for combination	$87.12[r]
Per clean-up bull		
	Total maintenance cost per clean-up bull over useful life	$2,400.00
	Total maintenance cost for all clean-up bulls over useful life	$4,800.00
	Total yearly maintenance cost for all clean-up bulls per heifer exposed	$12.00[s]
	Total cost per clean-up bull with interest	$4,240.00
	Total cost of all clean-up bulls with interest	$8,480.00
	Total yearly cost of all clean-up bulls with interest per heifer exposed	$21.20[s]
Financing		
	Total amount borrowed per conventional heifer	$86.20[t]
	Total amount borrowed per sexed heifer	$113.90[t]
	Total amount borrowed per combination heifer	$120.32[t]
	Total amount borrowed conventional per heifer with interest	$89.65

Table 5.4. Continued

Item	Calculation	Type of value
Financing (Continued)		
	Total amount borrowed sexed per heifer with interest	$118.45
	Total amount borrowed combination per heifer with interest	$125.13
Total expenses		
	Total cost per heifer exposed - conventional	$89.65[u]
	Total cost per heifer exposed - sexed	$118.45[u]
	Total cost per heifer exposed - combination	$125.13[u]
Income		
Per clean-up bull		
	Salvage value profit per clean-up bull	$1,260.00[v]
	Total income from sale of all clean-up bulls	$2,520.00
	Total income from sale of all clean-up bulls per heifer exposed	$6.30
Per Calf		
	Total income per TAI male calf - conventional semen	$868.00[w]
	Total income per TAI female calf - conventional semen	$704.90[w]
	Total income per TAI male calf - sexed semen	$868.00[w]
	Total income per TAI female calf - sexed semen	$804.90[w]
	Total income per TAI male calf - combination	$868.00[w]
	Total income per TAI female calf - combination	$804.90[w]
	Total income per male calf - natural service	$765.96[w]
	Total income per female calf - natural service	$617.35[w]
Total calf income		
	Total calf income - conventional	$39,715.73[x]
	Total calf income - sexed	$37,153.42[x]
	Total calf income - combination	$39,915.60[x]
	Total clean-up bull income - conventional	$27,320.36[x]
	Total clean-up bull income - sexed	$30,571.14[x]
	Total clean-up bull income - combination	$28,498.67[x]

Table 5.4. Continued

Item	Calculation	Type of value
Per heifer exposed		
	Total income per heifer exposed for conventional TAI	$397.16[y]
	Total income per heifer exposed for sexed TAI	$371.53[y]
	Total income per heifer exposed for combination TAI	$399.16[y]
	Total income per heifer exposed - conventional natural service	$273.20[y]
	Total income per heifer exposed - sexed natural service	$305.71[y]
	Total income per heifer exposed - combination natural service	$284.99[y]
Total income		
	Final income per heifer exposed to conventional semen	$695.56[z]
	Final income per heifer exposed to sexed semen	$702.45[z]
	Final income per heifer exposed to combination semen	$709.34[z]
Derived inputs Per Head		
Sex-sorted vs. conventional		
	Increased returns	$6.88[*]
	Decreased returns	$0.00[*]
	Decreased costs	$0.00[†]
	Increased costs	$28.80[†]
Combination vs. conventional		
	Increased returns	$13.78[*]
	Decreased returns	$0.00[*]
	Decreased costs	$0.00[†]
	Increased costs	$35.48[†]
Combination vs. sex-sorted		
	Increased returns	$6.90[*]
	Decreased returns	$0.00[*]
	Decreased costs	$0.00[†]
	Increased costs	$6.68[†]
Gain/loss per heifer exposed		
	Sex-sorted vs. conventional	-$21.92[‡]
	Combination vs. conventional	-$21.70[‡]
	Combination vs. sex-sorted	$0.22[‡]

[a] Cost per unit of product divided by no. of doses per unit.
[b] No. of cattle working d multiplied by no. of employees required multiplied by employee cost per d.
[c] PR/AI for the 7-d CO-Synch + CIDR protocol with conventional semen in the current study.
[d] Fixed value calculated from the difference in PR/AI between the 7-d CO-Synch + CIDR protocol with conventional semen and the PRE72 protocol and sex-sorted semen in the current study.
[e] Expected PR/AI for the 7-d CO-Synch + CIDR protocol with conventional semen minus 4.7%.
[f] Expected sex-ratio with sex-sorted semen.
[g] Results taken from the current study.
[h] No. of heifers in estrus or non-estrus multiplied by their respective PR/AI with their respective semen.
[i] PR/AI for conventional semen plus PR/AI for sex-sorted semen.
[j] No. of heifers in estrus or non-estrus multiplied by the cost of respective semen.
[k] No. of calves generated of the desired sex divided by the total no. of calves.
[l] Total head of heifers multiplied by the respective PR/AI.
[m] Expected calf crop multiplied by the respective sex ratio.
[n] Expected final pregnancy rate minus the PR/AI from each respective protocol, and multiplied by the sex ratio of conventional semen.
[o] Taken from Larson et al., 2006.
[p] Expected mean calf weight gain per d multiplied by mean no. of d younger than TAI calves.
[q] Mean expected weaning weight for each gender minus the mean expected decrease in natural service weaning weights.
[r] Sum of labor, estrus synchronization, semen, and AI technician costs.
[s] Total maintenance cost for all clean-up bulls over useful life divided by no. heifers divided by useful life.
[t] (Total cost per heifer plus total yearly cost of all clean-up bulls with interest per heifer exposed) multiplied by % costs borrowed.
[u] Total cost per heifer plus total yearly cost of all clean-up bulls with interest per heifer exposed.
[v] Salvage value multiplied by salvage weight.
[w] Mean expected weaning weight for each sex multiplied by the expected price of weaned calf.
[x] Expected number of calves per sex multiplied by total income per sex. Values for both sexes added together for each respective scenario.
[y] Total income divided by total no. of heifers.
[z] Sum of total income from sale of clean-up bulls, total calf income from TAI for each scenario, and total calf income from clean-up bull for each scenario divided by the total no. of heifers.
[*] Difference between final income for respective scenarios.
[†] Difference between total cost per heifer exposed for respective scenarios.
[‡] Increased returns plus decreased costs minus decreased returns and increased costs.

Table 5.5. Sensitivity analysis for heifers fixed-time artificially inseminated (TAI) with conventional or sex-sorted semen[a]

Item				Scenario[b]		
				Sex-Sorted vs. conventional	Combination vs. conventional	Combination vs. sex-sorted
Herd	No. heifers	PR/AI for CTRL-CNV, %[c]	Desired Sex	Gain or loss per heifer exposed, $[d]		
1	57	71.4	Male	66.52	-8.16	-64.68
2	364	41.5	Male	27.49	46.04	18.55
3	238	51.7	Female	-18.73	-12.70	6.02
4	170	68.2	Female	-15.48	-31.28	-15.80
5	145	63.2	Female	-17.22	-28.31	-11.09
6	141	50.0	Female	-20.59	-16.14	4.45
7	149	70.6	Female	-15.30	-35.00	-19.71
8	61	22.2	Female	-32.12	-6.04	26.08
9	51	50.0	Male	32.27	8.52	-23.75
10	373	38.8	Male	23.33	48.72	25.38
11	56	57.1	Female	-24.21	-41.68	-17.48
12	50	85.7	Female	-18.19	-72.54	-54.34
13	81	36.4	Female	-26.58	-12.43	14.15
14	72	25.0	Female	-30.17	-4.32	25.85
15	82	60.0	Female	-20.66	-34.54	-13.88
16	67	44.4	Female	-25.89	-24.52	1.37
17	94	66.7	Male	62.67	8.37	-54.31
18	87	36.4	Male	15.21	35.59	20.39
19	100	50.0	Female	-22.04	-21.22	0.82
20	96	66.7	Male	62.78	8.75	-54.03
21	145	57.1	Female	-18.73	-22.53	-3.80
22	114	25.0	Female	-27.62	4.62	32.24
23	62	40.0	Female	-27.58	-22.45	5.13
Overall Male	1,122	53.1	Male	46.43	38.29	-8.14
Overall Female	1,733	50.4	Female	-17.24	-5.14	12.10

[a] Values utilized from herds in current study. Default assumptions based on Table 5.2. Two clean-up bulls utilized in all models.

[b] Sex-sorted: All heifers receive sex-sorted semen. Conventional: all heifers receive conventional semen. Combination: heifers that express estrus prior to TAI receive sex-sorted semen whereas heifers that do not express estrus prior to TAI receive conventional semen.

[c] Pregnancy rates to TAI (PR/AI) for heifers exposed to the 7-d CO-Synch + CIDR protocol and inseminated with conventional semen.

[d] Calculated based on increased/decreased returns and increased/decreased costs for each scenario.

6. CONCLUSIONS

In experiment one, cows that expressed estrus (HIESTR and LWESTR) had greater physical activity during the proestrus and estrus periods, DFD at TAI, CL volume and plasma P4 concentration 7 d after TAI, and PR/AI compared to cows that did not express estrus (NOESTR). Furthermore, cows that expressed high-intensity estrus (HIESTR) based on physical activity had greater DFD on Day 0 and CL volume on Day 7 compared with cows that expressed low-intensity estrus (LWESTR). Estrus intensity did not impact PR/AI, though HIESTR cows had improved indicators of fertility, such as DFD on Day 0 and CL volume on Day 7, compared with LWESTR cows. Results reported herein corroborate our previous findings in beef cows exposed to an estradiol-based TAI protocol (Rodrigues et al, 2018). Together, expression of estrus near the time of TAI improves reproductive function and PR/AI, whereas estrus intensity influences important fertility markers in beef cows.

In experiment two, the probability of pregnancy increased as time of TAI increased in PG54 heifers but had little effect on other treatment groups. Presynchronization with PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol in conjunction with delayed TAI after CIDR removal resulted in a tendency for an increase in PR/AI in replacement beef heifers. In addition, presynchronization with both a CIDR insert and PGF increased PR/AI by 7.3%. Therefore, the PG-CIDR54 treatment may potentially be utilized to facilitate the use of TAI in beef heifers. However, future research is required to determine the effectiveness of the PG72 and PG-CIDR54 treatments in comparison to the 7-d CO-Synch + CIDR protocol.

In experiment three, PR/AI were reduced when sex-sorted semen was utilized as opposed to conventional semen. Delayed TAI without presynchronization did not improve PR/AI with either conventional or sex-sorted semen. However, by presynchronizing heifers with PGF 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol and delaying TAI to 72 h, PR/AI were increased by 8.3% with sex-sorted semen, and PR/AI with the PRE72-SEX protocol were similar to CTRL-CNV heifers. Therefore, the PRE72-SEX treatment may be utilized to facilitate the use of sex-sorted semen in replacement beef heifers. Furthermore, the primary factors that influence the gain or loss per heifer exposed include the expected premium for the desired sex, the cost of sex-sorted semen, the size of the herd, weaning weights, and the PR/AI of the CTRL-CNV treatment. According to our assumptions and values taken from each of the 23 herds included in this study, sex-sorted semen results in the greatest net return when male beef calves are selected for. In order for the selection of female beef calves to be more profitable, a perceived premium of greater than $154 per head is required.

Collectively, the results from these experiments may be utilized to develop strategies to improve fertility of beef females, and in doing so, may increase the reproductive efficiency, sustainability, and profitability of beef cattle production systems.