IMPROVING REPRODUCTIVE EFFICIENCY AND CALVING DISTRIBUTION OF HEIFERS THROUGH ESTRUS SYNCHRONIZATION, NATURAL SERVICE, AND FIXED-TIME ARTIFICIAL INSEMINATION

by

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ABSTRACT

Artificial insemination has is considered to be one of the most important innovations in cattle management. Fixed-time artificial insemination (FTAI) protocols have offered new opportunities to implement AI, however, these technologies have not been widely implemented in beef heifers. The FTAI pregnancy rates in beef heifers have been inconsistent, leading to frustration and often discontinuation of a program that offers significant genetic and economic potential. The FTAI conception rates in heifers have been especially variably as some heifers come into estrus and ovulate too early and some ovulate too late for FTAI to be effective. Despite these inconsistencies, a major benefit of a FTAI program in heifers is that more heifers calve earlier in the calving season creating "calving momentum." The calves conceived via AI have improved genetics resulting in improved carcass characteristics This improvement does come at some cost in terms of time, materials and labor.

While many protocols and recommendations regarding the timing of hormone administration have been given, little information in available on breeding protocols involving estrus synchronization protocols combining natural service and AI. The goal of this study was to identify a breeding protocol that will result in more heifers calving within the first 21 days of their first calving season, as well as identifying the calving distribution (AI vs. natural sired). In this study, a 7-day CO-Synch + CIDR® protocol was combined with immediate natural service to critically evaluate the effectiveness of a FTAI protocol in beef heifers in order to provide scientifically based recommendations regarding a FTAI combined with natural service in terms of conception, calving rate and sire distribution that will result in 85% of pregnant heifers calving in the first 30 days of the calving season.

Seventy-five pubertal heifers were randomly placed into 2 groups based on reproductive tract scores, ensuring that a relatively even allotment of peripubertal and pubertal heifers were in each group. They had free access to native forages, hay and water throughout gestation. The control Group (n=25 heifers; control) underwent a commonly used 7-day CO-Synch + CIDR® FTAI protocol, followed by introduction of a bull 10 days following AI. A 60-day breeding season was implemented. The experimental Group (n=50 heifers) underwent a similar 7-day CO-Synch + CIDR® protocol. Following CIDR removal at 7 days and FTAI on day 10, bulls were immediately placed with the heifers. Again, a 60-day breeding season was implemented. Three mature Angus bulls were used in the study, which were determined to be "satisfactory" on their breeding soundness examination. The experimental group was split into two groups (n=25) with one bull per group for logistic and management purposes. Initial pregnancy examinations were performed via trans-rectal ultrasound utilizing a 10 MHz probe at 120 days after the initiation of FTAI protocol. A follow-up pregnancy examination at five to six months of gestation was performed via trans-rectal palpation. Additionally, parentage testing of calves was performed on all calves to determine AI-sired calving distribution.

The goal was to identify a breeding protocol that resulted in 85% of the heifers calving within the first 30 days of their first calving season. The calving distribution (AI vs. natural sired) following the induction of the breeding protocol involving estrus synchronization, natural service, and fixed-time artificial insemination was evaluated. . The preliminary results indicated that total conception rates may be improved combining a FTAI protocol with immediate natural service in heifers. While the percentage of heifers calving in the first 30 days was similar between experimental and control groups, subsequent 30-day calving intervals may be improved by a combination of natural service and AI, utilizing synchronization protocols to more

efficiently tighten the calving season. Calving distribution in the experimental group drastically favored the natural service sire, which was expected. Thus, utilizing a FTAI protocol in beef heifers using the 7-day CO-Synch + CIDR® combined with immediate natural service may be a viable option for improving the pregnancy rates and increasing calving season momentum. However, if desiring a greater number of calves from AI sires, bulls should be turned out two weeks following the synchronization of estrus, as the percentage of AI to NS sired calves on the experimental compared to the control were 18% and 44% respectively.

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War Eagle!

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CHAPTER I: INTRODUCTION

Cow calf production is an important enterprise in the Southeastern United States. Of the 31.2 million beef cows that calved in 2017, 44% of those reside in the 13 southeastern states (Scaglia, Beck, Lalman, & Rouquette Jr, 2017). Florida alone ranks 13th in the nation in total numbers of beef cows, with a value exceeding $955 million each year (Moore, 2019). To continue to capitalize on the resources available and increase the profit margin for producers, we look to increase productivity through continued advancements in fertility.

Artificial insemination has been considered to be one of the greatest innovations in cattle management, yet only about 5% of southeastern cattle producers implement AI routinely (Whittier). Historically, effective estrus detection or the lack there of has been cited as the critical step for the success or failure of an AI program (Scaglia et al., 2017). Heat detection is not possible for many southeastern cattle producers that have full-time jobs outside the farm. Recently, fixed-time artificial insemination (FTAI) protocols have offered new opportunities to implement AI when effective heat detection is not possible. The FTAI pregnancy rates in beef heifers have been inconsistent, leading to frustration and often discontinuation of a program that offers significant genetic and economic potential. In the Southeast, FTAI use in beef heifers results in approximately a 50% AI conception rate (Whittier). There are a wide range of results and multiple reasons for the inconsistencies which have led to less than desirable FTAI conception rates in heifers. Heifers respond variably to the estrus synchronization protocols used for FTAI. Some heifers exhibiting estrus and ovulate too early and some ovulate too late for FTAI to be effective. Despite these inconsistencies, a major benefit of a FTAI program in heifers is that more heifers calve earlier in the calving season. There is an increased average daily gain of those calves born earlier in the breeding season, regardless of whether they conceived via AI or not.

The cost of natural service vs. artificial insemination per pregnancy are similar and hover right around $95.00 (Rodgers et al., 2012). However, this does not account for any of the genetic merit of an artificial insemination sired calf. The increased value per calf of a dam exposed to FTAI can be more than $100.00 (Rodgers et al., 2012). Heifers that calve early continue this trend of productivity throughout their lives. These early calving cows not only remain in the productive herd longer but also will wean more lifetime pounds of calf. Those calves born early in the calving season result in approximately $36 more per calf when compared to those calving later in the season (Sprott & Troxel, 1988). Additional benefits include an increase of approximately 10% or more pounds of beef sold, increases in cows retained in the herd by 11%, and decreases in the herd breakeven point by 18% (Adkins, Riley, Little, & Coatney, 2012).

The typical amount of time from calving to the resumption of fertile cycles (postpartum period) for 90% of a beef herd's mature cows is 60 to 80 days. For first-calf beef heifers the typical number of days post-calving for 90% to resume fertile cycles is closer to 100 to 120 days. By enrolling cows and first calf heifers in a synchronization protocol we can significantly decrease the postpartum anestrus interval, returning them to cyclicity sooner (Odde, 1990).

It is our desire to identify a breeding protocol that results in more heifers calving within the first 21 days of their first calving season. The goal is to identify the calving distribution (AI vs. natural sired) of a breeding protocol involving estrus synchronization, natural service, and fixed-time artificial insemination that will result in 85% of pregnant heifers calving in the first 30 days of the calving season. The objective of this project is to critically evaluate the effectiveness of a FTAI protocol in beef heifers using the 7-day CO-Synch + CIDR® combined with immediate natural service. This is done in an effort to provide southeastern cattle producers with scientifically based recommendations regarding a FTAI combined with natural service. Hence,

making FTAI a viable option and improving the pregnancy rates and genetic potential of southeastern cattle.

CHAPTER II: LITERATURE REVIEW

Ovarian Development

The initial step in sex determination occurs at fertilization when a sperm delivers either an X (female) or Y (male) chromosome to the oocyte (P. L. Senger, 2012). In the early embryo, when the yolk sack is still present, primordial germ cells originate from the base of the hindgut. These primordial germ cells migrate from the hind gut to the base of the allantois where they differentiate along the testis (male) or ovarian (female) developmental pathways. The bipotential gonad is located on the genital or gonadal ridge, which forms medial to the embryonic kidneys (mesonephros). During the time primordial germ cells are colonizing the genital ridges they are undergoing mitosis and their numbers increase significantly, resulting in the formation of the primitive sex cords. The proliferating sex cords cause the genital ridges to enlarge and push towards the mesonephros (Wartenberg, 1982).

During development, the embryo utilizes three distinct renal systems. The pronephros (pronephric kidney) is a non-functional remnant of the primitive kidney found in lower animals (P. L. Senger, 2012). The pronephros regresses and is replaced by the functional bilateral intermediate mesonephros, which forms urine and is drained by the mesonephric ducts (formerly called "Wolffian" ducts). The mesonephric ducts extend caudally and empty into the urogenital sinus. During the first trimester the metanephros (metanephric kidney) or functional form of the adult kidney will begin to appear. During the time that the mesonephros is developing, the paramesonephric ducts (Mullerian ducts) begin to form alongside them. At this time the embryo remains uncommitted or sexually indifferent (P. L. Senger, 2012).

Sexual differentiation is regulated by a single substance directed by a gene on the Sex-determining Region of the T Chromosome (SRY) (P. L. Senger, 2012). As the production of sperm and androgens is the main function of the testis, this genetic program is initiated by SRY, which directs somatic cell specification to Sertoli cells that orchestrates further development. The discovery Sry/SRY was the first step to identifying the downstream transcription factors and other molecular players that drive the bifurcation of Sertoli and granulosa cell differentiation.

Testis and ovarian development is different from other types of organogenesis as these cell types arise from bipotential precursors present in the genital ridge (P. L. Senger, 2012).

These cell types in the male depend on signals from Sertoli cells that differentiate from the influence of transcription factors SRY and SOX9 (P. L. Senger, 2012). The first somatic cells to differentiate are Sertoli cells, with blood endothelial cells migrating into the gonad to lay down a primitive arterial vasculature. Following Sertoli cell proliferation, fetal Leydig cells and peritubular myoid cells differentiate. The vasculature becomes more complete with additional development of venous vessels and lymphatic endothelial cells (P. L. Senger, 2012).

The female reproductive tract organs form and differentiate during the fetal and postnatal stages of development. The Paramesonephric or Mullerian Ducts will give rise to the oviducts, uterus, cervix and upper portion of the vagina that forms within the fetal kidneys or mesonephros (Vue et al., 2018). Upon birth, the uterus is composed of a lumen lined by a single layer of epithelial cells with a surrounding undifferentiated mesenchyme (Mullen & Behringer, 2014). The mesenchyme differentiates into an inner stromal compartment surrounded by an inner-circular and outer-longitudinal layers of smooth muscle which make up the myometrium. In the adult uterus, endometrial glands that produce factors required for uterine receptivity, embryo implantation, embryo survival and development will invade into the uterine stroma in a process called adenogenesis (Gray et al., 2001; Spencer, Hayashi, Hu, & Carpenter, 2005).

Timing of Mullerian Duct formation varies between species, but the process can be separated into three phases: initiation, invagination, and elongation(Mullen & Behringer, 2014). The initiation phase occurs when a thick placode-like structure forms on the anterior mesonephric epithelium near the Wolffian Duct. The invagination phase occurs when the cells in the placode-like structure become elongated and form tight junctions, resulting in a depression of

the mesonephric epithelium. As the depression becomes deeper, it transforms into funnel-like structure. As the Mullerian Duct cells move posteriorly, it enters the elongation phase, which at the end the tips of the Mullerian Duct will reach the urogenital sinus and fuse (Mullen & Behringer, 2014).

Mullerian Duct cell differentiation switches between mesenchymal and epithelial states during its formation (Mullen & Behringer, 2014). In the initiation phase, the specified Mullerian Duct cells are considered "mesoepithelial" and invade into the mesonephric mesenchyme. After elongation is completed, the Mullerian Duct cells in female fetus down regulates mesenchymal markers and upregulates epithelial molecular markers (Mullen & Behringer, 2014).

For proper sexual differentiation, the Wolffian Ducts (WD) must regress; this is regulated by the presence or absence of fetal gonadal hormones. The male gonad secretes androgens, which causes the WD to differentiate into the mature male reproductive tract organs (Vue et al., 2018). The absence of androgens in female fetuses leads to the elimination or regression of the Wolffian Duct. Wolffian Duct regression, was historically considered a passive process, where lack of androgens in female fetuses fails to support differentiation (Vue et al., 2018). Recently, it's been discovered that regression requires active signaling to promote cell death of the epithelium. MSX2 which is a transcription factor expressed in the WD epithelium, and orphan nuclear receptor chicken of albumin upstream promoter transcription factor II (COUP-TFII) found in the WD mesenchyme have both been identified as mediators of WD regression in female reproductive tract differentiation (Yin, Lin, & Ma, 2006). COUP-TFII, a mesenchyme specific transcriptional regulator, is required for WD regression during the differentiation of the female reproductive tract in the mouse. Loss of COUP-TFII, results in retention of the WD

independent of androgen signaling. In fetal males, androgens secreted from the testis antagonize COUP-TFII function and prevent WD regression (Zhao et al., 2017).

The oviduct is the conduit for oocyte and embryo transfer to the uterus and is the site of fertilization (Vue et al., 2018). The ovulated oocyte enters the oviduct through the infundibulum and travels through the ampulla, which contains numerous longitudinal epithelial folds and abundant cilia to aid in oocyte transport. Upon fertilization, the zygote will travel through the isthmus region of the oviduct. When leaving the oviduct, the zygote travels through the uterotubal junction then into the uterine horn/body. A bursa surrounds the oviduct and ovary. The mammalian female reproductive organs, including the oviduct, uterus, cervix, and anterior vagina, are all derived along the anterior-posterior axis of the Mullerian Duct during embryonic development. The most anterior aspect of the Mullerian Duct gives rise to the oviduct (Vue et al., 2018).

The Transforming Growth Factor Beta (TFGB), Wingless (WNT) and Mammalian Target of Rapamycin (mTOR) signaling pathways have been found to be potential regulators of oviduct development. TGFB plays a role in controlling cell proliferation, differentiation and apoptosis during oviduct development (Conery et al., 2004; Elliott & Blobe, 2005; Li et al., 2011). The WNT pathway appears to play a direct role in oviduct development and is associated with the appearance of coiling and initial formation of the anterior region of the Mullerian Duct (Vue et al., 2018). The mTOR signaling appears to play a key role in smooth muscle differentiation and function in the oviduct (Daikoku et al., 2013; Tanaka et al., 2012).

In mammals the majority of the development and differentiation of the female reproductive tract is completed by birth (Vue et al., 2018). The endometrium of the uterus consists of two epithelial cell types; luminal epithelium (LE) and glandular epithelium (GE), and

two stratified stromal compartments including a densely organized stromal zone, blood vessels and immune cells (Vue et al., 2018). The myometrium consists of smooth muscle layers of the uterine wall, the inner circular layer and an outer longitudinal layer (Gray et al., 2001).

Morphogenic events common to morphogenesis of the uterus include: (1) organization and stratification of the endometrial stroma, (2) differentiation and growth of the myometrium and (3) coordinated development of the endometrial glands (Vue et al., 2018). The LE will invaginate into the stroma to generate the GE (endometrial or uterine glands), resulting in an extensive network of glands that extends towards the myometrium (Gray et al., 2001; Spencer et al., 2005). Uterine adenogenesis is the process of endometrial gland formation from the LE. Prenatal urogenital tract development in female mammals is an ovary (hormonal) independent process (Gray et al., 2001). While uterine development and endometrial adenogenesis can proceed in the absence of the ovary for varying periods of time during early postnatal development. However, gland morphogenesis remains mediated by diverse mechanisms including hormonal, cellular and molecular events (Vue et al., 2018).

Oogenesis & Folliculogenesis

The ovarian primordium develops from an elongated gonadal ridge on the ventral aspect of the embryonic kidney or mesonephros (Adams & Singh, 2014). In bovine ovaries, the ovarian primordium does not initially have a distinct surface epithelium with a basement membrane separating it from the underlying stroma but is composed of "gonadal ridge epithelial like" (GREL) cells. The GREL cells are precursors of both granulosa cells of future follicles of the future ovary. Primordial germ cells (PGC) migrate from the yolk sac and developing hindgut to colonize the gonadal ridge. Once at the gonadal ridge, PGCs undergo mitotic proliferation and co-mingle with the GREL cells (Adams & Singh, 2014). These cells invade the stroma and mesonephros, penetrating the gonadal ridge or ovarian primordium, separating into clumps of PGC and associated GREL cells into ovigerous cords in what will become the ovarian medulla at the base of the ovary (Rahe, Owens, Fleeger, Newton, & Harms, 1980).

The developing medulla contains stroma, blood vessels, and mesonephric tubules that will persist in the adult ovary (Adams & Singh, 2014). As the ovary continues to develop, the ovigerous cords break down into smaller groups of PGC and GREL cells, which result in the formation of primordial follicles. Upon primordial follicle formation, mitotic division of the oogonium ceases, it this enlarges, and becomes arrested in the early stages of Meiosis I. Later in of ovarian development, when preantral and antral follicles appear, the cells of the ovarian surface become single-layered and the stroma underlying the surface thickens to become the tunica albuginea (Adams & Singh, 2014).

Folliculogenesis is the developmental process in which an activated primordial follicle develops to a preovulatory size through the growth and differentiation of the oocyte and its surrounding granulosa cells (Adams & Singh, 2014). Ovarian follicles are generally, categorized

as primordial, primary, secondary, or tertiary or antral. Initiation of follicular growth or activation, begins with the transformation of pre-granulosa cells of the primordial follicle into a single layer of cuboidal granulosa or follicular cells, at which point the follicle is referred to as a primary follicle (Adams & Singh, 2014).

As granulosa cells proliferate there is an increase in the number of layers around the oocyte (Adams & Singh, 2014). A follicle with two to six layers of granulosa cells is referred to as a secondary follicle, and a follicle with more than six layers of granulosa cells and a fluid-filled antrum is referred to as a tertiary or antral follicle. Antral or Graafian follicles become the ovulatory follicle following the preovulatory gonadotropin surge (Adams & Singh, 2014).

Follicular Growth & Dynamics of Ovarian Cyclicity

Follicular growth and regression is a continuous process and is independent of the phase of the reproductive cycle (Adams & Singh, 2014). Follicular waves are first detectable as 4–5 mm follicles, around days 0 and 10 for two-wave interovulatory intervals and on approximately days 0, 9 and 16 for three-wave interovulatory intervals (Adams & Singh, 2014). Follicular wave emergence in cattle is characterized by the sudden growth of small follicles (Adams & Singh, 2014). The growth rate is similar between follicles in the wave for about 2 days, after which one follicle is then selected to continue growth, which is known as the dominant follicle. The remaining follicles become atretic and regress. In both two- and three-wave estrous cycles, the emergence of the first follicular wave occurs consistently on the day of ovulation. Emergence is preceded by a surge in plasma FSH concentrations. Follicular products, particularly from the dominant follicle, suppresses FSH release and subsequent emergence of the next follicular wave (Adams & Singh, 2014).

Estradiol and Inhibin-A and-B are the principal follicular products responsible for suppressing FSH (Singh, Pierson, & Adams, 1998). Inhibin-A, produced by the small growing follicles of the wave, appears to be the most important suppressor of FSH during initial wave emergence. Estradiol from the dominant follicle is the most important FSH suppressor afterwards (Singh et al., 1998).

Selection is the process by which a single follicle becomes functionally and morphologically dominant over other follicles within the same wave (Adams & Singh, 2014). In cattle, the mechanism of selection of the dominant follicle is based on reliance of other follicles within a wave on FSH, and their responsiveness to LH. The transient rise in FSH permits

sufficient follicular growth so that some follicles acquire LH responsiveness. The ability to respond to LH allows the follicle the ability to survive without FSH (Adams & Singh, 2014).

Granulosa cells from the dominant follicle have an increased ability to bind LH compared with the subordinate follicles and have greater LH receptor mRNA expression (Adams & Singh, 2014). Production of intrafollicular-fluid factors of dominant versus subordinate follicles is associated with the development of greater sensitivity to LH and FSH in the developing dominant follicle. Intrafollicular concentrations of insulin-like growth factor (IGF-1) remain elevated and estradiol begins to increase in the incipient dominant follicle while both begin to decrease in the incipient subordinate follicles (Adams & Singh, 2014).

Ovulation involves a series of events that culminates in the evacuation of contents from an antral follicle and the initiation of the development of a corpus luteum (Adams & Singh, 2014). The decrease in circulating concentrations of progesterone at the time of luteolysis removes the negative feedback effects on the pituitary and hypothalamus, resulting in an increase in LH pulse frequency and amplitude (Adams & Singh, 2014).

The availability of LH results in final maturation of the dominant follicle with an increase in production of estradiol and upregulation of the LH receptors on granulosa cells (Adams & Singh, 2014). Preovulatory increases in LH result in remodeling include: vascular changes, disruption of the basement membrane and rupture of the follicular wall, cumulus cell expansion, luteinization, and resumption of meiosis by the oocyte. During estrus, the high circulating concentrations of estradiol in the absence of progesterone are responsible for the LH surge and estral-like behavior. Although LH receptors are present on both granulosa and theca cells, LH receptors are highly expressed in those layers closer to the basement membrane in granulosa cells. To affect the entire follicle, LH needs second messengers to be able facilitate the

relationship among granulosa cells and cumulus cells and the oocyte through gap junctions. The two main secondary messengers for LH are cyclic AMP and intracellular calcium (Adams & Singh, 2014).

The binding of LH to G-protein receptors on granulosa cells surface results in activation of adenyl cyclase and an increase in cyclic AMP, stimulating protein kinase A (PKA) and C PKC) pathways (Adams & Singh, 2014). Stimulation of PKA and PKC causes phosphorylation and activation of transcription factors within a few hours of the LH surge. The LH surge, indirectly induces activation of the progesterone and prostaglandin pathways that modify fibroblast function to produce chemokines that will recruit inflammatory cells and growth factors that initiate angiogenesis (Adams & Singh, 2014).

There are two groups of enzymes that govern the degradation of the ovarian epithelium during ovulation: metalloproteinases and plasminogen activators, both of which expression are induced by the LH surge (Adams & Singh, 2014). These enzymes play a role in remodeling and a major role in follicle rupture during ovulation in cattle. Paracrine factors, proinflammatory cytokines and families of protease enzymes act in a coordinated effort with each other leading to degradation of extracellular connective tissue and ultimately follicle rupture after the LH surge (Adams & Singh, 2014).

Meiotic progression of the oocyte is arrested at prophase I, which is the first mitotic arrest during the fetal development of primordial follicles (Adams & Singh, 2014). The resumption of meiosis occurs when half the set of chromosomes are extruded as a polar body. Nuclear maturation progresses to metaphase II following the LH surge. Soon after the LH surge, gap junctions between cumulus cells and the oocyte deteriorate, which removes the inhibitory

signaling, and the oocyte can then resume meiosis. Meiosis is paused again in the second meiotic arrest called metaphase II until fertilization, when meiosis is finally complete. Cyclic AMP and GMP are considered the primary inhibitors of meiotic resumption in the oocyte. Cyclic GMP which is produced by granulosa cells, enters the oocyte via gap junctions between the oocyte and cumulus cells to maintain low levels of cAMP phosphodiesterase 3A (PDE3A) in the oocyte. This low level of PDE3A maintains the high levels of cAMP produced within the oocyte and maintains meiotic arrest. LH exposure, decreases cGMP production from granulosa cells, uncoupling the gap junctions via mitogen-activated protein kinase (MAPK). The reduced production and presence of cyclic GMP into the oocyte increases PDE3A, which hydrolyzes cyclic AMP and enables the resumption of meiosis (Adams & Singh, 2014).

Onset of Puberty in the Female

Puberty is a complex process involving both physical and behavioral changes that culminate in the functional potential to become pregnant (Castellano, Heras, Ruiz-Pino, & Tena-Sempere, 2018). Neuroendocrine puberty is by definition the maturity of hypothalamic centers responsible for basal and surge release of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) in response to the negative and positive feedback of estradiol-17b (Amstalden, Alves, Liu, Cardoso, & Williams, 2011). Puberty is initiated by the pulsatile release of gonadotropin releasing hormone (GnRH) triggering the release of both LH and FSH (Ojeda & Skinner, 2006). This pubertal increase in GnRH/LH secretion is primarily an ovarian-independent phenomenon in females. The "Central Drive Hypothesis" states that the increase in pulsatile GnRH secretion at the onset of female puberty emerges from changes in the activity of the central pathways controlling GnRH neurons (Ojeda & Skinner, 2006). The pubertal activation of GnRH neurons is brought on by changes to the inputs to the GnRH neuronal network, consisting of a reduction of inhibitory influences and an increase in stimulatory signals. A significant fraction of the stimulatory inputs that modulate GnRH secretion is provided by neurons that synthesize glutamate, kisspeptin, and neurokinin B (NKB) (Lomniczi, Wright, Castellano, Sonmez, & Ojeda, 2013).

Kisspeptin is one of the most powerful neuropeptide activators of the gonadotropin system during the pubertal transition (Pinilla, Aguilar, Dieguez, Millar, & Tena-Sempere, 2012). Kisspeptins are a family of structurally related peptides, encoded by KISS1, that act through the binding and activation of the G protein-coupled receptor, Gpr54, which is also termed kisspeptin receptor or Kiss1R (Oakley, Clifton, & Steiner, 2009). The activation of Kiss1 during the pubertal transition involves a hypothalamic rise of Kiss1 during the juvenile–pubertal transition

which then activates the GnRH/LH system (V. Navarro et al., 2004). Kisspeptin's role in controlling puberty has been demonstrated when synthetic kisspeptin analogs were given to prepubertal female mice resulting in the precocious puberty onset; this is in contrast to when kisspeptin antagonists were administer which led to delayed uterine cyclicity and reduced uterine and ovarian weights (Decourt et al., 2016; Pineda et al., 2010).

There are two major hypothalamic populations of kisspeptin neurons in the brain, one in the rostral periventricular area of the 3rd ventricle (RP3V) and one in the arcuate nucleus (ARC) (García-Galiano, Pinilla, & Tena-Sempere, 2012). The RP3V kisspeptin neurons respond to estrogen by increasing production of kisspeptin, thereby demonstrating a positive feedback relationship resulting in the generation of the preovulatory LH surge. In contrast, the synthesis of kisspeptin is inhibited by estrogen in the ARC demonstrating a negative feedback effect and resulting in an inhibitory effect of estrogen on GnRH/gonadotropin release. As a result, these two effects are crucial for the tonic pulsatile secretion of gonadotropins (J. T. Smith, Cunningham, Rissman, Clifton, & Steiner, 2005). Elimination of kisspeptin neurons during the early juvenile period will prevent normal pubertal maturation and cause infertility (Mayer & Boehm, 2011).

The KNDy neurons are composed of kisspeptin, neurokinin B (NKB), and dynorphin. These neurons can be acted on by steroid hormones, such as estradiol, to regulate the pattern of activity of GnRH. NKB and Dynorphin A (Dyn) are co-regulators of kisspeptin neurons and are expressed in some ARC kisspeptin neurons (Castellano et al., 2018). Since the kisspeptin neuronal population expresses NKB and Dyn, they have been termed KNDy neurons. The KNDy hypothesis proposes that the release of kisspeptin from the ARC kisspeptin neurons onto GnRH neurons is regulated by the reciprocal stimulatory and inhibitory actions of NKB and Dyn, respectively (V. M. Navarro et al., 2012). When a NKB agonist was administered to

prepubertal female rats it stimulated the secretion of LH (V. M. Navarro et al., 2012). The inhibitory action of Dyn on kisspeptin release declines when puberty approaches and the stimulatory effect of NKB on kisspeptin secretion gradually increases during this transition (Castellano et al., 2018).

Leptin also plays a role with kisspeptin, an interaction which controls the onset of puberty (Castellano et al., 2018). Leptin acts as less of a trigger and more like a buffer, allowing puberty to proceed once acceptable levels are achieved and acting indirectly on kisspeptin neurons in the control of female puberty (Castellano et al., 2018).

Estradiol can have either positive or negative feedback mechanisms which will increase or decrease, respectively, the release of GnRH to the median eminence. High levels of Estrogen during the pre-ovulatory stage causes a switch of estradiol action from negative to positive feedback with ovarian estradiol (E2) acting as the main signal. This will induce elevated GnRH neuronal activity and cause the preovulatory surge of GnRH followed by LH release to initiate ovulation. When estrogen is increased, kisspeptin is increased in the Anteroventral Periventricular Nucleus (AVPV) and decreased in the Arcuate Nucleus. Estradiol will reduce the excitatory effect of NKB and enhance the inhibitory effect of dynorphin on KNDy firing rate (Jacobs, Veitch, & Chappell, 2016).

Estrogen Receptor α (ERα) is this most prominent type of KISSr in both the AVPV and ARC. The ERα works through both positive and negative feedback mechanisms and project onto GnRH neurons. The AVPV kisspeptin neurons are regulated by E2 to increase activity during the LH surge. When the AVPV is damaged both the pre-ovulatory and E2l induced LH surges are blocked. This suggests that ERα in kisspeptin is required for the positive feedback by E2 (Wang & Moenter, 2020).

Folliculogenesis, Dynamics & Dominance

Follicular development is by nature a dynamic process. Follicles are selected and then proceed through series of developmental events on their path to ovulation. These events involve multiple endocrinological and physiological mechanisms. Follicular growth and development is initiated by the recruitment of a group of follicles which are responsive to gonadotrophins and stimulated by slight elevations in FSH as well as other intrafollicular signaling mechanisms (P. L. Senger, 2012). The question that needs to be answered is: what makes these follicles become dominate?

Follicular or cyclic recruitment refers to the activation of primordial follicles to begin their growth as a follicular wave. The follicles in each wave increase in size for several days and then one follicle is "selected" for further growth, also referred to as the dominant follicle (DF) (Ginther, Beg, Bergfelt, Donadeu, & Kot, 2001). There are 2-3 follicular waves per cycle in ruminants. It is the DF of the final wave of the cycle that ovulates (Ginther et al., 2001).

Atresia of follicles occurs at every stage of follicular development, but the rate of atresia is highest late in antral follicular development (Hirshfield, 1991). The purpose of antral follicles is to nurture the oocyte and maintain its meiotic and developmental capacities, allowing the oocytes to grow to a size with an adequate number of theca and granulosa cells. The theca cells enable the granulosa cells to secrete the appropriate amount of E2, triggering the surge or release of gonadotropins from the anterior pituitary and stimulating ovulation (J. E. Fortune, 2018). Many antral follicles reach a particular size at which atresia is their normal fate. These follicles cannot develop any further. So, despite having basal hormonal (gonadotropin) levels sufficient to sustain follicular growth, regression still happens.

In relation to the recruitment of "cohorts" of follicles into follicular waves most species develop a small rise in circulating FSH accompanied by the initiation of each follicular wave (J. E. Fortune, 2018). Cattle are no exception as they exhibit a secondary surge of FSH around the beginning of the first follicular wave of the cycle and small rises in FSH were detected just prior to the later follicular waves of the same estrous cycle. The temporal relationships between increases in circulating FSH and the initiation of follicular waves suggest that the two events are related. In cattle, a secondary surge of FSH occurs 1 day after the LH/FSH surge. Injection of bovine follicular fluid (containing inhibin which suppresses FSH) delays the first wave of follicular development for the duration of FSH suppression (Ginther et al., 2001).

The number of follicles recruited into waves is typically greater than the number of follicles that are ovulated. A key characteristic of dominant follicles, compared to other subordinate follicles, is their increased capacity to secrete E2 which is a hallmark of preovulatory follicles. Dominant follicles already have a higher capacity for E2 production at the time when their growth track changes from that of subordinate follicles. As E2 is a negative feedback regulator of FSH, DF's are essentially starving their subordinate cohorts of FSH to insufficient levels (Ginther et al., 2001).

Estradiol production is a key characteristic of emerging dominant follicles. Estradiol exerts negative feedback on FSH secretion by the anterior pituitary, depriving the subordinate follicles of sufficient FSH. Ovarian follicles produce E2 via interactions between the inner granulosa cells, which line the antrum of the follicle, and the outer theca cells, which are outside the basement membrane between the theca and granulosa cells (J. E. Fortune, 2018).

At very early stages of follicular development, theca cells have LH receptors and granulosa cells have FSH receptors. Theca cells are stimulated by LH to convert cholesterol to

progestins and then to androgens, but they lack the aromatase enzyme to convert androgens to estrogens (E2 and estrone) (J. Fortune, Rivera, & Yang, 2004). Granulosa cells cannot synthesize androgens, but they are able to convert androgens that diffuse from the theca across the basement membrane into estrogens. Granulosa cells acquire LH receptors as follicles develop towards ovulation. Levels of mRNAs for LH receptors, FSH receptors, aromatase, and P450 17a-hydroxylase in follicular cells of DF vs. the largest subordinate follicle revealed that they were all higher in the DF. This did not occur until a day after the difference in E2l was detected (J. Fortune, Rivera, Evans, & Turzillo, 2001).

The role that Insulin-Like Growth Factor (IGF) plays in the selection of dominate follicles occurs before divergence in follicular size, which enables the increased production of E2 (Spicer, 2004). In bovine follicles, FSH induces Insulin-like Growth Factor Binding Protein 4 and 5 (IGFBP-4/-5) and the protease PAPP-A (pregnancy-associated plasma protein A). An increase in PAPP-A is one of the earliest changes seen. This increase is followed by a decrease in IGFBP-4 and -5 and an increase in free IGF-1 with a subsequent increase in E2, proving that the DF is biochemically selected prior to size deviation (J. Fortune et al., 2004). Due to the increase in PAPP-A, IGF works synergistically with FSH to increase E2 production which rapidly suppresses basal FSH to levels that are insufficient to support similar changes in other follicles of the cohort. It has been shown that a low level of IGFBP-4 in the follicular fluid of a follicle in a recruited wave, prior to selection of DF, was more predictive of future dominance than follicular diameter or concentration of E2 in follicular fluid (J. Fortune et al., 2004).

Nonovulatory DF's lose function before they lose morphological dominance (J. Fortune, Sirois, Turzillo, & Lavoir, 1991). A DF at the time of luteal regression continues to grow and to increase its capacity to produce E2. This is stimulated by the decrease in circulating progesterone

during luteal regression which causes an increase in LH (Ginther et al., 2001). The change or increase in LH pulse frequency when the negative feedback effects of luteal progesterone are removed, coupled with the low levels of circulating FSH, were once thought of a shift in the DF from FSH to LH-dependence. Estradiol made by the DF decreases basal FSH, but the DF is still dependent on basal levels of FSH and will undergo regression if FSH is depressed completely (J. Fortune et al., 1991).

The increase in basal LH during the latter part of the follicular phase is important to convert the DF into a pre-ovulatory follicle capable of secreting E2 levels sufficient to elicit a gonadotropin surge. The major effect of the increased circulating LH on theca cells is to increase production of androgen precursors, which has been induced and maintained by FSH for the conversion to E2 by granulosa cells (Hampton et al., 2004).

The preovulatory surge of LH and FSH is induced when there is a positive feedback on the hypothalamus to estradiol and the negative feedback of progesterone is eliminated with regression of the CL.. The gonadotropin surge converts pre-ovulatory follicles into peri-ovulatory follicles. The changes induced lead to meiotic maturation of the oocyte, cumulus expansion, and the structural and functional changes that produce the rupture of the DF (J. E. Fortune, 2018).

Luteolysis

The corpus luteum (CL) is a transitory endocrine gland that forms on the ovary from the granulosa and thecal cells that remain in the postovulatory follicle (P. L. Senger, 2012). Its primary function is to secrete progesterone, preparing the uterus for implantation, as well as maintaining pregnancy by promoting uterine quiescence. If fertilization does not occur, or if embryonic signaling is insufficient, the CL will regress (P. L. Senger, 2012). Luteal regression (luteolysis) results in pregnancy failure, but also removes the negative feedback of progesterone on gonadotropin release, allowing for maturation and ovulation of a new oocyte and another opportunity to establish a pregnancy. (P. Senger, 1997)

Luteolysis is the process by which functional and structural changes culminate in the rapid degradation and eventually the complete disappearance of the CL (P. L. Senger, 2012). The hallmark of luteolysis is the functional change that occurs which is the cessation of steroid production (Pate, 2018). This is followed by the somewhat more gradual structural changes, such as breakdown of extracellular matrix, cellular death, and removal of cellular debris. The focus of this discussion is on the pathways leading to luteal regression or rescue at the level of the CL (Pate, 2018).

Luteolysis is sometimes subdivided into functional and structural components. The rapid decrease in progesterone production begins prior to onset of cell death, meaning that death of steroidogenic cells is not the initial cause of the decline in progesterone production but that PGF2α directly decreases progesterone (P. L. Senger, 2012). The specific actions of PGF2α on each cell type in the CL, which ultimately result in luteal regression, will be discussed as a whole rather than subdividing these actions into functional and structural actions (P. Senger, 1997).

In the case of the rescue of the CL in pregnancy, an essential event called the “maternal recognition of pregnancy” causes the continuation of luteal progesterone production which maintains the pregnancy (P. L. Senger, 2012). In species in which uterine PGF2α initiates luteolysis, rescue of the CL involves changes in uterine secretion of PGF2α. Interferon Tau, which is the signal for maternal recognition of pregnancy in the bovine acts on endometrial cells to inhibit expression of estrogen receptors. The lack of estrogen action in the endometrium results in decreased expression of oxytocin receptors which disrupts the normal pulsatile release of PGF2α that is necessary to cause luteolysis (P. L. Senger, 2012). Additionally, rescue of the CL involves mechanisms within the tissue to maintain steroidogenesis and capillary integrity (Pate, 2018). During maternal recognition of pregnancy, the CL becomes less sensitive to the luteolytic effects of PGF2α. Ultimately the signal from the embryo results in maintenance of survival genes and prevents upregulation of death pathway-related genes, protecting the integrity of the luteal vasculature and maintaining steroidogenesis. This event is critical for successful reproduction (Hughes & Pate, 2019).

Oxytocin plays a key role in luteolysis as the endometrial expression of oxytocin receptors parallels the uterus’ responsiveness to oxytocin. This is why the uterus becomes more sensitive to the effects of oxytocin around the time of luteolysis (Pate, 2018). After stimulation by oxytocin, the oxytocin receptors are internalized and recycled to the plasma membrane, coinciding with the frequency of PGF2α pulses from the endometrium. Oxytocin serves as a pulse generator for uterine PGF2α release. In the ruminant, large concentrations of oxytocin are synthesized and stored in the CL and are released into the ovarian vein in response to PGF2α. The initial release of PGF2α from the uterus is likely triggered by pituitary release of oxytocin (Pate, 2018).

Luteolysis is initiated in the CL as PGF2α binds to a G protein coupled receptor in the plasma membrane (PTGFR). This initiates luteolysis by activating phospholipase C which cleaves inositol triphosphate and diacylglycerol from membrane phospholipids, ultimately leading to an increase in intracellular free calcium and activation of protein kinase C (Pate, 2018). The functional effects of PGF2α are mediated by activation of the protein kinase C pathway. The activation of protein kinase C induced by PGF2α leads to activation of mitogen-activated protein kinase (MAPK) signaling pathways and upregulation of the transcription factors. Activation of MAPK11 and MAPK8 have been linked to the regulation of apoptosis and autophagy as well as the stimulation of interleukin IL8 which attracts neutrophils during luteolysis (Pate, 2018).

Upon initiation of luteolysis by PGF2α, there is auto-amplification of PGF2α signaling in luteal cells (Hughes & Pate, 2019). PGF2α stimulates the enzymes that metabolize arachidonic acid and downregulate the enzymes that catabolize PGF2α to inactive metabolites. PGE2 activates the cAMP pathway in luteal cells, stimulating progesterone production. Conversion of PGE2 toPGF2α is important to maintain the decline in steroidogenesis. The direct action of PGF2α to initiate luteolysis is the inhibition of steroidogenesis (Hughes & Pate, 2019).

The CL is comprised of a heterogeneous population of cells that participate in the luteolytic process. The population includes capillary endothelial cells which are particularly abundant, a network of fibroblasts that develop the supporting extracellular matrix, and immune cells scattered throughout the luteal tissue (Hughes & Pate, 2019). PGF2α stimulates production of endothelin-1 (EDN1) by capillary endothelial cells which inhibits progesterone production by steroidogenic cells (Pate, 2018). Cooperative interaction among steroidogenic cells, immune

cells, and endothelial cells occurs to ensure the progression of luteolysis by recruiting additional immune cells and orchestrating the destruction of the tissue itself (Pate, 2018).

Cellular changes occur in the CL during luteolysis. Luteal endothelial cells (LEC) produce EDN1 which acts on luteal small and large steroidogenic cells (LSC) to decrease progesterone production (Pate, 2018). Endothelial cell expression of chemokines and adhesion molecules result in recruitment of immune cells and extravasation into the luteal parenchyma where they can be in intimate contact with LSC (Hughes & Pate, 2019). There is decreased expression of growth factors within the CL resulting in destabilization of blood vessels and disruption of blood flow (Henkes et al., 2008). Thrombospondin 1 (THBS1) is produced by both LEC and LSC and has multiple effects in the CL during luteolysis, leading to activation of caspase 3 which is part of the apoptotic cascade, and leading to an increased expression of transforming growth factor beta 1 (TGFβ1). TGFβ1 increases synthesis of SERPINE1, which facilitates the breakdown of the extracellular matrix (ECM) (Yang et al., 2016). TGFβ1 also causes transformation of fibroblasts into myofibroblasts which leads to the formation of the arteriovenous anastomoses in the CL during luteolysis, decreasing oxygen availability to the luteal cells. Activated immune cells in the CL produce cytokines that inhibit LH-stimulated progesterone production and stimulate PGF2α production in LSC, which promote the production of reactive oxygen species (ROS) and ECM breakdown, causing apoptosis of LSC (Pate, 2018).

Lutenization

The corpus luteum (CL) of pregnancy is a transient endocrine gland that produces progesterone (Hughes & Pate, 2019). The processes regulating luteal regression or luteolysis in the absence of pregnancy is quite complicated. Luteolysis is necessary in order for another ovulation to occur, allowing the female another opportunity to conceive. The mechanism for the initiation of luteal regression begins with the acquisition of luteolytic capacity. The CL regresses in response to PGF2α produced by the endometrium on the uterus late in the cycle, but fails to regress in response to the same concentration of PGF2α earlier in the cycle (Diaz, Crenshaw, & Wiltbank, 2000). The acquisition of luteolytic capacity, while observed in a in a variety of species, from a practical standpoint, has allowed for the development of efficient synchronization protocols for livestock (Diaz et al., 2000).

The bovine CL does not regress in response to a luteolytic injection of PGF2α prior to day 6 of the estrous cycle (Henricks, Long, Hill, & Dickey, 1974). Later in the cycle, this same concentration of PGF2α will cause complete luteal regression to occur and allow another ovulation to proceed (Henricks et al., 1974). There is no difference in prostaglandin F receptor (PTGFR) expression or affinity for PGF2α in the CL of differing responsiveness to PGF2α in the bovine CL (M. Wiltbank, Shiao, Bergfelt, & Ginther, 1995). Prior to and after the acquisition of luteolytic capacity, the early CL possesses the ability to respond to PGF2α, but they do not regress (Tsai & Wiltbank, 1998). Luteolytic capacity may be mediated by the ability of the CL to synthesize intraluteal prostaglandins is a requirement for structural regression (Niswender et al., 2007).

As the CL acquires luteolytic capacity it gains the ability to be activated by PGF2α and regulates downstream effects, including the production of progesterone (Hughes & Pate, 2019).

Luteal cytokine production, and infiltration of immune cells, in response to PGF2α is also different in the early CL (Hughes & Pate, 2019). PGF2α is able to induce tissue infiltration of monocytes in the mature CL, which include functions related to immune regulation and apoptosis (Tsai, Juengel, & Wiltbank, 1997). Luteal vasculature also responds differently to PGF2α after acquisition of luteolytic capacity, as there is a rapid increase in blood flow for the first 2h following injection of PGF2α (Acosta, Yoshizawa, Ohtani, & Miyamoto, 2002). For luteal regression to occur, responses to PGF2α must include: altered prostaglandin synthesis and metabolism, immune cell chemoattraction and activation, alteration of vasculature, and specific cell signaling events (Hughes & Pate, 2019).

In most domestic animals, the uterus is required for luteal regression, as the CL is maintained for a long period of time in the hysterectomized animal (J. Wiltbank & Casida, 1956). For complete luteal regression to occur with regularity; repeated pulsatile release of PGF2α from the uterus is required, as well as a functional loss of progesterone and the structural changes including cell death and involution that must occur (Ginther, Araujo, Palhao, Rodrigues, & Beg, 2009; Hughes & Pate, 2019). Ultimately, alteration of transcription factors and changes in gene expression in the regressing CL, will result in a reduction of progesterone production by large luteal cells (LLC) and LH-stimulated progesterone from small luteal cells (SLC) (Chen, Fong, & Davis, 2001). This causes the downstream accumulation of free intracellular calcium which mediates PGF2α-induced cell death in LLC (M. Wiltbank, Diskin, & Niswender, 1991). PGF2α also has the ability to inhibit luteal cells from utilizing lipoproteins for steroid hormone biosynthesis (Nett, McClellan, & Niswender, 1976).

The CL has a rich blood supply and PGF2α has actions on the endothelial cells as marked angio-regression occurs during luteolysis (Augustin, Braun, Telemenakis, Modlich, & Kuhn,

1995). Luteal endothelial cells are the first to undergo apoptosis, as the changes in luteal blood flow occur very early in luteal regression (Sawyer, Niswender, Braden, & Niswender, 1990). Endothelial cells themselves are rich sources of (Endothelin 1) EDN1, which is a potent vasoconstrictor and contributes to the loss of blood flow seen during luteal regression (J. S. Davis, Rueda, & Spanel-Borowski, 2003). Nitric Oxide (NO) is also an important component of the luteolytic cascade (T. El-Sherry, Derar, & Bakry, 2013). NO is a vasodilator and helps to modulate the transient increase in blood flow of the CL during early luteolysis (T. M. El-Sherry, Senosy, Mahmoud, & Wasfy, 2013).

Immune cells and cytokines also play a role in luteolysis, as they are produced in greater abundance in luteal regression than during the estrous cycle (Hughes & Pate, 2019). One function of cytokine production and the regulation of luteolysis is the induction of intraluteal prostaglandin production, which necessary for structural regression (Niswender et al., 2007). A lesser-known cytokine, chemerin, has been demonstrated to be a potential regulator of luteal progesterone production as well (Yang et al., 2016). Cytokines are key regulators of cell death and the loss of progesterone production during luteal regression, as they induce prostaglandin production (Hughes & Pate, 2019).

Luteolysis involves extensive cell death, however, apoptosis is not the only mechanism of cell death during luteolysis (Hughes & Pate, 2019). Necroptosis is an orderly, programmed form of necrosis that is not necessarily associated with acute tissue damage (Hughes & Pate, 2019). This is another mechanism that may be activated during luteolysis as well as autophagy, which is a stress response wherein cells recycle unneeded organelles that lead to cell breakdown and death (Hughes & Pate, 2019).

A discussion about luteolysis isn't complete without covering luteal rescue and the maternal recognition of pregnancy (MRP), which is the process by which the reproductive system of the dam recognizes that the conceptus is present and must maintain the pregnancy (P. L. Senger, 2012). An essential event during MRP is rescue of the CL and continuation of luteal progesterone production (P. L. Senger, 2012).

In the ruminant, the MRP signal is a trophoblast-derived interferon, IFNT. It is produced by the trophectoderm of the conceptus beginning around day 14 to 15 in cattle (C. Farin et al., 1990). The specific timing of the MRP signal results from the necessity of rescuing the CL from luteolytic pulses of uterine PGF2α (P. L. Senger, 2012). The action of conceptus-derived hormones on the uterus and the CL, are necessary for luteal rescue. Interferon response controls the expression of estrogen receptor 1 (ESR1), which in turn regulates expression of oxytocin receptor (OXTR) expression as well as the pulsatile release of PGF2A from the uterus (Spencer & Bazer, 1996). IFNT, via the MAPK signaling pathway also inhibits the function of the prostaglandin transporter protein, which mediates the release of prostaglandins from cells, in endometrial luminal epithelial cells (Lee, Stanley, McCracken, Banu, & Arosh, 2014). In summary IFNT alters prostaglandin release and transport, which is an important component of the antiluteolytic mechanism mediated by IFNT (Hughes & Pate, 2019).

Estrus Synchronization & Fixed Time Artificial Insemination

Estrus-synchronization (ES) in combination with AI have been available for the synchronization of the estrous cycle for more than 40 years (G. Lamb, Dahlen, Larson, Marquezini, & Stevenson, 2010). Estrus synchronization involves administering a series of hormones to induce a group of cows or heifers to be fertile at a chosen time period, which facilitates heat detection and AI (Islam, 2011). Estrus synchronization with AI in beef cattle offers multiple advantages over entirely natural mating systems or AI without estrus synchronization (Islam, 2011). Estrus or "Heat" detection is made easier with estrus synchronization by reducing the number of days necessary to observe for signs of estrus (Dickinson et al., 2019). Proper estrus detection is critical for successful artificial insemination if fixed-time artificial insemination (FTAI) protocols are not implemented (Diskin & Kenny, 2016).

The majority of estrus synchronization protocols use one or a combination of three basic methods that work with the physiology of the cow's normal estrous cycle (P. Senger, 1997). Early ES protocols focused on the regressing CL with an injection of PGF2α administered to cause CL regression and standing heat in approximately 72 h (Islam, 2011; G. Lamb et al., 2010). If the cow or heifer is in the first 5 to 7 days of her estrous cycle, or metestrus when her CL is unresponsive PGF2α (Islam, 2011). Progesterone or progestins, from either a Controlled Internal Drug Release (EAZI-BREED™ CIDR®) inserts or ingested in the feed by feeding melengestrol acetate (MGA®), mimic the effects of the cow's natural progesterone by preventing estrus from occurring as long as they are present in the body (Islam, 2011). Later protocols combined the use of PGF2α and progestins (G. Lamb et al., 2010). It was discovered that ovarian follicles in cattle occur in wave-like patterns, with generally one follicle becoming

dominant. The idea of controlling follicular waves with a single injection of GnRH at random stages of the estrous cycle to induce the release of LH, leading to ovulation or luteinization of the dominant follicles (≥10 mm) was developed (J. Fortune, Sirois, & Quirk, 1988; Sartori, Fricke, Ferreira, Ginther, & Wiltbank, 2001). A new follicular wave is initiated 1.5 to 2 d after GnRH administration in females in which the dominant follicle ovulated or luteinized in response to GnRH-induced LH release (G. Lamb et al., 2010). Luteal tissue forming after GnRH administration is capable of undergoing PGF2α-induced luteolysis 6 to 7 d later (Twagiramungu, Guilbault, & Dufour, 1995). These advances facilitated research to develop reliable protocols that rely solely on FTAI and ensure that systems were successful in anestrous or peripubertal and estrous-cycling females at any stage of the estrous cycle (G. Lamb et al., 2010).

Artificial insemination (AI) is a reproductive management tool that allows cattle producers to use proven sires of high genetic merit, including important attributes such as growth, maternal, and carcass traits, and increased calving ease (G Perry). This is not a new concept, as efforts to establish AI as a practical procedure were attempted before the turn of the 20th century by Ivanoff in Russia using domestic farm animals (Foote, 2010). AI is one of the most widely available reproductive biotechnologies available for producers to infuse superior genetic traits into their cattle at costs far below that of actually purchasing a sire of similar quality (Seidel Jr, 1995). The process of AI in cattle using conventional AI methods involves the deposition of semen; typically cryopreserved in the uterine body (López-Gatius, 2000).

The average estrous cycle, for the bovine female is 21 days with a range of 18-24 days (2 and 3 wave cows) (P. L. Senger, 2012). The cycle "begins" on Day 1 when ovulation occurs. The oocyte then migrates to the oviduct where if viable sperm are present, fertilization will occur are the ampullary isthmus junction (P. L. Senger, 2012). Regardless of whether the egg is fertilized,

by approximately Day 5-7, the site of ovulation on the ovary develops from a corpus hemorrhagicum (CH) into a corpus luteum (CL) producing progesterone which maintains the pregnancy (P. L. Senger, 2012). While the CL is secreting progesterone, the cow will not display estrus whether pregnancy has occurred or not (P. L. Senger, 2012). At approximately day 17, if the cow is not pregnant, the uterus releases the PGF2α that causes the CL to regress in about 3-5 days (GA Perry, Dalton, & Geary, 2011). While the CL is regressing, a new egg-containing follicle is developing that secretes E2, causing the cow to come into standing heat on about day 20 or 21 of the estrous cycle (Islam, 2011).

Cattle should be inseminated near the end of standing heat to provide enough time for the sperm to undergo capacitation, giving them the ability to fertilize the egg (P. L. Senger, 2012). Mature cattle ovulate approximately 27+/- 3 h after the onset of estrus (Diskin & Kenny, 2016). Insemination approximately 12 h after standing estrus was first observed provides time for the sperm to undergo capacitation (Diskin & Kenny, 2016). Traditional artificial insemination has followed the "AM-PM rule," as animal first observed in heat in the morning should be inseminated that evening (12 h later) (Riaz et al., 2018). As the sperm is greater in number has a longer lifespan in the female reproductive tract, it is better to have the sperm waiting on the egg, rather than the egg waiting on the sperm (P. L. Senger, 2012).

FTAI, is a program which combines the administration of a series of hormones, so that timed artificial insemination (TAI) can occur at a predetermined time following an appropriate synchronization program without the need for estrus detection (Diskin & Kenny, 2016). Improvements in FTAI and estrus synchronization protocols have increased pregnancy rates, while allowing for easier scheduling of labor resources and less cattle handling. The administration of GnRH during the estrus cycle causes regression or ovulation of the dominate

follicle and initiates a new wave of follicular growth. Ovulation of the dominate follicle occurs consistently, however ovulation of dominate follicles in the static or regressing stages occurs rarely, 33% and 0%, respectively (Pursley, Mee, & Wiltbank, 1995). In intital work, Pursley et al. reported the range in timing of ovulation in lactating dairy cows to be 84 to 120 h following a GnRH and PGF2α treatment. It was demonstrated that the range of ovulation could be reduced to 8 hours in a second injection of GnRH was administered 48 h after PFG2α in the GnRH-PGF2α (Ov-synch) treatment group. While management and experience with ES and AI protocols improve conception results a realistic goal of a 50% is expected when implementing these protocols (G. C. Lamb & Mercadante, 2016). In a commercial setting ES and AI are used for one AI service, and then clean up bulls are commonly introduced 10-14 d later for the remainder of the breeding season.

Estrus Synchronization & Effect of Day of Cycle

The development of methods to synchronize estrus evolved as a better physiologic understanding of the estrus cycle, led to the ability to control and manipulate with different aspects. The physiological basis for ES followed the discovery that progesterone inhibited ovulation and preovulatory follicular maturation (Nellor & Cole, 1956). The CL was the first structure that was able to be manipulated to consistently control of its' lifespan and the luteolytic and luteotropic mechanisms which are associated with it, thereby limiting the lifespan of the luteal phase (Thimonier, Chupin, & Pelot, 1975). Progesterone was discovered to prolong the luteal phase of the estrous cycle and aided in establish an artificial luteal phase by administering exogenous progesterone to maintain a cow in the luteal phase for an extended period of time (G. Lamb et al., 2010). Treatments combining progesterone with PGF2α, gave nearly complete autonomy to both maintain and control the end of the luteal phase of the estrous cycle (G. Lamb et al., 2010).

Transrectal ultrasonography yielded the ability to monitor ovarian follicles and corpora luteal over time and begin to understand the changes that occur during a follicular wave. This is where the understanding that growth of follicles in cattle occurs in distinct wave-like patterns, with a new follicular waves occurring approximately every 10 d (6 to 15 d range) (G. Lamb et al., 2010). This allowed for more precise controlling of estrus cycles as the manipulation of both follicular wave and luteal lifespan was possible (G. Lamb et al., 2010). A single injection of GnRH to cows at random stages of their estrus cycles causes the release of LH, which leads to synchronized ovulation or luteinization of most large, dominant follicles (G. Lamb et al., 2010). A new follicular wave is initiated in all cows within two to three days of GnRH administration and the luteal tissue that forms after GnRH is capable of undergoing PGf2α-induced luteolysis

six to seven days later (G. Lamb et al., 2010). While the use of a GnRH-PGF2α in protocols increased estrus synchronization rate, a drawback was that approximately 5 to 15% of the cows are detected in estrus on or before the day of PGF2α injection, thus reducing the proportion of females that are detected in estrus and inseminated during the synchronized period (Kojima et al., 2000).

This resulted in the use of progestogen-GnRH-PGF2α protocols for inducing and synchronizing a fertile estrus in postpartum beef cows and replacement heifers in which the GnRH-PG protocol is preceded by either short- or long-term progestin treatment. The progestin exposure enhanced the response to estrus synchronization, increased the pregnancy rate to AI during the synchronized period, and allowed insemination at a fixed time (Kojima et al., 2000). The GnRH-PG-GnRH protocol was developed to synchronize follicular waves and timing of ovulation for FTAI resulting in the development of a preovulatory follicle that ovulates in response to a second GnRH-induced LH surge 48 h after PG injection (Ovsynch) (Pursley et al., 1995). Timing of ovulation with Ovsynch occurs between 24 to 32 h after the second GnRH injection and is synchronized in 87 to 100% of lactating dairy cows (Pursley et al., 1997). Pregnancy rates among cows inseminated at a fixed time following Ovsynch ranged from 32 to 45% (Pursley et al., 1997). The Ovsynch protocol, did not effectively synchronize estrus and ovulation in dairy heifers (35% pregnancy rate compared with 74% in PGF2α controls (Pursley et al., 1997). The lack of response to the protocols was likely due to potential differences in follicular wave patterns including: the relative length of time to ovulation from the second GnRH injection; the anticipated range in timing of ovulation; and the degree of ovulation synchrony that occurs (G. Lamb et al., 2010).

Control of Estrus & Ovulation in Beef Heifers

As previously mentioned, beef heifers present a special set of challenges when attempting to facilitate synchronization of estrus.Induction of an ovulatory estrus in peripubertal heifers is possible when utilizing synchronization programs that include progesterone or progestins. ES is an effective means of increasing the proportion of females that become pregnant early in the breeding period, resulting in shorter calving seasons and more uniform calf crops (D. J. Patterson, Thomas, Martin, Nash, & Smith, 2013). Females that conceive earlier in the calving season, wean calves that are on average 13 days older and 21 lb (9.5 kg) heavier than calves from nonsynchronized females (Schafer, Brinks, & LeFever, 1990). Effective ES programs offer cycling heifers a predicted time that facilitates AI, as well as eliminates the time required to detect estrus, thus decreasing labor expense allowing FTAI to become much more practical (D. J. Patterson et al., 2013). The inability to predict the time of estrus for individual heifers in a group often makes AI alone impractical to use, because of the labor required for detection of estrus (D. J. Patterson et al., 2013).

Progestins were initially used to induce estrus in peripubertal heifers and were combined with E2 to mimic changes that occur in the concentrations of these hormones at puberty (D. J. Patterson et al., 2013). Progesterone increases during the initiation of puberty in heifers, and before resumption of normal ovarian cyclicity in postpartum suckled beef cows (D. J. Patterson et al., 2013). Progestins stimulate an increase in follicular growth that results in increased production of E2 by ovarian follicles (Henricks, Hill, & Dickey, 1973). MGA and CIDR have been shown to initiate estrous cyclicity in peripubertal beef heifers and are associated with increased LH pulse frequency (R. Smith & Day, 1990). The stimulatory effects of progestins on

LH secretion are greatest after removal of the steroid and improvement in the prepubertal induction response increases with age. (Hall et al., 1997; Imwalle, Patterson, & Schillo, 1998).

The increase in pulsatile release of LH which occurs in response to progestin treatment in peripubertal heifers results in a decrease in estrogen receptors within neuronal systems that mediate the negative feedback actions of estradiol on GnRH secretion (Anderson, McDowell, & Day, 1996). It is known that the age of onset of puberty is a heritable trait, and that induction of puberty in replacement heifers, might result in a situation whereby attainment of puberty would be difficult without hormone treatment (D. J. Patterson et al., 2013). This should not be over looked, but considered in cattle that are later-maturing, but of sufficient age and weight at the time of treatment to permit successful application (D. J. Patterson et al., 2013).

Comparison of Protocols in Cycling and Prepubertal Beef Heifers

There are numerous protocols for the induction of ES, but we will review some of the most common protocols and the management decisions that are affected by them. MGA is a commonly used orally active progestin used to suppress estrus and prevent ovulation in order to facilitate ES in beef heifers (D. J. Patterson et al., 2013). The duration of feeding may vary among protocols, but the level of feeding is consistent and critical to success (D. J. Patterson et al., 2013). This also presents an issue as animals that fail to consume the required amount of MGA (0.5 mg/animal/day) on a daily basis may return to estrus prematurely reducing the estrous response during the synchronized period (D. J. Patterson et al., 2013). Heifers will exhibit estrus beginning 48 h after MGA withdrawal, and this will continue for 6 to 7 d. It is generally recommended that females exhibiting estrus during this period not be inseminated or exposed for natural service, because of the reduced fertility experienced by females at the first heat after MGA withdrawal (D. J. Patterson et al., 2013).

MGA is sometimes used in conjunction with Natural Service (NS) and is one of the simplest methods involving the use bulls to breed synchronized groups of females, it's also useful in helping producers make a transition from NS to AI. Heifers typically receive a 14 d feeding period of MGA and are then exposed to fertile bulls 10 d after MGA withdrawal (D. J. Patterson et al., 2013). This system is effective if consideration of bull to female ratio (15 to 20 synchronized females be exposed per bull) is observed (D. J. Patterson et al., 2013). A combination of MGA with PGF2α, as luteal regression can be induced when PGF2α is administered in the presence of a functional CL during days 6 to 16 of the estrous cycle (D. J. Patterson et al., 2013). PGF2α should be administered 19 d after the last MGA feeding, placing

all animals in the late luteal stage of the estrous cycle at the time of PGF2α injection (D. J. Patterson et al., 2013).

The MGA Select protocol changed the day of PGF2α injection from day 31 to day 33 of treatment and included the addition of GnRH on day 26 of treatment (D. J. Patterson et al., 2013). The addition of GnRH on day 26 of the MGA-PG protocol induced luteal tissue formation and initiated a new follicular wave on approximately day 28 in estrous-cycling beef heifers, which significantly increased the proportion of heifers with synchronized follicular waves on day 33 (D. J. Patterson et al., 2013). Pregnancy rates resulting from AI remained adequate for both (MGA Select) MGA-GnRH-PG and MGA-PG treated heifers (75% and 72%, respectively) (D. J. Patterson et al., 2013).

The development of the 7-d CIDR-PG protocol was a great advancement for heifers as it yield an improved pregnancy rate in prepubertal beef heifers treated with the CIDR protocol (D. J. Patterson et al., 2013). Initially the drawback of this protocol was that PGF2α was administered on day 6 after CIDR insertion, which required an additional day of handling the heifers. It was later determined that heifers that received PGF2α on day 6 (1 day before CIDR removal) exhibited estrus earlier than heifers that received PG on day 7 (at CIDR removal), but there were no differences between groups, so to simplify treatment administration, PGF2α is in most cases now administered at the time of CIDR removal (D. J. Patterson et al., 2013).

A study designed to compare long-term progestin-based estrous synchronization protocols in beef heifers, utilizing presynchronization with MGA (MGA Select) or CIDR (CIDR Select: 14-d treatment with MGA or CIDR, followed 12 or 9 d later, respectively, with an injection of GnRH, and PG 7 days after GnRH) was compared on the basis of estrous response, timing of AI, and pregnancy rate in beef heifers (D. J. Patterson et al., 2013). No differences

were found in terms of estrous response between MGA Select and CIDR Select heifers; however, CIDR Select–treated heifers showed improved synchrony of estrus, conception, and pregnancy rates during the synchronized period (D. J. Patterson et al., 2013). This improvement was associated with the CIDR Select treatment's reduced interval to estrus and improved synchronization of follicular waves after CIDR removal rather than at the end of MGA feeding (D. J. Patterson et al., 2013).

This concept supports the idea that presynchronization before initiation of a GnRH and PGF2α protocol may be of greater importance in heifers, and significant in relation to success of the CIDR Select protocol (D. J. Patterson et al., 2013). Pregnancy rates following administration of the CIDR Select protocol were comparable, whether heifers were inseminated on the basis of observed estrus or at a predetermined fixed time (D. J. Patterson et al., 2013). In an additional study, there is a significant difference in the mean interval to estrus after PGF2α between estrous-cycling (62.4 ± 2.4 hours) and prepubertal heifers (52.4 ± 4.4 hours) assigned to the MGA-PG protocol, but no difference between estrous-cycling and prepubertal heifers assigned to 14-day CIDR-PG (55.4 ± 2.4 and 57.0 ± 4.4 hours, respectively) (D. J. Patterson et al., 2013).

Pregnancy rates resulting from FTAI from heifers receiving one of two CIDR-based protocols have been evaluated. The first group of heifers assigned to the CIDR Select received a CIDR insert from days 0 to 14 followed by GnRH 9 days after CIDR removal and PG 7 days after GnRH treatment (D. J. Patterson et al., 2013). The second group of heifers was assigned to CO-Synch + CIDR protocol, were administered GnRH and received a CIDR insert, and PG and CIDR removal 7 days later. AI was performed at predetermined fixed times for heifers in both treatments at 72 or 54 hours after PG for the CIDR Select and CO-Synch + CIDR groups, respectively. All heifers were administered GnRH at the time of AI. Pregnancy rates resulting

from FTAI were significantly greater following the CIDR Select protocol (62%) compared with the CO-Synch + CIDR protocol (47%). The CIDR Select protocols success likely stemmed from a greater and more synchronous estrous response (D. J. Patterson et al., 2013).

Stage of cycle differences among estrous-cycling heifers at CIDR insertion can explain the potential for reduced synchrony of estrus following CIDR removal in comparison with prepubertal heifers (D. J. Patterson et al., 2013). Both estrous-cycling and prepubertal heifers assigned to CIDR-PGF2α protocols have a more highly synchronized estrus than those assigned to the CIDR Select protocol (D. J. Patterson et al., 2013). Presynchronization with a progestin before GnRH and PGF2α appears to be effective in synchronizing estrus and may aid in inducing cyclicity in prepubertal or peripubertal heifers (D. J. Patterson et al., 2013).

Heifer Reproductive Tract Scoring & Pre-breeding Examination

The development of replacement heifers represents a management period which will have a large influence on the reproductive success of a beef herd for years. Herd productivity increases when a high percentage of heifers become pregnant early in the first portion of the breeding season and a high percentage of these first calf heifers or primiparous cows conceive earlier in the breeding season subsequent for a second pregnancy (Larson, White, & Laflin, 2016). There are multiple factors that go into the selection of replacement heifers including reproductive tract scoring, onset of puberty, age and weight (Larson et al., 2016).

The onset of puberty is primarily influenced by age and weight within a given breed, the average age at puberty for Bos taurus and Bos taurus–crossbred heifers in around 10 to 14 months, with crossbred heifers reaching puberty at a slightly younger age than purebred heifers due to a lack heterosis (Larson et al., 2016). In order for primiparous cows (first-calf heifers) to give birth to their first calf by 22 to 23 months of age so that they have 90 to 100 days between calving and the start of the following breeding season, they must become pregnant by 12.5 to 13.5 months of age(Larson et al., 2016). This requires them to reach puberty at least 21 days before the first day of the breeding season or 12 to 13 months of age (Larson et al., 2016). Nutrition is also a critical component in meeting the average daily requirement from the period from weaning to breeding in critical, as weight is also an important factor in determining the onset of puberty and ensuring a successful heifer development program (Larson et al., 2016). The target weight to reach puberty is based on the ratio between the average weight of heifers in a cohort divided by the average mature weight of the multiparous cows in the herd that produced the heifers (Larson et al., 2016). Heifer cohorts fed diets to reach approximately 55% to 65%

have better reproductive performance than heifer cohorts fed to reach lower weight ratios (D. Patterson, Corah, Kiracofe, Stevenson, & Brethour, 1989).

Body condition score (BCS) during the peripartum period also affects fertility. When comparing poor BCS to adequate BCS there was 23% increase in the proportion of cows pregnant to TAI (G. C. Lamb & Mercadante, 2016). Cows which calved in poor BCS experienced longer postpartum intervals to first estrus than those cows calving in moderate to good BCS (G. C. Lamb & Mercadante, 2016). The quantity of fat tissue is also an indicator of BCS and energy reserve, but also an important endocrine organ that synthesizes leptin, which regulates body energy metabolism and food intake. Concentrations of leptin have been positively correlated with amplitude and frequency of LH pulses, as well as, the interval from parturition to first ovulation.

Reproductive Tract Scoring (RTS) and evaluation of the reproductive soundness or readiness is also a crucial portion of the heifer examination. The optimum timing of a reproductive soundness examination will occur between 10-12 months of age and 6 weeks before the breeding season or ES date (G. C. Lamb & Mercadante, 2016). This offers time to correct low BCS, and corresponds well with optimal timing of prebreeding vaccinations, allows for the removal of free-martins and provides very accurate information about the percentage of cycling heifers (Larson et al., 2016). Potential replacement heifers should undergo a thorough physical examination, determining body weight, BCS and palpation of the reproductive tract(Larson et al., 2016). Palpation of the reproductive tract to determine the presence of a CL or large follicles on the ovaries and to estimate the size of the uterus is done in order to determine if a heifer is cycling (Larson et al., 2016). Pelvimetry or pelvic area measurement at 1 year of age has been described, because the major cause of dystocia is a disproportionately large calf compared with

the heifer's pelvic area (Larson et al., 2016). Pelvic area measurement should be used to select a minimum pelvic size as a culling criterion, such as 130 to 150 cm2 at 1 year of age (Larson et al., 2016). Pelvic area tends to increase rapidly near the time of puberty, or more so than during the prepubertal period and should be taken into account with the heifer's age.

The practice of RTS was originally developed to assist beef producers with selection of potential herd replacements and support timing of estrus synchronization programs (Andersen, LeFever, Brinks, & Odde, 1991). A RTS system was developed to estimate pubertal status using subjective estimates of sexual maturity, based on ovarian follicular development and palpable size of the uterus (Andersen et al., 1991). A RTS of one was assigned to heifers with infantile tracts, usually small, toneless uterine horns and small ovaries devoid of significant structures. Heifers assigned a RTS of two were closer to puberty, primarily due to larger uterine horns and ovaries. Those heifers assigned a RTS of three are thought to be on the verge of estrous cyclicity based on uterine tone and palpable follicles. Heifers assigned a score of four were considered to be estrous cycling as indicated by uterine tone and size, coiling of the uterine horns, as well as presence of a preovulatory size follicle. Heifers assigned a score of four do not have an easily distinguished corpus luteum. Heifers with RTS of five are similar to those with a score of four, except for the presence of a palpable corpus luteum. Prebreeding examinations that included RTS furnished the opportunity to assess reproductive development, but provide insight to aberrant situations that may detract from a heifer's subsequent reproductive potential such as freemartism and other genetic anomalies (Andersen et al., 1991).

Kansas State University developed a replacement heifer evaluation system combining several of these assessments into a single three-point classification system (ready, intermediate, and problem) to facilitate communication between the veterinarian and producer concerning

heifer breeding management (Larson et al., 2016). “Ready” is a heifer of adequate weight and body condition, with no structural flaws that impede fertility or longevity, a palpable CL or large follicle with good uterine tone consistent with normal estrous cycles, and a normally shaped pelvis with a minimum pelvic area of 130 cm2 (Larson et al., 2016). “Intermediate” criterion includes heifers of adequate weight and body condition, no structural flaws that impede fertility or longevity, some uterine tone and small palpable follicles but may not be cycling at the start of the breeding season (Larson et al., 2016). “Problem” heifers are not adequately heavy or their frame size that does not meet herd goals, structural flaws that impede fertility or longevity, very immature reproductive tracts, ovarian abnormalities, eye lesions that impede vision, heifers with an abnormally shaped pelvis, freemartins, or pregnant heifers (Larson et al., 2016).

In summary to avoid problems when using ES, heifers should be selected for a program when the following conditions are met: (1) replacement heifers are developed to prebreeding target weights of at least 65% of their projected mature weight; and (2) RTS are assigned to heifers < 2 weeks before ES treatment begins (scores of two or higher on a scale of one to five), and at least 50% of the heifers are assigned an RTS of four or five (D. J. Patterson et al., 2013).

Spermatogenesis

Before spermatozoa can be produced there are certain endocrine requirements that must be met including: adequate GnRH from the hypothalamus, FSH and LH secretion from the anterior lobe of the pituitary (AP) and secretion of gonadal steroids; Testosterone (T) and Estrogen (E2) (P. L. Senger, 2012). The hypothalamus produces GnRH in frequent intermittent episodes and acts upon the AP. GnRH expression causes the immediate discharge of LH in episodic fashion every 10 to 20 minutes and occur four to eight times per day. FSH concentrations are lower, but pulsations are longer because of the constant secretion of inhibin from the adult testis. LH acts on the Leydig cells, which synthesize P4, most of which is converted to T. This entire process is very quick and its' pulsatile nature is helpful, as it prevents the Leydig cells from becoming refractory. Normal intratesticular concentrations of T are 100 to 500 times higher than in systemic circulation, this is important as the systemic concentration of T is far below the threshold which would cause a down-regulation in the GnRH/LH feedback loop (P. L. Senger, 2012).

Testosterone secreted by the Leydig cells is transported into the Sertoli cells, where it is converted to Dihydrotestosterone (DHT) and E2. Then, T and E2 are transported by the blood to the hypothalamus where they exert a negative feedback effect on the GnRH neurons (P. L. Senger, 2012). LH binds the receptors in the interstitial cells of Leydig and FSH binds to Sertoli cells. Leydig cells secrete T that is transported to the adjacent vasculature and the sertoli cells where T is converted to DHT (P. L. Senger, 2012).

Spermatogenesis takes place entirely within the seminiferous tubules and consists of all cell divisions and morphologic changes that occur to developing germ cells (P. L. Senger, 2012). Spermatogenesis can be divided into three phases: proliferation, meiotic and differentiation

phases. In the proliferation phase, all mitotic divisions of spermatogonia occur. Several generations of A-spermatogonia undergo mitotic divisions, generating a numerous B-spermatogonia. Stem cell renewal is an important part of the proliferation phase. The loss of intercellular bridges allows some spermatogonia to revert back to stem cells, providing a continual renewal of stem cells from which now spermatogonia can develop (A. Barth & R. Oko, 1989).

The meiotic phase begins with primary spermatocytes. During meiosis I, genetic diversity is guaranteed by DNA replication and the crossing over during production of secondary spermatocytes (A. Barth & R. Oko, 1989). The meiotic phase and second meiotic division concludes with the production of haploid (lN) spermatids. Differentiation is the third phase of spermatogenesis, in which no further cell divisions take place, but the spherical undifferentiated spermatid undergoes a transformation that results in the production of a fully differentiated, highly specialized spermatozoon containing a head, containing nuclear material, a flagellum including a midpiece; with a mitochondrial helix and a principal piece. Spermatogonia, the most immature of the germ cells are located at the periphery of the seminiferous tubule near the basement membrane (A. Barth & R. Oko, 1989). As germ cells proliferate, they move toward the lumen. Groups of spermatogonia, spermatocytes or spermatids are connected by intercellular bridges, so that the cytoplasm of an entire cohort is interconnected. These intercellular bridges help to provide communication between cells that contribute to the synchronized development of a cohort (A. Barth & R. Oko, 1989; P. L. Senger, 2012).

Spermatogonia (2N) are the most primitive cells encountered in the seminiferous epithelium, located in the basal compartment (Thibault & Levasseur, 2001). Spermatogonia undergo several mitotic divisions with the last division resulting in primary spermatocytes.

There are three types of spermatogonia: A-spermatogonia, I-spermatogonia (intermediate) and B-spermatogonia. A-spermatogonia undergo several mitotic divisions in which they progress mitotically. A pool of stem cells is also maintained so that the process can continue indefinitely. Stem cells divide mitotically to provide a continual source of A-spermatogonia allowing spermatogenesis to continue without interruption for years (Thibault & Levasseur, 2001).

During spermatogenesis the number of chromosomes in the gamete is reduced to the haploid state, via meiosis (P. L. Senger, 2012; Thibault & Levasseur, 2001). The mitotic divisions of B-spermatogonia result in the formation of primary spermatocytes. These primary spermatocytes immediately enter the first meiotic prophase (Thibault & Levasseur, 2001). The meiotic prophase consists of five stages: preleptotene, leptotene, zygotene, pachytene, and diplotene (P. L. Senger, 2012).

Primary spermatocytes must progress through the steps before the first meiotic division can occur (P. L. Senger, 2012). During the preleptotene phase, complete DNA replication occurs, forming tetrads; which fuse at random points known as "chiasmata" and crossing-over of DNA material takes place. The prophase of the first meiotic division ensures genetic heterogeneity so that each secondary spermatocyte and subsequently each spermatid will be genetically unique. Prophase of the first meiotic division is an extended process, as the lifespan of the primary spermatocyte is the longest of all germ cell types found in the seminiferous epithelium. In the bull, the lifespan of the primary spermatocyte is 18 to 19 days, with the total duration of spermatogenesis lasting 61 days in bulls (Barth, 1989). The secondary spermatocyte, which results from the first meiotic division of a primary spermatocyte lasts only 1-2 days depending on the species, but rapidly undergoes the second meiotic division, resulting in haploid spherical spermatids (Thibault & Levasseur, 2001)

To form cells that are capable of fertilization, spermatids must undergo changes in which the nucleus becomes highly condensed and the acrosome is formed as the cell becomes potentially motile (A. Barth & R. Oko, 1989). Motility requires the development of a flagellum and the mitochondrial helix; which will serve as the metabolic powerplant of the spermatozoa (P. L. Senger, 2012). The Golgi phase is characterized by the development of the acrosome (P. L. Senger, 2012). The spermatid contains a large, highly developed Golgi apparatus located near the nucleus consisting of many small vesicles (Thibault & Levasseur, 2001). The Golgi, in a spermatid, will give rise to the subcellular organelle known as the acrosome (Thibault & Levasseur, 2001). Proacrosomic vesicles form and fuse, generating a larger vesicle that resides adjacent to the nucleus known as the acrosomic vesicle (P. L. Senger, 2012). Smaller Golgi vesicles are continually added to the larger vesicle increasing its size. While the acrosomic vesicle is being formed, the centrioles migrate from the cytoplasm to the base of the nucleus, where the proximal centriole will give rise to an implantation apparatus that allows the flagellum to be anchored to the nucleus (Thibault & Levasseur, 2001). The distal centriole gives rise to the developing axoneme, which is the central portion of a flagellum and eventual principal piece of the sperm tail (P. L. Senger, 2012).

During the cap phase the acrosome forms a cap over the anterior portion of the nucleus (P. L. Senger, 2012). At this point, the Golgi has performed its function of packaging the acrosomal contents and membranes and moves away from the nucleus toward the caudal end of the spermatid. The primitive flagellum and eventual tail, formed from the distal centriole, begins to project from the spermatid toward the lumen of the seminiferous tubule (P. L. Senger, 2012).

During the acrosomal phase, the acrosome spreads until it covers two-thirds of

the anterior nucleus, at which point the nucleus begins to elongate (A. D. Barth & R. Oko, 1989). A system of microtubules known as the manchette develops near the posterior nucleus. Portions of the manchette attach to the region of the nucleus just posterior to the acrosome, which will become the post-nuclear cap (P. L. Senger, 2012). During the acrosomal phase, spermatids become embedded in Sertoli cells with their tails protruding toward the lumen of the seminiferous tubule (Thibault & Levasseur, 2001). Mitochondria migrate toward and cluster around the flagellum in the region posterior to the nucleus and are quickly assembled around the flagellum from the base of the nucleus to the anterior one third of the tail in a spiral fashion, forming the middle piece of the fully differentiated spermatozoa (P. L. Senger, 2012). The entire spermatozoon is covered with a plasma membrane and the integrity of the plasma membrane is required for the survival and function of spermatozoa (A. Barth & R. Oko, 1989).

The release of spermatozoa from the Sertoli cells into the lumen of the seminiferous tubule is referred to as spermiation (P. L. Senger, 2012). During spermiogenesis, nuclear histones of the haploid sperm nucleus are replaced by protamines, which are arginine-rich nuclear proteins essential for DNA condensation (Thibault & Levasseur, 2001). The sufihydryl groups of protamines form disulfide bonds, which are the basis for nuclear condensation that result in a highly compact, stable nucleus that forms the sperm head (P. L. Senger, 2012). At this point in spermiogenesis, transcription and translation stops because the transcriptional machinery no longer has access to the nuclear DNA (Thibault & Levasseur, 2001).

The DNA within the sperm head remains inert until fertilization and helps to prevent damage to the DNA between spermiation and fertilization (P. L. Senger, 2012). During fertilization, the process is reversed by glutathione in the cytoplasm of the oocyte, which dissolves the disulfide cross-links within the sperm nucleus (P. L. Senger, 2012). The protamines

are replaced with histones from the oocyte's cytoplasm resulting in nuclear decondenation and formation of the male pronucleus, characterized by keratinization and reversal of DNA stability, but only after the sperm has entered the oocyte's cytoplasm (Thibault & Levasseur, 2001).

The anterior portion of the nucleus is covered by the acrosome, which is bound by lysosomes that contains hydrolytic enzymes (P. L. Senger, 2012). These enzymes are required for penetration of the zona pellucida of the ovulated oocyte (Thibault & Levasseur, 2001). During fertilization the acrosome undergoes an ordered, highly specialized exocytosis, known as the acrosome reaction, which allows release of the enzymes that are packaged in it to digest and penetrate the zona pellucida (P. L. Senger, 2012). During this time there are noticeable changes in the energy metabolism and the oxygen uptake, however spermatozoa can only undergo the acrosome reaction if they have undergone capacitation prior to initiation (A. D. Barth & R. Oko, 1989).

The membrane component posterior to the acrosome is the post-nuclear cap and the tail is composed of the: capitulum, middle piece, and principal piece (P. L. Senger, 2012). The capitulum fits into the implantation socket, which is a depression in the posterior nucleus (Thibault & Levasseur, 2001). The anterior portion of the tail consists of laminated columns that give the neck region flexibility when it becomes motile, so the tail can move laterally and side-to-side during the flagellar beat (P. L. Senger, 2012). The axonemal component of the tail originates from the distal centriole and is composed of nine pairs of microtubules that are arranged radially around two central filaments (Thibault & Levasseur, 2001). Surrounding this nine plus nine plus two arrangement of microtubules are nine coarse fibers unique to the flagellum of spermatozoa (A. Barth & R. Oko, 1989). The mitochondrial sheath is arranged in a helical pattern around the outer coarse fibers of the tail and contributes to the mid-piece (A.

Barth & R. Oko, 1989). The annulus is the junction between the middle piece and the principal piece, which makes up the majority of the tail where only the microtubules end in the terminal piece (A. Barth & R. Oko, 1989).

The male produces gametes continually and uniformly throughout his reproductive lifespan, with the exception to seasonal breeders that produces spermatozoa during the breeding season only (P. L. Senger, 2012). There is a 2 to 4 week delay before the effects of deleterious events such as: heat stress, shipping, fever, exposure to certain toxins are observed on the ejaculate (P. L. Senger, 2012). Sometimes as many as 6 to 12 weeks are required before the restoration of normal spermatogenesis can be accomplished after such an event (P. L. Senger, 2012).

Interpretation of ejaculate characteristics requires knowledge of the timing of spermatogenesis in the species being evaluated (P. L. Senger, 2012). The cycle of the seminiferous epithelium is the progression through a series of stages at a location along a seminiferous tubule (Thibault & Levasseur, 2001). The time required for this progression is the duration of the cycle of the seminiferous epithelium. At any given point along the seminiferous tubule, one can observe four or five concentric "layers" of germ cells, these cells in each layer comprise a generation or cohort and develop in a synchronous group (P. L. Senger, 2012). Each generation or cycle will give rise to a more advanced generation. The more immature cell types are generally located near the basement membrane and the more advanced cells reside in the adluminal compartment (Thibault & Levasseur, 2001).

At any instance in time, cross-sections at different locations along the seminiferous tubule will show different generations of cells (Thibault & Levasseur, 2001). Some cells in each section are actively engaged in spermatogenesis, but only one cross-section may be ready to

release spermatozoa into the lumen (Thibault & Levasseur, 2001). Along the length of any seminiferous tubule there are only certain cross-sections where spermatozoa are released at any given point in time, however all other zones or stages are preparing to release spermatozoa, but have not reached the appropriate stage of maturity for spermiation to occur (P. L. Senger, 2012). Different sections or zones along a seminiferous tubule contain different cellular associations, or stages of the cycle of the seminiferous epithelium (P. L. Senger, 2012). Eight stages in the cycle of the seminiferous epithelium are described by Senger et al, whoever other descriptions include as many as 14 stages (P. L. Senger, 2012). Below, the cellular composition of each stage of the seminiferous epithelium will be briefly described. Stage I may contains one generation of A-spermatogonia, two generations of primary spermatocytes and one generation of spermatids. The entire progression of one cycle of the seminiferous epithelium from stage I through stage VIII requires about 13.5 days in the bull (A. Barth & R. Oko, 1989).

The complete process of spermatogenesis from A-spermatogonia to the formation of fully differentiated spermatozoa takes 61 days, in which the seminiferous epithelium proceeds through 4.5 cycles (A. Barth & R. Oko, 1989). The actual cycle of the germinal elements of the seminiferous epithelium have different lifespans (P. L. Senger, 2012). A primary spermatocyte exists for about 21 days while a secondary spermatocyte exists for only 1.5 days in the bull. Spermatogonia, primary and secondary spermatocytes all divide and generate many spermatids. During spermatogenesis many of the proliferating spermatogonia die and never become primary spermatocytes. There is also is death of primary spermatocytes, although most spherical spermatids go on to form spermatozoon (P. L. Senger, 2012).

The spermatogenic wave refers to the differences at any given instant in time along the length of the seminiferous tubule (Thibault & Levasseur, 2001). As the distance between these

spermiation sites is relatively constant, each stage of the seminiferous epithelium transitions to a successively more advanced stage (P. L. Senger, 2012). The physiologic importance of the spermatogenic wave is to provide a constant supply of spermatozoa to the epididymis, creating a pool for ejaculation (A. Barth & R. Oko, 1989).

Capacitation & Fertilization

Following insemination and deposition within the female reproductive tract, many spermatozoa are lost from the female reproductive tract via retrograde transport and phagocytized by the numerous leukocytes present within the caudal female reproductive tract (P. L. Senger, 2012). Spermatozoa remaining must then traverse the cervix and uterus before entering the oviduct. Capacitation must occur before a spermatozoa can fertilize the oocyte. Once sperm encounter the egg they must be ready to undergo the acrosomal reaction for fertilization to take place (P. L. Senger, 2012). In the bovine, the male ejaculates semen into the cranial vagina (A. Barth & R. Oko, 1989).

The degree to which spermatozoa are lost from the female tract depends upon the physical nature of the ejaculate and the site of seminal deposition (P. L. Senger, 2012). When the female reproductive tract is under the influence of estradiol, neutrophils are sequestered in the mucosa of the tract and more specifically in the vagina and uterus. Neutrophils attack all foreign materials that are introduced into the female reproductive tract at insemination, as copulation is not a sterile procedure (P. L. Senger, 2012). While leukocyte infiltration is an important contributor to post-insemination spermatozoa losses, they are also important for the prevention of reproductive tract infections (Thibault & Levasseur, 2001).

Post-insemination transport of spermatozoa following copulation can be divided into two phases: Rapid transport and Sustained transport phases (P. L. Senger, 2012). Minutes after copulation, spermatozoa can be found in the oviducts. The rapid phase of transport represents a burst of activity brought about by contraction of the muscularis layer of the female tract in conjunction with copulation. The more important component of transport is the sustained phase in which spermatozoa are transported to the oviducts from reservoirs in the cervix and the

uterotubal junction (P. L. Senger, 2012). In the sustained transport phase, sperm move into the isthmus and attach to the oviductal epithelium. Sperm temporarily attach to the epithelium of the lower isthmus near the uterotubal junction because this is the first oviductal region they encounter (P. L. Senger, 2012). This attachment, sometimes called "docking" elicits a signal cascade in the sperm that promotes viability (P. L. Senger, 2012).

Estradiol helps to stimulate contractions of the muscularis, particularly the myometrium (Thibault & Levasseur, 2001). Semen produces its own prostaglandins PGF2α and PGE1, which cause increased tone and motility of the uterus and the oviduct (P. L. Senger, 2012). Fluids secreted into the lumen of the female tract also serve as a vehicle for transport. During estrus secretion of sulfomucins from the apical port1on of the cervical mucosa produces sheets of viscous mucus. Secretion is toward the lumen and flows in a caudal direction. Less viscous sialomucins are produced in the basal crypts of the cervix, spermatozoa in theses basal regions are orientated in the same direction and traverse the cervix towards the uterus through these privileged pathways of low viscosity (P. L. Senger, 2012). Spermatozoa encountering the viscous sulfomucin are washed out of the tract (P. L. Senger, 2012).

Spermatozoa acquire maturity during epididymal transit, however the maturational changes that occur in the epididymis do not render spermatozoa completely fertile (Thibault & Levasseur, 2001). Spermatozoa must reside in the female reproductive tract for a minimum period of time, to undergo changes known as spermatozoal capacitation, that allow them to become fertile (A. Barth & R. Oko, 1989). Capacitation sites vary among species, but in species where spermatozoa are deposited in the cranial vagina, capacitation begins as sperm pass through the cervix (P. L. Senger, 2012). Capacitation can be reversed by returning capacitated spermatozoa to seminal plasma, where they become decapacitated and require additional

capacitation time in the female reproductive tract before they can regain their fertility (P. L. Senger, 2012). Seminal plasma components appear to coat the plasma membrane with surface substances that prevent interactions of spermatozoa with the egg (Thibault & Levasseur, 2001). In the oviduct, as capacitation is completed, the motility patterns of spermatozoa become hyperactive and switch to a progressive, linear motility (P. L. Senger, 2012).

Spermatozoa contain specific proteins on their plasma membrane surfaces overlying the acrosome that bind specifically to zona pellucida proteins (P. L. Senger, 2012). Zona binding proteins on the plasma membrane must be exposed during the capacitation process before binding to the zona pellucida can occur (Thibault & Levasseur, 2001). The zona pellucida of the oocyte consists of three glycoproteins named zona proteins 1, 2 and 3 (ZPl, ZP2 and ZP3) (P. L. Senger, 2012). Zona proteins 1 and 2 are structural proteins providing integrity to the zona. Zona protein 3 is similar to a receptor for a hormone and binds to the proteins on the spermatozoal membrane (P. L. Senger, 2012). In order to gain the ability to bind to the ZP, it must first go through changes during epididymal maturation in which the spermatozoa is bathed in a myriad of lipids and proteins which contribute to the acquisition of zona binding affinity (Reid, Redgrove, Aitken, & Nixon, 2011).

The sperm plasma membrane contains two zona binding sites (P. L. Senger, 2012). The first binding site, referred to as the primary zona binding region is responsible for the adherence of spermatozoa to the zona pellucida, the second binding site is involved in the acrosome reaction promoting ligand. (Reid et al., 2011). When binding occurs between this region and the ZP3 molecule, a signal transduction occur and this binding initiates the acrosomal reaction (P. L. Senger, 2012). The purpose of the acrosomal reaction is to first, enable the spermatozoa to penetrate the zona pellucida and secondly to modify the equatorial segment so that it can later

fuse with the plasma membrane of the oocyte (Thibault & Levasseur, 2001). The acrosomal reaction begins when the plasma membrane of the spermatozoon forming multiple fusion sites with the outer acrosomal membrane, this process is called vesiculation (P. L. Senger, 2012). After vesiculation, the acrosomal contents are dispersed and the sperm nucleus is left with the inner acrosomal membrane surrounding it. Vesiculation characterizes the acrosomal reaction and distinguishes it from a damaged acrosome (P. L. Senger, 2012). Damage to the acrosome membrane and plasma membranes are irreversible and brought about by changes in osmotic pressure, sudden cooling, followed by heating and marked changes in pH (A. Barth & R. Oko, 1989).

Following the attachment to the zona pellucida, the acrosome reaction allows the release of a variety of enzymes including Acrosin, which hydrolyzes zona proteins as well as enhances the sperm's ability to bind to the zona. Acrosin has an inactive form known as proacrosin, which has a strong affinity for the zona and aids in binding the spermatozoon to the zona as the acrosomal reaction proceeds (P. L. Senger, 2012). As proacrosin is converted to acrosin, the sperm begins to penetrate and make its way through the zona pellucida (A. Barth & R. Oko, 1989). The mechanical force generated by the flagellar action of the tail must maintain sperm contact with the zona pellucida (P. L. Senger, 2012). Maintenance of an intact zona pellucida is important because it prevents blastomeres in the early embryo from separating during embryogenesis (P. L. Senger, 2012). Once the spermatozoon completely penetrates the zona and reaches the perivitelline space, the plasma membrane of the oocyte fuses with the membrane of the equatorial segment and the fertilizing spermatozoon is engulfed (Thibault & Levasseur, 2001).

After membrane fusion, the oocyte undergoes a series of changes that prepare it for early embryogenesis (P. L. Senger, 2012). During the first and second meiotic divisions of oogenesis, small, dense cortical granules consisting of mucopolysaccarides, proteases, plasminogen activator, acid phosphatase and peroxidase move to the periphery of the oocyte cytoplasm (P. L. Senger, 2012). After membrane fusion between the oocyte and spematozoon, the cortical granules undergo exocytosis and their contents are released into the perivitelline space, resulting in the zona block, a process whereby the zona pellucida undergoes biochemical changes so that additional spermatozoa cannot penetrate, whereby preventing polyspermia (P. L. Senger, 2012).

After the sperm nucleus has entered the cytoplasm of the egg, it becomes the male pronucleus (P. L. Senger, 2012). Before the pronucleus can be formed, however the sperm must undergo changes within the oocyte cytoplasm. After the fertilizing spermatozoon enters the oocyte cytoplasm the nucleus must "decondense" so that the male chromosomes may pair up with the chromosomes of the female pronucleus. This decondensation requires the reduction of the disulfide cross-links of the sperm (P. L. Senger, 2012). Glutathione is the primary reducing agent in the cytoplasm of the oocyte used to break these disulfide cross-links (A. Barth & R. Oko, 1989). Upon bond reduction the sperm nucleus decondenses and the nuclear material is available for interaction with the female nuclear material (A. Barth & R. Oko, 1989). The final step of fertilization is the fusion or syngamy of the male and female pronuclei, following syngamy, the zygote enters the first stages of embryogenesis (P. L. Senger, 2012).

Serving Capacity & Multisire Units

Sexual behavior in the bull is typically defined as the behavior associated with the detection and service of estrous females (Chenoweth, 1997). Libido, or sex drive, is usually defined as the "willingness and eagerness" of a bull to attempt to mount and service; mating ability refers to the ability of the bull to fulfill this aspiration (Chenoweth, 1997). The serving capacity is a measure of the number of services achieved by a bull under stipulated conditions and thus includes aspects of both libido and mating ability (Chenoweth, 1997).

Multiple factors can influence a bull's sex drive and therefore serving capacity including: age, social standing, libido, experience, energy levels and production traits (Chenoweth, 1997). Bull-to-Female Ratios (BFR) have been looked at for many years to achieve the most ideal reproductive efficiency. It has become apparent that individual bull reproductive capability has a more important effect on reproductive success than did BFR or single versus multi-sire breeding combinations (Chenoweth, 1997). In a comparison of the reproductive performance of young bulls mated with estrus-synchronized females at BFRs of either 1:20 or 2:40 indicated that single-sire mating is most efficient (P. Farin, Chenoweth, Mateos, & Pexton, 1982). Social ranking appears to be most influential in older bulls (those 3.5 to 4 years of age or older) and appears to be more related to seniority than to age or body weight (Blockey, 1979).

Bulls with higher libido and serving capacity scores tended to have a higher number of services and serviced a higher percentage of estrus heifers (P. Farin et al., 1982). However, the percentage of heifers that became pregnant is not different between single- and multi-sire breeding groups (P. Farin et al., 1982). It is interesting that in multi-sire groups, pregnancy rates are higher for heifers observed serviced by multiple bulls than for heifers observed serviced by only one bull (P. Farin et al., 1982). From this we can conclude that single-sire groups are more

efficient in sire usage than multi-sire groups but multi-sire groups do provide an increase chances of settling heifers (P. Farin et al., 1982). This is most likely due to overlap in servicing in multi-sire groups (P. Farin et al., 1982).

Steroid Hormone Regulation in the Male and Mating Behavior

Sexual reproduction requires sexually differentiated behaviors to ensure attraction between partners and mating (P. Senger, 1997). These behaviors are a key factor in the success of sexual reproduction, on which species survival depends. Males and females adopt different behaviors and postures during attraction and mating. Males readily express sexual behavior whenever a receptive female is present. Behaviors such as chemo-investigation, displaying an olfactory preference, generating vocalizations, among others are all done to convey the male's motivational state and help attract a female partner (P. Senger, 1997). Reproductive and associated hormones are regulated by an interplay between the nervous and endocrine system to coordinated and initiate all reproductive functions. The fundamental responsibility of the nervous system is to translate external stimuli into neural signals that bring about a change in the reproductive organs and tissues. Sexual behavior is tightly controlled by these neural processes, which begin during development and are regulated by gonadal hormones (P. Senger, 1997).

There are three critical periods of hormonal regulation: perinatal, prepubertal, and adulthood (P. L. Senger, 2012). The perinatal period begins in late gestational and the early neonatal period. Testosterone released from the fetal and neonatal testes permanently potentiates male (masculinization) behavioral and anatomic characteristics while inhibiting female (defeminization) characteristics and behavior. In the prepubertal/pubertal period androgens (e.g., testosterone) are secreted into the blood. Testosterone travels to the brain where it is converted to E2 and dihydrotestosterone (DHT) by aromatase and 5α reductase, respectively . At adulthood in males, gonadal testosterone acts on the male neural circuitry to stimulate sexual behavior. Male sexual stimulation is reduced or inhibited by castration but can be restored by hormonal

supplementation. Testosterone and E2 regulate the signaling pathways which play an important role in displays of sexual behavior (P. L. Senger, 2012).

Sex steroids account for sexual dimorphism because they are responsible for the establishment of primary and secondary sexual characteristics, which are under the control of androgens and estrogens in males and females (P. L. Senger, 2012). Both estrogen excess and estrogen deficiency influence male sexual development, testis function, the hypothalamic-pituitary-testis axis, spermatogenesis, and ultimately male fertility. Estrogens as well as androgens are responsible for crucial physiological functions in men such as fertility, reproduction, and bone health (P. L. Senger, 2012).

In dominate males, testosterone is associated with chasing and courtship directed exclusively at the community of females and subordinate males (O'Connell & Hofmann, 2012). In subordinate males, testosterone in association with the other steroid hormones expressed are consistently decreased, suggesting that circulating steroid hormones are reflective of gonadal state (O'Connell & Hofmann, 2012). Mating behavior occurs in all species and preference is not only limited to ruminants. One study observed sexually naïve, naturally cycling female rats during behavioral estrous that were given the opportunity to mate with two males simultaneously (Winland et al., 2012). One male rat in each pair was categorized as the preferred mate. Each male in the pairs was categorized as “attractive” or “non-attractive.” During 76% of these mating tests the same male rat in each pair was preferred by different female rats. Overall attractiveness of individual male rats predicted reproductive success in the present study. Interestingly, “attractive” males sired significantly fewer pups than “non-attractive” males. Neither behavioral nor physiological measures (body weight, urinary testosterone levels) of male rats predicted their reproductive success. The results indicate that certain features of some males are more attractive

to females, but attractive males are not always the most prolific in terms of number of progeny sired (Winland et al., 2012).

Heterospermic Insemination

Heterospermic Insemination (HI) is the situation in which the sperm of two or more males are present in the female reproductive tract prior to fertilization occurring. HI is not uncommon, except in purely monogamous species (PJ, 1996). It is advantageous for a male to mate with the maximum proportion of females because a male's impact on the next generation is directly related to the proportion of that generation that he sires. The influence a female has on a population is influenced by the number of living offspring she produces, which is why it is advantageous to mate with the most fertile males and at the most fertile period (Winland et al., 2012). Aggressive behavior or libido aren't clear indications of fertility in mice or most other species and have little correlation with fecundity (Winland et al., 2012).

Production livestock management systems impose limits on the exact time of insemination and monogamy to ensure paternity for pedigrees and for economy of few males to many females by natural or artificial insemination (PJ, 1996). Therefore, it is crucial to assess the potential fertility of the male via semen quality and proper handling at every opportunity. Measures of fertility are used to evaluate the past record of fertility but are not a direct indication of future fertility. These tests of fertility of the male include sperm motility estimates, sperm metabolic measures, sperm morphology, in vitro penetration of oocytes, non-return rates, conception rates, litter sizes, etc. These tests of fertility are subjective. Objective tests include conception rates, proportion of inseminated females giving birth, and litter size and are considered to be the ultimate standards by which to compare fertility (PJ, 1996). Mixing equal numbers of sperm from two different males and inseminating the mixtures seems intuitively to give a more accurate and objective measure of relative fertility of each male (Beatty, Bennett, Hall, Hancock, & Stewart, 1969).

HI is a competition between sperm at one time point and within one female rather than between groups of females. Hence, allowing the uncertainty of the female's physiological status, microenvironment, and management factors to be taken into question. This makes for a much more sensitive test than homospermic insemination because with homospermic competition there is no competition. The capability of a male to sire offspring competitively has been examined for rabbits, chickens, swine, and cattle (Beatty, 1960; Beatty et al., 1969; Ferreira et al., 2015; Martin & Dziuk, 1977). Differences among fertile males that were obvious in heterospermic comparisons were often small or undetectable after homospermic AI (Amann, Saacke, Barbato, & Waberski, 2018).

The makeup of the sperm pool at the oviduct site is different from what is present near the uterotubal junction (UTJ) or uterus (Saacke, 1988). The compensable subpopulation of sperm are incapable of reaching an oocyte or interacting therewith to induce blockage of the zona reaction (Saacke, 1988). Uncompensable subpopulation of sperm enter the oocyte and induce the zona reaction but fail to fertilize the oocyte and provide sustained embryogenesis (Amann et al., 2018). Transit of spermatozoa moving distally up the oviduct is under the influence of motility, velocity, and differential responses of sperm to female signals. All of these factors are important aspects of enabling sperm passage of oviductal barriers (Amann et al., 2018).

Distinct events lead to sperm-egg fusion, but cell-cell adhesion is a precursor step, progressing from interaction of the membranes to the close apposition of the membranes. Sperm binding to the egg starts with loose attachments and progresses to firm adhesion where sperm readily attach to the zona pellucida (ZP) (Evans, 2012). The zona pellucida favors entry of only the most competent sperm, while discriminating against sperm with a misshapen heads or other abnormalities. The fully competent sperm subpopulation competing at the oocyte(s) is highly

skewed, with few sperm typically present. This subpopulation consists of sperm that have traversed the uterus and oviduct, entered and departed from sperm storage reservoirs, undergo gradual capacitation, fertilize an oocyte, and contribute to the embryo (Evans, 2012).

Fresh spermatozoa from bulls has been mixed with zona-free hamster eggs. Bulls differed in their egg penetration rates; heterospermic competitive tests were highly correlated with fertility (A. Davis, Graham, & Foote, 1987). Male HI performance can be altered by adjusting the proportion (ratio) of sperm from the competing males in the AI dose in a linear fashion (Martin, Reimers, Lodge, & Dziuk, 1974). Sperm morphology affects outcome because abnormal flagellum or head and mid-piece attachments are excluded from colonizing the oviduct sperm reservoir (Petrunkina, Gehlhaar, Drommer, Waberski, & Topfer-Petersen, 2001).

One of the best predictors of parentage after heterospermic AI is low DNA-fragmentation index for a male's sperm (Kasimanickam et al., 2006). Sperm motility parameters have been assessed by computer assisted sperm analysis (CASA), flow cytometry analysis of DNA Fragmentation Index (DFI), and Plasma Membrane Integrity (PMI) to evaluate the relationship to Competitive Fertilization Index (CFI). The DFI was negatively correlated to CFI, whereas the PMI and total progressive motility were positively correlated to CFI. Bulls with less DFI and higher PMI had higher probabilities of siring calves (Kasimanickam et al., 2006).

The mechanisms by which surface molecules of the sperm interact with oviduct epithelial cells and understanding their role in sperm joining one of the subpopulations whose sperm enter the oocyte rather than the compensable subpopulation is still unclear (Suarez, 2016). Sperm-associated proteins help sperm stay out of the noncompensable subpopulation through roles in oviduct transit and sperm-oocyte binding. When bovine caudal epididymal sperm were mixed in vitro with seminal fluids from bulls differing in fertility, sperm penetration was enhanced by

exposure to fluids from higher-fertility bulls (Henault, Killian, Kavanaugh, & Griel Jr, 1995). With swine, certain seminal plasma proteins have a strong effect on success or failure of boar sperm in heterospermic AI (Flowers, Deller, & Stewart, 2016).

Single Nucleotide Polymorphisms (SNPs) for Individual Identification and Parentage Testing

DNA markers have become extremely important in animal breeding and have been successfully used in bovine identification, parentage testing and to establish relationships between two or more individuals, as well as to trace meat through the entire food chain (Fernández et al., 2013). Microsatellites or short tandem repeats (STRs) or Simple Sequence Repeat (SSR) have been the genetic markers of choice for more than two decades, although they are highly polymorphic, informative and interspersed throughout the entire genome, so STR are not always consistent because of inconsistent allele size calling and errors in size determination and not to mention extremely time consuming to analyze (Baumung, Simianer, & Hoffmann, 2004). STRs also require intact DNA and are prone to errors or "stutter" products (Baumung et al., 2004). Advances in high-throughput DNA sequencing, computer software and bioinformatics have made the use of Single Nucleotide Polymorphisms (SNPs) more popular (Heaton et al., 2002). A SNP is a substitution of a single nucleotide at a specific location in the genome.

This measures a small change or point mutations at individual positions on the chromosome in DNA coding and as these changes are rare, they only happen once and seldom back mutate (Heaton et al., 2002). SNPs have a greater mapping resolution and a lower error rate, with the advantage of being greater in abundance, as well as more genetically stable in mammals, plus have a simpler nomenclature and are more suited to automated analysis and data interpretation (Heaton et al., 2002; Markovtsova, Marjoram, & Tavaré, 2000).

SNPs have been successful in the discovery of quantitative trait loci (QTL) and the association of genes with specific productive traits and in the identification of individuals and breeds (Fernández et al., 2013). The prerequisite for development of efficient SNP-based

identification systems is the description of a minimal set with sufficient power to uniquely identify individuals and their parents in a variety of popular breeds and crossbred populations (Heaton et al., 2002). Most of the routine work in livestock genetic laboratories includes the analysis of closely related animals (Fernández et al., 2013). The Parentage Recording Working Group of the International Committee for Animal Recording (ICAR) has developed a cattle consensus panel of 99 SNPs, to certify laboratories worldwide (Fernández et al., 2013).

Next Generation Sequencing (NGS) offers several options in SNP testing. In "sequencing" all bases along the Y chromosome are read, so both muted and unmuted mutations will appear and this allows for the identification of novel and private SNPs (Cleary et al., 2014). Single SNP tests read a short portion of a known sequence to see if a particular SNP exists at a known location; this is used for validating new discoveries. Therefore, SNP Panels allow testing of large sets of SNPs at once (Cleary et al., 2014). This does not lead to the discovery of novel SNPs, but it only confirms if they are present of not (Cleary et al., 2014). An efficient SNP identification system requires a marker set with enough power to identify individuals and their parents (Fernández et al., 2013).

CHAPTER III: JOURNAL ARTICLE

ABSTRACT

Artificial insemination has is considered to be one of the greatest innovations in cattle management. Fixed-time artificial insemination (FTAI) protocols have offered new opportunities to implement AI. However, these technologies have not been widely implemented in beef heifers. FTAI pregnancy rates in beef heifers have been inconsistent, leading to frustration and often discontinuation of a program that offers significant genetic and economic potential. FTAI conception rates in heifers have been especially variably as some heifers come into estrus and ovulate too early and some ovulate too late for FTAI to be effective. Despite these inconsistencies, a major benefit of a FTAI program in heifers is that more heifers calve earlier in the calving season creating "calving momentum." Calves conceived via AI have improved carcass characteristics and improved genetics. This improvement does come at some cost in terms of time materials and labor.

Many protocols and recommendations regarding timing of hormone administration have been detailed by Lamb et al. 2010, but little information in available on breeding protocols involving estrus synchronization protocols combining natural service and AI. The objective of this study was to identify a breeding protocol that will result in more heifers calving within the first 21 days of their first calving season, as well as identifying the calving distribution (AI vs. natural sired). The hypothesis was that implementation of a 7-day CO-Synch + CIDR® protocol combined with immediate natural service would provide an effective FTAI protocol in beef heifers that would result in 85% of pregnant heifers calving in the first 30 days of the calving season.

Seventy-five pubertal heifers were randomly placed into 2 groups based on reproductive tract scores, ensuring that a relatively even allotment of peri-pubertal and pubertal heifers were

in each group. All heifers had free access to native forages, hay and water throughout gestation. The control Group (n=25 heifers) underwent a commonly used 7-day CO-Synch + CIDR® FTAI protocol, followed by introduction of a bull 10 days following AI. A 60-day breeding season was implemented. The experimental Group (n=50 heifers) underwent a similar 7-day CO-Synch + CIDR® protocol. Following CIDR removal at 7 days and FTAI on day 10, bulls were immediately placed with the heifers. Again, a 60-day breeding season was implemented. Three mature Angus bulls were used in the study. All bulls were determined to be "satisfactory" on their breeding soundness examination. The experimental group was split into two groups (n=25) with one bull per group for logistic and management purposes. Initial pregnancy examinations were performed via trans-rectal ultrasound utilizing a 10 MHz probe at 120 days after the initiation of FTAI protocol. A follow-up pregnancy examination at five to six months of gestation was performed via trans-rectal palpation. Additionally, parentage testing of calves was performed on calves to determine AI-sired calving distribution.

The preliminary results indicated that total conception rates may be improved combining a FTAI protocol with immediate natural service in heifers. While the percentage of heifers calving in the first 30 days was similar between experimental and control groups, subsequent 30 day calving intervals may be improved by a combination of natural service and aid in the tightening of a calving window. Calving distribution in the experimental group drastically favored the natural service sire, which was expected. Thus, utilizing a FTAI protocol in beef heifers using the 7-day CO-Synch + CIDR® combined with immediate natural service may be a viable option for improving the pregnancy rates and increasing calving season momentum.

INTRODUCTION

Cow calf production is an important enterprise in the Southeastern United States. Of the 31.2 million beef cows that calved in 2017, 44% of those reside in the 13 southeastern states (Scaglia et al., 2017). Florida alone ranks 13th in the nation in total numbers of beef cows, with a value exceeding $955 million each year (Moore, 2019). To continue to capitalize on the resources available and increase the profit margin for producers, we look to increase productivity through continued advancements in fertility.

Artificial insemination has been considered to be one of the greatest innovations in cattle management, yet only about 5% of southeastern cattle producers implement AI routinely (Whittier). Historically, effective estrus detection or the lack there of has been cited as the critical step for the success or failure of an AI program (Scaglia et al., 2017). Heat detection is not possible for many southeastern cattle producers that have full-time jobs outside the farm. Recently, fixed-time artificial insemination (FTAI) protocols have offered new opportunities to implement AI when effective heat detection is not possible. The FTAI pregnancy rates in beef heifers have been inconsistent, leading to frustration and often discontinuation of a program that offers significant genetic and economic potential. In the Southeast, FTAI use in beef heifers results in approximately a 50% AI conception rate (Whittier). There are a wide range of results and multiple reasons for the inconsistencies, which have led to less than desirable FTAI conception rates in heifers. Heifers respond variably to the estrus synchronization protocols used for FTAI. Some heifers exhibiting estrus and ovulate too early and some ovulate too late for FTAI to be effective. Despite these inconsistencies, a major benefit of a FTAI program in heifers is that more heifers calve earlier in the calving season. There is an increased average daily gain

of those calves born earlier in the breeding season, regardless of whether they conceived via AI or not.

The cost of natural service vs. artificial insemination per pregnancy are similar and hover right around $95.00 (Rodgers et al., 2012). However, this does not account for any of the genetic merit of an artificial insemination sired calf. A calf produced though AI can have an increased value of more than $100.00 above calves produced through natural service (Rodgers et al., 2012). Heifers that calve early continue this trend of productivity throughout their lives. These early calving cows not only remain in the productive herd longer but will wean more lifetime pounds of calf. Those calves born early in the calving season result in approximately $36 more per calf when compared to those calving later in the season (Sprott & Troxel, 1988). Additional benefits include an increase of approximately 10% or more pounds of beef sold, increases in cows retained in the herd by 11%, and decreases in the herd breakeven point by 18% (Adkins et al., 2012).

The cows and first calf heifers are benefited by a decreased anestrus post-partum interval, which in turn returns them to cyclic estrus sooner (Odde, 1990). The typical amount of time from calving to the resumption of fertile cycles (postpartum period) for 90% of a beef herd's mature cows is 60 to 80 days. For first-calf beef heifers, the typical number of days post-calving for 90% to resume fertile cycles is closer to 100 to 120 days (Odde, 1990). By enrolling them in a synchronization protocol we can significantly decrease this time (Odde, 1990).

It is our desire to identify a breeding protocol that results in more heifers calving within the first 21 days of their first calving season. The goal is to identify the calving distribution (AI vs. natural sired) of a breeding protocol involving estrus synchronization, natural service, and fixed-time artificial insemination that will result in 85% of pregnant heifers calving in the first 30

days of the calving season. The objective of this project is to critically evaluate the effectiveness of a FTAI protocol in beef heifers using the 7-day CO-Synch + CIDR® combined with immediate natural service. Our hypothesis was that implementation of a FTAI protocol in beef heifers using the 7-day CO-Synch + CIDR® combined with immediate natural service would result in 85% of the pregnant heifers calving in the first 30 days of the calving season. This information gathered from this study will provide southeastern cattle producers with scientifically based recommendations regarding a FTAI combined with natural service. Hence, making FTAI a viable option and improving the pregnancy rates and genetic potential of southeastern cattle.

STATEMENT OF RESEARCH OBJECTIVES

The purpose of this study was to (1) identify a practical, easy to implement breeding protocol involving a combination of estrus synchronization, natural service, and fixed-time artificial insemination that will result in 85% of pregnant heifers calving in the first 30 days of the calving season and (2) collect preliminary information regarding the distribution of AI-sired calves and natural service-sired calves if bulls were introduced before FTAI.

Materials & Methods

The animals used in this study were from the Auburn University College of Veterinary Medicine (AUCVM) research and teaching herds. All procedures were approved by the Auburn University Institutional Care and Use Committee (PRN 2017-3022). A total of 75 pubertal heifers, raised and sourced from North Auburn University Beef Unit were randomly placed into 2 groups based on reproductive tract scores. Heifers were scored and randomly placed in two groups in accordance with the selection protocol established by Patterson and bullock 1995, ensuring that a relatively even allotment of peri-pubertal and pubertal heifers would be placed in each group. The groups remained on pasture in accordance to Auburn University ICAUC guidelines. They had free access to native forages, hay and water throughout gestation. The control Group (n=25 heifers; control) underwent a commonly used 7-day CO-Synch + CIDR® FTAI protocol, followed by introduction of a bull 10 days following AI and a 60-day breeding season implemented. The experimental Group (n=50 heifers) underwent a similar 7-day CO-Synch + CIDR® protocol. Following CIDR removal at 7 days and FTAI on day 10, bulls were immediately placed with the heifers. Again, a 60-day breeding season was implemented. Three mature Angus bulls originating from the North AUCVM Beef Unit were used for this study. All bulls used in the study were determined to be "satisfactory" on their breeding soundness examination. The experimental group was split into two groups (n=25) with one bull per group for logistic and management purposes. Initial pregnancy examinations were performed via trans-rectal ultrasound (Ibex Evo Pro, E.I. Medical, Loveland, CO) utilizing a 10 MHz probe at 120 days after the initiation of FTAI protocol. A follow-up pregnancy examination at five to six months of gestation was performed via trans-rectal palpation. Additionally, parentage testing (Neogen SireSeek™, Lincoln, NE) of calves was performed to determine AI-sired calving

distribution. A power analysis was performed to determine the appropriate sample size for statistical significance.. Significance was set at a p value of less than 0.05 and power at 80%.

Parentage Sampling

The SeekSire™ Test (Neogen, Lincoln, NE) using the Illumina Infinium Chemistry with a combination of *Bos taurus, Bos indicus,* USDA and ISAG parentage markers was utilized to perform the parentage testing analysis with blood collected from the jugular vein and submitting in EDTA tubes.

Pregnancy Diagnosis

Initial pregnancy examinations were performed via trans-rectal ultrasound (Ibex Evo Pro, E.I. Medical, Loveland, CO) utilizing a 10 MHz probe at approximately 120 days after the initiation of FTAI protocol. A follow-up pregnancy examination at five to six months of gestation was performed via trans-rectal palpation.

Statistical Analysis

Evaluation was performed using online T-Test software (Quantitaiveskills.com). The alpha or significance level was considered as 0.05. Therefore, any p-value less than or equal to 0.05, represents the significance of the corresponding variable and any $p<0.1$ was indicative of a trend.

This study was conducted from November 2018 to March 2020. The 3 groups are compared (table 1).

Results

The display of estrus-like behavior for the control and experimental groups at the time of AI if illustrated in (Figure 1). The estrus response rate to the synchronization protocol, determined by the visualization/exhibition of estrus behavior and confirmed by a worn (>50% orange) “Heat Watch” patch was 56% and 74% for the control and experimental groups, respectively. The conception rate was determined via trans-rectal ultrasound at approximately 120 days after the initiation of FTAI protocol. The conception rates for the control and experimental groups were 72% and 78%, respectively. The conception per cycle was determined by the calving date. The control group per cycle conception rates were as follows: cycle 1: 32%, cycle 2: 41%, cycle 3: 20% and cycle 4: 13%. The experimental group per cycle conception rates were as follows: cycle 1: 48%, cycle 2: 35%, cycle 3, 29% and cycle 4: 8% (Figure 2). Figure 3 illustrates the calving season broken into 30 day intervals determined from the beginning of the calving season using recoded calving dates (Control n=25, Experimental n=50). The experimental and control group calving percentages were similar in the first 30 days of the calving season at 77% and 78% respectively. The second 30- day interval saw a decline in the control (16%) to the experimental (23%) group. In this study, 85% of the heifers in the experimental group did not calve in the first 30 days of the breeding season. Additionally, there was no significant difference found between the percentage of heifers calving in within the first 30 days of the calving season for the control and experimental groups so the null hypothesis was accepted. The control group had 6% of the heifers calve within the last 30 days of the calving season.

Reproductive tract scoring (RTS), based on Anderson and Bullock 1995 (1 to 5 scoring system) was completed prior to the initiation of synchronization protocols. The number of

pubertal and peripubertal heifers in each group was randomized for consistency. Pregnancy rate was determined via trans-rectal ultrasound and compared to the RTS. None of the animals in the study had a RTS <2. All 4 and 5 RTS heifers in both the control and experimental groups had a >90% conception rate (Figure 4).

The sire distribution for AI vs NS was determined on all calves. Parentage Testing for Sire distribution was performed on suckling calves prior to weaning. SeekSire (Neogen, Lincoln, NE) using the Illumina Infinium Chemistry with a combination of *Bos taurus, Bos indicus*, USDA and ISAG parentage markers were used to perform the parentage testing analysis with blood collected from the jugular vein and submitting in EDTA tubes. The sire distribution in the control group; 7 Day Co-synch+CIDR with the introduction of a cleanup bull 14 days after FTAI was 44% AI sired calves or consistent with a 44 % AI conception rate. The sire distribution of the experimental group; 7 Day Co-synch+CIDR with immediate introduction of a cleanup bull, prior to FTAI yielded an 18% AI sire conception rate (Figure 5). Statistical evaluation was performed using T-Test software with a significance level of 0.05. Comparison of the AI percentage of the control group versus that of the experimental groups was found to be significant with a P-value of 0.03.

Figure 1. The figure illustrates the comparison of the percentage of heifers displaying estrus in the control (56%) and the experimental group (74%).

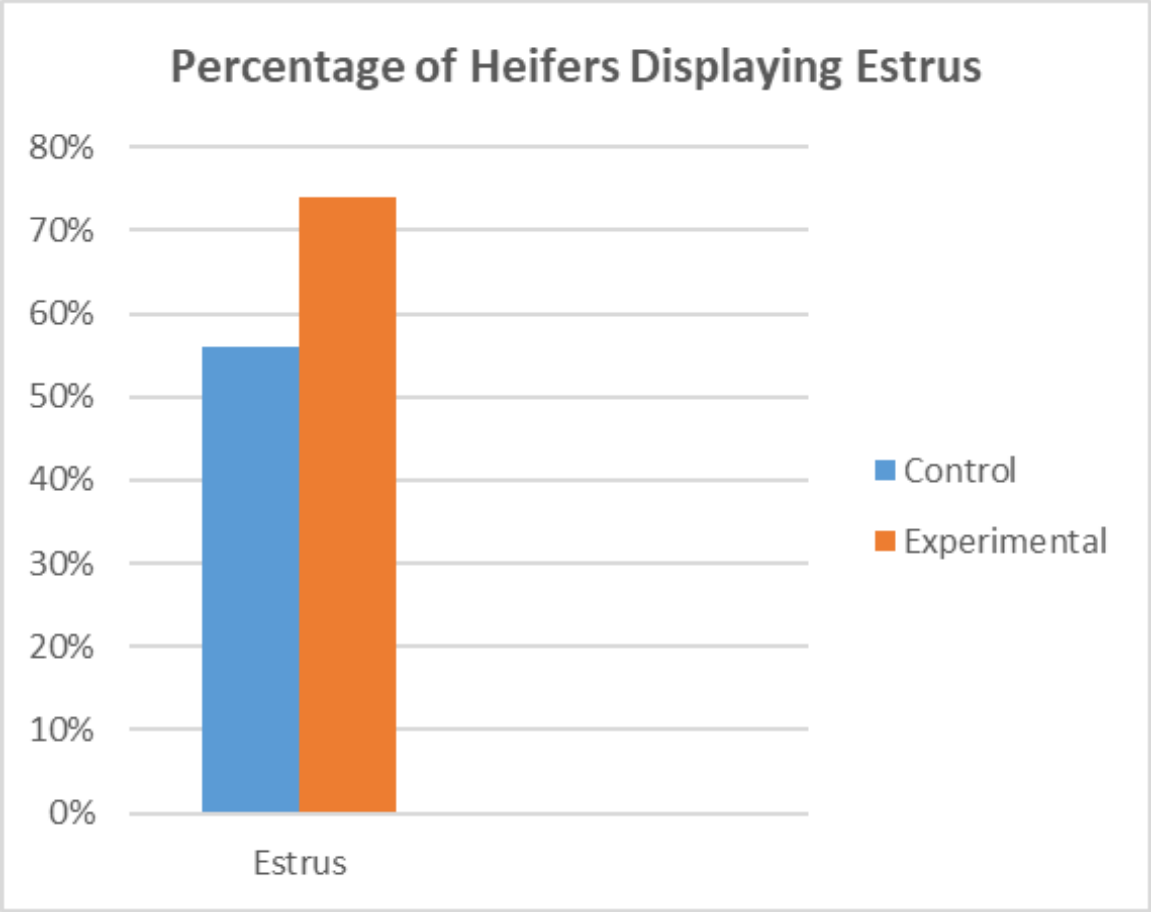


Figure 2. The figure illustrates the total conception and the conception rates per cycle for the control (n=25) and the experimental groups (n=50) during the breeding season.

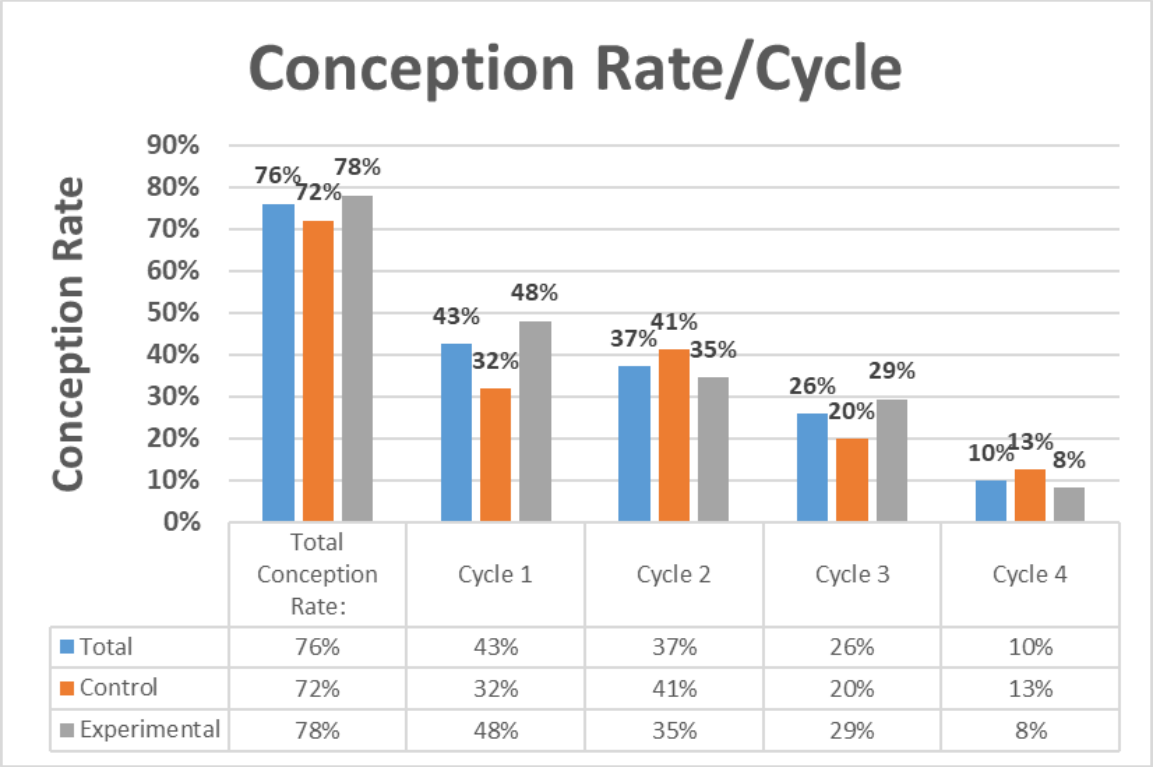


Figure 3. The figure illustrates the calving season for the control (n=25) and the experimental (n=50) groups broken into 30-day intervals determined from the beginning of the calving season using recorded calving dates.

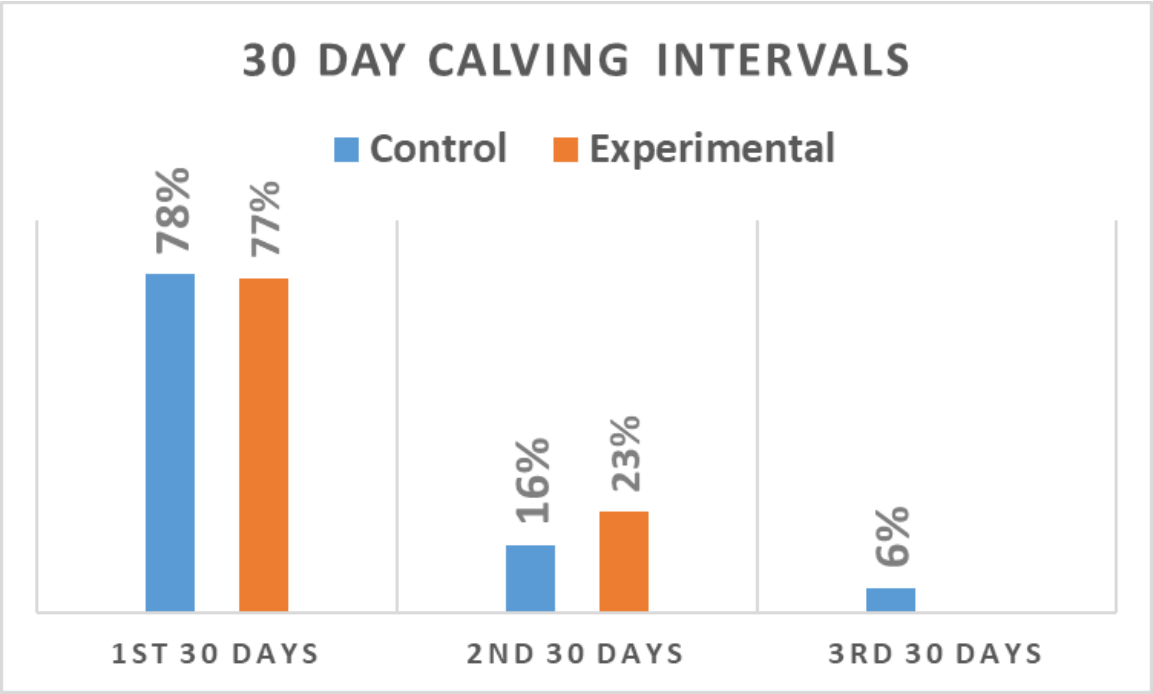


Figure 4. Illustration of the pregnancy Rate versus the. reproductive tract score (RTS) of heifers in the experimental and control group.

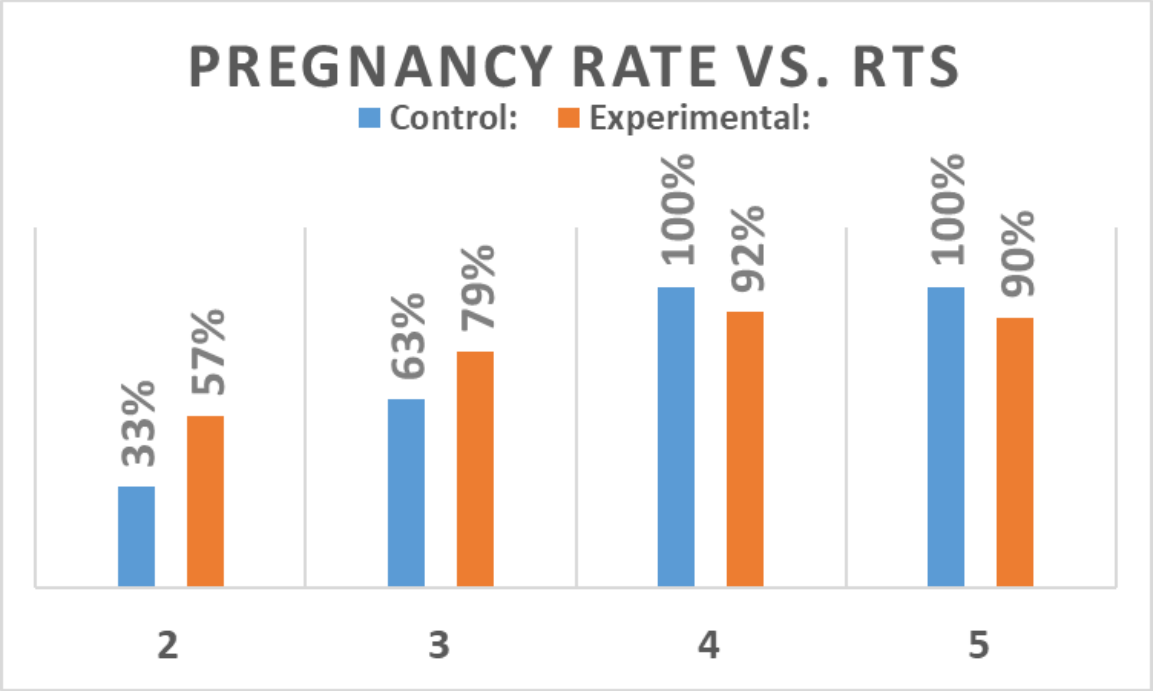
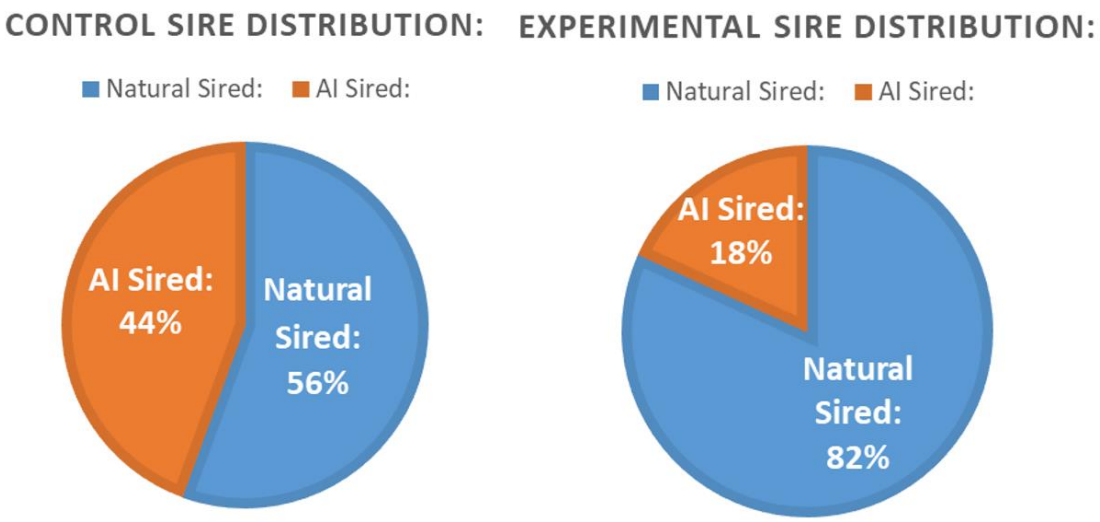


Figure 5. The sire distribution for the calves in the control and experimental groups are represented in the respective pie charts.



Discussion:

The purpose of this study was to identify and evaluate a breeding protocol that would result in more heifers calving within the first 21 days of their first calving season, as well as explore the calving distribution; AI vs. naturally sired calves using a combination of ES, AI and natural service (NS) that would result in 85% of pregnant heifers calving in the first 30 days of the calving season. The estrus response rate to the synchronization protocol was determined to be 68% when both groups were combined and 56% and 74%, for the control and experimental groups, respective. The range of heifers detected in heat is consistent with reported results (Lamb 2006). However, it was interesting that the control group had a lower observed response rate even though the groups were sorted randomly after RTS and BCS scoring. It is likely that the immediate addition of the bulls to the experimental group after CIDR removal could have increased this portion of heifers displaying estrus, similar to how the "buck effect" is utilized in other species (Senger 2012).

Several factors have affected the adoption and utilization of ES programs. Initially programs failed to address the limitation of ES, but they also failed to take into account the nature or lack thereof synchrony in a heifer's estrus cycle. Initial unpublished work at from Auburn University's College of Veterinary have found marked differences in the time from CIDR® removal to display of estrus. Using the conclusions from a 2007 study by Busch et al., which evaluated the interval from CIDR® removal to the expression of estrus, a noticeable decrease in the time interval between CIDR® removal and displayed estrus behavior was identified in this study. The mean interval from CIDR® removal to estrus in AUCVM heifers was approximately 42 hours, compared to 52 hours in the previous study by Busch et al., 2007. Groups of heifers either showing no response or exhibiting estrus much earlier than expected and thus ovulating too early for FTAI to be effective would specifically benefit from a protocol combining FTAI and NS. It is this lack of synchrony of estrus

and ovulation that have ultimately precluded FTAI from reaching acceptable pregnancy rates and popularity among southeastern beef producers. If an ES program fails in managing follicular waves, it results in less synchronous control of follicular maturation and CL regression. As a result, the onset of estrus occurs over a varied period of time during the synchronization period, leading to lower conception rates with conventional FTAI programs.

The conception rate was determined via trans-rectal ultrasound at approximately 120 days after the initiation of ES protocol. Conception rate is defined as the number of females exposed compared to the number of conceptions achieved. The conception rate for the control was 72% and the experimental group was 78%. These preliminary results indicate that total conception rates may be improved combining a FTAI protocol with immediate NS in heifers. While the percentage of heifers calving in the first 30 days was similar between experimental and control groups, subsequent 30-day calving intervals may be improved by utilizing NS in concert with AI. This kind of protocol was found to be beneficial in the tightening the calving window. The last 30-day window showed 6% of the control group calving during this time making a negative impact on the pounds of calf sold at weaning. The results of this study provide evidence that implementation of NS in concert with AI might aid in prevention of a strung-out calving season.

Reproductive tract scoring, based on Anderson and Bullock 1995, was completed prior to the initiation of the synchronization protocols. Examinations are to be performed when heifers are between 12 to 14 months of age and have reached approximately 65% of their mature body weight (D. Patterson & Bullock, 1995). The correlation between RTS and pregnancy rates remained consistent with reported results (Randle, 2002). The reproductive performance of heifers with an RTS of 2 or less was decreased as these heifers are less likely to be cycling at the

beginning of the breeding season and therefore less likely to respond to an ES protocol until later in the breeding season.

The sire distribution for AI vs NS was determined on all calves using SeekSire (Neogen, Lincoln, NE) parentage testing prior to weaning. The calving distribution in the experimental group favored the NS sire substantially, which was expected as the volume of ejaculate and total number of sperm from the NS was much higher at the time of insemination. The quality of the NS sperm hadn't undergone cryopreservation, so the motility and morphology were also improved (Schenk, Suh, Cran, & Seidel Jr, 1999). It is also not uncommon for one male to sire more offspring in a multisire unit even using similar numbers of spermatozoa per insemination (Beatty et al., 1969). Additional studies have found pronounced differences, in the pregnancy results of fresh verses semen that was frozen prior to insemination (Dziuk, 1996). However, a combination of fresh or fresh chilled would have most likely facilitated benefit from heterospermic insemination in this study. Swine litter sizes and fertility rates have improved with heterospermic insemination and it seems to be much more practical to utilize this technology for an increased benefit in litter bearing species (Hammitt, Martin, & Callanan, 1989). Thus, utilizing a FTAI protocol in beef heifers using the 7-day CO-Synch + CIDR® with fresh and or chilled semen from a higher quality bull combined with immediate natural service may result in more calves sired by the better bull then was seen in this study.

Bull fertility is an important evaluation when implementing a protocol involving NS and must be evaluated prior to the initiation of the breeding season. All bulls used to breed heifers (and cows for that matter) should be evaluated, with thorough breeding soundness examinations to evaluate semen quality, structural soundness, and overall health prior start of the breeding season. Once the breeding season of protocol is initiated, producers must spend time observing

activity in the breeding pasture to make sure that bulls are performing adequately. It is extremely important to visually evaluate bull performance during the first 30 days of the breeding season.

If heifers have reached puberty and ES system has applied appropriately, 70% or more of heifers should display estrus within the time window predicted by the ES system (Larson et al., 2016). Beef heifers that are bred with AI at an appropriate time, relative to ovulation of a fertile oocyte have a 60% to 80% probability of establishing a pregnancy (Laflin & White, 2016). This means that if greater than 80% of the heifers are cycling at the start of the breeding season, on average 5% should be bred each day. If results do not meet this goal, the percentage of pubertal heifers, accuracy of ES, and the success of administration should be investigated. If estrous response to ES is poor, alternate ES protocols can be used, but also, the period of NS and AI can be altered as well. In published results 50 to 60% of heifers identified in estrus and bred artificially should become pregnant with AI (Larson et al., 2016). Failure to meet these goals likely indicates inaccuracies in ES, poor semen delivery of handling, or poor BCS of heifers.

In conclusion, when combining ES with AI and NS it comes down to a producer's goals. AI conceived calves should have improved carcass characteristics and improved genetics, but this improvement does come at some cost in terms of time, materials and often a reduced conception rate when used alone. Placing significant selection pressure on heifers that are able to achieve and maintain a pregnancy from an AI mating or with natural service during a shortened breeding season, enables producers to select heifers that will not only reach puberty earlier, but also continue this early calving trend into their productive lives.

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APPENDIX

Control (Group 1) & Experimental (Groups 2 & 3)									
Dam	Calf	Calving Date	Sire	Wt. @ Tract Score	Tract Score	BCS @ Tract Score	Birth Wt.	Displayed Estrus	Group
Column	Column	Column15	Column14	Column3	Column	Column5	Column6	Column62	Column
1779	9107	08-26-2019	Horizon	600	4	6	55	Yes	1
7318	9108	08-29-2019	Horizon	615	5	5	65	Yes	1
7278	9109	08-30-2019	Horizon	530	4	5	65	Yes	1
7292	9111	09-01-2019	Horizon	605	5	5	60	Yes	1
17133	9112	09-02-2019	Horizon	640	3	5	55	No	1
1787	9113	09-02-2019	Horizon	640	3	5.5	65	No	1
7300	9114	09-02-2019	Horizon	595	4	5	50	Yes	1
17112	9116	09-02-2019	Horizon	570	5	5.5	60	Yes	1
1785	9135	09-18-2019	6729D	545	3.5	5	50	Yes	1
7348	9137	09-18-2019	6729D	620	2	5	55	No	1
17136	9138	09-18-2019	6729D	565	4	5	55	Yes	1
7370	9140	09-20-2019	6729D	655	4	5	75	No	1
7406X	9146	09-22-2019	6729D	670	5	5.5	65	Yes	1
7251	9147	09-25-2019	6729D	665	5	5	70	Yes	1
7264	9159	10-06-2019	6729D	575	3	5	65	Yes	1
7404	9162	10-18-2019	6729D	475	4	5	60	Yes	1
7340	9168	10-25-2019	6729D	535	3	5	60	No	1
7332	9175	11-04-2019	6729D	550	2	5	70	No	1
7249	open			495	3	5		Yes	1
7334	open			550	3	5		No	1
7349	open			530	2	5		No	1
7378	open			520	2	5		No	1
7397	open			495	2	5		Yes	1
7400	open			510	3	5		No	1
7403	open			505	2	5		No	1
7263	9154	09-30-2019	619	515	4	5	50	Yes	2
17146	9110	09-01-2019	635	645	3	5	55	No	2
7313	9115	09-02-2019	635	525	3	5	55	No	2
7261	9117	09-03-2019	635	585	4	6	60	Yes	2
1796	9119	09-04-2019	635	600	5	5	55	Yes	2
1781	9121	09-05-2019	635	665	4	6	65	Yes	2
1782	9134	09-17-2019	635	620	4	5	55	Yes	2
7241	9142	09-21-2019	635	590	3	5	60	No	2
7255	9145	09-22-2019	635	505	2	5	65	Yes	2
7305	9157	10-02-2019	635	500	2	5	55	No	2
17149	9160	10-12-2019	635	625	5	5	70	Yes	2
7341	9165	10-20-2019	635	545	4	5	70	Yes	2
1775	9179	11-10-2019	635	630	5	5	65	Yes	2
7302	9122	09-05-2019	Horizon	525	5	5.5	60	Yes	2
7243	9129	09-12-2019	Horizon	520	2	5	55	No	2
17159	9131	09-12-2019	Horizon	505	3.5	5	50	Yes	2
7322	100 D PG	PG Loss		535	3	5		Yes	2
7259	60 D PG	PG Loss		540	2.5	5		No	2
17119	90 D PG	PG Loss		560	3	5		No	2
7272	Open	Open		560	2	5		No	2
7316	Open	Open		540	2	5		No	2
7333	Open	Open		515	4	5		Yes	2
7344	Open	Open		550	2	5		Yes	2
17176	Open	Open		630	3	5		Yes	2
1769X	Open	Open		665	5	6		Yes	2
17156	9118	09-03-2019	619	600	3	5.5	65	Yes	3
7280	9124	10-06-2019	619	495	3.5	5	55	Yes	3
7396	9125	09-10-2019	619	530	4	5	55	Yes	3
7311	9128	09-12-2019	619	575	5	5	60	Yes	3
1789	9130	09-12-2019	619	710	5	5.5	60	Yes	3
7339	9132	09-12-2019	619	540	3	5	70	Yes	3
7262	9133	09-14-2019	619	695	4	5	70	No	3
1767	9136	09-18-2019	619	630	5	5.5	55	Yes	3
7281	9148	09-25-2019	619	565	3	5	60	Yes	3
1799	9149	09-25-2019	619	510	4	5	60	Yes	3
7394	9161	10-15-2019	619	535	3	5.5	65	Yes	3
17137	9163	10-18-2019	619	505	2	5	60	No	3
17109	9167	10-25-2019	619	610	5	5.5	60	Yes	3
7331	9170	10-27-2019	619	510	2	5	65	Yes	3
7240	9120	09-04-2019	Horizon	550	4	5	55	Yes	3
17179	9123	10-06-2019	Horizon	585	2	5	55	Yes	3
7266	9126	09-10-2019	Horizon	550	2	5	55	No	3
17113	100 D PG	PG Loss		570	4	5		Yes	3
17139	110 D PG	PG Loss		550	5	5		Yes	3
7279	75 D PG	PG Loss		640	4	5		Yes	3
1797	Open	Open		670	3.5	6		No	3
7323	Open	Open		515	3	5		Yes	3
7357	Open	Open		540	2	5		Yes	3
7393	Open	Open		500	2	5		Yes	3
17155	Open	Open		480	2.5	5		Yes	3

Control (Group 1) Estrus Response Rate:	**56%**
Control (Group 1) Conception Rate:	**72%**
Control (Group 1) Calving Rate:	**72%**
Control (Group 1) AI Sired Calves:	**44%**
Experimental Estrus Response Rate:	**74%**
Experimental Conception Rate:	**78%**
Experimental Calving Rate:	**68%**
Experimental AI Sired Calves:	**18%**

Group 1 (Control)

Tag		Calving Date	Sire	Wt. @ Tract Score	Tract Score	BCS @ Tract Score	Birth Wt.	Displayed Estrus	Group
Columi	Column16	Column15	Column14	Column3	Columi	Column5	Columi	Column62	Columi
1779	9107	08-26-2019	Horizon	600	4	6	55	Yes	1
7318	9108	08-29-2019	Horizon	615	5	5	65	Yes	1
7278	9109	08-30-2019	Horizon	530	4	5	65	Yes	1
7292	9111	09-01-2019	Horizon	605	5	5	60	Yes	1
17133	9112	09-02-2019	Horizon	640	3	5	55	No	1
1787	9113	09-02-2019	Horizon	640	3	5.5	65	No	1
7300	9114	09-02-2019	Horizon	595	4	5	50	Yes	1
17112	9116	09-02-2019	Horizon	570	5	5.5	60	Yes	1
1785	9135	09-18-2019	6729D	545	3.5	5	50	Yes	1
7348	9137	09-18-2019	6729D	620	2	5	55	No	1
17136	9138	09-18-2019	6729D	565	4	5	55	Yes	1
7370	9140	09-20-2019	6729D	655	4	5	75	No	1
7406X	9146	09-22-2019	6729D	670	5	5.5	65	Yes	1
7251	9147	09-25-2019	6729D	665	5	5	70	Yes	1
7264	9159	10-06-2019	6729D	575	3	5	65	Yes	1
7404	9162	10-18-2019	6729D	475	4	5	60	Yes	1
7340	9168	10-25-2019	6729D	535	3	5	60	No	1
7332	9175	11-04-2019	6729D	550	2	5	70	No	1
7249	open			495	3	5		Yes	1
7334	open			550	3	5		No	1
7349	open			530	2	5		No	1
7378	open			520	2	5		No	1
7397	open			495	2	5		Yes	1
7400	open			510	3	5		No	1
7403	open			505	2	5		No	1

Group 1 Estrus Response Rate:	**56%**
Group 1 Conception Rate:	**72%**
Group 1 Calving Rate:	**72%**
Group 1 AI Sired Calves:	**44%**

Experimental (Groups 2 & 3)									
Dam	Calf	Calving Date	Sire	Wt. @ Tract Score	Tract Score	BCS @ Tract Score	Birth Wt.	Displayed Estrus	Group
Column	Column	Column15	Column14	Column3	Column	Column5	Column6	Column62	Column
7263	9154	09-30-2019	619	515	4	5	50	Yes	2
17146	9110	09-01-2019	635	645	3	5	55	No	2
7313	9115	09-02-2019	635	525	3	5	55	No	2
7261	9117	09-03-2019	635	585	4	6	60	Yes	2
1796	9119	09-04-2019	635	600	5	5	55	Yes	2
1781	9121	09-05-2019	635	665	4	6	65	Yes	2
1782	9134	09-17-2019	635	620	4	5	55	Yes	2
7241	9142	09-21-2019	635	590	3	5	60	No	2
7255	9145	09-22-2019	635	505	2	5	65	Yes	2
7305	9157	10-02-2019	635	500	2	5	55	No	2
17149	9160	10-12-2019	635	625	5	5	70	Yes	2
7341	9165	10-20-2019	635	545	4	5	70	Yes	2
1775	9179	11-10-2019	635	630	5	5	65	Yes	2
7302	9122	09-05-2019	Horizon	525	5	5.5	60	Yes	2
7243	9129	09-12-2019	Horizon	520	2	5	55	No	2
17159	9131	09-12-2019	Horizon	505	3.5	5	50	Yes	2
7322	100 D PG	PG Loss		535	3	5		Yes	2
7259	60 D PG	PG Loss		540	2.5	5		No	2
17119	90 D PG	PG Loss		560	3	5		No	2
7272	Open	Open		560	2	5		No	2
7316	Open	Open		540	2	5		No	2
7333	Open	Open		515	4	5		Yes	2
7344	Open	Open		550	2	5		Yes	2
17176	Open	Open		630	3	5		Yes	2
1769X	Open	Open		665	5	6		Yes	2
17156	9118	09-03-2019	619	600	3	5.5	65	Yes	3
7280	9124	10-06-2019	619	495	3.5	5	55	Yes	3
7396	9125	09-10-2019	619	530	4	5	55	Yes	3
7311	9128	09-12-2019	619	575	5	5	60	Yes	3
1789	9130	09-12-2019	619	710	5	5.5	60	Yes	3
7339	9132	09-12-2019	619	540	3	5	70	Yes	3
7262	9133	09-14-2019	619	695	4	5	70	No	3
1767	9136	09-18-2019	619	630	5	5.5	55	Yes	3
7281	9148	09-25-2019	619	565	3	5	60	Yes	3
1799	9149	09-25-2019	619	510	4	5	60	Yes	3
7394	9161	10-15-2019	619	535	3	5.5	65	Yes	3
17137	9163	10-18-2019	619	505	2	5	60	No	3
17109	9167	10-25-2019	619	610	5	5.5	60	Yes	3
7331	9170	10-27-2019	619	510	2	5	65	Yes	3
7240	9120	09-04-2019	Horizon	550	4	5	55	Yes	3
17179	9123	10-06-2019	Horizon	585	2	5	55	Yes	3
7266	9126	09-10-2019	Horizon	550	2	5	55	No	3
17113	100 D PG	PG Loss		570	4	5		Yes	3
17139	110 D PG	PG Loss		550	5	5		Yes	3
7279	75 D PG	PG Loss		640	4	5		Yes	3
1797	Open	Open		670	3.5	6		No	3
7323	Open	Open		515	3	5		Yes	3
7357	Open	Open		540	2	5		Yes	3
7393	Open	Open		500	2	5		Yes	3
17155	Open	Open		480	2.5	5		Yes	3
		Experimental Estrus Response Rate:	**74%**						
		Experimental Conception Rate:	**78%**						
		Experimental Calving Rate:	**68%**						
		Experimental AI Sired Calves:	**18%**						