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Efficacy of Sequential Estrus Synchronization and Timed Artificial Insemination in Beef Cattle Beginning 19 days after an Initial Timed Artificial Insemination

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To the Graduate Council:

I am submitting herewith a thesis written by Courtnie Carter Bridges entitled "Efficacy of Sequential Estrus Synchronization and Timed Artificial Insemination in Beef Cattle Beginning 19 days after an Initial Timed Artificial Insemination." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Efficacy of Sequential Estrus Synchronization and Timed Artificial Insemination in Beef Cattle Beginning 19 days after an Initial Timed Artificial Insemination

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ABSTRACT

A breeding program utilizing single fixed-timed artificial insemination (AI) was compared to a program utilizing resynchronization for a second fixed-timed AI. Angus and Angus cross primiparous, and multiparous cows were randomly assigned to one of two treatment regimens; either single TAI (CON; n=468) or two sequential TAI (RS; n=473). The main objectives were to assess pregnancy rates and analyze fetal age to estimate calving distribution rates between the two treatments groups. The 7-day CO-synch + CIDR protocol was utilized in both treatment groups to synchronize estrus for TAI-1 at 60h - 66h post- Prostaglandin injection (PGf2α). Animals in the RS treatment groups received an additional 7-day CO-synch + CIDR protocol 19 d after TAI-1. On d 26, both CON and RS animals were pregnancy checked by way of transrectal ultrasonography. At this time, RS animals that were diagnosed not pregnant received an injection of PGf2α, and an Estrotect® estrous detection aid was applied. All non-pregnant RS cows received a second TAI (TAI-2) and GnRH injection 60-66h after PGf2α (day 29). Additional pregnancy checks were initiated at the following days relative to TAI-1, d 60, and d 120.There was no difference in pregnancy rates post TAI-1 ($P<0.55$; RS = 61.67 ± 1.4%, CON = 59 ± 1.4%). The probability of pregnancy to AI 60 d post TAI-1 was higher ($P< 0.01$) for RS (82%) than for CON cows (57%). Overall pregnancy rate on d 120 post-TAI-1 did not differ between treatments (92%; $P< 0.823$). The probability of pregnancy to AI on d 120 was higher ($P< 0.0001$) for RS (78%) than for CON cows (50%). By way of design, the probability of pregnancy sired by natural service was higher ($P< 0.03$) for CON (41%) than RS (15%). Mean fetal age at 120d pregnancy diagnosis did not differ ($P< 0.5547$) between treatments (RS, 94 days: CON, 96 days). In conclusion, re-synchronization for a second TAI beginning 19 d after the initial TAI increased total AI-sired pregnancies and has the potential to reduce the required number of natural service sires without negatively influencing calving distribution.

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CHAPTER ONE

INTRODUCTION AND REVIEW OF PERTINENT LITERATURE

INTRODUCTION

Profitability in beef cattle production is directly influenced by the producer's management practices. The lack of vertical integration in the beef cattle industry promotes variation in the management practices and success between operations. Reproductive efficiency is the primary factor affecting profitability in commercial beef operations [6]. Since reproductive performance is a rate-limiting metric for profitability, economic success is more probable when management is consistently focused on the factors that influence reproduction. Pregnancy rate, weaning rate, and calving distribution are three response variables used to assess the status and initiate changes in reproductive management. Reproductive performance can be measured by the number of cows that conceive in each breeding season (i.e., pregnancy rate). However, this measurement falls short of accounting for pregnancy, neonatal, and pre-weaning mortality. Weaning rate is an ideal indicator of reproductive performance in the production setting since farmers and ranchers can more easily access it. While weaning rate includes factors not directly associated with the reproductive function (i.e., postnatal immune function), it is more dramatically influenced by fecundity than any other factor. Moreover, since the primary product of beef cow-calf operations is weaned calves [17], weaning rate is a readily available metric at the production level of analysis.

Calving distribution, the interval between the first and last calf born in a single calving season, also dramatically influences profitability. The authors demonstrated that shifting from year-round to a 90-day calving season increased the resulting calf revenue by $3 per cwt price premium for calves from a controlled breeding season [16]. Calving distribution can be expressed as a measurement of the number of calves born in each 21-day interval of the calving season. Calving season length varies between operations from a stringent 45-day to a more standard 60-day or a 90-day calving season. Decreasing calving season duration increases profits and maximizes net returns in beef cow-calf systems by increasing weaning weights and age in weaned calves [6]. This review will discuss the physiological events that occur in the bovine

estrous cycle and the ability to control these events with synchronization protocols to utilize AI and optimize productivity and efficiency.

The Bovine Estrous Cycle

The estrous cycle is defined as the interval between consecutive periods of estrus. The average length of the estrous cycle in cattle is 21 days and ranges from 14 – 24 days. The estrous cycle can be divided into four distinct phases: proestrus, estrus, metestrus, and diestrus. The follicular phase includes proestrus (days 17 – 21) and estrus (day 0), while the luteal phase includes metestrus (days 1 – 4) and diestrus (days 5 – 17). The estrous cycle is regulated by hormones secreted by the hypothalamus, pituitary, ovary, and uterus.

Estrus

Estrus is the period when the female is sexually receptive to the male and is considered the beginning of the estrous cycle. This phase usually lasts from 12 to 18 hours in cattle. During estrus, progesterone is very low (> 1 ng/ml; [61]), and elevated estradiol production from the Graafian follicle stimulates an increase in the release of gonadotropin-releasing hormone (GnRH) pulses from the hypothalamus and, in turn, luteinizing hormone (LH) secretion from the pituitary. This surge of LH then initiates the cascade of events that lead to ovulation (rupture of the follicle and release of the oocyte). Elevated concentrations of estradiol are also associated with the display of behavioral estrus. The display of behavioral estrus usually occurs 24 hours after the rise in estradiol and is accompanied by the LH surge, and ovulation typically takes place 30 hours later [12]. It has been reported that environmental changes or disruption of the endocrine events of the estrous cycle may lead to a change in the timing of the preovulatory LH surge [35], behavioral estrus [34], and ovulation [2]. If the timing of these events is altered, and there is an increased interval between displayed estrus and ovulation, the number of viable sperm cells at the ampullary-isthmus junction at the time of oocyte deposition could be too few to ensure normal fertility [13].

Metestrus

Transitional events after estrus and ovulation characterize metestrus. The transition of follicular tissue to luteal tissue is referred to as luteinization, and this process is dependent on LH [4]. During ovulation, the basement membrane separating the theca and granulosa cells degenerate. Shortly after ovulation, the theca and granulosa cells mix uniformly. The luteal tissue cells are

referred to as large and small luteal cells. One of the most widely accepted theories for CL formation is that large luteal cells originate from the granulosa cells of the follicle and small luteal cells arise from the theca cells.

Circulating concentrations of progesterone begin to increase, and estradiol decreases as the corpus luteum (CL) is formed. The size of the CL, and its capacity for progesterone production, are correlated to ovulatory follicle size [49,58]. However, changes in the environment can alter the life span and steroidogenic capacity of the CL [8]. For example, heifers housed under heat stress conditions had smaller diameter CL and lower serum progesterone concentrations than those housed in a thermoneutral environment. The end of metestrus usually occurs five days after ovulation, at which time plasma progesterone concentration will reach an average of 7.5 ng/ml until the beginning of proestrus [46].

Diestrus

The most prolonged period of the estrous cycle is differentiated by relatively high circulating concentrations of progesterone and low concentrations of estradiol. This period usually lasts 10 – 14 days in cattle. The episodic patterns of LH and follicle stimulating hormone (FSH) typically fluctuate to accommodate follicular growth and regression but are inhibited by the negative feedback of progesterone and estradiol at the hypothalamus [50].

Progesterone and estradiol are steroid hormones arising from the same biochemical pathway beginning with cholesterol as the primary substrate. Both share a common molecular nucleus composed of three phenanthrene rings and one cyclopentane ring. The biological activity of a steroid hormone can be predicted by the number of carbons present. A 21-carbon steroid will have progesterone properties, and an 18-carbon steroid will have estrogenic activity. The compound structure is an essential factor when considering the effects of exogenous stimuli that may impact reproduction via endocrine regulation.

Progesterone is produced by both large and small luteal cells. As previously mentioned, this process requires cholesterol and is LH-dependent. Luteinizing hormone initiates the protein kinase A (PKA) second messenger system in small luteal cells responsible for internalizing the cholesterol complex into the cell. The mitochondria are responsible for the series of enzymatic events that convert cholesterol to pregnenolone. Pregnenolone exits the mitochondria and is converted to progesterone.

Diestrus ends with a regression of the corpus luteum (luteolysis). The most widely accepted theory is that luteolysis is governed by two hormones, oxytocin and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) [37]. According to McCracken, oxytocin from the CL stimulates the production of prostaglandin F2α (PGF2α) by the uterus. The $PGF_2\alpha$ is then transferred across the countercurrent plexus of the utero-ovarian vein and the ovarian artery, reaching its specific receptors on the CL. Additionally, McCracken and coworkers (1999) stated that, when bound to its receptors on the luteal cell, $PGF_2\alpha$ structurally degrades the cell and inhibits further progesterone production.

Prostaglandin $F_2\alpha$ is not localized to any particular tissue but is considered a uterine hormone as it pertains to reproductive endocrinology. All prostaglandins are 20-carbon unsaturated hydroxyl fatty acids with a cyclopentane ring. Arachidonic acid is the precursor of $PGF_2\alpha$ as well as prostaglandin E_2 (PGE_2).

Proestrus

As the female enters proestrus, the circulating concentration of progesterone begins to decline, and estradiol increases. The dominant follicle of the final follicular wave is responsible for the estradiol increase as it grows larger and becomes more steroidogenic. Pulsatility of LH becomes more frequent (3-8 pulses/6h; [53]), which drives the larger follicle's maturation and prepares it for ovulation.

The follicle produces estradiol through an interaction of the theca and granulosa cells. The theca interna cells have LH receptors that, when bound, initiate the enzymatic pathway that yields testosterone from cholesterol. This testosterone then crosses the basement membrane and diffuses into the granulosa cell. The granulosa cells contain aromatase that converts testosterone to estradiol in response to the binding of FSH to receptors on the cell surface. This process is commonly referred to as the "two-cell two-gonadotropin model."

The gonadotropins (LH and FSH) are produced and released from the anterior pituitary. They are glycoproteins with an identical alpha subunit and a unique beta subunit. Their release is governed by gonadotropin-releasing hormone (GnRH) produced in neurosecretory cells of the hypothalamus and carried to the anterior pituitary via a portal vascular complex. As mentioned above, gonadotropins support ovarian structural activity and steroid production. The nature of LH and FSH release patterns will be discussed in further detail during the review of follicular dynamics.

Folliculogenesis

Folliculogenesis is defined as the formation, and maturation, of follicles and involves the development of a primordial follicle into a preovulatory follicle and ovulation. Follicular growth during the estrous cycle of the bovine female occurs in wave-like patterns. During the estrus cycle, two to four waves of follicles undergo recruitment, selection, dominance, and atresia, with the final dominant follicle ovulating rather than becoming atretic [21,53]. Differences in the number of follicular waves occurring during an estrous cycle can impact the fertility of the resulting ovulation [57]. A preovulatory follicle arising from the second wave of a two-wave cycle will have developed longer than one arising from the third wave of a three-wave cycle and will release an oocyte that is older and more likely to degenerate prior to reaching the site of fertilization [29]. Thus, environmental impacts that lead to the ovulation of an older follicle (>9 days; [29]) could result in altered fertility.

Folliculogenesis begins and ends with the process of ovulation. Ovulation of the dominant follicle is how the oocyte is released through the destruction of the follicle tissue. The cascade of events that leads to the follicle rupture is set in motion by the preovulatory LH surge. The LH surge is prompted by estradiol binding to receptors on neurons in the hypothalamus that synapse with GnRH neurons. Estradiol also binds directly in the pituitary as part of the surge initiation. These GnRH neurons release their hormone into the hypothalamohypophyseal portal plexus of the stalk median eminence. From this point, the GnRH is moved to the anterior pituitary, where it prompts the release of LH.

Two significant phenomena associated with ovulation are increased blood flow to the ovary and the release of lysosomal enzymes. Hyperemia, stimulated by histamine and prostaglandin E_2, facilitates increased hydrostatic pressure in the follicle. Additionally, the shift from estradiol to progesterone production initiates collagenase synthesis by the theca interna. Collagenase digests the tunica albuginea as hydrostatic pressure increases, and the stigma begins to protrude. Prostaglandin $F_2\alpha$ stimulates contractions of the ovarian smooth muscle and coupled with the increased pressure and enzymatic activity, the follicle ruptures, and the oocyte is released.

After ovulation, a new wave of primordial follicles (approximately 8 in heifers; [20]) is recruited. A surge of FSH is a component of the preovulatory surge of gonadotropins, and a transient increase in FSH occurs before each follicular wave. This may serve as the initiator of

follicular waves [1]. Until the point of selection, the newly recruited cohort of follicles relies on the previously mentioned co-activity of LH and FSH for growth and survival. The fact that these primary follicles are dependent on both gonadotropins is essential in understanding the selection process, as the future dominant follicle will eventually gain the ability to thrive without FSH. Selection, or deviation, is the process by which a single follicle begins to exert dominance on the subordinate follicles of a cohort (dominance refers to the inhibition of new follicle recruitment by the selected follicle). The growth rate of the newly recruited follicles is not identical, and as they approach selection, the eventual dominant follicle will be larger [49]. One of the most widely accepted theories suggests that, as FSH concentrations decline due to increased estradiol and inhibin, the largest follicle can continue growth because it has begun to acquire LH receptors on the granulosa cells that will stimulate androgen aromatization. This ability to thrive with less FSH may also be due, in part, to local effects of estradiol and insulin-like growth factor 1 (IGF-1), increased by the attainment of LH receptors in the future dominant follicle [49]. The remaining subordinates do not develop to the point of utilizing the lower levels of FSH and therefore regress.

The average life span of the dominant follicle during an estrous cycle with two follicular waves is 5 to 7 days [34]. At the end of this period, the follicle becomes atretic if it arose from the first wave or is ovulated if it developed from the ovulatory wave. As the steroidogenic capacity of the dominant follicle increases with size, estradiol 17-β (E_2) further inhibits FSH release from the pituitary. Since the granulosa cells of only the dominant follicle gain LH receptors, it is the only follicle able to thrive without FSH stimulation. The suppression of recruitment continues until the follicle regresses or ovulates because of the preovulatory LH surge.

Tools to Improve Reproductive Efficiency

Applied reproductive management technologies, such as estrus synchronization (ES) and artificial insemination (AI), have been shown to increase herd profitability by shortening the calving to conception interval and the potential to improve calf genetics [11,22,33].

Fixed-time artificial insemination (FTAI) can be used to eliminate the demand for natural heat detection and facilitate a defined time to breed females. Generally, 50 to 60% of those females can be expected to conceive to a single FTAI, with slight variations in rates depending on several

factors. Those factors include the general management level, environmental stress factors (ex., heat stress, and animal handling methods), body condition score, days postpartum at insemination, prior selection pressure for fertility, and health status. The remaining females that do not conceive to the FTAI require additional efforts to achieve pregnancy. In most management scenarios, the most common method of rebreeding the remaining open females is utilizing natural service sires [5,26]. Some operations opt to utilize artificial insemination for follow-up breeding to take advantage of the genetically superior sires available [18]. However, in situations where a second AI is desired, the primary method is through an observed return to estrus. This method can be labor-intensive and is cited as the major deterrent to producer adoption of these technologies [18,26]. Additionally, most resynchronization protocols currently available utilize heat detection for the second insemination [18,31,36]. Implementing a resynchronization protocol for FTAI has the potential to reduce the labor associated with heat detection and allow precise scheduling for the follow-up inseminations.

Estrous Synchronization

One of the most common methods of estrus synchronization in cattle involves the use of a progestin (progesterone), such as Melengestrol acetate (MGA) or a controlled internal drug-releasing device (CIDR). Exogenous progestins work to prevent estrus expression during the duration of progestin administration [34]. Progestins can also reduce the postpartum anestrus period by reducing or eliminating the occurrence of a short luteal phase and inducing cyclicity [1,34, 55]. Several studies have also shown the use of progestin to reduce the variation of return to estrus after a previous synchronization [35,62]. However, progestin alone is not sufficient in manipulating the follicular wave for FTAI. Most TAI programs utilize the administration of gonadotropin-releasing hormone (GnRH), prostaglandin F2α (PGF), as well as a source of progesterone. GnRH works to induce ovulation or luteinization of the dominant follicle to “reset” the follicular wave and initiate follicular growth. Hence, a dominant follicle is present when the second dose of GnRH is initiated at the time of breeding, and ovulation can be stimulated. PGF functions to initiate luteal regression, allowing for maturation of the dominant follicle, an increase in estradiol levels, and the resulting LH surge to initiate ovulation [12]. The 7-day CO-Synch program (Figure 1) is one of the most widely utilized TAI programs [14]. The 7-day CO-

Synch program involves giving a GnRH injection and placing a progestin on d -7, PGF on d 0, and an additional GnRH injection concurrent with AI (Figure 1).

Re-synchronization

Implementing a resynchronization protocol for a second FTAI may help bridge the gap between net returns and input cost. Effective execution of resynchronization protocols aims for early detection of open females and re-insemination as soon as possible [5]. Additionally, reducing the time and labor associated with a second AI breeding may increase the adoption of this profitable reproductive technology since those are the major deterrents. One way to mitigate the required time and animal handling period is to omit estrus detection and instead use a resynchronization protocol with FTAI. Bo et al. (2016) described success when including two FTAI periods rather than estrus detection. In their study, animals received a CIDR insert on day 16 after the TAI-1 and were administered GnRH on day 21. Ultrasonography was conducted on day 28, and any non-pregnant females received PGF2α and were given estradiol benzoate (EB) or GnRH on day 29 or 30, respectively. The second FTAI occurred 30 hours after EB or 12 hours after GnRH administration. They reported an overall pregnancy rate of 80% after two TAIs. [5]. Results like these show the efficacy of utilizing resynchronization for FTAI to optimize productivity and reduce input costs associated with heat detection. However, with modern advancements in reproductive technologies, there is also room for the advancement of resynchronization protocols for beef cattle.

Ultrasound as a Tool

Improvements in modern ultrasound technology allow for more accurate, early pregnancy detection, thus more efficient use of resynchronization. Accurately identifying those females that do not breed to the initial insemination has proven to be a limitation in utilizing resynchronization for FTAI. The utilization of ultrasonography provides real-time results and eliminates the necessity of lab analysis to determine pregnancy status. Real-time early pregnancy detection allows for more precise control of the onset of estrus by enabling prostaglandin administration in the resynchronization protocol without risking pregnancy loss. Together, these tools present the opportunity to increase the percentage of pregnancies established by FTAI while minimizing the associated time and labor.

Statement of the Problem

There is ample opportunity available for beef operations to improve herd profitability. ES, AI, and pregnancy detection technologies allow for more concentrated breeding and calving seasons. However, there is still room for developing a feasible resynchronization protocol that implements early pregnancy detection and does not negatively influence calving distribution. The following experiment was designed to implement early pregnancy detection in a resynchronization protocol in a way that applies to producers without negatively affecting calving distribution.

CHAPTER TWO
MATERIALS AND METHODS

All procedures were approved by the University of Tennessee Knoxville Institute Animal Care and Use Committee (IACUC).

Animals and Sample Collection

Angus and Angus cross primiparous, and multiparous cows (n= 941) were used for this multi-state collaborative study. All cattle were synchronized using a 7-day CO-Synch plus controlled intravaginal drug release (CIDR; Zoetis) protocol (Figure 1.1) and timed artificially inseminated (TAI) on d 0. Animals were assigned evenly based on parity (mature cows and three-year-olds) to one of two treatments (CON or RS). Cattle in the RS treatment groups were resynchronized using the same protocol 19 d after the first TAI. These animals received an injection of GnRH (100 µg as 2cc of Cystorelin i.m.; Boehringer Ingelheim Animal Health; Duluth, GA) and had a CIDR inserted intravaginally on d 19. Following the 7-day CO-Synch protocol, the CIDR was removed 7 days later on d 26. At this time, transrectal ultrasonography was performed to determine pregnancy status. Non-pregnant animals received an injection of PGF2α (25 mg as 5cc of Lutalyse i.m.; Parsippany, NJ) and a heat detection patch was applied to their tail head (Estrotect®, Western Point, Inc., Apple Valley, MN). All RS cows receiving PGF2α received the second TAI 54-66 hrs later. Pregnancy was also determined (transrectal ultrasound) for cows in the CON groups on d 26 to compare rates.

Animals in both treatment groups were transrectally ultrasounded with an Ibex EVO ultrasound machine fitted with a linear probe 26 d after the initial TAI. A skilled technician assessed for the presence or absence of a fetus in the animals' uterus. Additional ultrasounds were performed at d 60, 90, and 120 to assess pregnancy status and the occurrence of embryonic and/or fetal loss. Data collection concluded after the 120 d pregnancy diagnosis.

Blood samples were collected from both treatment groups on d 19 and d 26 from a subset of the animals (TN cattle; n= 461). Blood was collected from the tail vein into red top, 3 mL Vacutainer tube. Samples were centrifuged at 3,000 rpm for 30 minutes at 4 ° C. Plasma samples were stored at - 70° C for at least 24 hrs and then shipped to Texas A&M University for analysis

of PAG concentration. It should be noted that the PAG data will not be included in the results of this study.

Locations

This study was a multi-state collaborative effort. Cattle from the following universities were involved in the study: The University of Tennessee Knoxville, The University of Kentucky, and The University of Georgia. The following are the locations of animals in each respected state; Highland Rim Research and Education Center (Springfield, TN), Ames Plantation (Bolivar, TN), University of Kentucky C. Oran Little Research Center (Versailles, KY), University of Kentucky Research and Education Center (Princeton, KY), NW Georgia Experiment Station (Calhoun, GA). All procedures and experimental methods were similar across all locations.

Statistical Analysis

Data were statistically analyzed as a completely randomized design with two blocking factors, parity (i.e., mature cows and first-calf heifers) and location. Differences in treatments were determined using mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute, Cary, NC, USA) in relation to pregnancy rates at d 26, d 60, and d 120. The GLIMMIX procedure was also used to evaluate embryonic loss between the treatments. Location and treatment x location were used as random effects in all models. Differences in treatments were determined by using F-protected least significant differences. The MEANS procedure was used to test the treatment difference in multiple variables at day 120. The GLIMMIX procedure was also utilized to analyze treatment difference on distribution of variables at day 120. Body Condition Score (BCS) at the time of AI was used as a covariate in the model. To assess the ability of utilizing transrectal ultrasonography to detect pregnancy at d 26, the FREQ procedure was utilized on the CON treatment group comparing AI pregnancy diagnosis between d 26 and d 60. Cohen's Kappa statistics were calculated in SAS, and the Kappa scoring scale was used to analyze results. The Kappa scoring scale reads as follows: Very good= 0.80–1.00, Good = 0.60–0.80, Moderate = 0.40–0.60, Fair = 0.20–0.40. It should be noted that one location, KY, was not included in the d 120 analysis due to issues with data transfer but is included in all other analyses.

CHAPTER THREE

RESULTS

There is probability of pregnancy to AI on d 26 did not differ between treatments (P<0.55; RS = 61.67 ± 1.4%, CON = 59 ± 1.4%). Mean fetal age at d 60 did not differ between treatments (P<0.7556; RS = 42.54 ± 1.97 d, CON = 41.84 ± 1.97 d) when open cows were included in the analysis, but there was a significant difference in the number of AI-sired pregnancies at d 60 (P< 0.0115; RS = 82.42 ± 2.1, CON = 57.50 ± 1.9). Similarly, the mean fetal age at d 60 (P< 0.7478; RS = 51.09 ± 1.89 d, CON = 50.70 ± 1.89 d) did not differ between treatments when open cows were excluded from the analysis. Figure 2.1 depicts the percentage of animals pregnant to AI on d 60 between the CON and RS treatments. Treatment significantly affected the percentage of natural service pregnancies at d 60 (P< 0.0070; CON = 24%, RS = 66%), as depicted in Figure 3.1. There was a significant difference between treatments for the probability of pregnancy to AI on d 120 (P < 0.0001; RS = 78%, CON = 50%). Likewise, the probability of natural service-sired pregnancy on d 120 differed between treatments (P< 0.03; CON 43%, RS 15%). Pregnancy rates by treatment on d 120 are depicted in Figure 4.1. Mean fetal age at d 120 did not differ between treatment groups when open cows were included in the analysis (P<0.5547; RS = 96.50 ± 2.7 d, CON = 94.19 ± 2.7 d). Similarly, the mean fetal age did not differ when open cows were excluded from the analysis on 120 d (P< 0.7745; RS = 102.26 ± 3 d, CON = 103.39 ± 3 d). Additional descriptive statistics regarding fetal age at day 120 are noted in table 1.1. The distribution of fetal age at day 120 was also not statistically significant (P< 0.9157). There was no difference in the probability of a cow being open on d 120 (P < 0.4611; CON = 4.3%, RS 3%).

Pregnancy Detection at 26 d

Based on the Kappa score (0.82), “very good” agreement was found when comparing the 26 d and 60 d pregnancy diagnoses in CON treatment groups.

Embryonic loss

There was no difference between treatments when comparing the frequency of embryonic loss between 26 d and 60 d (P< 0.8024; RS = 5.15 ± 3.6, CON = 5.75 ± 3.5).

CHAPTER FOUR
DISCUSSION

The results described here address primary hurdles to adopting estrus resynchronization in U.S. beef cattle operations. The data suggest that it is possible to initiate a resynchronization protocol in beef cattle of unknown pregnancy status beginning 19 d after the first TAI. Pregnancy success at d 26 was not affected by the estrus resynchronization protocol used here. These findings were expected based on previous research showing that GnRH and progestin administration is not detrimental to existing pregnancies [9,54]. This outcome was further substantiated by comparing the 26 d to the 60-d pregnancy diagnosis, in which there was no indication of a difference in embryonic mortality between RS and CON animals.

Resynchronization is an efficient strategy for increasing the number of animals conceiving to AI, and it can be utilized strategically when the number of natural service sires is insufficient [48]. An efficient resynchronization protocol aims to initiate ovulation of nonpregnant females in a defined window, enabling an additional insemination without extending the interval to conception. Sá Filho et al. (2014) reported reduced conception rates to AI when GnRH was utilized in a resynch protocol beginning 22 days after an initial AI. The reduced fertility was attributed to the likelihood that a majority of the heifers in their study had already returned to estrus and ovulated by day 22 [48]. It is heavily reported that GnRH administration after ovulation would be insufficient in establishing a new follicular wave emergence [20-21, 34, 49, 53]. Thus, based on the pregnancy results achieved in this study, better follicular control was achieved by initiating the resynch protocol on d 19 post the initial TAI. Resynchronized females in this study had a total of 82.4% of cows bred by AI at d 60 (first and second TAI), compared to 57.5% in the CON group. The novel approach in this study is the use of prostaglandins (PGF2α) In the resynchronization protocol. One of the biggest hurdles in utilizing resynchronization is early and accurately identifying which females are open, thus limiting the use of prostaglandins in most cases. However, the vast improvements in modern ultrasound technology provide a solution to this. It has been reported, and shown in the results of this research, that pregnancy can

be diagnosed rapidly and accurately as early as 26 d post AI with transrectal ultrasonography [23].

It is well known and reported that increasing the number of females conceiving to AI accelerates the rate of genetic progress [3,14,18]. Larden et al. (2020) compared a commercial beef cow-calf operation utilizing natural service (NS) solely as the breeding method to an operation utilizing one round of FTAI. They showed that even though the NS program had a lower input cost (less than ~$38/cow), the FTAI program generated an increase in net profit ($284/cow) [33]. Additionally, differences in lifetime productivity have been reported. Females conceiving to AI as a yearling heifer exhibit greater longevity in the herd, and they tend to wean heavier calves when compared to their cohorts that conceived to NS [22].

In conclusion, the onset of a resynchronization protocol on 19 d after an initial TAI, specifically the 7-day CO-Synch + CIDR, successfully increased pregnancies sired by artificial insemination without affecting the distribution of expected calving as compared to traditional clean-up natural service breeding. Pre-established pregnancies were not affected by the resynchronization.

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APPENDIX A: FIGURES

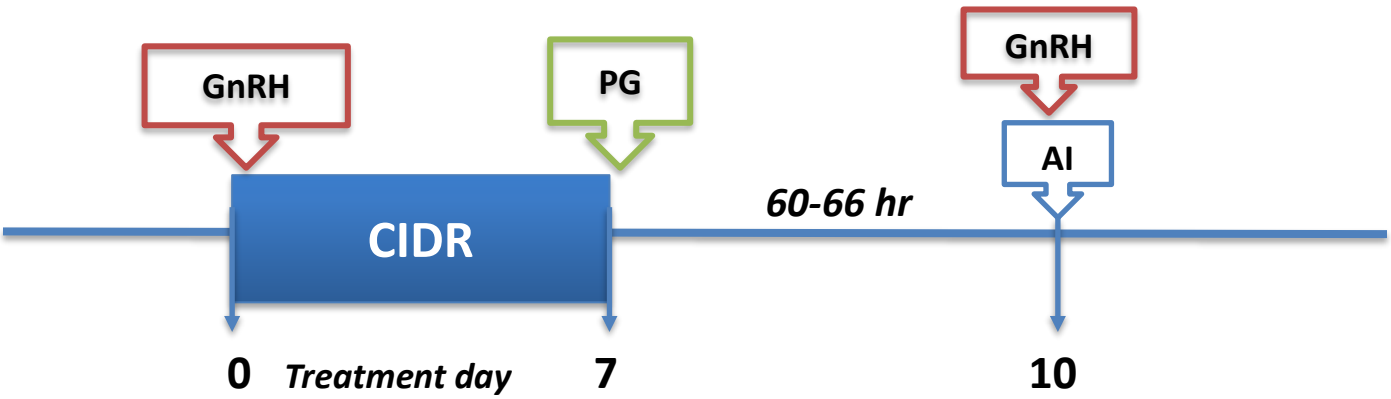

Figure 1.1 Treatment schedule for cows managed in 7 d CO-Synch + CIDR for TAI protocol. On d 0 cows received CIDR insertion and were administered GnRH.7 days later, the CIDR was removed, and cows were administered $PGF_{2\alpha}$. Approximately 66 hours later, GnRH is administered, and cattle are bred by FTAI.

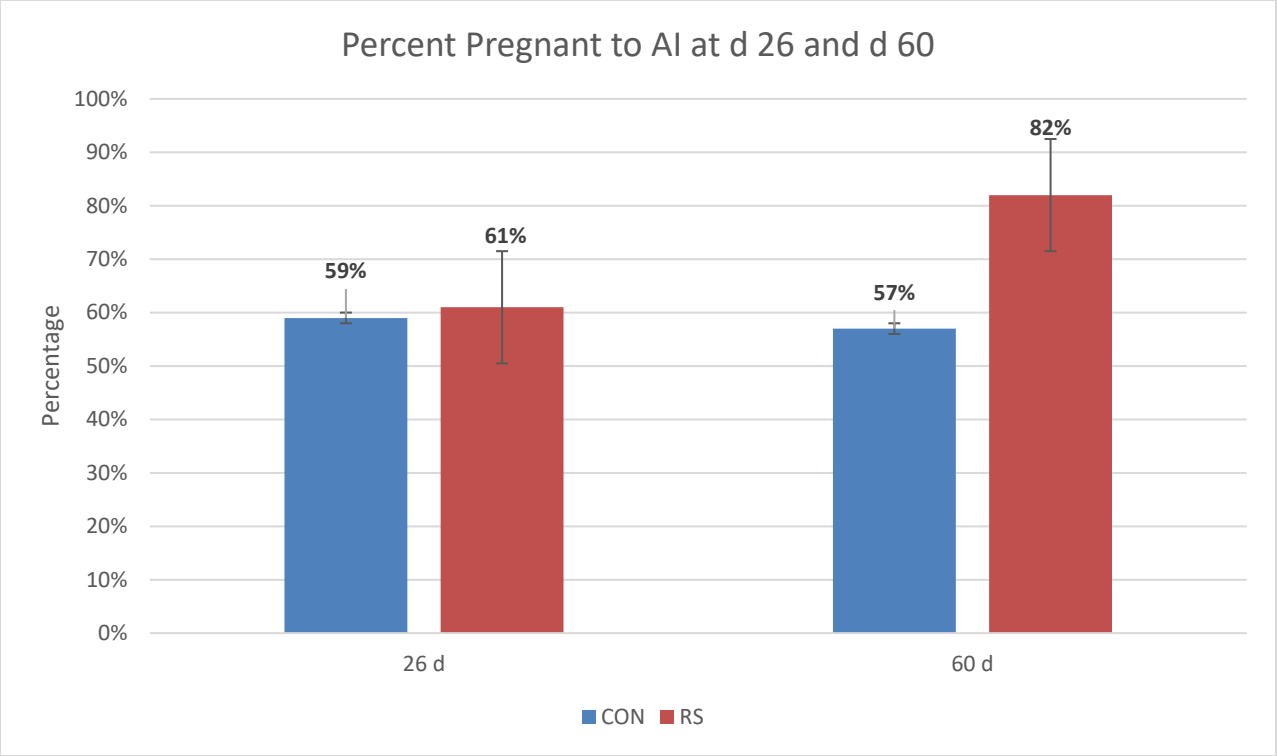

Figure 2.1 Effect of treatment on pregnancy to AI after two inseminations in the RS group.

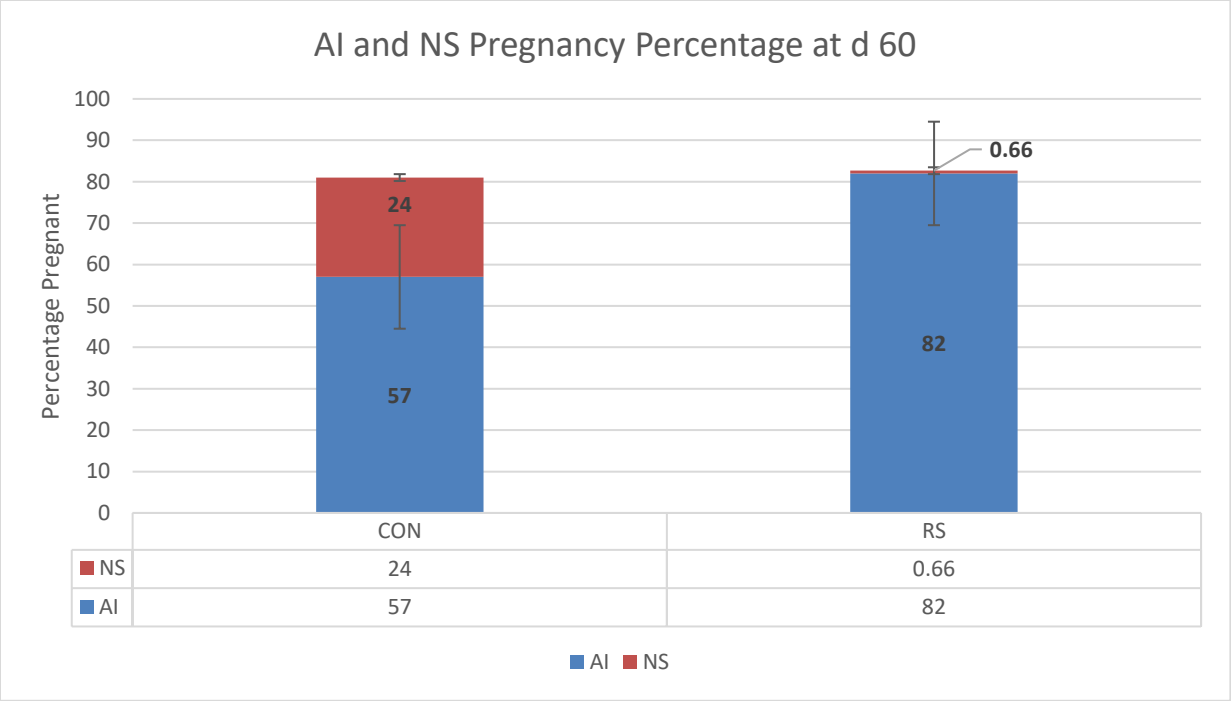

Figure 3.1 Effect of treatment on the percentage pregnant to AI (P< 0.0115) and NS (P< 0.0070) at day 60.

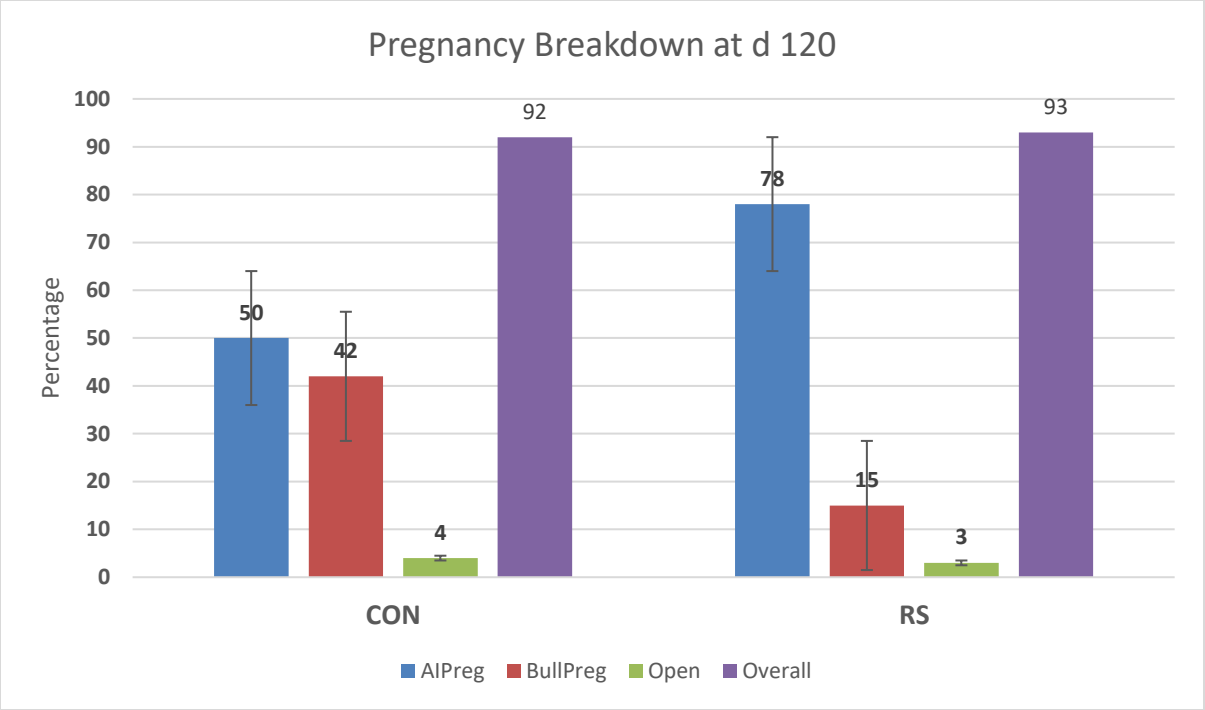

Figure 4.1 Effect of treatment on pregnancy to AI, natural service, overall, and percentage open at d 120.

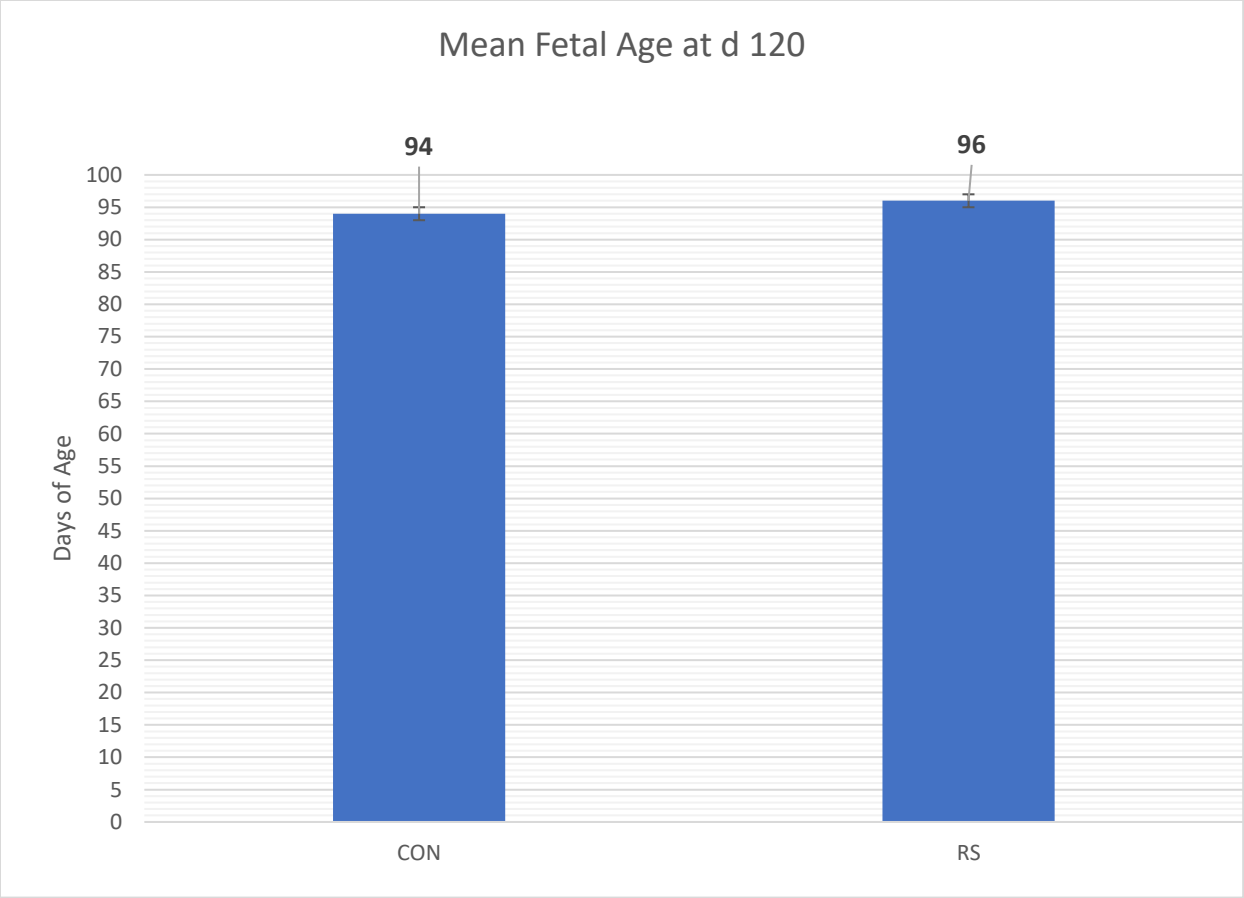

Figure 5.1 Effect of treatment on mean fetal age including open cows at 120 d post TAI-1.

APPENDIX B: TABLES

Table 1.1 Descriptive Statistics comparing the difference between treatment groups on day 120 excluding open cows

Treatment	(n)	Mean	Std. Deviation	Median	Range	Max	Min
CON	443	103.91	21.72	116	96	124	28
RS	449	103.63	23.07	116	96	124	28

VITA

Courtnie Bridges (Carter) was born in Wartrace, TN. She Studied at Troy University for three years, and then completed her B.S. in Basic and Applied Sciences at Middle Tennessee State University. Then, under the mentorship of Dr. Justin Rhinehart, Courtnie started working full time in the AgResearch and Extension arm of The University of Tennessee while also pursuing a M.S. in reproductive physiology. Upon completion of coursework in May 2022, Courtnie will begin a career with Trans Ova Genetics.