NOVEL APPROACHES TO INCREASE THE NUMBER OF PREGNANCIES GENERATED VIA ARTIFICIAL INSEMINATION IN BEEF HERDS

by

SAMIR BURATO

(Under the Direction of Pedro Levy Piza Fontes)

ABSTRACT

The objective of this research was to evaluate two different strategies to increase the number of artificial insemination (**AI**) pregnancies in beef herds. The first study evaluated the impact of early progesterone removal on pregnancy rates to fixed-time artificial insemination (**FTAI**) in presynchronized beef cows. Pregnancy rates of cows enrolled in the 7&7 Synch were compared with pregnancy rates of cows enrolled in a similar protocol combined with an early progesterone withdraw prior to FTAI. There were no differences in estrus expression and pregnancy rates between treatments. The second study evaluated the fertility of an early resynchronization strategy (**ER**) associated with color doppler ultrasonography (**CD**). Pregnancy, conception and cumulative pregnancy rates were compared between cows exposed to natural service as a second service strategy, and cows exposed to ER and FTAI as the second service. Early resynchronization resulted in fewer pregnancies compared to natural service as the second service strategy.

INDEX WORDS: fixed-time artificial insemination, presynchronization, color doppler ultrasonography, early resynchronization.

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"Be joyful in hope,
Patient in affliction,
Faithful in prayer"
(Rom.12.12).

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CHAPTER 1

INTRODUCTION

The United States beef production in 2023 was 26.9 billion pounds with the total cattle inventory estimated at 89.3 million heads (USDA, 2024). In 2022, the total cash receipts from marketing of meat animals were $117 billion, where cattle and calves accounted for 74% of this total (USDA, 2023). While United States has a distinguished position in the global food supply, recent data warned for the crescent global population and alarming food demand increase in the next decades. A meta-analysis forecasting global food demand estimated an increase of 35% to 56% in total food requirements in the next 4 decades (Van Dijk, et al., 2021), highlighting the need for development of sustainable and efficient strategies to enhance the global food production.

Beef cattle production in the state of Georgia is predominantly grazing cow-calf operations. Georgia cattle inventory totaled 1.03 million head in 2023, being the second largest commodity produced in the state (USDA, 2023). Notably, beef cows accounted for roughly 46% of this number. In a cow-calf scenario, in addition to becoming pregnant, the timing of conception within the breeding season has a major effect on the cow herd efficiency (Oosthuizen et al., 2018). Thus, reproductive strategies that add value to the calf crop and increase the percentage of cows weaning a heavy calf each year are highly recommended in order to improve the economic returns to the cow-calf producer.

Estrus synchronization (**ES**) is an efficient tool to enhance cow herd fertility and increase the number of females exposed to biotechnologies such as fixed-time artificial insemination (**FTAI**) and embryo transfer. Fixed-time artificial insemination overcomes the postpartum anestrus and allows producers to breed a greater number of cows earlier in the breeding season, while eliminating the need for estrus detection. Rodgers, et al. (2012) reported that the economic advantage FTAI compared to natural service were associated with the greater number of calves being born at the beginning of the calving season and the greater genetic merit added to the herd by utilizing proven genetics. Moreover, studies demonstrated that females that become pregnant earlier in the breeding season wean heavier calves (Funston, et al., 2012) and have greater longevity in the herd (Cushman, et al., 2013).

In the past 15 years, different strategies have been evaluated to facilitate the use of FTAI and increase the number of artificial insemination (**AI**) pregnancies. Presynchronization strategies aim to improve pregnancy rates to FTAI through increased ovulatory response to ES protocols, leading to more uniform subsequent follicular development (Bonacker et al., 2020; Perry et al., 2012), and increase fertility outcomes (Perry, et al., 2012; Oosthuizen, et al., 2020; Andersen et al., 2021). Also, increasing proestrus length and decreasing circulating concentrations of progesterone earlier in the development of the ovulatory follicle has been shown to increase pregnancy rates to FTAI (Bridges, et al., 2008; Bridges, et al., 2010). Additionally, other studies established resynchronization strategies to expose females that did not conceive to FTAI to a second attempt of AI without the need of estrus detection (Sa Filho, et al., 2014; Baruselli, et al., 2018).

In order to decrease the interbreeding interval in conventional resynchronization strategies, more recent data evaluated early resynchronization programs using the color doppler ultrasonography (**CD**; Pugliesi, et al., 2019; Motta, et al., 2020; Palhão, et al., 2020). Collectively, these strategies represent tools to enhance cattle reproductive efficiency; yet, further research is warranted to improve outcomes of current technologies. Therefore, the objective of this thesis is to evaluate the effects of manipulating follicular dominance in presynchronization programs and establish an early resynchronization protocol combined with the CD in *Bos taurus* beef cows.

References

Andersen, C. M., Bonacker, R. C., Smith, E. G., Spinka, C. M., Poock, S. E., & Thomas, J. M. (2021). Evaluation of the 7 & 7 Synch and 7-day CO-Synch + CIDR treatment regimens for control of the estrous cycle among beef cows prior to fixed-time artificial insemination with conventional or sex-sorted semen. Animal Reproduction Science, 235, 106892. https://doi.org/10.1016/j.anireprosci.2021.106892

Baruselli, P. S., Ferreira, R. M., Sá Filho, M. F., & Bó, G. A. (2018). Review: Using artificial insemination v. natural service in beef herds. Animal, 12, s45–s52. https://doi.org/10.1017/S175173111800054X

Bonacker, R. C., Stoecklein, K. S., Locke, J. W. C., Ketchum, J. N., Knickmeyer, E. R., Spinka, C. M., Poock, S. E., & Thomas, J. M. (2020). Treatment with prostaglandin F2α and an intravaginal progesterone insert promotes follicular maturity in advance of gonadotropin-releasing hormone among postpartum beef cows. Theriogenology, 157, 350–359. https://doi.org/10.1016/j.theriogenology.2020.08.018

Bridges, G. A., Helser, L. A., Grum, D. E., Mussard, M. L., Gasser, C. L., & Day, M. L. (2008). Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology, 69(7), 843–851. https://doi.org/10.1016/j.theriogenology.2007.12.011

Bridges, G. A., Mussard, M. L., Burke, C. R., & Day, M. L. (2010). Influence of the length of proestrus on fertility and endocrine function in female cattle. Animal Reproduction Science, 117(3–4), 208–215. https://doi.org/10.1016/j.anireprosci.2009.05.002

Cushman, R. A., Kill, L. K., Funston, R. N., Mousel, E. M., & Perry, G. A. (2013). Heifer calving date positively influences calf weaning weights through six parturitions1. Journal of Animal Science, 91(9), 4486–4491. https://doi.org/10.2527/jas.2013-6465

Funston, R. N., Musgrave, J. A., Meyer, T. L., & Larson, D. M. (2012). Effect of calving distribution on beef cattle progeny performance. Journal of Animal Science, 90(13), 5118–5121. https://doi.org/10.2527/jas.2012-5263

Motta, I. G., Rocha, C. C., Bisinotto, D. Z., Melo, G. D., Ataide Júnior, G. A., Silva, A. G., Gonzaga, V. H. G., Santos, J. A., Freitas, B. G., Lemes, K. M., Madureira, E. H., & Pugliesi, G. (2020). Increased pregnancy rate in beef heifers resynchronized with estradiol at 14 days after TAI. Theriogenology, 147, 62–70. https://doi.org/10.1016/j.theriogenology.2020.02.009

Oosthuizen, N., Fontes, P. L. P., Sanford, C. D., Ciriaco, F. M., Henry, D. D., Canal, L. B., DiLorenzo, N., & Lamb, G. C. (2018). Estrus synchronization and fixed-time artificial insemination alter calving distribution in Bos indicus influenced beef heifers. Theriogenology, 106, 210–213. https://doi.org/10.1016/j.theriogenology.2017.10.028

Oosthuizen, N., Fontes, P. L. P., Porter, K., & Lamb, G. C. (2020). Presynchronization with prostaglandin F2α and prolonged exposure to exogenous progesterone impacts estrus expression and fertility in beef heifers. Theriogenology, 146, 88–93. https://doi.org/10.1016/j.theriogenology.2020.02.010

Palhão, M. P., Ribeiro, A. C., Martins, A. B., Guimarães, C. R. B., Alvarez, R. D., Seber, M. F., Fernandes, C. A. C., Neves, J. P., & Viana, J. H. M. (2020). Early resynchronization of non-pregnant beef cows based in corpus luteum blood flow evaluation 21 days after Timed-AI. Theriogenology, 146, 26–30. https://doi.org/10.1016/j.theriogenology.2020.01.064

Perry, G. A., Perry, B. L., Krantz, J. H., & Rodgers, J. (2012). Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success1. Journal of Animal Science, 90(2), 489–494. https://doi.org/10.2527/jas.2011-4319

Pugliesi, G., Bisinotto, D. Z., Mello, B. P., Lahr, F. C., Ferreira, C. A., Melo, G. D., Bastos, M. R., & Madureira, E. H. (2019). A novel strategy for resynchronization of ovulation in Nelore cows using injectable progesterone (P4) and P4 releasing devices to perform two timed inseminations within 22 days. Reproduction in Domestic Animals, 54(8), 1149–1154. https://doi.org/10.1111/rda.13475

Rodgers, J. C., Bird, S. L., Larson, J. E., Dilorenzo, N., Dahlen, C. R., DiCostanzo, A., & Lamb, G. C. (2012). An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows1. Journal of Animal Science, 90(11), 4055–4062. https://doi.org/10.2527/jas.2011-4836

Sá Filho, M. F., Marques, M. O., Girotto, R., Santos, F. A., Sala, R. V., Barbuio, J. P., & Baruselli, P. S. (2014). Resynchronization with unknown pregnancy status using progestin-based timed artificial insemination protocol in beef cattle. Theriogenology, 81(2), 284–290. https://doi.org/10.1016/j.theriogenology.2013.09.027

USDA. (2023). Southern Region News Release Livestock and Milk Production and Income. Retrieved from Livestock and Milk Production and Income 04/28/2023 (usda.gov)

USDA. (2024). Livestock, Dairy, and Poultry Outlook: January 2024. Retrieved from: Livestock, Dairy, and Poultry Outlook: January 2024 (usda.gov)

Van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food, 2(7), 494–501. https://doi.org/10.1038/s43016-021-00322-9

CHAPTER 2

LITERATURE REVIEW

Reproductive physiology and estrus behavior in beef cows

Estrous cycle is a series of physiological events beginning at estrus and culminating in another estrus and ovulation. Cows are considered non-seasonal polyestrous animals, having multiple chances to conceive throughout the year. The estrous cycle is coordinated by neuro-endocrine events through the activity of the hypothalamus-pituitary-gonadal axis (SENGER, 2012). Onset of the estrous cycle occurs after puberty, when the hypothalamus achieves capacity to secrete gonadotropin-release hormone (**GnRH**) at the appropriate frequency and quantity to stimulate gonadotropin release by the pituitary. Estrous cycle in cattle is approximately 21 days and can be divided in two phases: follicular phase and luteal phase. Follicular phase can be further divided into proestrus and estrus, whereas the luteal phase can be divided into metestrus and diestrus.

Follicular phase

Follicular phase is marked by a period of increased estradiol secretion by ovarian follicles and decreased circulating progesterone concentrations. Progesterone is produced by the ovarian corpus luteum (**CL**) and has negative feedback on GnRH hypothalamic secretion. Therefore, after CL regression, a progressive decrease in circulating concentrations of progesterone alleviates its negative feedback on the hypothalamus. Under greater GnRH stimulus, the frequency of the luteinizing hormone (**LH**) pulses is increased, allowing the dominant follicle to progressively grow. Proestrus is considered the period from luteolysis to the LH ovulatory surge. During proestrus and estrus, the dominant follicle achieve maximum growth and estradiol secretion reaches a peak, leading to the preovulatory LH surge. The model proposed to explain the follicular synthesis and secretion of estradiol is referred to the 2-cell-2-gonadotropin model (Armstrong, et al., 1977). Ovarian Graafian follicles possess a fluid cavity, the cumulus oocyte complex and two populations of cells, granulosa and theca cells. According to the 2-cell-2-gonadotropin model, gonadotropins secreted by the anterior pituitary stimulate the follicular synthesis and secretion of estradiol. First, within the theca cells, LH binds to protein G receptors to increase the production of intracytoplasmic cyclic adenosine monophosphate (**cAMP**). Intracytoplasmic cyclic adenosine monophosphate activates protein kinase A, which induces steroidogenic enzymes involved in androgen synthesis. In a multi-step reaction, cholesterol is converted to testosterone, which is then transferred to the granulosa cells. Follicle stimulating hormone (**FSH**) binds to granulosa G-coupled protein receptors that activates aromatase. Finally, aromatase catalyzes the conversion of testosterone to estradiol within the granulosa cells.

Biotechnologies such as ES and FTAI manipulate the estrous cycle via exogenous hormonal treatments that alter follicular dynamics. A compelling body of evidence highlighted the relationship between follicle diameter, estradiol concentration, and fertility during proestrus of cows exposed to ES programs. In dairy cows, ovulation of smaller follicles decreased CL size and plasma concentrations of progesterone during early gestation, resulting in decreased pregnancy rates to AI (Vasconcelos, et al., 2001). Beef cows with follicles greater than 12 mm had greater pregnancy rates than cows with follicles $\leq$ 12 mm after a CO-Synch ES protocol (Lamb, et al., 2001). Greater pregnancy rates were reported in heifers with larger follicles, regardless of estrus expression (Perry, et al., 2007). In a large-scale trial utilizing *Bos indicus* cows exposed to estradiol and progestin-based protocols, there was positive linear relationship between follicular diameter and pregnancy success (Sa Filho, et al., 2010). Conversely, when *Bos taurus* beef cows were inseminated after spontaneous ovulation, follicle diameter did not affect conception rates (Perry, et al., 2005). Preovulatory follicle diameter was only a source of variation in conception rates when cows were induced to ovulate after GnRH injection.

In addition to follicle diameter, plasma concentrations of estradiol during proestrus plays a major role in fertility, positively influencing oocyte quality (Read et al., 2022), promoting sperm transportation in the oviduct (Hawk, 1983), and modulating uterine function to facilitate subsequent pregnancy establishment during the luteal phase (Ozturk and Demir, 2010; Madsen, et al., 2015). Several experiments have evaluated the impact of manipulating proestrus length on fertility and estradiol secretion in beef and dairy females (Day, 2015). Decreasing the interval between follicular wave emergence and luteolysis in ES protocols and prolonging proestrus length improved fertility of lactating dairy cows exposed to FTAI (Santos et al., 2010). In beef cows, extending proestrus length from 66 to 72 h increased pregnancy rates to FTAI (Bridges, et al., 2008) and preovulatory plasma estradiol concentration (Bridges et al., 2010). Conversely, studies experimentally increasing proestrus length reported no differences in pregnancy rates. Abreu et al., (2013, 2014) administrated PG either 2 days or 6 days after follicular wave emergence to create a difference of approximately 3 days in ovulatory follicle age between young (**YF**) and mature follicle (**MF**) treatment group. Although the YF treatment had longer proestrus and shorter period of dominance compared with the MF treatment, conception rates to AI based on estrus detection (Abreu, et al., 2013), or FTAI (Abreu, et al., 2014) did not differ between treatments. Further research is warranted to understand the factors that generated this inconsistency in the literature.

Preceding ovulation, the greater estradiol concentration leads to estrus expression. The behavior of estrus is characterized by visible signs of sexual receptivity and it lasts approximately 12 to 16 h in cows (Allrich, 1994). The relevant signs to estrus detection are increased activity, decreased dry matter intake, mucous vaginal discharge, and acceptance to mount. Previous studies extensively demonstrated that estrus expression is a major factor to fertility in reproductive biotechnologies such as AI and embryo transfer programs (Pereira et al., 2016; Rodrigues et al., 2018; Oosthuizen et al., 2020). Estrus behavior occurs as a consequence of the estradiol secretion by the dominant follicle above a certain threshold. Therefore, the increased fertility observed in females that express estrus is partially explained by mechanisms associated with estradiol secretion. In order to investigate the influence of reproductive steroidal hormones during embryo development, ovariectomized ewes were divided to receive or not injections of estradiol and progesterone to simulate the endogenous hormonal profile during estrus. Ewes that did not receive estradiol prior to embryo transfer had lower pregnancy success, indicating the importance of estradiol secretion for pregnancy establishment (Miller, et al., 1977). Atkins, et al. (2013) proposed a multi-factor pathway where estradiol concentration and follicle diameter are key factors in oocyte competence and embryo survival. Using a reciprocal embryo transfer approach, hormonal concentrations were monitored in donor cows and recipients to investigate factors influencing pregnancy establishment. Accordingly, increased estradiol concentration at insemination in the donor cow increased fertilization success. In recipient cows, pregnancy maintenance was greater when peri-ovulatory plasma concentrations of estradiol were increased and, circulating concentrations of progesterone on the day of the transfer were greater. In another study, cows standing in estrus had increased preovulatory circulating concentrations of estradiol and reduced uterine pH in the first 4 h of activity, which is associated with a better uterine environment for semen transport activity (Perry and Perry, 2008).

Luteal Phase

The luteal phase is characterized by CL formation and elevated concentrations of progesterone. It initiates immediately after ovulation and terminates when luteolysis occur. In the metestrus phase, cells of the ovulated dominant follicle undergo luteinization, where the theca and granulosa cells after structural remodeling become small luteal cells and large luteal cells, respectively (Donaldson and Hansel, 1965; Hansel and Convey, 1983). Corpus luteum is a transient gland responsible for progesterone secretion throughout gestation, therefore, it is of major relevance for pregnancy maintenance. Subsequent to these initial cellular modifications, luteal cells undergo hypertrophy and increases in volume (Yoshioka et al., 2013). Morphological studies of the CL revealed an exceptional capillary net and an intense process of angiogenesis during its formation (Augustin et al., 2000). Moreover, there is a rapid decline in intra-luteal blood perfusion during the CL regression. Acosta, et al. (2002) monitored the luteal blood perfusion via CD following PG administration in cows. Accordingly, hours after PG injection, luteal blood perfusion largely decreased, following a similar pattern of progesterone, as expected during luteolysis. Interestingly, PG injection over the beginning of the estrous cycle did not decrease blood flow and progesterone concentration, indicating the refractory period during the early luteal phase, as previously described (Levy, et al., 2000).

Following ovulation, multiple small follicles are recruited to grow under FSH stimulation. Real-time ultrasonography analysis allowed the determination of the wave-pattern growth of the ovarian follicles (Pierson and Ginther, 1986; Sirois and Fortune, 1988; Knopf, et al., 1989). Follicles grow under FSH and LH stimulation. At certain points of follicular growth, the dominant follicle secretes inhibin, which induces atresia in subordinate follicles. Inhibin, in a feedback system, inhibits the secretion of FSH, causing follicular regression. The dominant follicle; however, expresses LH receptors that allow growth during periods of low circulating concentrations of FSH. During diestrus, luteal concentrations of progesterone exert negative feedback on LH secretion. Thus, the dominant follicle also becomes atretic. Once all follicles have regressed, inhibin production decreases, and FSH recruits a new follicular wave that will follow a similar growth pattern. The dominant follicle only attains ovulatory competence following luteolysis and a decrease in circulating concentrations of progesterone. Due to the relevance of luteolysis and luteal blood flow to one of the experiments reported herein, this review provides more details about the complex physiological events occurring during luteal regression.

Luteolysis

Luteolysis is a remarkable physiological event that causes CL regression and allows for the pre-ovulatory LH surge. In mammals, PG is considered the main luteolytic factor (McKracken, et al., 1999). The role of PG as the luteolytic hormone in the normal estrous cycle has been extensively described (Knickerbocker, et al., 1988; Bonnin, et al., 1999;

Wiltbank, et al., 2018). Kindahl, et al. (1976) observed increased pulses of PG plasma metabolite, 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ (PMGF) in the last three days of the estrous cycle, followed by a large decrease in progesterone levels. Exogenous injection of 30 mg of PG administered during diestrus caused decrease in the luteal diameter and circulating concentration of progesterone from 4.0 ± 0.4 ng/ml to 1.5 ± 0.2 ng/ml (Louis, et al., 1973) and intrauterine infusions of PG in Holstein cows caused luteolysis followed by increased estrogen secretion, an ovulatory surge of LH (Louis, et al., 1974). Moreover, immunization against PG in ewes prevented normal resumption of the estrous cycle (Scaramuzzi, et al., 1973).

Studies in sheep and cattle described the role of the uterus in PG secretion (Anderson et al., 1969). Complete hysterectomy in cows and ewes extended the CL lifespan up to 100 days (Wiltbank and Casida, 1956). However, when the horn ipsilateral to the CL was maintained, the CL lifespan was not prolonged, indicating a local utero-ovary effect of PG action (Moor and Rowson, 1966). It has been defined that the site of PG synthesis is the epithelial cells of the endometrium (Tithof, et al., 2007). This biochemical process initiates with the formation of the arachidonic acid from the endometrium phospholipids. Arachidonic acid, the main precursor of prostaglandins, is converted to PG via cyclooxygenase (COX) enzymatic activity (Arosh, et al., 2004). The timing of luteolysis in cattle is expected to be around day 15 after ovulation (Ginther, et al., 2007). Mechanisms regulating the timing of luteolysis are coordinated by the expression of oxytocin receptors, estradiol secretion, and downregulation of progesterone receptors (Silvia, et al., 1991). In the normal estrous cycle, pulsatile release of oxytocin from the neurohypophysis is followed by PG pulses (SENGER, 2012). Other studies showed the contribution of luteal oxytocin to PG pulses (Kotwica, et al., 1993). Uterine response to oxytocin stimulus is modulated by the expression of oxytocin receptors after increased estradiol secretion by the dominant follicle (Fleming, et al., 2006). During late-diestrus, the long exposure to high progesterone concentration results in the downregulation of progesterone receptors. Downregulation of progesterone receptors results in the upregulation of estrogen receptors. Activation of estrogen receptors in the uterine luminal epithelium results in the upregulation of oxytocin receptors, allowing for oxytocin to elicit PG production (SENGER, 1997).

Initial indication of luteolysis is the diminished ability of luteal cells to secrete progesterone, characterized as functional luteolysis. Modulation of functional luteolysis involves intricate interactions among steroidogenic cells, endothelial factors, and immune cells (Korzekwa, et al., 2008). Studies demonstrated the role of endothelin-1 as a driver of steroidogenic action loss. Endothelin-1 is an amino acid produced by endothelial cells that has a potent vasoconstrictor effect. In cows exposed to PG analogue (Cloprostenol), it was observed a rapid increase in endothelin-1 and its receptors, followed by a decrease in the CL steroidogenic capacity (Levy, et al., 2000). Similarly, administration of 100 µg endothelin-1 reduced plasma progesterone concentrations in ewes during diestrus (Hinckley and Milvae, 2001). Endothelial cells count for more than 50% of the luteal cell population (O'Shea, et al., 1989), therefore, luteal blood restraint is an important mechanism of luteal cell death. Authors have also emphasized the role of leukotrienes, cytokines, and nitric oxide as modulators of luteal regression (Skarzynski and Okuda, 2010). Following functional luteolysis, the CL undergoes structural luteolysis, a process characterized by a progressive reduction in volume. Structural regression of the CL occurs through cellular apoptosis. Studies have indicated that the apoptosis of luteal cells is mediated by TNF-α and IFN-γ, produced by T lymphocytes and macrophages. Reactive oxygen species, discharged through the activation of protein kinase C also contributes to the apoptosis of luteal cells (Sugino and Okuda, 2007).

Pharmacological control of the estrous cycle

One of the biggest limitations of the use of reproductive biotechnologies is the ability to accurately detect estrus. The difficulty is even more pronounced in beef cattle systems, where producers need to gather cows and bring them to handling facilities randomly according to the individual time of estrus in each animal. Therefore, research aimed to establish a method to synchronize estrus and ovulation of cows. The first attempts to synchronize the estrous cycle dated from the early 1970's. Administration of PG during random stages of the estrous cycle induced estrus and ovulation within 3 days (Louis, et al., 1973; Lauderdale, et al., 1974). Moreover, a single administration of PG in heifers was effective in inducing estrus in only 60% of them; but, a second injection 12 days apart induced estrus in 88% of animals (Hafs, et al., 1975). Although fertility in cows inseminated after PG injection was acceptable (Hafs, et al., 1975), this strategy had two main limitations; first, PG only induced heat in cycling animals after the refractory period of the CL, which corresponds to the first 5 days of the estrous cycle (Levy et al., 2000). Second, the relatively long window of time where cows show heat (within 4 days) made the estrus synchrony not precise enough for FTAI. Therefore, more research was needed to increase the percentage of cows responding to the protocol and decrease the window of time where cows come in estrus. More recent studies reported better synchronization rates and synchrony precision by combining PG injection with GnRH analogues (Thatcher, et al., 1989; Twagiramungu, et al., 1992), indicating that for a better synchronization protocol, luteal regression must be associated with induction of ovulation via GnRH. It was only in 1995 that an ES protocol was combined with FTAI in dairy cows and yielded acceptable pregnancy rates (Pursley, et al., 1995). In this study, a GnRH injection was administered to induce ovulation of a dominant follicle. Seven days later, administration of PG induced luteal regression to decrease circulating concentrations of progesterone and allow greater secretion of gonadotropins to promote follicular development. Another injection of GnRH was performed 48 h after PG injection to induce preovulatory LH surge, and cows were timed breed 24 h later. This protocol is referred to OvSynch, and is considered the first ES protocol to yield acceptable pregnancy rates to artificial insemination, while eliminating the need for estrus detection. In beef cows, administration of the second GnRH concurrently with FTAI (COSynch) in order to eliminate one trip to the chute, yielded similar conception rates to the regular OvSynch (Geary, et al., 2001).

The limitation of these GnRH and PG based protocols is the relatively high lack of response to the 1st GnRH. As a result, a percentage of cows that did not ovulate to the 1st GnRH can potentially express estrus between the GnRH administration and the PG injection, compromising the synchrony of the protocol. In addition, exposure to exogenous progesterone can be used to overcome the postpartum anestrus (Perry et al., 2004), thus, several studies investigated the utilization of progestins to improve fertility in FTAI protocols (Lamb and Mercadante, 2016). When a single injection of PG was compared with insertion of a controlled internal progesterone release device (**CIDR**) for 7 days, and PG on the day before CIDR removal, it was reported better estrus expression and pregnancy rate in beef females treated with CIDR (Lucy, et al. 2001). Similarly, Lamb, et al. (2001) compared the COSynch protocol versus COSynch associated with CIDR for 7 days. In this study, anestrous cows exposed to CIDR had greater pregnancy rates compared to anestrous cows with no exogenous progesterone. Accordingly, treatment with a CIDR device resumed cyclicity in postpartum anestrous cows (Perry, et al., 2004; Meneghetti, et al., 2009). Stevenson, et al. (2000), reported increased estrus detection in cows exposed to a synthetic analogue of progesterone (Norgestomet). Moreover, this study reported better pregnancy rates when cows were synchronized and inseminated after estrus detection over cows that were FTAI. This synchronization protocol that utilizes estrus detection and clean-up AI at 84h was called Select Synch. In a large-scale trial, the COSynch + CIDR protocol yielded similar pregnancy rates compared to the Select Synch and proved to be a reliable FTAI protocol for beef cows (Larson, et al., 2006) and heifers (Lamb, et al., 2006).

In order to optimize fertility to FTAI protocols, other studies investigated the effects of the period of dominance and proestrus length to fertility (Day, 2015). Shortening the period of progesterone exposure (5-day CIDR) and extending the period from **PG** to insemination to 72 h increased fertility in beef cows (Bridges, et al., 2008). Also, the same group reported increased estradiol concentrations as the proestrus length was extended (Bridges, et al., 2010).

Currently, there are different variations of FTAI protocols utilizing GnRH, PG and progesterone to induce cyclicity and synchronize ovulation. Regardless of the protocol, the use of FTAI in beef herds have consistently increased the reproductive efficiency and largely incremented the genetic merit of commercial cow-calf operations.

Yet, novel strategies aim to enhance synchronization rates via presynchronization and, augment the number of AI pregnancies through a timed rebreeding program.

Recent alternatives to maximize the number of pregnancies generated via artificial insemination

Presynchronization

The primary hormones utilized in FTAI protocols in United States are GnRH, PG and progesterone. Although current protocols yield acceptable pregnancy rates, it has been reported that only 66% of cows at random stages of the estrous cycle ovulate after a GnRH analogue injection (Geary, et al., 1998), leading to suboptimal follicular growth synchrony (Thompson, et al., 1999). Several studies aimed to understand the factors influencing GnRH ovulatory response in synchronization protocols. Vasconcelos, et al. (1999) reported that cows induced to ovulate after a GnRH injection in the first 4 days of the estrous cycle had lower (23%) ovulatory response compared to cows on days 5-9 (96%), 10-16 (54%) and 17-21 (77%), suggesting an ideal stage of the estrous cycle to start the OvSynch protocol. In another study, ovulation rates following a GnRH injection in heifers were 0%, 100%, 25%, 60%, and 100% on days 2, 5, 10, 15, and 18 of the estrous cycle, respectively (Moreira, et al., 2000). Similarly, ovulatory response to GnRH significantly varied according to the day of the estrous cycle when GnRH was injected (Martinez, et al., 1999). In a series of experiments, Sartori, et al. (2001) demonstrated that follicular diameter is another factor influencing follicular ovulatory response. In this study, cows were injected with purified LH at known days of the estrous cycle or at known follicular diameter. Ovulation was induced in all follicles greater than 12 mm; however, no ovulation occurred in follicles smaller than 10 mm after administration of 4 mg of purified LH. Moreover, increasing the LH dose to 40 mg induced ovulation in 10 mm follicles, although follicles of 7.0 and 8.5 mm still failed to ovulate under greater gonadotropin stimulus, indicating that LH dose can partially influence ovulatory response. As previous described, the ovarian follicular dynamics follow a wave-like pattern, having a growing, static, and regressing phase (Ghinter, et al., 1996). Thus, depending on the day of the estrous cycle, dominant follicles may have a different physiological maturity, varying in size and number of gonadotropins receptors. Accordingly, the levels of LH receptor mRNA in theca cells of antral follicles increased with follicular size and, LH receptors were only expressed in granulosa cells in follicles greater than 9 mm (Xu, et al., 1995). Similarly, the number of LH receptors in the granulosa cells were greater for dominant follicles after day 4 of the follicular wave compared to subordinate follicles (Evans and Fortune, 1997). Furthermore, another group reported the effects of plasma progesterone concentrations on ovulation after GnRH administration (Colazo, et al., 2008). In this study, the ovulatory response of heifers with different plasma progesterone concentrations at the time of the GnRH injection were evaluated. Heifers with decreased plasma concentrations of progesterone had greater ovulatory response after GnRH administration compared with heifers with greater concentrations of progesterone. Moreover, there was a linear relationship between peak plasma LH and the plasma progesterone concentration after GnRH injection. Collectively, these data suggest that the dominant follicle achieve ovulatory capacity after achieving a certain maturity threshold and, factors such as follicle diameter, stage of the cycle, and circulating concentrations of progesterone impact the ovulatory response to exogenous GnRH analogues.

To enhance follicular synchrony and conception rates in FTAI protocols, several studies attempted to develop presynchronization strategies to increase the percentage of cows with a GnRH responsive follicle at the beginning of the FTAI protocol. Progestin exposure prior to GnRH increased fertility in beef females (Patterson, et al., 1990; Stegner, et al., 2004). Heifers presynchronized with CIDR for 14 days prior to a GnRH injection (CIDR Select) and FTAI had significant greater conception rates compared to heifers submitted to the 7 Day CO-Synch + CIDR protocol (Busch, et al., 2007). In another study, presynchronization with a low progestin dose and PG injection consistently increased ovulation rate to GnRH ($P < 0.01$); however, pregnancy rates were not affected by treatment (Small, et al., 2009). Greater ovulation rate reported in this study was likely associated with the formation of a large dominant follicle responsive to the GnRH because of the sub-luteal concentrations of progesterone prior to the initiation of the protocol. Accordingly, maintenance of the dominant follicle and follicular overgrowth has been well-documented in cows with a sub-luteal plasma progesterone concentration (Taylor, et al., 1993; Stock and Fortune, 1993). Recently, studies reported greater pregnancy rates and estrus expression in beef females presynchronized with CIDR for 7 days and PG at CIDR insertion. The 7&7 Synch is a PG and progestin-based presynchronization strategy that has been adopted in ES programs in beef cattle for both replacement heifers (Oosthuizen, et al., 2018, 2020) and postpartum cows (Andersen, et al., 2021). The underlying rationale of this strategy is to increase the proportion of cows with a dominant follicle at the time of the first GnRH injection, leading to a greater response to the initial GnRH and increased subsequent follicular growth synchrony (Bonacker et al., 2020).

Resynchronization of ovulation

Resynchronization protocols have been developed to expose females that did not conceive to the first FTAI to a second AI service while eliminating the need for estrus detection (Sa Filho, et al., 2014). Generally, cycling cows that failed to conceive express estrus approximately 15 to 25 days after the first AI (Larson et al., 2009). Thus, non-pregnant cows exposed to natural service conceive during this time range. Strategies that utilize estrus detection yield similar pregnancy rates compared to natural service and, are efficient tools to increase the number of pregnancies in the beginning of the breeding season (Larson et al., 2009); however, estrus detection is time consuming and labor intensive, thus, it might not be feasible for certain operations. To optimize rebreeding strategies following estrus, studies attempted to concentrate estrus detection via utilization of exogenous progestin devices (Van Cleef, et al., 1996; Chenault, et al., 2003; Larson, et al., 2009). In Stevenson, et al. (2003), insertion of a previously used progestin device on day 13 after insemination successfully concentrated the estrus return of beef heifers. Similarly, resynchronization with a CIDR increased the estrus synchrony in inseminated beef heifers with unknown pregnancy status and yielded acceptable conception rates (Colazo, et al., 2006). Conversely, other studies aimed to establish a FTAI rebreeding programs to completely eliminate estrus detection. In a large-scale study, *Bos indicus* females resynchronized and timed bred following a pregnancy diagnosis 30 days apart from the initial FTAI yielded up to 85% cumulative AI pregnancy rates (Marques, et al. 2015). In this study, although the considerably high conception rates, the 40 days interval between the two breeding events failed to generate pregnancies in the first estrus after AI, delaying the timing of conception within the breeding season. Therefore, to decrease the timing of conception through a timed rebreeding protocol, *Bos indicus* females were resynchronized 22 days after the initial FTAI utilizing a progestin-based protocol (Sa Filho, et al., 2014). In this study, Nelore heifers with unknown pregnancy status were submitted to one of two treatments; Resynch: heifers received a norgestomet ear implant and estradiol benzoate injection 22 days after insemination. Seven days later, pregnancy diagnosis was performed and heifers diagnosed as non-pregnant, received PG and FTAI 48h later; Control: heifers were submitted to natural service from day 15 to 30 after the initial FTAI. Resynchronization substantially increased the number of AI pregnancies compared to natural service, without detrimental effects to the pre-established pregnancy, measured through pregnancy losses between the two groups and pregnancy rates to the first FTAI. In this strategy, the timing between the first and second FTAI was decreased by 8 days compared to the strategy reported in Marques, et al. (2015), allowing for a tighter breeding season. However, these traditional resynchronization protocols still require a pregnancy diagnosis to detect non-pregnant females, which can only be accurately performed after 26 days of gestation with the brightness (B)-mode conventional ultrasonography (Pieterse, et al., 1990; Quintela, et al., 2012). Consequently, the limiting factor is the inability to detect the non-pregnant animals during the time range where cows return in estrus.

Pregnancy diagnosis based on luteal blood perfusion

To decrease the time necessary to confirm gestation in cattle, a set of studies aimed to validate the CD as a tool to diagnose pregnancy earlier than conventional methods (Fontes and Oosthuizen, 2022). Currently, the gold-standard method to diagnose pregnancy early in cows relies on the visualization of a conceptus with a heartbeat through transrectal ultrasonography. According to Quintela, et al. (2012), the accuracy of the B-mode ultrasonography for pregnancy diagnosis ranged from 85.7% on day 24-26 of pregnancy to 95% on days 30-35. Similarly, maximum sensitivity and negative predictive value for pregnancy diagnosis by transrectal ultrasound was found to be on day 26 for heifers, and day 29 for cows (Romano, et al., 2006). Color doppler ultrasonography allows for the estimation of blood flow that can be used to indicate the functionality of body structures. Blood flow is estimated through the principles of the Doppler effect, characterized by the change in frequency of a wave when the wave observer or wave source and the target object move towards or away from one another. In the context of blood vessels, the movement of the red blood cells causes changes in the wave frequency that is received by the transducer (Ginther and Utt, 2004). Color doppler mode allows for the visualization of these changes in frequency as color signals superimposed over a conventional B-mode image. Higher frequencies occur when the blood cells are moving towards the transducer and are generally indicated by colors ranging from yellow to red. Alternatively, lower frequencies occur when blood cells are moving away from the transducer and are generally indicated by colors ranging from green to blue (Fontes and Oosthuizen, 2022).

Analysis of plasma progesterone concentrations are the gold standard method to assess luteal function. However, plasma progesterone measurements are not rapidly accessible chute-side, limiting its commercial application in cattle reproductive management. Alternatively, an extensive body of literature has shown that blood perfusion is associated with CL lifespan and functionality (Miyamoto, et al., 2005;

Acosta, et al., 2002) and the percentage of blood perfusion signals on the CL is a reliable strategy to access luteal function (Pugliesi, et al., 2023). In Guinter, et al. (2007), progesterone concentrations and percentage of CL with blood perfusion measured via CD were monitored daily in heifers following spontaneous luteolysis. Percentage of colored pixels on the CL surface decreased and was associated with progesterone decline during luteolysis. Yet, when luteolysis was induced systemically with 500 µg cloprostenol, there was a slight increase in blood perfusion within 0.5 h after cloprostenol injection, followed by a consistent decrease 6-12 h afterwards. In another experiment, progesterone concentrations, percentage of blood perfusion, and circulating concentrations of PGFM were monitored in 12-h intervals. PGFM concentrations started to increase 60 to 24 h before the progesterone concentrations were < 1ng/ml. Progesterone concentrations started to decrease 48 h before reaching the setpoint of 1 ng/ml at 0 h. Ultimately, percentage of the CL with blood perfusion signals started to decrease 24 h before circulating progesterone reached 1 ng/mL, ranging from a 60% decrease at 24 h before, to a 15% decrease 24 h after progesterone reached 1 ng/ml. In another study, ultrasonography evaluations and progesterone sampling over the normal estrous cycle revealed that luteal blood perfusion and progesterone concentrations decreased in 80% within a period of 48 h during the regression phase (Herzog, et al., 2010). Remarkably, luteal size had a delayed decline compared to progesterone concentrations. Thus, the authors concluded that luteal blood perfusion during luteolysis was a better indicator of luteal function compared to luteal size (Herzog, et al., 2010).

Due to the greater correlation among progesterone concentration and luteal blood perfusion during luteolysis, studies aimed to evaluate the use of blood perfusion estimates via CD to diagnose pregnancy earlier than industry-standard methods. Early attempts to validate the CD for pregnancy diagnosis on days 17 and 19 after insemination concluded to be a less reliable method due to the low accuracy and specificity (Utt, et al., 2009; Herzog, et al., 2011). When dairy cows were examined for luteal blood perfusion on day 20 after insemination, more promising results were reported, with an accuracy of 74.8% and 99% sensitivity (Siqueira, et al., 2013). These differences among studies were attributed to the early stages after insemination when CD was performed in Utt, et al. (2009) and Herzog, et al. (2011), leading to greater variation in luteal blood perfusion among cows that failed to conceive. In another study performed with *Bos indicus* beef cows, both luteal tissue area and luteal blood perfusion were considered reliable methods to detect non-pregnant cows on day 20 after AI. The best results were found when both methods were combined (Pugliesi, et al., 2014). Recently, our research group validated CD for early pregnancy diagnosis in *Bos taurus* beef females (Holton, et al., 2022a,b). In the first set of works (Holton, et al., 2022a), 178 beef heifers were FTAI on day 0 and pregnancy confirmed via CD on day 20 and 22. Conventional ultrasonography was performed on days 29 and 94 to confirm pregnancy status. Pregnant heifers had greater CL diameter, area, volume, and blood perfusion when compared with nonpregnant heifers on days 20 and 22. Accuracy of CD on days 20 and 22, were 91% and 94%, respectively. No false-negative results were observed for CD on both days 20 and 22 (negative predicted value = 100%) and false-positive results represented 8% and 6% of the diagnoses. Similarly, in beef cows (Holton, et al., 2022b), pregnant cows had larger and more vascularized CL compared with nonpregnant cows, accuracy for CD on days 20 and 22 were 87% and 92%, respectively and, no false negative (FN) results were observed.

Collectively, these data suggest that CD is a reliable tool to access luteal function and to diagnose gestation after the expected period of luteolysis, due to its relatively high accuracy and convenience to be performed chute-side. Moreover, the absence of false-negative results allows for early resynchronization protocols, minimizing the risk of inducing abortions due to prostaglandin administration.

Early Resynchronization via Color Doppler Pregnancy Diagnosis

Resynchronization strategies followed by FTAI has been largely utilized in beef herds in South America, where the vast areas and the drastic anestrus postpartum of *Bos indicus* cows in tropical countries limit estrus detection strategies (Baruselli, et al., 2018); however, the major drawback of conventional resynchronization protocols is the long interval between reproductive services. As discussed, pregnancy can be only accurately detected 26-29 days of gestation, therefore, resynchronization involving luteolytic drugs cannot be performed before this period. In an efficient cow-calf operation, in addition to the calf crop percentage, timing of conception plays a key role in the profitability of the system. Several studies demonstrated the benefits of a short breeding season and early calving (Funston, et al., 2012; Cushman, et al., 2013). Thus, the utilization of CD to diagnose pregnancy at 20 days represents an alternative to timed early rebreeding strategies.

Recently, several studies have evaluated strategies combining an estradiol and progesterone-based resynchronization protocol and CD. Pugliesi, et al. (2019) compared conception rates to FTAI in Nelore heifers submitted to an early resynchronization protocol with or without a long-acting progesterone (P4-LA). In this study, heifers received progesterone 12 days after the initial FTAI. At day 20, cows identified as non-pregnant via CD received a PG injection, estradiol cypionate and equine chorionic gonadotropin and FTAI 48 h later. The LA-P4 had greater conception rates in the second round of insemination (60.9 vs 44.6%) and the cumulative pregnancy rates did not differ among groups (76%). In a previous report, estradiol benzoate was utilized in the beginning of the resynchronization protocol, however, cows injected with estradiol during early gestation had decreased pregnancy establishment, suggesting a negative effect of administering estradiol benzoate in cows with unknow pregnancy status (Vieira, et al., 2014). Therefore, in Pugliesi, et al. (2019), only progesterone was utilized to synchronize the follicular wave. Conventional FTAI protocols synchronize ovulation through follicular atresia administering estradiol in combination with progesterone (Meneghetti et al., 2009; Bo et al., 2019), or ovulation of the dominant follicle (Larson, et al 2006) with GnRH. Generally, progesterone administration by itself can regress dominant follicles and recruit a follicular wave. However, only high doses of progesterone can induce atresia in follicles before divergence (Cavalieri, 2018). Thus, a long-acting progesterone source associated with a CIDR was utilized in Pugliesi, e al. (2019). Conversely, other studies utilizing estradiol to resynchronize 12-14 days after insemination reported acceptable pregnancy rates and no harmful effect to the established gestation (Stevenson, et al., 2003; Mota, et al., 2020). In Palhao, et al. (2020), a resynchronization protocol starting 13 days after the initial FTAI and rebreeding on day 23 successfully increased the number of AI pregnancies in the beginning of the breeding season, yielding 70.5% cumulative conception rate in the first 23 days. Similarly, others reported acceptable conception rates and cumulative AI pregnancies in early resynchronization strategies in *Bos indicus* cattle (Andrade, et al., 2020; Motta, et al., 2020; Ataide Junior, et al., 2021; Silva et al., 2022). Accordingly, these data suggests that early resynchronization associated with CD can effectively increase the number of AI pregnancies and decrease the rebreeding interval.

Although extensive literature studied early resynchronization protocols in *Bos indicus* cattle, these strategies have never been investigated in GnRH-progestin-based protocols in *Bos taurus* cows. In United States, the only FDA-approved drugs for ES are GnRH, PG and progesterone. Therefore, synchronization is achieved through induction of ovulation after GnRH administration. In the context of early resynchronization in GnRH-progestin-based protocols, inducing a newly accessory CL would compromise the accuracy of the CD, which is based on luteal viability. Thus, strategies to resynchronize cows with unknow pregnancy status must rely on progestin protocols only. Further research is required to evaluate the fertility of *Bos taurus* cows exposed to early resynchronization strategies without the use of GnRH at CIDR insertion.

References

Abreu, F. M., Cruppe, L. H., Maquivar, M., Utt, M. D., Madsen, C. A., Vasconcelos, J. L. M., Mussard, M. L., Day, M. L., & Geary, T. W. (2014). Effect of follicle age on conception rate in beef heifers1. Journal of Animal Science, 92(3), 1022–1028. https://doi.org/10.2527/jas.2013-6761

Abreu, F. M., Geary, T. W., Cruppe, L. H., Madsen, C. A., Jinks, E. M., Pohler, K. G., Vasconcelos, J. L. M., & Day, M. L. (2014). The effect of follicle age on pregnancy rate in beef cows1. Journal of Animal Science, 92(3), 1015–1021. https://doi.org/10.2527/jas.2013-6760

Acosta, T. J., Yoshizawa, N., Ohtani, M., & Miyamoto, A. (2002). Local Changes in Blood Flow Within the Early and Midcycle Corpus Luteum after Prostaglandin F2α Injection in the Cow1. Biology of Reproduction, 66(3), 651–658. https://doi.org/10.1095/biolreprod66.3.651

Allrich, R. D. (1994). Endocrine and Neural Control of Estrus in Dairy Cows. Journal of Dairy Science, 77(9), 2738–2744. https://doi.org/10.3168/jds.S0022-0302(94)77216-7

Alves, R. L. O. R., Silva, M. A., Consentini, C. E. C., e Silva, L. O., Folchini, N. P., Oliva, A. L., Prata, A. B., Gonçalves, J. R. S., Wiltbank, M. C., & Sartori, R. (2021). Hormonal combinations aiming to improve reproductive outcomes of Bos indicus cows submitted to estradiol/progesterone-based timed AI protocols. Theriogenology, 169, 89–99. https://doi.org/10.1016/j.theriogenology.2021.04.007

Andersen, C. M., Bonacker, R. C., Smith, E. G., Spinka, C. M., Poock, S. E., & Thomas, J. M. (2021). Evaluation of the 7 & 7 Synch and 7-day CO-Synch + CIDR treatment regimens for control of the estrous cycle among beef cows prior to fixed-time artificial insemination with conventional or sex-sorted semen. Animal Reproduction Science, 235, 106892. https://doi.org/10.1016/j.anireprosci.2021.106892

ANDERSON, L. L., BLAND, K. P., & MELAMPY, R. M. (1969). Comparative Aspects of Uterine-Luteal Relationships. In Proceedings of the 1968 Laurentian Hormone Conference (pp. 57–104). Elsevier. https://doi.org/10.1016/B978-0-12-571125-8.50005-3

Andrade, J. P. N., Gomez-León, V. E., Andrade, F. S., Carvalho, B. P., Lacouth, K. L., Garcia, F. Z., Jacob, J. C. F., Sales, J. N. S., Wiltbank, M. C., & Mello, M. R. B. (2020). Development of a novel 21-day reinsemination program, ReBreed21, in Bos indicus heifers. Theriogenology, 155, 125–131. https://doi.org/10.1016/j.theriogenology.2020.04.021

Armstrong, D. T., & Dorrington, J. H. (1977). Estrogen biosynthesis in the ovaries and testes. Advances in Sex Hormone Research, 3, 217–258.

Arosh, J. A., Banu, S. K., Chapdelaine, P., Madore, E., Sirois, J., & Fortier, M. A. (2004). Prostaglandin Biosynthesis, Transport, and Signaling in Corpus Luteum: A Basis for Autoregulation of Luteal Function. Endocrinology, 145(5), 2551–2560. https://doi.org/10.1210/en.2003-1607

Ataide Junior, G. A., Kloster, A., de Moraes, É. G., Motta, I. G., Claro Junior, I., Vasconcelos, J. L. M., Ferraz, P. A., de Paula Nogueira, G., & Pugliesi, G. (2021). Early resynchronization of follicular wave emergence among Nelore cattle using injectable and intravaginal progesterone for three timed artificial inseminations. Animal Reproduction Science, 229, 106759. https://doi.org/10.1016/j.anireprosci.2021.106759

Atkins, J. A., Smith, M. F., MacNeil, M. D., Jinks, E. M., Abreu, F. M., Alexander, L. J., & Geary, T. W. (2013). Pregnancy establishment and maintenance in cattle1. Journal of Animal Science, 91(2), 722–733. https://doi.org/10.2527/jas.2012-5368

Augustin, H. G. (2000). Vascular morphogenesis in the ovary. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *14*(6), 867–882. https://doi.org/10.1053/beog.2000.0132

Baruselli, P. S., Ferreira, R. M., Sá Filho, M. F., & Bó, G. A. (2018). Review: Using artificial insemination v. natural service in beef herds. Animal, 12, s45–s52. https://doi.org/10.1017/S175173111800054X

Bó, G., Cutaia, L., Peres, L., Pindnato, D., Maraña, D., & Baruselli, P. (2019). Technologies for fixed-time artificial insemination and their influence on reproductive performance of Bos indicus cattle. Bioscientifica Proceedings. https://doi.org/10.1530/biosciprocs.6.014

Bonacker, R. C., Stoecklein, K. S., Locke, J. W. C., Ketchum, J. N., Knickmeyer, E. R., Spinka, C. M., Poock, S. E., & Thomas, J. M. (2020). Treatment with prostaglandin F2α and an intravaginal progesterone insert promotes follicular maturity in advance of gonadotropin-releasing hormone among postpartum beef cows. Theriogenology, 157, 350–359. https://doi.org/10.1016/j.theriogenology.2020.08.018

Bonnin, P., Huynh, L., L’Haridon, R., Chene, N., & Martal, J. (1999). Transport of uterine PGF2 to the ovaries by systemic circulation and local lymphovenous-arterial diffusion during luteolysis in sheep. Reproduction, 116(1), 199–210. https://doi.org/10.1530/jrf.0.1160199

Bridges, G. A., Helser, L. A., Grum, D. E., Mussard, M. L., Gasser, C. L., & Day, M. L. (2008). Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology, 69(7), 843–851. https://doi.org/10.1016/j.theriogenology.2007.12.011

Bridges, G. A., Mussard, M. L., Burke, C. R., & Day, M. L. (2010). Influence of the length of proestrus on fertility and endocrine function in female cattle. Animal

Reproduction Science, 117(3–4), 208–215. https://doi.org/10.1016/j.anireprosci.2009.05.002

Busch, D. C., Wilson, D. J., Schafer, D. J., Leitman, N. R., Haden, J. K., Ellersieck, M. R., Smith, M. F., & Patterson, D. J. (2007). Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers1. Journal of Animal Science, 85(8), 1933–1939. https://doi.org/10.2527/jas.2006-845

Cavalieri, J. (2018). Effect of treatment of Bos indicus heifers with progesterone 0, 3 and 6 days after follicular aspiration on follicular dynamics and the timing of oestrus and ovulation. Animal Reproduction Science, 193, 9–18. https://doi.org/10.1016/j.anireprosci.2018.03.026

Cerri, R. L. A., Rutigliano, H. M., Chebel, R. C., & Santos, J. E. P. (2009). Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. REPRODUCTION, 137(5), 813–823. https://doi.org/10.1530/REP-08-0242

Chenault, J. R., Boucher, J. F., Dame, K. J., Meyer, J. A., & Wood-Follis, S. L. (2003). Intravaginal Progesterone Insert to Synchronize Return to Estrus of Previously Inseminated Dairy Cows. Journal of Dairy Science, 86(6), 2039–2049. https://doi.org/10.3168/jds.S0022-0302(03)73793-X

Colazo, M. G., Kastelic, J. P., Mainar-Jaime, R. C., Gavaga, Q. A., Whittaker, P. R., Small, J. A., Martinez, M. F., Wilde, R. E., Veira, D. M., & Mapletoft, R. J. (2006). Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology, 65(3), 557–572. https://doi.org/10.1016/j.theriogenology.2005.06.001

Colazo, M. G., Kastelic, J. P., Davis, H., Rutledge, M. D., Martinez, M. F., Small, J. A., & Mapletoft, R. J. (2008). Effects of plasma progesterone concentrations on LH release and ovulation in beef cattle given GnRH. Domestic Animal Endocrinology, 34(1), 109–117. https://doi.org/10.1016/j.domaniend.2006.11.004

Cushman, R. A., Kill, L. K., Funston, R. N., Mousel, E. M., & Perry, G. A. (2013). Heifer calving date positively influences calf weaning weights through six parturitions1. Journal of Animal Science, 91(9), 4486–4491. https://doi.org/10.2527/jas.2013-6465

Day, M. (2015). State of the art of GnRH-based timed AI in beef cattle. Animal Reproduction, 12(3), 473–478.

Donaldson, L., & Hansel, W. (1965). Histological Study of Bovine Corpora Lutea. Journal of Dairy Science, 48(7), 905–909. https://doi.org/10.3168/jds.S0022-0302(65)88360-6

Evans, A. C. O., & Fortune, J. E. (1997). Selection of the Dominant Follicle in Cattle Occurs in the Absence of Differences in the Expression of Messenger Ribonucleic Acid for Gonadotropin Receptors*. Endocrinology, 138(7), 2963–2971. https://doi.org/10.1210/endo.138.7.5245

Fleming, J. G. W., Spencer, T. E., Safe, S. H., & Bazer, F. W. (2006). Estrogen Regulates Transcription of the Ovine Oxytocin Receptor Gene through GC-Rich SP1 Promoter Elements. Endocrinology, 147(2), 899–911. https://doi.org/10.1210/en.2005-1120

Fontes, P. L. P., & Oosthuizen, N. (2022). Applied Use of Doppler Ultrasonography in Bovine Reproduction. Frontiers in Animal Science, 3. https://doi.org/10.3389/fanim.2022.912854

Funston, R. N., Musgrave, J. A., Meyer, T. L., & Larson, D. M. (2012). Effect of calving distribution on beef cattle progeny performance. Journal of Animal Science, 90(13), 5118–5121. https://doi.org/10.2527/jas.2012-5263

Geary, T. W., Whittier, J. C., Downing, E. R., LeFever, D. G., Silcox, R. W., Holland, M. D., Nett, T. M., & Niswender, G. D. (1998). Pregnancy rates of postpartum beef cows that were synchronized using Syncro-Mate-B or the Ovsynch protocol. Journal of Animal Science, 76(6), 1523. https://doi.org/10.2527/1998.7661523x

Geary, T. W., Whittier, J. C., Hallford, D. M., & MacNeil, M. D. (2001). Calf removal improves conception rates to the Ovsynch and CO-Synch protocols. Journal of Animal Science, 79(1), 1. https://doi.org/10.2527/2001.7911

Ginther, O. J., & Utt, M. D. (2004). Doppler ultrasound in equine reproduction: principles, techniques, and potential. Journal of Equine Veterinary Science, 24(12), 516–526. https://doi.org/10.1016/j.jevs.2004.11.005

Ginther, O. J., Silva, L. A., Araujo, R. R., & Beg, M. A. (2007). Temporal Associations among Pulses of 13,14-Dihydro-15-keto-PGF2alpha, Luteal Blood Flow, and Luteolysis in Cattle1. Biology of Reproduction, 76(3), 506–513. https://doi.org/10.1095/biolreprod.106.057653

Guimarães da Silva, A., Nishimura, T. K., Rocha, C. C., Motta, I. G., Laurindo Neto, A., Ferraz, P. A., Bruni, G. A., Orlandi, R. E., Massoneto, J. P. M., & Pugliesi, G. (2022a). Comparison of estradiol benzoate doses for resynchronization of ovulation at 14 days after timed-AI in suckled beef cows. Theriogenology, 184, 41–50. https://doi.org/10.1016/j.theriogenology.2022.02.025

Hafs, H. D., Manns, J. G., & Drew, B. (1975). Onset of oestrus and fertility of dairy heifers and suckled beef cows treated with prostaglandin F 2α. Animal Science, 21(1), 13–20. https://doi.org/10.1017/S0003356100030361

Hansel, W., & Convey, E. M. (1983). Physiology of the estrous cycle. Journal of Animal Science, 57 Suppl 2, 404–424.

Hawk, H. W. (1983). Sperm Survival and Transport in the Female Reproductive Tract. Journal of Dairy Science, 66(12), 2645–2660. https://doi.org/10.3168/jds.S0022-0302(83)82138-9

Herzog, K., Brockhan-Lüdemann, M., Kaske, M., Beindorff, N., Paul, V., Niemann, H., & Bollwein, H. (2010). Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. Theriogenology, 73(5), 691–697. https://doi.org/10.1016/j.theriogenology.2009.11.016

Herzog, K., Voss, C., Kastelic, J. P., Beindorff, N., Paul, V., Niemann, H., & Bollwein, H. (2011). Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows. Theriogenology, 75(3), 549–554. https://doi.org/10.1016/j.theriogenology.2010.09.024

Hinckley, S. T., & Milvae, R. A. (2001). Endothelin-1 Mediates Prostaglandin F2α-Induced Luteal Regression in the Ewe1. Biology of Reproduction, 64(6), 1619–1623. https://doi.org/10.1095/biolreprod64.6.1619

Holton, M. P., Oosthuizen, N., Melo, G. D. de, Davis, D. B., Stewart, R. L., Pohler, K. G., Lamb, G. C., & Fontes, P. L. P. (2022). Luteal color doppler ultrasonography and pregnancy-associated glycoproteins as early pregnancy diagnostic tools and predictors of pregnancy loss in Bos taurus postpartum beef cows. Journal of Animal Science, 100(2). https://doi.org/10.1093/jas/skac018

Ketchum, J. N., Perry, G. A., Quail, L. K., Epperson, K. M., Ogg, M. A., Zezeski, A. L., Rich, J. J. J., Zoca, S. M., Kline, A. C., Andrews, T. N., Ortega, M. S., Smith, M. F., & Geary, T. W. (2023). Influence of preovulatory estradiol treatment on the maintenance of pregnancy in beef cattle receiving in vivo produced embryos. Animal Reproduction Science, 255, 107274. https://doi.org/10.1016/j.anireprosci.2023.107274

Kindahl, H., Edqvist, L.-E., Bane, A., & Granstrom, E. (1976). BLOOD LEVELS OF PROGESTERONE AND 15-KETO-13,14-DIHYDRO-PROSTAGLANDIN F2 DURING THE NORMAL OESTROUS CYCLE AND EARLY PREGNANCY IN HEIFERS. European Journal of Endocrinology, 82(1), 134–149. https://doi.org/10.1530/acta.0.0820134

Knickerbocker, J. J., Wiltbank, M. C., & Niswender, G. D. (1988). Mechanisms of luteolysis in domestic livestock. Domestic Animal Endocrinology, 5(2), 91–107. https://doi.org/10.1016/0739-7240(88)90011-2

Knopf, L., Kastelic, J. P., Schallenberger, E., & Ginther, O. J. (1989). Ovarian follicular dynamics in heifers: Test of two-wave hypothesis by ultrasonically monitoring individual follicles. Domestic Animal Endocrinology, 6(2), 111–119. https://doi.org/10.1016/0739-7240(89)90040-4

Kotwica, J., & Skarzynski, D. (1993). Influence of oxytocin removal from the corpus luteum on secretory function and duration of the oestrous cycle in cattle. Reproduction, 97(2), 411–417. https://doi.org/10.1530/jrf.0.0970411

Lamb, G. C., Stevenson, J. S., Kesler, D. J., Garverick, H. A., Brown, D. R., & Salfen, B. E. (2001). Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. Journal of Animal Science, 79(9), 2253. https://doi.org/10.2527/2001.7992253x

Lamb, G. C., & Mercadante, V. R. G. (2016). Synchronization and Artificial Insemination Strategies in Beef Cattle. Veterinary Clinics of North America: Food Animal Practice, 32(2), 335–347. https://doi.org/10.1016/j.cvfa.2016.01.006

Larson, J. E., Lamb, G. C., Stevenson, J. S., Johnson, S. K., Day, M. L., Geary, T. W., Kesler, D. J., DeJarnette, J. M., Schrick, F. N., DiCostanzo, A., & Arseneau, J. D. (2006). Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2α, and progesterone1. Journal of Animal Science, 84(2), 332–342. https://doi.org/10.2527/2006.842332x

Larson, J. E., Thielen, K. N., Funnell, B. J., Stevenson, J. S., Kesler, D. J., & Lamb, G. C. (2009). Influence of a controlled internal drug release after fixed-time artificial insemination on pregnancy rates and returns to estrus of nonpregnant cows1. Journal of Animal Science, 87(3), 914–921. https://doi.org/10.2527/jas.2008-1443

Lauderdale, J. W., Seguin, B. E., Stellflug, J. N., Chenault, J. R., Thatcher, W. W., Vincent, C. K., & Loyancano, A. F. (1974). Fertility of Cattle Following PGF2α Injection. Journal of Animal Science, 38(5), 964–967. https://doi.org/10.2527/jas1974.385964x

Levy, N., Kobayashi, S., Roth, Z., Wolfenson, D., Miyamoto, A., & Meidan, R. (2000). Administration of Prostaglandin F2α During the Early Bovine Luteal Phase Does

Not Alter the Expression of ET-1 and of Its Type A Receptor: A Possible Cause for Corpus Luteum Refractoriness. Biology of Reproduction, 63(2), 377–382. https://doi.org/10.1095/biolreprod63.2.377

Lima, F. S., Ribeiro, E. S., Bisinotto, R. S., Greco, L. F., Martinez, N., Amstalden, M., Thatcher, W. W., & Santos, J. E. P. (2013). Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers. Journal of Dairy Science, 96(11), 7054–7065. https://doi.org/10.3168/jds.2013-7093

Louis, T. M., Hafs, H. D., & Seguin, B. E. (1973). Progesterone, LH, Estrus and Ovulation after Prostaglandin F2 in Heifers. Experimental Biology and Medicine, 143(1), 152–155. https://doi.org/10.3181/00379727-143-37274

Louis, T. M., Hafs, H. D., & Morrow, D. A. (1974). Intrauterine Administration of Prostaglandin F2α in Cows: Progesterone, Estrogen, LH, Estrus and Ovulation. Journal of Animal Science, 38(2), 347–353. https://doi.org/10.2527/jas1974.382347x

Lucy, M. C., Billings, H. J., Butler, W. R., Ehnis, L. R., Fields, M. J., Kesler, D. J., Kinder, J. E., Mattos, R. C., Short, R. E., Thatcher, W. W., Wettemann, R. P., Yelich, J. V, & Hafs, H. D. (2001). Efficacy of an intravaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. Journal of Animal Science, 79(4), 982. https://doi.org/10.2527/2001.794982x

Madsen, C. A., Perry, G. A., Mogck, C. L., Daly, R. F., MacNeil, M. D., & Geary, T. W. (2015). Effects of preovulatory estradiol on embryo survival and pregnancy establishment in beef cows. Animal Reproduction Science, 158, 96–103. https://doi.org/10.1016/j.anireprosci.2015.05.006

Marques, M. de O., Morotti, F., da Silva, C. B., Júnior, M. R., da Silva, R. C. P., Baruselli, P. S., & Seneda, M. M. (2015). Influence of category-heifers, primiparous and multiparous lactating cows-in a large-scale resynchronization fixed-time artificial insemination program. Journal of Veterinary Science, 16(3), 367. https://doi.org/10.4142/jvs.2015.16.3.367

Martinez, M. F., Adams, G. P., Bergfelt, D. R., Kastelic, J. P., & Mapletoft, R. J. (1999). Effect of LH or GnRH on the dominant follicle of the first follicular wave in beef heifers. Animal Reproduction Science, 57(1–2), 23–33. https://doi.org/10.1016/S0378-4320(99)00057-3

McCracken, J. A., Custer, E. E., & Lamsa, J. C. (1999). Luteolysis: A Neuroendocrine-Mediated Event. Physiological Reviews, 79(2), 263–323. https://doi.org/10.1152/physrev.1999.79.2.263

Meneghetti, M., Filho, O. G. S., Peres, R. F. G., Lamb, G. C., & Vasconcelos, J. L. M. (2009). Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: Basis for development of protocols. Theriogenology, 72(2), 179–189. https://doi.org/10.1016/j.theriogenology.2009.02.010

Miller, B., Moore, N., Murphy, L., & Stone, G. (1977). Early Pregnancy in the Ewe: Effects of Oestradiol and Progesterone on Uterine Metabolism and on Embryo Survival. Australian Journal of Biological Sciences, 30(4), 279. https://doi.org/10.1071/BI9770279

Miyamoto, A., Shirasuna, K., Wijayagunawardane, M. P. B., Watanabe, S., Hayashi, M., Yamamoto, D., Matsui, M., & Acosta, T. J. (2005). Blood flow: A key regulatory component of corpus luteum function in the cow. Domestic Animal Endocrinology, 29(2), 329–339. https://doi.org/10.1016/j.domaniend.2005.03.011

MOOR, R. M., & ROWSON, L. E. A. (1966). LOCAL UTERINE MECHANISMS AFFECTING LUTEAL FUNCTION IN THE SHEEP. Reproduction, 11(2), 307–310. https://doi.org/10.1530/jrf.0.0110307

Moreira, F., de la Sota, R. L., Diaz, T., & Thatcher, W. W. (2000). Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. Journal of Animal Science, 78(6), 1568. https://doi.org/10.2527/2000.7861568x

Motta, I. G., Rocha, C. C., Bisinotto, D. Z., Melo, G. D., Ataide Júnior, G. A., Silva, A. G., Gonzaga, V. H. G., Santos, J. A., Freitas, B. G., Lemes, K. M., Madureira, E. H., & Pugliesi, G. (2020). Increased pregnancy rate in beef heifers resynchronized with estradiol at 14 days after TAI. Theriogenology, 147, 62–70. https://doi.org/10.1016/j.theriogenology.2020.02.009

Oosthuizen, N., Fontes, P. L. P., Sanford, C. D., Ciriaco, F. M., Henry, D. D., Canal, L. B., DiLorenzo, N., & Lamb, G. C. (2018). Estrus synchronization and fixed-time artificial insemination alter calving distribution in Bos indicus influenced beef heifers. Theriogenology, 106, 210–213. https://doi.org/10.1016/j.theriogenology.2017.10.028

Oosthuizen, N., Fontes, P. L. P., Porter, K., & Lamb, G. C. (2020). Presynchronization with prostaglandin F2α and prolonged exposure to exogenous progesterone impacts estrus expression and fertility in beef heifers. Theriogenology, 146, 88–93. https://doi.org/10.1016/j.theriogenology.2020.02.010

Oosthuizen, N., Fontes, P. L. P., Oliveira Filho, R. V., Dahlen, C. R., Grieger, D. M., Hall, J. B., Lake, S. L., Looney, C. R., Mercadante, V. R. G., Neville, B. W., Perry, G. A., Powell, J. G., Prezotto, L. D., Seidel, G. E., Walker, R. S., Cardoso, R. C., Pohler, K. G., & Lamb, G. C. (2021a). Pre-synchronization of ovulation timing and delayed fixed-time artificial insemination increases pregnancy rates when sex-sorted semen is used for insemination of heifers. Animal Reproduction Science, 226, 106699. https://doi.org/10.1016/j.anireprosci.2021.106699

Oosthuizen, N., Melo, G. D., Seidel, G. E., Stewart, R. L., Rowden, L., Lamb, G. C., & Fontes, P. L. P. (2021b). Effects of prolonging the interval from progestin removal to prostaglandin F2α injection from 16 to 17 d in a long-term estrus synchronization protocol in beef heifers. Translational Animal Science, 5(2). https://doi.org/10.1093/tas/txab062

O’Shea, J. D., Rodgers, R. J., & D’Occhio, M. J. (1989). Cellular composition of the cyclic corpus luteum of the cow. Reproduction, 85(2), 483–487. https://doi.org/10.1530/jrf.0.0850483

Ozturk, S., & Demir, R. (2010). Particular functions of estrogen and progesterone in establishment of uterine receptivity and embryo implantation. Histology and Histopathology, 25(9), 1215–1228. https://doi.org/10.14670/HH-25.1215

Palhão, M. P., Ribeiro, A. C., Martins, A. B., Guimarães, C. R. B., Alvarez, R. D., Seber, M. F., Fernandes, C. A. C., Neves, J. P., & Viana, J. H. M. (2020). Early resynchronization of non-pregnant beef cows based in corpus luteum blood flow evaluation 21 days after Timed-AI. Theriogenology, 146, 26–30. https://doi.org/10.1016/j.theriogenology.2020.01.064

Patterson, D. J., Corah, L. R., & Brethour, J. R. (1990). Response of prepubertal and × heifers to melengestrol acetate with or without gonadotropin-releasing hormone. Theriogenology, 33(3), 661–668. https://doi.org/10.1016/0093-691X(90)90543-3

Pereira, M. H. C., Wiltbank, M. C., & Vasconcelos, J. L. M. (2016). Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. *Journal of Dairy Science*, *99*(3), 2237–2247. https://doi.org/10.3168/jds.2015-9903

Perry, G. A., & Geary, T. W. (2004). Ability of CIDRs or Melengestrol Acetate to Initiate Estrous Cycles in Early Postpartum Beef Cows. http://openprairie.sdstate.edu/sd_beefreport_2004http://openprairie.sdstate.edu/sd_beefreport_2004/16

Perry, G. A., Smith, M. F., Lucy, M. C., Green, J. A., Parks, T. E., MacNeil, M. D., Roberts, A. J., & Geary, T. W. (2005). Relationship between follicle size at insemination and pregnancy success. Proceedings of the National Academy of Sciences, 102(14), 5268–5273. https://doi.org/10.1073/pnas.0501700102

Perry, G. A., Smith, M. F., Roberts, A. J., MacNeil, M. D., & Geary, T. W. (2007). Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. Journal of Animal Science, 85(3), 684–689. https://doi.org/10.2527/jas.2006-519

Perry, G. A., & Perry, B. L. (2008). Effect of preovulatory concentrations of estradiol and initiation of standing estrus on uterine pH in beef cows. Domestic Animal Endocrinology, 34(3), 333–338. https://doi.org/10.1016/j.domaniend.2007.09.003

Perry, G. A., Perry, B. L., Krantz, J. H., & Rodgers, J. (2012). Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success1. Journal of Animal Science, 90(2), 489–494. https://doi.org/10.2527/jas.2011-4319

Peterson, C., Alkar, A., Smith, S., Kerr, S., Hall, J. B., Moore, D., & Kasimanickam, R. (2011). Effects of one versus two doses of prostaglandin F2alpha on AI pregnancy rates in a 5-day, progesterone-based, CO-Synch protocol in crossbred beef heifers. Theriogenology, 75(8), 1536–1542. https://doi.org/10.1016/j.theriogenology.2010.12.017

Pierson, R. A., & Ginther, O. J. (1984). Ultrasonography of the bovine ovary. Theriogenology, 21(3), 495–504. https://doi.org/10.1016/0093-691X(84)90411-4

Pieterse, M. C., Szenci, O., Willemse, A. H., Bajcsy, C. S. A., Dieleman, S. J., & Taverne, M. A. M. (1990). Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. Theriogenology, 33(3), 697–707. https://doi.org/10.1016/0093-691X(90)90547-7

Pugliesi, G., Miagawa, B. T., Paiva, Y. N., França, M. R., Silva, L. A., & Binelli, M. (2014). Conceptus-Induced Changes in the Gene Expression of Blood Immune Cells and the Ultrasound-Accessed Luteal Function in Beef Cattle: How Early Can We Detect Pregnancy?1. Biology of Reproduction, 91(4). https://doi.org/10.1095/biolreprod.114.121525

Pugliesi, G., Bisinotto, D. Z., Mello, B. P., Lahr, F. C., Ferreira, C. A., Melo, G. D., Bastos, M. R., & Madureira, E. H. (2019). A novel strategy for resynchronization of ovulation in Nelore cows using injectable progesterone (P4) and P4 releasing devices to perform two timed inseminations within 22 days. Reproduction in Domestic Animals, 54(8), 1149–1154. https://doi.org/10.1111/rda.13475

Pugliesi, G., Guimarães da Silva, A., Viana, J. H. M., & Siqueira, L. G. B. (2023). Review: Current status of corpus luteum assessment by Doppler ultrasonography to diagnose non-pregnancy and select embryo recipients in cattle. Animal, 17, 100752. https://doi.org/10.1016/j.animal.2023.100752

Pursley, J. R., Mee, M. O., & Wiltbank, M. C. (1995). Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology, 44(7), 915–923. https://doi.org/10.1016/0093-691X(95)00279-H

Quintela, L., Barrio, M., Peña, A., Becerra, J., Cainzos, J., Herradón, P., & Díaz, C. (2012). Use of Ultrasound in the Reproductive Management of Dairy Cattle. Reproduction in Domestic Animals, 47(s3), 34–44. https://doi.org/10.1111/j.1439-0531.2012.02032.x

Read, C. C., Edwards, J. L., Schrick, F. N., Rhinehart, J. D., Payton, R. R., Campagna, S. R., Castro, H. F., Klabnik, J. L., & Moorey, S. E. (2022). Preovulatory serum estradiol concentration is positively associated with oocyte ATP and follicular fluid metabolite abundance in lactating beef cattle. Journal of Animal Science, 100(7). https://doi.org/10.1093/jas/skac136

Rodgers, J. C., Bird, S. L., Larson, J. E., Dilorenzo, N., Dahlen, C. R., DiCostanzo, A., & Lamb, G. C. (2012). An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows1. Journal of Animal Science, 90(11), 4055–4062. https://doi.org/10.2527/jas.2011-4836

Rodrigues, A. D., Cooke, R. F., Cipriano, R. S., Silva, L. G. T., Cerri, R. L. A., Cruppe, L. H., Meneghetti, M., Pohler, K. G., & Vasconcelos, J. L. M. (2018). Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in Bos indicus-influenced beef cows1. *Journal of Animal Science*, *96*(1), 236–249. https://doi.org/10.1093/jas/skx043

Romano, J. E., Thompson, J. A., Forrest, D. W., Westhusin, M. E., Tomaszweski, M. A., & Kraemer, D. C. (2006). Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology, 66(4), 1034–1041. https://doi.org/10.1016/j.theriogenology.2006.02.044

Sá Filho, M. F., Crespilho, A. M., Santos, J. E. P., Perry, G. A., & Baruselli, P. S. (2010). Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Animal Reproduction Science, 120(1–4), 23–30. https://doi.org/10.1016/j.anireprosci.2010.03.007

Sá Filho, M. F., Marques, M. O., Girotto, R., Santos, F. A., Sala, R. V., Barbuio, J. P., & Baruselli, P. S. (2014). Resynchronization with unknown pregnancy status using progestin-based timed artificial insemination protocol in beef cattle. Theriogenology, 81(2), 284–290. https://doi.org/10.1016/j.theriogenology.2013.09.027

Santos, J. E. P., Narciso, C. D., Rivera, F., Thatcher, W. W., & Chebel, R. C. (2010). Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. Journal of Dairy Science, 93(7), 2976–2988. https://doi.org/10.3168/jds.2009-2870

Sartori, R., Fricke, P. M., Ferreira, J. C. P., Ginther, O. J., & Wiltbank, M. C. (2001). Follicular Deviation and Acquisition of Ovulatory Capacity in Bovine Follicles1. Biology of Reproduction, 65(5), 1403–1409. https://doi.org/10.1095/biolreprod65.5.1403

Scaramuzzi, R. J., Baird, D. T., Boyle, H. P., Land, R. B., & Wheeler, A. G. (1977). The secretion of prostaglandin F from the autotransplanted uterus of the ewe. Reproduction, 49(1), 157–160. https://doi.org/10.1530/jrf.0.0490157

Senger, PL. Pathways to Pregnancy & Parturition. 3rd ed., Current Conceptions, 2012.

Silvia, W. J., Lewis, G. S., Mccracken, J. A., Thatcher, W. W., & Wilson, L. (1991). Hormonal Regulation of Uterine Secretion of Prostaglandin F2α during Luteolysis in Ruminants1. Biology of Reproduction, 45(5), 655–663. https://doi.org/10.1095/biolreprod45.5.655

Siqueira, L. G. B., Areas, V. S., Ghetti, A. M., Fonseca, J. F., Palhao, M. P., Fernandes, C. A. C., & Viana, J. H. M. (2013). Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. Journal of Dairy Science, 96(10), 6461–6472. https://doi.org/10.3168/jds.2013-6814

Sirois, J., & Fortune, J. E. (1988). Ovarian Follicular Dynamics during the Estrous Cycle in Heifers Monitored by Real-Time UItrasonograph1. Biology of Reproduction, 39(2), 308–317. https://doi.org/10.1095/biolreprod39.2.308

Skarzynski, D., & Okuda, K. (n.d.). Inter-and intra-cellular mechanisms of prostaglandin F2α action during corpus luteum regression in cattle. In M. Lucy, J. Pate, M. Smith, & T. Spencer (Eds.), Reproduction in Domestic Ruminants VII (pp. 305–324). Nottingham University Press. https://doi.org/10.7313/UPO9781907284991.025

Small, J. A., Colazo, M. G., Kastelic, J. P., & Mapletoft, R. J. (2009). Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. Theriogenology, 71(4), 698–706. https://doi.org/10.1016/j.theriogenology.2008.09.045

Stegner, J. E., Kojima, F. N., Ellersieck, M. R., Lucy, M. C., Smith, M. F., & Patterson, D. J. (2004). A comparison of progestin-based protocols to synchronize estrus in postpartum beef cows1. Journal of Animal Science, 82(4), 1016–1021. https://doi.org/10.2527/2004.8241016x

Stevenson, J. S., Thompson, K. E., Forbes, W. L., Lamb, G. C., Grieger, D. M., & Corah, L. R. (2000). Synchronizing estrus and(or) ovulation in beef cows after combinations of GnRH, norgestomet, and prostaglandin F2alpha with or without timed insemination. Journal of Animal Science, 78(7), 1747. https://doi.org/10.2527/2000.7871747x

Stevenson, J. S., Johnson, S. K., Medina-Britos, M. A., Richardson-Adams, A. M., & Lamb, G. C. (2003). Resynchronization of estrus in cattle of unknown pregnancy status using estrogen, progesterone, or both1. Journal of Animal Science, 81(7), 1681–1692. https://doi.org/10.2527/2003.8171681x

Stock, A. E. (1993). Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology, 132(3), 1108–1114. https://doi.org/10.1210/en.132.3.1108

SUGINO, N., & OKUDA, K. (2007). Species-Related Differences in the Mechanism of Apoptosis During Structural Luteolysis. Journal of Reproduction and Development, 53(5), 977–986. https://doi.org/10.1262/jrd.19047

Taylor, C., Rajamahendran, R., & Walton, J. S. (1993). Ovarian follicular dynamics and plasma luteinizing hormone concentrations in norgestomet-treated heifers. Animal Reproduction Science, 32(3–4), 173–184. https://doi.org/10.1016/0378-4320(93)90089-A

Thatcher, W. W., Macmillan, K. L., Hansen, P. J., & Drost, M. (1989). Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology, 31(1), 149–164. https://doi.org/10.1016/0093-691X(89)90572-4

Thompson, K. E., Stevenson, J. S., Lamb, G. C., Grieger, D. M., & Löest, C. A. (1999). Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and prostaglandin F2alpha. Journal of Animal Science, 77(7), 1823. https://doi.org/10.2527/1999.7771823x

Tithof, P. K., Roberts, M. P., Guan, W., Elgayyar, M., & Godkin, J. D. (2007). Distinct phospholipase A2 enzymes regulate prostaglandin E2 and F2alpha production by bovine endometrial epithelial cells. Reproductive Biology and Endocrinology, 5(1), 16. https://doi.org/10.1186/1477-7827-5-16

Twagiramungu, H., Guilbault, L. A., Proulx, J., & Dufour, J. J. (1992). Synchronization of estrus and fertility in beef cattle with two injections of buserelin and prostaglandin. Theriogenology, 38(6), 1131–1144. https://doi.org/10.1016/0093-691X(92)90126-C

Utt, M. D., Johnson, G. L., & Beal, W. E. (2009). The evaluation of corpus luteum blood flow using color-flow Doppler ultrasound for early pregnancy diagnosis in bovine embryo recipients. Theriogenology, 71(4), 707–715. https://doi.org/10.1016/j.theriogenology.2008.09.032

Van Cleeff, J., Macmillan, K. L., Drost, M., Lucy, M. C., & Thatcher, W. W. (1996). Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to service. Theriogenology, 46(7), 1117–1130. https://doi.org/10.1016/S0093-691X(96)00284-1

Van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. Nature Food, 2(7), 494–501. https://doi.org/10.1038/s43016-021-00322-9

Vasconcelos, J. L. M., Silcox, R. W., Rosa, G. J. M., Pursley, J. R., & Wiltbank, M. C. (1999). Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology, 52(6), 1067–1078. https://doi.org/10.1016/S0093-691X(99)00195-8

Vasconcelos, J. L. M., Sartori, R., Oliveira, H. N., Guenther, J. G., & Wiltbank, M. C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, 56(2), 307–314. https://doi.org/10.1016/S0093-691X(01)00565-9

Wiltbank, J. N., & Casida, L. E. (1956). Alteration of Ovarian Activity by Hysterectomy. Journal of Animal Science, 15(1), 134–140. https://doi.org/10.2527/jas1956.151134x

Wiltbank, M. C., Mezera, M. A., Toledo, M. Z., Drum, J. N., Baez, G. M., García-Guerra, A., & Sartori, R. (2018). Physiological mechanisms involved in maintaining the corpus luteum during the first two months of pregnancy. Animal Reproduction, 15(Suppl. 1), 805–821. https://doi.org/10.21451/1984-3143-AR2018-0045

Xu, Z., Allen Garverick, H., Smith, G. W., Smith, M. F., Hamilton, S. A., & Youngquist, R. S. (1995). Expression of Follicle-Stimulating Hormone and Luteinizing Hormone Receptor Messenger Ribonucleic Acids in Bovine Follicles during the First Follicular Wave1. Biology of Reproduction, 53(4), 951–957. https://doi.org/10.1095/biolreprod53.4.951

Yoshioka, S., Abe, H., Sakumoto, R., & Okuda, K. (2013). Proliferation of Luteal Steroidogenic Cells in Cattle. PLoS ONE, 8(12), e84186. https://doi.org/10.1371/journal.pone.0084186

CHAPTER 3

INFLUENCE OF EARLY PROGESTERONE REMOVAL ON FOLLICULAR DEVELOPMENT, ESTRUS EXPRESSION, AND PREGNANCY RATES IN PRESYNCHRONIZED BEEF COWS[1]

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Abstract

The objective of this study was to evaluate the impact of early progesterone removal on pregnancy rates to fixed-time artificial insemination (FTAI) in presynchronized beef cows. Postpartum beef cows (n = 882) were randomly assigned to 1 of 2 treatments: 1) 7&7 Synch: Cows received a controlled internal drug release insert (CIDR) and a 25-mg injection of prostaglandin F2α (PG1) on day 0. Cows received 100 μg of Gonadotropin release hormone (GnRH) on day 7 and the CIDR was removed seven days later, concurrently with a second prostaglandin F2α (PG2) injection (day 14). Cows received a second injection of GnRH and were FTAI 60-66 h after PG2 (day 17); 2) 7&6 Synch: Cows received the same treatment as 7&7 Synch cows; however, CIDR removal and PG2 were performed on day 13, and cows were FTAI 60-66 h after PG2. Ovarian ultrasonography was performed to determine follicle diameter at PG2 and FTAI in a subset of cows (n = 40) for both treatments. Cows exposed to the 7&7 Synch tended to have larger follicle diameter at PG2 compared with 7&6 Synch cows (P = 0.09); however, there were no differences in follicle diameter at FTAI. There were no differences between treatments for estrus expression (7&7 Synch: 77.1 ± 5.91; 7&6 Synch: 74.6 ± 6.28; P = 0.50) nor pregnancy rates to FTAI (7&7 Synch: 60.1 ± 4.32; 7&6 Synch: 58.2 ± 4.26; P = 0.69). In conclusion, early removal of progesterone did not impact pregnancy rates in presynchronized beef cows.

Introduction

Estrus synchronization is an important tool to overcome anestrus postpartum and alter calving distribution in beef herds (Rodgers, et al 2012). Additionally, estrus synchronization facilitates the use of biotechnologies that result in faster genetic progress, such as artificial insemination (AI) and embryo transfer. Fixed-time artificial insemination (FTAI) protocols manipulate follicular development in order to induce ovulation in a predicted window of time (Pursley, et al., 1995; Geary, et al., 1998; Larson, et al., 2006). The primary hormones utilized in FTAI protocols in the United States are gonadotropin-release hormone (GnRH), prostaglandin F2α (PG) and progesterone. Exogenous GnRH analogues induce follicle turnover through the induction of ovulation, resulting to the emergence of a synchronized new follicular wave (Macmillan and Thatcher, 1991); however, only approximately 60% of postpartum beef cows at random stages of the estrous cycle ovulate after a GnRH analogue injection (Geary, et al., 1998), leading to suboptimal subsequent follicular growth synchrony (Thompson, et al., 1999). Studies demonstrated that ovulatory response to GnRH varies according to various factors, including dominant follicle diameter and circulating concentrations of progesterone (Vasconcelos, et al., 1999; Colazo, et al., 2008; Rojas, et al., 2023). Therefore, several studies attempted to develop presynchronization protocols to increase the percentage of cows with a GnRH responsive follicle at the beginning of the FTAI protocol (Busch, et al., 2007; Small, et al., 2009; Perry, et al., 2012; Oosthuizen, et al., 2018; Oosthuizen, et al., 2020).

The 7&7 Synch is a PG and progestin-based presynchronization strategy that has been adopted in estrus synchronization programs for replacement heifers (Oosthuizen, et al., 2018, 2020) and postpartum cows (Andersen, et al., 2021). The underlying rationale of this strategy is to increase the proportion of cows with a dominant follicle at the time of the first GnRH injection, leading to a greater response to the initial GnRH and increased subsequent follicular growth synchrony (Bonacker et al., 2020). This is accomplished by the insertion of an intravaginal progesterone device concurrently with a PG analogue injection 7 days prior to the first GnRH injection. This presynchronization approach resulted in greater estrus expression and pregnancy to FTAI in beef heifers (Oosthuizen et al., 2020; 2022) and cows (Andersen et al., 2021) when compared to non-presynchronized controls.

Reducing the period between follicular wave emergence and luteolysis has also been shown to increase fertility of beef cows exposed to short-term FTAI protocols (Day, 2015). Bridges, et al. (2008) observed an increase in pregnancy rates when the interval between the first GnRH and PG was reduced from 7 to 5 days and the interval between PG and FTAI was increased from 60 to 72h. Reducing the interval between follicular wave emergence and luteolysis was also associated with increased fertility in beef cows exposed to estradiol and progesterone-based protocols (Meneghetti et al., 2009). Similar results have also been observed in dairy cows when the interval between the first GnRH and PG was reduced from 7 to 5 days (Santos et al., 2010).

Reducing the interval between GnRH and PG exposes younger follicles to a low progesterone endocrine milieu and young follicles have been shown to have greater capacity to synthesize estradiol compared to older non-atretic follicles (Valdez, et al., 2005). Plasma concentrations of estradiol during follicular development plays a major role in fertility, positively influencing oocyte quality, promoting sperm transportation in the oviduct, and modulating uterine function to facilitate subsequent pregnancy establishment during the luteal phase (Richardson et al., 2016). Therefore, inducing a decrease in circulating progesterone earlier during follicular growth has been proposed as an alternative to increase follicular steroidogenesis and optimize follicular development (Minela et al., 2023). In fact, reducing the interval between GnRH and PG from 7 to 6 days resulted in earlier onset of estrus expression and increased the proportion of beef cows in estrus prior to FTAI (Fontes et al., 2019). Similarly, reducing the interval between GnRH and PG in lactating dairy cows increased estrus intensity based on automated activity monitors (Minela et al., 2023), which is associated with pregnancy rates to FTAI and embryo transfer (Madureira et al.,2019; 2022). While reducing the interval between follicular wave emergence and luteolysis has been shown to increase pregnancy rates to FTAI in beef cows that have not been exposed to presynchronization, its impact on fertility in presynchronized cows remains unknown. We hypothesized that decreasing the interval between follicular wave emergence and luteolysis from 7 to 6 days would increase the fertility of presynchronized beef cows. Thus, the objective of this study was to evaluate the impact of early progesterone removal and PG administration on follicular development, estrus expression, and pregnancy rates in presynchronized beef cows.

Materials and methods

All cows were handled in accordance with procedures approved by the University of Georgia's Animal Care and Use Committee (A2023 01-043-Y1-A0).

Animals and treatments

A total of 882 lactating beef cows (body condition score [BCS] = 4.9 ± 0.77; days postpartum [DPP] = 76.5 ± 20.28) from 7 locations in 3 different states (Georgia, South Dakota, and Tennessee) were enrolled in a completely randomized design. Within location, cows were stratified by breed and days postpartum before being randomly assigned to 1 of 2 treatments: 1) 7&7 Synch: Cows received a controlled internal drug release insert (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health, Parsippany, NJ) and a 25-mg injection of PG (PG1; day 0; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health). Cows received 100 µg of GnRH (GnRH1; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) on day 7 and the CIDR was removed seven days later (day 14), concurrently with a second PG injection (PG2). Cows received a second injection of GnRH and were FTAI 60-66 h after PG2 (day 17); 2) 7&6 Synch: Cows received the same treatment as 7&7 Synch cows; however, CIDR removal and PG2 were performed on day 13, and cows were FTAI 60-66 h after CIDR removal. For one location (SD1; n = 75), DPP data was not available and cows and randomly assigned to treatment without being stratified by DPP. Estrus detection patches were applied at CIDR removal for both treatments and utilized to evaluate estrus expression between PG2 and FTAI (Estrotect Breeding Indicators; Rockway Inc., Spring Valley, WI). Cows were considered to have expressed estrus prior to FTAI when at least 75% of the rub-off coating was removed from the patch at FTAI. Cows that lost patches were considered to have expressed estrus. One herd within location 5 (n = 45) had over 30% of estrus detection patch loss; therefore, estrus detection data was not utilized for that herd. Within location, AI technicians and sires were equally distributed among treatments. Within each location, cows from both treatments were comingled throughout the experiment and exposed to the same nutritional program and environmental conditions.

Ovarian Ultrasonography and Pregnancy Diagnosis

Ovarian ultrasonography was performed to determine follicle diameter and CL presence at PG2, FTAI and 7 days after FTAI (day 24) in a subset of cows (n = 40) in one location. Follicles with a diameter ≥ 4 mm were recorded. Follicular diameter was measured at the widest point and at a right angle to the first measurement. Follicular diameter was calculated as the average of the 2 measurements. Daily follicular growth rate was calculated by subtracting follicle diameter at PG2 from the follicle diameter at FTAI, divided by the number of days between PG2 to FTAI. Seven days after FTAI (day 24), ovaries were evaluated to confirm ovulation of the dominant follicle. Ovulation was defined as the formation of a new corpus luteum ipsilaterally to the previously recorded dominant follicle. Pregnancy diagnosis was performed 28-35 days after FTAI using transrectal ultrasonography (Sonoscape S8EXP; SonoScape Medical Corp). Within location, pregnancy diagnosis was performed on the same day for cows in both treatments. A final pregnancy diagnosis was performed at the end of the breeding season to confirm overall pregnancy rates. In 5 locations (n = 380 cows), fetal age was determined for each pregnant cow at the final pregnancy diagnosis based on fetal morphometries (Fontes et al., 2019). Cows that became pregnant to FTAI and were open in the final pregnancy diagnosis were classified as undergoing late embryonic/early fetal mortality (LEEFM). Cows that become pregnant to FTAI and were classified as pregnant by natural service in the final pregnancy diagnosis were also classified as undergoing LEEFM. Thirteen cows were culled prior to the final pregnancy diagnosis and not included in the analyses of final pregnancy rates and LEEFM.

Statistical analysis

All data were analyzed using the SAS Statistical Package (Version 9.4; SAS/STATS, SAS Inst. Inc. Cary, NC, USA) as completely randomized designed where cow was considered the experimental unit. All continuous data (BCS, DPP, follicle diameter, and follicular growth rate) were analyzed using the MIXED procedure of SAS, whereas binary data (estrus expression, pregnancy rates, and presence of a CL) were analyzed using the GLIMMIX procedure of SAS. For descriptive statistics (BCS, and DPP), models included the fixed effects of treatment and the random effects of location and treatment × location, whereas models utilized to evaluate ovarian response variables included the only fixed effects of treatment. Model utilized to analyze estrus expression included the fixed effects of treatment, BCS, DPP, and the two-way interactions between treatment and BCS, as well as treatment and DPP. When analyzing pregnancy rates to FTAI, final pregnancy rates, and LEEFM, models included the fixed effects of treatment, BCS, DPP, estrus, and the two-way interactions between treatment and each of the abovementioned explanatory variables. Models for estrus expression, pregnancy rates to FTAI, final pregnancy rates, and LEEFM included the random effects of location and the treatment × location interaction. Both DPP and BCS were divided into two categories (DPP: >72 or ≤72; BCS: adequate (BCS > 4) or low (BCS ≤ 4) when utilized as an explanatory variable for pregnancy rates and estrus expression. When analyzing the response variables estrus expression, pregnancy rates, and LEEFM, a backward stepwise selection approach was utilized where explanatory variables with the greatest P-value were gradually removed from the model. Variables with $P \leq 0.20$ were maintained in the final model and the main effect of treatment was also maintained in the model because of its relevance to the hypothesis tested herein.

Significance was declared at $P \leq 0.05$ and $0.05 > P \leq 0.10$ was considered a statistical tendency. All data is reported as least square means and SEM unless otherwise indicated.

Results

Data describing the population of cows enrolled in this study is summarized in Table 1. There were no differences in DPP (7&7 Synch: 75.7 ± 2.78 and 7&6 Synch: 74.0 ± 2.77; $P = 0.38$), BCS (7&7 Synch: 5.5 ± 0.08 and 7&6 Synch: 5.5 ± 0.08; $P = 0.90$), percentage of cows classified as being > 72 DPP (7&7 Synch: 66.3 ± 7.44 and 7&6 Synch: 55.4 ± 7.45%; $P = 0.20$), and percentage of cows classified as having adequate BCS (7&7 Synch: 68.3 ± 15.16 and 7&6 Synch: 68.3 ± 15.16; $P = 0.99$). Overall estrus expression across locations was 62.6% (546/872), ranging between 58.2% (Location 2) and 81.3% (Location 7). Cows with greater DPP (> 72) had a 19.4% increase ($P < 0.01$) in estrus expression compared with cows with fewer DPP (≤ 72; Figure 2). However, there were no differences between treatments in the proportion of cows expressing estrus

(*P* = 0.36; Figure 2) and there were no 2-way interactions between treatment and the other explanatory variables ($P \geq 0.41$).

Overall pregnancy rates to FTAI across locations were 60.8% (536/822), ranging from 53.4% (Location 5) to 64.1% (Location 4). Similar to the estrus expression results, there were no effects ($P = 0.37$) of treatment on pregnancy rates to FTAI (Figure 3). There was an interaction ($P = 0.05$) between treatment and BCS; however, the least square mean differences comparisons were not significant ($P \geq 0.36$), except for the comparison between 7&6 Synch cows with adequate or low BCS. Within 7&6 Synch,~~;~~ cows with low BCS tended ($P = 0.09$) to have decreased conception rates compared with 7&6 Synch cows with adequate BCS (49.0 ± 6.36% vs. 65.0 ± 3.81%, respectively). The remaining 2-way interactions between treatment and other explanatory variables were not significant ($P \leq 0.52$). Within both treatments, there was an effect of estrus ($P = 0.05$), where cows that expressed estrus between PG2 and FTAI had an 8.2% increase pregnancy rates compared with cows that failed to express estrus (Figure 3). Final pregnancy rates across treatments and locations were 90.9% (790/869) and the percentage of cows undergoing LEEFM was 6.1% (23/380). There was no effect of treatment ($P = 0.87$) on final pregnancy rates (7&7 Synch: 91.9 ± 2.18%; 7&6 Synch: 92.5 ± 2.06%), and no interaction between treatment and the remaining 2-way interactions ($P \geq 25$). There was also no main effect of treatment on LEEFM ($P = 0.84$; 7&7 Synch: 6.1 ± 3.23%; 7&6 Synch: 5.4 ± 3.49%). A tendency for a treatment × BCS interaction ($P = 0.10$) was observed for LEEFM; however, there were no differences in least square means comparisons ($P \geq 0.15$).

Results for the ovarian response variables are summarized in Table 2. Cows exposed to the 7&7 Synch tended to have larger follicle diameter at PG2 compared with 7&6 Synch cows (*P* = 0.09); however, there were no differences in follicle diameter at FTAI (*P* = 0.62). Cows that expressed estrus tended to have larger follicle diameter at FTAI compared with cows that failed to express estrus (*P* = 0.10). Yet, follicle diameter at PG2 and FTAI were not influenced by the interaction between treatment and estrus expression ($P \geq 0.71$). Follicular growth between PG2 and FTAI, percentage of cows with a corpus luteum at PG2, and ovulation rate were not impacted by treatment, estrus expression, or the interaction between treatment × estrus expression ($P \geq 0.52$).

Discussion

Since the establishment of GnRH and progesterone-based short-term estrus synchronization protocols for FTAI, such as the 7-day CO-Synch + CIDR (Lamb et al., 2006; Larson et al., 2006), two main independent adaptations to this protocol have been proposed to increase pregnancy rates. Presynchronization strategies aim to improve pregnancy rates to FTAI through increased ovulatory response to the initial GnRH injection (GnRH1), leading to more uniform subsequent follicular development (Bonacker et al., 2020; Perry et al., 2012), and increase fertility outcomes (Perry, et al., 2012; Oosthuizen, et al., 2020; Andersen et al., 2021). In addition, increasing proestrus length and decreasing circulating concentrations of progesterone earlier in the development of the ovulatory follicle has been shown to increase circulating concentrations of estradiol during final stages of follicular growth (Bridges, et al., 2010), improve subsequent embryo quality (Cerri, et al. 2009), and increase pregnancy rates to

FTAI (Bridges, et al., 2008; Bridges, et al., 2010). These studies were performed without the use of a presynchronization strategy; therefore, it is unknown if inducing an earlier decrease in circulating concentrations of progesterone also increases pregnancy rates in postpartum beef cows that have been previously presynchronized. In the present study, we hypothesized that early progesterone withdrawal and PG2 injection would optimize follicular development and increase pregnancy rates in presynchronized beef cows. Hence, the intravaginal progesterone device was removed and PG2 was administered on day 13 (6 days after the GnRH1) for cows enrolled in the 7&6 Synch treatment, exposing younger follicles (4-5 days after the expected follicular wave emergence) to a low progesterone environment compared with the 7&7 Synch treatment (5-6 days after follicular wave emergence).

Contrary to our hypothesis, there were no differences in pregnancy rates to FTAI in the present study. Bridges et al., (2008) reported an increase in fertility when cows were exposed to a 5-day CO-Synch + CIDR and FTAI was performed 72 h after PGF injection. Similar results were observed in dairy cows (Santos, et al., 2010). Nevertheless, exposing cows to the 5-day CO-Synch + CIDR protocol followed by FTAI 60 h after PGF failed to increase fertility when compared to 7-day CO-Synch + CIDR protocol (Bridges et al., 2008). Therefore, the use of a 60-66 h interval between PG2 and FTAI in the 7&6 Synch treatment might explain the lack of differences in pregnancy rates observed in the present study.

When utilizing the 5-day CO-Synch + CIDR protocol, intravaginal progesterone device removal and PG injection are performed approximately 3-4 days after follicular wave emergence. Although this strategy improves pregnancy rates to FTAI (Bridges et al., 2008; Bridges et al., 2010), two injections of PG 12 h apart were utilized to induce luteolysis (Bridges et al., 2008; Santos et al., 2010). The luteolytic response to PG at progesterone removal in cows that are exposed to the 5 d CO-Synch + CIDR program is suboptimal because of the transient inability of bovine corpus luteum to undergo luteolysis during early diestrus (Levy et al., 2000; Zalman et al., 2012). Although conflicting data is available in the literature (Whittier et al., 2010), studies have shown a decrease in estrus expression and pregnancy rates in postpartum beef cows and replacement beef and heifers when PG was only administered concurrently with intravaginal progesterone device removal (Kasimanickam et al., 2009; Peterson et al., 2011; Lima et al., 2013). Presynchronization already demands one extra handling event compared to conventional protocols and labor is one of the main challenges for the adoption of estrus synchronization in beef herds. Therefore, the 6-day interval between GnRH1 and PG2 was utilized in the 7&6 Synch treatment to avoid corpus luteum refractoriness while still inducing an earlier decrease in circulating concentrations of progesterone compared with the 7&7 Synch. A relatively similar interval between GnRH and PG has been previously utilized and shown to result in greater pregnancy rates compared with the 5-day CO-Synch + CIDR protocols (Perry et al., 2012). However, hastening intravaginal progesterone device removal and PG injection by one day was not enough to alter pregnancy rates in the present study.

Cows that expressed estrus had greater pregnancy rates to FTAI in the present study. These results corroborate with several studies that reported an increase in pregnancy rates in cows that express estrus in response to estrus synchronization (Richardson et al., 2016; Rodrigues, et al., 2018; Oosthuizen, et al., 2020b). Estrus expression is triggered by an increase in circulating concentrations of estradiol preceding ovulation. The mechanisms by which estradiol impacts fertility are associated with improved sperm transportation (Hawks, 1983), optimized uterine environment during the luteal phase (Ozturk and Demir, 2010), and increased embryo survival (Madsen, et al., 2015; Ketchum et al., 2023). Previous studies indicated that follicles have greater capacity to secrete estradiol during early stages of development (Rhodes, et al.,1995; Valdez, et al., 2005). Although estradiol concentration was not evaluated in this study, estrus expression was expected to be greater in the 7&6 Synch as a consequence of greater steroidogenic activity within the granulosa cells of the dominant follicles exposed to a low progesterone milieu earlier in development. When the interval between GnRH and PG was reduced from 7 to 5 days in lactating dairy cows, earlier administration of PG resulted a greater rate of increase in serum concentrations of estradiol and delayed, but more intense estrus expression based on activity monitors (Minela et al., 2023). Nevertheless, there were no differences between treatments in the percentage of cows expressing estrus prior to FTAI in the present study. The lack of differences in estrus expression might be related to the fact that both treatments had the same interval between luteolysis and FTAI.

Diameter of the ovulatory follicle is another factor influencing fertility in FTAI programs. Studies have shown the relationship between diameter of the ovulatory follicle and circulating concentrations of estradiol prior to FTAI (Vasconcelos, et al., 2001; Jinks, et al., 2013). Beef cows with follicles greater than 12 mm at FTAI had greater pregnancy rates than those with follicles ≤ 12 mm (Lamb, et al., 2001). Similar results have also been reported in *Bos indicus* females exposed to estradiol and progesterone-based estrus synchronization protocols (Sa Filho, et al., 2010; Alves et al., 2021). Nonetheless, preovulatory follicle diameter was only associated with conception rates when cows were induced to ovulate after GnRH injection. When cows were inseminated after spontaneous ovulation, follicle diameter did not affect conception rates (Perry, et al., 2005). Similarly, Bridges, et al. (2010) reported better pregnancy rates in cows with higher estradiol concentration, regardless of follicle size, indicating there are factors beyond follicle size influencing fertility. In the current study, there was a tendency for increased follicular diameter at PG2 in the 7&7 Synch treatment, due to the longer time allowed for follicular growth to occur between GnRH and PG2 compared to the 7&6 Synch. However, follicle diameter at FTAI and follicle growth rate did not differ between treatments. Therefore, we reject the hypothesis that early progesterone removal enhances follicular development and estrus expression when progesterone removal and PG2 were performed 6 days after GnRH1 in presynchronized beef cows. Decreasing the interval between GnRH1 and PG2 to 5 days and increasing the interval between PG2 and FTAI might be required in presynchronized beef cows to elicit changes in follicular growth dynamics associated with an early decrease early in circulating concentrations of progesterone.

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References

Abreu, F. M., Cruppe, L. H., Maquivar, M., Utt, M. D., Madsen, C. A., Vasconcelos, J. L. M., Mussard, M. L., Day, M. L., & Geary, T. W. (2014). Effect of follicle age on conception rate in beef heifers1. Journal of Animal Science, 92(3), 1022–1028. https://doi.org/10.2527/jas.2013-6761

Abreu, F. M., Geary, T. W., Cruppe, L. H., Madsen, C. A., Jinks, E. M., Pohler, K. G., Vasconcelos, J. L. M., & Day, M. L. (2014). The effect of follicle age on pregnancy rate in beef cows1. Journal of Animal Science, 92(3), 1015–1021. https://doi.org/10.2527/jas.2013-6760

Alves, R. L. O. R., Silva, M. A., Consentini, C. E. C., e Silva, L. O., Folchini, N. P., Oliva, A. L., Prata, A. B., Gonçalves, J. R. S., Wiltbank, M. C., & Sartori, R. (2021). Hormonal combinations aiming to improve reproductive outcomes of Bos indicus cows submitted to estradiol/progesterone-based timed AI protocols. Theriogenology, 169, 89–99. https://doi.org/10.1016/j.theriogenology.2021.04.007

Andersen, C. M., Bonacker, R. C., Smith, E. G., Spinka, C. M., Poock, S. E., & Thomas, J. M. (2021). Evaluation of the 7&7 Synch and 7-day CO-Synch + CIDR treatment regimens for control of the estrous cycle among beef cows prior to fixed-time artificial insemination with conventional or sex-sorted semen. Animal Reproduction Science, 235, 106892. https://doi.org/10.1016/j.anireprosci.2021.106892

Bleach, E. C. L., Glencross, R. G., & Knight, P. G. (2004). Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. Reproduction, 127(5), 621–629. https://doi.org/10.1530/rep.1.00190

Bonacker, R. C., Stoecklein, K. S., Locke, J. W. C., Ketchum, J. N., Knickmeyer, E. R., Spinka, C. M., Poock, S. E., & Thomas, J. M. (2020). Treatment with prostaglandin F2α and an intravaginal progesterone insert promotes follicular maturity in advance of gonadotropin-releasing hormone among postpartum beef cows. Theriogenology, 157, 350–359. https://doi.org/10.1016/j.theriogenology.2020.08.018

Bridges, G. A., Helser, L. A., Grum, D. E., Mussard, M. L., Gasser, C. L., & Day, M. L. (2008). Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology, 69(7), 843–851. https://doi.org/10.1016/j.theriogenology.2007.12.011

Bridges, G. A., Mussard, M. L., Burke, C. R., & Day, M. L. (2010). Influence of the length of proestrus on fertility and endocrine function in female cattle. Animal Reproduction Science, 117(3–4), 208–215. https://doi.org/10.1016/j.anireprosci.2009.05.002

Busch, D. C., Wilson, D. J., Schafer, D. J., Leitman, N. R., Haden, J. K., Ellersieck, M. R., Smith, M. F., & Patterson, D. J. (2007). Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers1. Journal of Animal Science, 85(8), 1933–1939. https://doi.org/10.2527/jas.2006-845

Cerri, R. L. A., Rutigliano, H. M., Chebel, R. C., & Santos, J. E. P. (2009). Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. REPRODUCTION, 137(5), 813–823. https://doi.org/10.1530/REP-08-0242

Colazo, M. G., Kastelic, J. P., Davis, H., Rutledge, M. D., Martinez, M. F., Small, J. A., & Mapletoft, R. J. (2008). Effects of plasma progesterone concentrations on LH release and ovulation in beef cattle given GnRH. Domestic Animal Endocrinology, 34(1), 109–117. https://doi.org/10.1016/j.domaniend.2006.11.004

Day, M. (2015). State of the art of GnRH-based timed AI in beef cattle. Animal Reproduction, 12(3), 473–478.

Geary, T. W., Whittier, J. C., Downing, E. R., LeFever, D. G., Silcox, R. W., Holland, M. D., Nett, T. M., & Niswender, G. D. (1998). Pregnancy rates of postpartum beef cows that were synchronized using Syncro-Mate-B or the Ovsynch protocol. Journal of Animal Science, 76(6), 1523. https://doi.org/10.2527/1998.7661523x

Jinks, E. M., Smith, M. F., Atkins, J. A., Pohler, K. G., Perry, G. A., MacNeil, M. D., Roberts, A. J., Waterman, R. C., Alexander, L. J., & Geary, T. W. (2013). Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows1. Journal of Animal Science, 91(3), 1176–1185. https://doi.org/10.2527/jas.2012-5611

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

Ketchum, J. N., Perry, G. A., Quail, L. K., Epperson, K. M., Ogg, M. A., Zezeski, A. L., Rich, J. J. J., Zoca, S. M., Kline, A. C., Andrews, T. N., Ortega, M. S., Smith, M. F., & Geary, T. W. (2023). Influence of preovulatory estradiol treatment on the maintenance of pregnancy in beef cattle receiving in vivo produced embryos. Animal Reproduction Science, 255, 107274. https://doi.org/10.1016/j.anireprosci.2023.107274

Lamb, G. C., Larson, J. E., Geary, T. W., Stevenson, J. S., Johnson, S. K., Day, M. L., Ansotegui, R. P., Kesler, D. J., DeJarnette, J. M., & Landblom, D. G. (2006). Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2α, and progesterone1. Journal of Animal Science, 84(11), 3000–3009. https://doi.org/10.2527/jas.2006-220

Lamb, G. C., Stevenson, J. S., Kesler, D. J., Garverick, H. A., Brown, D. R., & Salfen, B. E. (2001). Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. Journal of Animal Science, 79(9), 2253. https://doi.org/10.2527/2001.7992253x

Larson, J. E., Lamb, G. C., Stevenson, J. S., Johnson, S. K., Day, M. L., Geary, T. W., Kesler, D. J., DeJarnette, J. M., Schrick, F. N., DiCostanzo, A., & Arseneau, J. D. (2006). Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2α, and progesterone1. Journal of Animal Science, 84(2), 332–342. https://doi.org/10.2527/2006.842332x

Levy, N., Kobayashi, S., Roth, Z., Wolfenson, D., Miyamoto, A., & Meidan, R. (2000). Administration of Prostaglandin F2α During the Early Bovine Luteal Phase Does Not Alter the Expression of ET-1 and of Its Type A Receptor: A Possible Cause for Corpus Luteum Refractoriness. Biology of Reproduction, 63(2), 377–382. https://doi.org/10.1095/biolreprod63.2.377

Lima, F. S., Ribeiro, E. S., Bisinotto, R. S., Greco, L. F., Martinez, N., Amstalden, M., Thatcher, W. W., & Santos, J. E. P. (2013). Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers. Journal of Dairy Science, 96(11), 7054–7065. https://doi.org/10.3168/jds.2013-7093

Macmillan, K. L., & Thatcher, W. W. (1991). Effects of an Agonist of Gonadotropin-Releasing Hormone on Ovarian Follicles in Cattle1. Biology of Reproduction, 45(6), 883–889. https://doi.org/10.1095/biolreprod45.6.883

Madsen, C. A., Perry, G. A., Mogck, C. L., Daly, R. F., MacNeil, M. D., & Geary, T. W. (2015). Effects of preovulatory estradiol on embryo survival and pregnancy establishment in beef cows. Animal Reproduction Science, 158, 96–103. https://doi.org/10.1016/j.anireprosci.2015.05.006

Madureira, A. M. L., Polsky, L. B., Burnett, T. A., Silper, B. F., Soriano, S., Sica, A. F., Pohler, K. G., Vasconcelos, J. L. M., & Cerri, R. L. A. (2019). Intensity of estrus following an estradiol-progesterone-based ovulation synchronization protocol influences fertility outcomes. Journal of dairy science, 102(4), 3598–3608. https://doi.org/10.3168/jds.2018-15129

Madureira, A. M. L., Burnett, T. A., Marques, J. C. S., Moore, A. L., Borchardt, S., Heuwieser, W., Guida, T. G., Vasconcelos, J. L. M., Baes, C. F., & Cerri, R. L. A. (2022). Occurrence and greater intensity of estrus in recipient lactating dairy cows improve pregnancy per embryo transfer. Journal of dairy science, 105(1), 877–888. https://doi.org/10.3168/jds.2021-20437

Minela, T., Gibb, P., McBeth, S. et al. Reduced period from follicular wave emergence to luteolysis generated greater steroidogenic follicles and estrus intensity in dairy cows. Sci Rep 13, 22818 (2023). https://doi.org/10.1038/s41598-023-50001-x

Oosthuizen, N., Canal, L. B., Fontes, P. L. P., Sanford, C. D., DiLorenzo, N., Dahlen, C. R., Seidel, G. E., & Lamb, G. C. (2018). Prostaglandin F2α 7 d prior to initiation of the 7-d CO-synch + CIDR protocol failed to enhance estrus response and pregnancy rates in beef heifers. Journal of Animal Science, 96(4), 1466–1473. https://doi.org/10.1093/jas/sky058

Oosthuizen, N., Cooke, R. F., Schubach, K. M., Fontes, Pedro. L. P., Brandão, A. P., Oliveira Filho, R. v., Colombo, E. A., Franco, G. A., Reese, S., Pohler, K. G., & Lamb, G. C. (2020). Effects of estrous expression and intensity of behavioral estrous symptoms on variables associated with fertility in beef cows treated for fixed-time artificial insemination. Animal Reproduction Science, 214, 106308. https://doi.org/10.1016/j.anireprosci.2020.106308

Oosthuizen, N., Fontes, P. L. P., Porter, K., & Lamb, G. C. (2020). Presynchronization with prostaglandin F2α and prolonged exposure to exogenous progesterone impacts estrus expression and fertility in beef heifers. Theriogenology, 146, 88–93. https://doi.org/10.1016/j.theriogenology.2020.02.010

Oosthuizen, N., Fontes, P. L. P., Oliveira Filho, R. V., Dahlen, C. R., Grieger, D. M., Hall, J. B., Lake, S. L., Looney, C. R., Mercadante, V. R. G., Neville, B. W., Perry, G. A., Powell, J. G., Prezotto, L. D., Seidel, G. E., Walker, R. S., Cardoso, R. C., Pohler, K. G., & Lamb, G. C. (2021). Pre-synchronization of ovulation timing and delayed fixed-time artificial insemination increases pregnancy rates when sex-sorted semen is used for insemination of heifers. Animal Reproduction Science, 226, 106699. https://doi.org/10.1016/j.anireprosci.2021.106699

Ozturk S, Demir R. Particular functions of estrogen and progesterone in establishment of uterine

Perry, G. A., Perry, B. L., Krantz, J. H., & Rodgers, J. (2012). Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success1. Journal of Animal Science, 90(2), 489–494 https://doi.org/10.2527/jas.2011-4319

Perry, G. A., Smith, M. F., Lucy, M. C., Green, J. A., Parks, T. E., MacNeil, M. D., Roberts, A. J., & Geary, T. W. (2005). Relationship between follicle size at insemination and pregnancy success. Proceedings of the National Academy of Sciences, 102(14), 5268–5273. https://doi.org/10.1073/pnas.0501700102

Peterson, C., Alkar, A., Smith, S., Kerr, S., Hall, J. B., Moore, D., & Kasimanickam, R. (2011). Effects of one versus two doses of prostaglandin F2alpha on AI pregnancy rates in a 5-day, progesterone-based, CO-Synch protocol in crossbred beef heifers. Theriogenology, 75(8), 1536–1542. https://doi.org/10.1016/j.theriogenology.2010.12.017

Pursley, J. R., Mee, M. O., & Wiltbank, M. C. (1995). Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology, 44(7), 915–923. https://doi.org/10.1016/0093-691X(95)00279-H

receptivity and embryo implantation. Histol Histopathol. 2010;25(9):1215-28. PMid:20607663.

Rhodes, F. M., Fitzpatrick, L. A., Entwistle, K. W., & Kinder, J. E. (1995). Hormone concentrations in the caudal vena cava during the first ovarian follicular wave of the oestrous cycle in heifers. Reproduction, 104(1), 33–39. https://doi.org/10.1530/jrf.0.1040033

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

Rodgers, J. C., Bird, S. L., Larson, J. E., Dilorenzo, N., Dahlen, C. R., DiCostanzo, A., & Lamb, G. C. (2012). An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows1. Journal of Animal Science, 90(11), 4055–4062. https://doi.org/10.2527/jas.2011-4836

Rodrigues, A. D., Cooke, R. F., Cipriano, R. S., Silva, L. G. T., Cerri, R. L. A., Cruppe, L. H., Meneghetti, M., Pohler, K. G., & Vasconcelos, J. L. M. (2018). Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in Bos indicus-influenced beef cows1. Journal of Animal Science, 96(1), 236–249. https://doi.org/10.1093/jas/skx043

Rojas Canadas, E., Battista, S. E., Kieffer, J. D., Wellert, S. R., Mussard, M. L., & Garcia-Guerra, A. (2023). GnRH dose at initiation of a 5-day CO-Synch + P4 for fixed time artificial insemination in suckled beef cows. Animal Reproduction Science, 250, 107210. https://doi.org/10.1016/j.anireprosci.2023.107210

Sá Filho, M. F., Crespilho, A. M., Santos, J. E. P., Perry, G. A., & Baruselli, P. S. (2010). Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Animal Reproduction Science, 120(1–4), 23–30. https://doi.org/10.1016/j.anireprosci.2010.03.007

Santos, J. E. P., Narciso, C. D., Rivera, F., Thatcher, W. W., & Chebel, R. C. (2010). Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. Journal of Dairy Science, 93(7), 2976–2988. https://doi.org/10.3168/jds.2009-2870

Small, J. A., Colazo, M. G., Kastelic, J. P., & Mapletoft, R. J. (2009). Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. Theriogenology, 71(4), 698–706. https://doi.org/10.1016/j.theriogenology.2008.09.045

Thompson, K. E., Stevenson, J. S., Lamb, G. C., Grieger, D. M., & Löest, C. A. (1999). Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and prostaglandin F2alpha. Journal of Animal Science, 77(7), 1823. https://doi.org/10.2527/1999.7771823x

Valdez, K. E., Cuneo, S. P., Gorden, P. J., & Turzillo, A. M. (2005). The role of thecal androgen production in the regulation of estradiol biosynthesis by dominant bovine follicles during the first follicular wave1,2. Journal of Animal Science, 83(3), 597–603. https://doi.org/10.2527/2005.833597x

Vasconcelos, J. L. M., Sartori, R., Oliveira, H. N., Guenther, J. G., & Wiltbank, M. C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, 56(2), 307–314. https://doi.org/10.1016/S0093-691X(01)00565-9

Vasconcelos, J. L. M., Silcox, R. W., Rosa, G. J. M., Pursley, J. R., & Wiltbank, M. C. (1999). Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology, 52(6), 1067–1078. https://doi.org/10.1016/S0093-691X(99)00195-8

Whittier, W. D., Kasimanickam, R. K., Currin, J. F., Schramm, H. H., & Vlcek, M. (2010). Effect of timing of second prostaglandin F2α administration in a 5-day, progesterone-based CO-Synch protocol on AI pregnancy rates in beef cows. Theriogenology, 74(6), 1002–1009. https://doi.org/10.1016/j.theriogenology.2010.04.029

Zalman, Y., Klipper, E., Farberov, S., Mondal, M., Wee, G., Folger, J. K., Smith, G. W., & Meidan, R. (2012). Regulation of Angiogenesis-Related Prostaglandin F2alpha-Induced Genes in the Bovine Corpus Luteum1. Biology of Reproduction, 86(3). https://doi.org/10.1095/biolreprod.111.095067

Tables and Figures

Table 3.1. Descriptive data by location for postpartum cows enrolled in the study.

Location	State	No. of cows	Breed	DPP[1]	BCS[2]	Estrus expression, %[3]	PR/AI, %[4]
1	Tennessee	155	Angus	86.7 ± 14.91	-	75.5 (117/155)	62.6 (97/155)
2	Georgia	55	*Bos indicus*-influenced	78.1 ± 23.70	3.7 ± 0.78	58.2 (32/55)	60.0 (33/55)
3	Georgia	157	Angus	65.8 ± 20.34	5.6 ± 0.69	63.1 (99/157)	61.8 (97/157)
4	Georgia	78	Angus, Angus×Hereford	80.0 ± 19.62	4.9 ± 0.56	64.0 (126/197)	64.1 (50/78)
5	Georgia	204	Angus, Angus×Hereford	77.9 ± 21.38	4.7 ± 0.48	73.9 (125/169)	53.4 (109/204)
6	Georgia	158	Angus	86.7 ± 14.91	3.7 ± 0.78	65.8 (104/158)	64.0 (106/158)
7	South Dakota	75	Angus	-	-	81.3 (61/75)	58.7 (44/75)

[1] DPP: day postpartum at the time of fixed-time artificial insemination. Reported as mean ± SD.

[2] BCS: body condition score (Wagner et al., 1988). Reported as mean ± SD.

[3] Estrus expression between progesterone insert withdrawal and fixed-time artificial insemination.

[4] Combined pregnancy rates for both treatments.

Table 3.2. Impact of estrus synchronization treatment on ovarian response variables in a subset of animals.

	Treatment							
	7&6 Synch		7&7 Synch			P-value		
Item	Non-estrus	Estrus	Non-estrus	Estrus	SEM	TRT	Estrus	T × E
Follicle diameter, mm								
PG2	10.74	11.3	11.45	12.33	0.48	0.09	0.15	0.74
FTAI	13.06	14.23	13.80	14.54	0.62	0.37	0.10	0.71
Follicular growth[2], mm	2.31	2.86	2.35	2.10	0.66	0.56	0.81	0.52
Corpus luteum at PG2[3], %	100.0	66.67	70.00	80.00	14.49	0.98	0.98	0.98
Ovulation[4], %	100.0	83.33	70.00	100.00	14.49	0.99	0.99	0.96

[1] **7&7 Synch**: Cows received a controlled internal drug release insert (CIDR) and a 25-mg injection of prostaglandin $F_{2\alpha}$ (PG1; day 0). Cows received 100 µg of GnRH on day 7 and CIDR was removed seven days later concurrently with a second PG (PG2) injection. Cows received a second injection of GnRH and were fixed-time artificial insemination (FTAI) 60-66 h after PG2 (day 17). **7&6 Synch**: Cows received the same treatment; however, CIDR removal and PG2 and FTAI were performed on day 13 and 16, respectively.

[2] Defined as difference in follicle diameter between days -3 and 0.

[3] Cows with corpus luteum present at PG2.

[4] Cows were considered to when a CL was present on day 24.

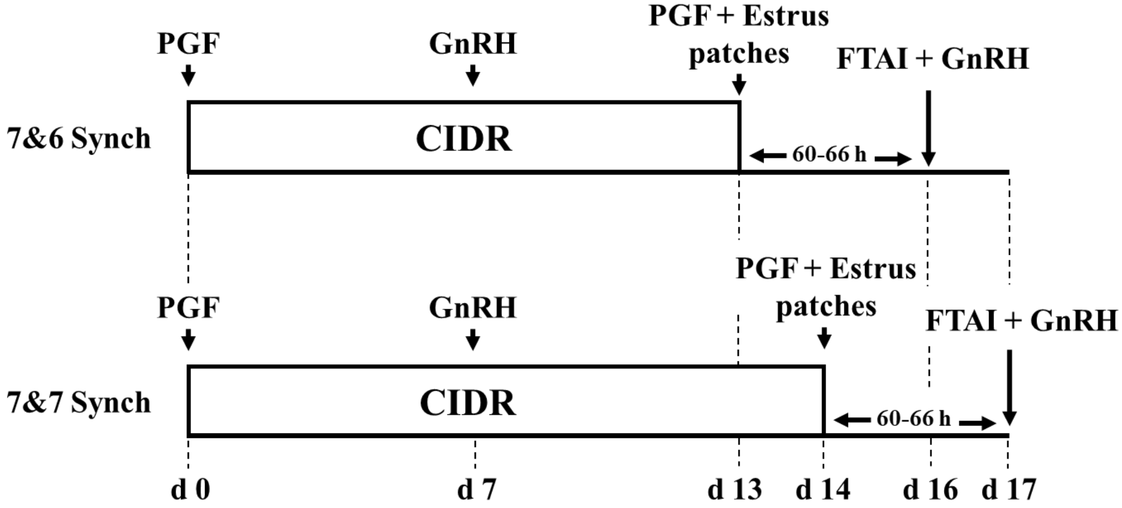

Figure 3.1. Schematics of experimental treatments. Postpartum beef cows (n = 882) from 7 locations were randomly assigned to either the 7&6 Synch or the 7&7 Synch treatments. GnRH: gonadotropin-releasing hormone. PGF: Prostaglandin $F_{2\alpha}$. FTAI: fixed-time artificial insemination. D: day. H: hours.

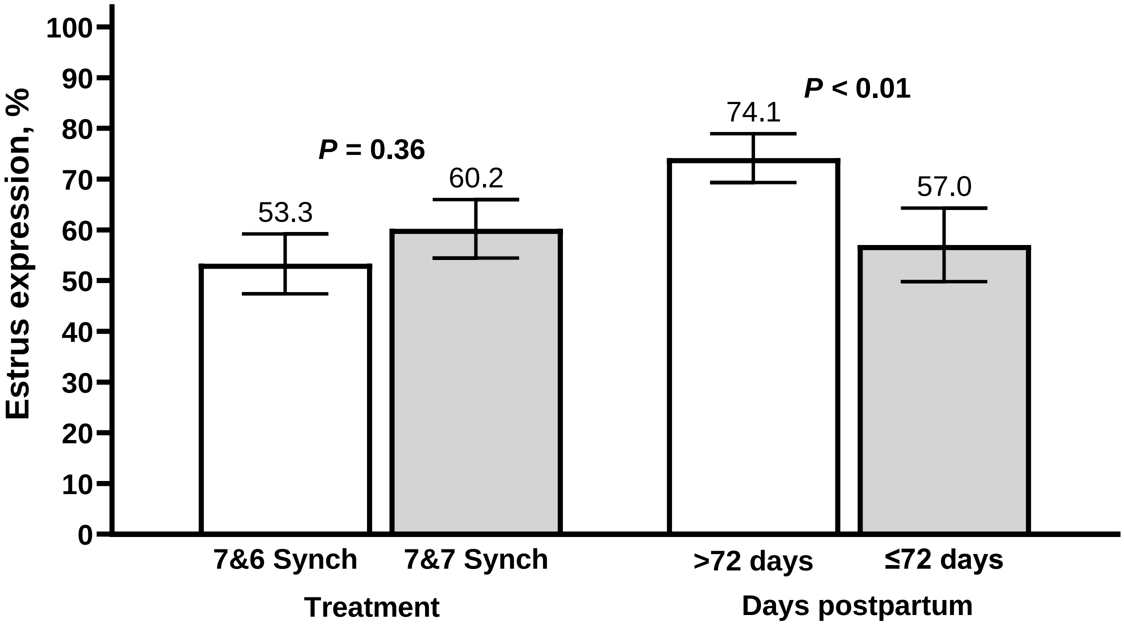

Figure 3.2. Impact of treatment and days postpartum (DPP) category on estrus expression of beef cows exposed to presynchronization. Postpartum beef cows (n = 882) were randomly assigned to either the 7&6 Synch or the 7&7 Synch treatments. Estrus detection patches were applied on the day of progesterone intravaginal device (CIDR) removal and utilized to evaluate estrus expression between the second prostaglandin $F_{2\alpha}$ and fixed-time artificial insemination (FTAI). Cows were considered to have expressed estrus prior to FTAI when at least 50% of the rub-off coating was removed from the patch at FTAI.

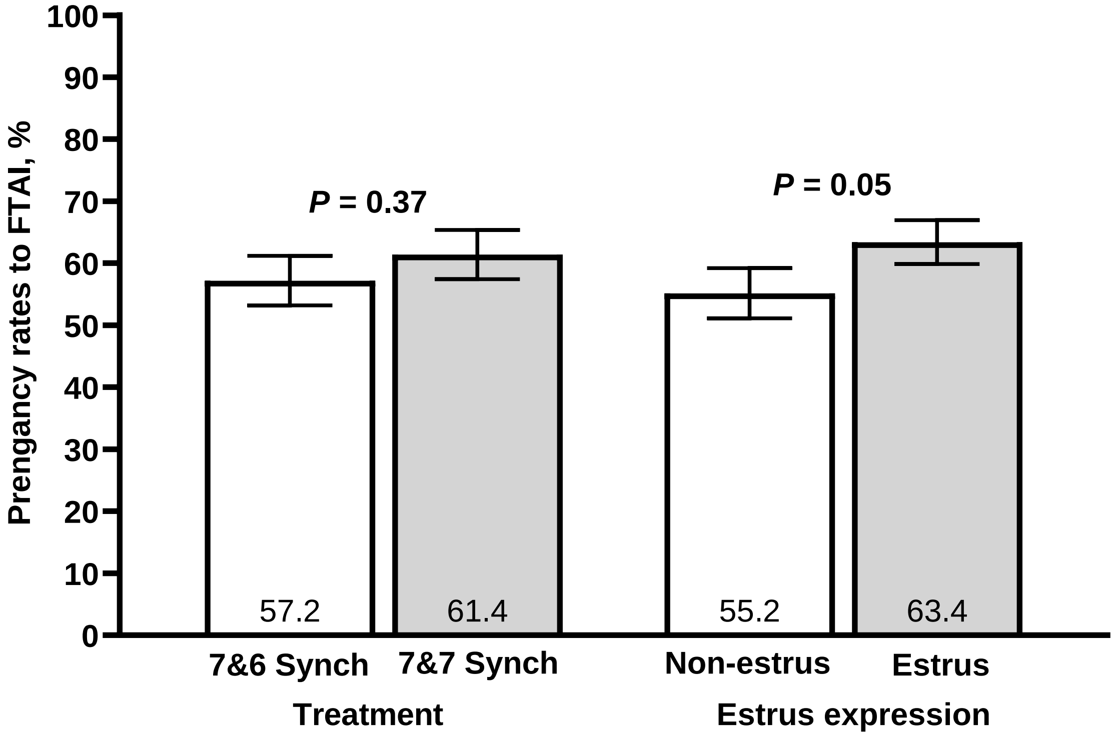

Figure 3.3. Effects of treatment and estrus expression on pregnancy rates to fixed-time artificial insemination (FTAI) in postpartum beef cows (n = 882) exposed to the 7&6

CHAPTER 4

IMPACT OF EARLY RESYNCHRONIZATION IN COMBINATION WITH COLOR DOPPLER ULTRASONOGRAPHY ON CUMULATIVE PREGNANCY RATES IN POSTPARTUM BEEF COWS

Introduction

Pregnancy rates and calf crop percentage are the main profitability driver in a cow-calf operation. In addition, timing of conception within the breeding season has a major effect on the cow herd efficiency (Oosthuizen et al., 2018). Studies demonstrated that females that conceive earlier wean heavier calves (Funston et al., 2012) and are more likely to remain in the herd (Cushman et al., 2013). Moreover, estrus synchronization (**ES**) and fixed-time artificial insemination (**FTAI**) are efficient tools to increase the proportion of cows conceiving in the beginning of the breeding season, leading to greater economic benefits when compared to natural service (Rodgers et al., 2012).

Cycling cows that failed to conceive to FTAI return to estrus 15 to 25 days after the artificial insemination (AI; Larson et al., 2009). Thus, non-pregnant cows exposed to natural service breeding after the first FTAI are serviced during this range of time. Nevertheless, utilization of proven sires in AI often add value to the calf-crop when compared to natural service sired offspring. Strategies that utilize progestin to facilitate estrus detection yield acceptable pregnancy rates and are efficient tools to increase the number of AI pregnancies earlier in the breeding season (Stevenson et al., 2003; Colazo et al., 2006). However, estrus detection is time consuming, labor intensive, and not feasible for extensive beef cattle operations. In order to overcome the need of estrus detection during a second AI service, resynchronization protocols were developed to expose females that fail to conceive to the first FTAI to a second FTAI (Sa Filho et al., 2014). Resynchronization has been largely utilized in beef herds in South America, where the extensive nature of cow-calf operations combined with the challenges associated with postpartum anestrus in *Bos indicus* cows limit use of estrus detection strategies for second service breeding (Baruselli, et al., 2018). Nonetheless, a major drawback of conventional resynchronization protocols is the long interval between the first and second FTAI, when utilizing conventional ultrasonography for early pregnancy diagnosis (Pieterse et al., 1990; Quintela et al., 2012).

Several studies combined an estradiol and progesterone-based resynchronization protocol with an early pregnancy diagnosis using color doppler ultrasonography (**CD**) to diagnose pregnancy early and decrease the interval between FTAI events (Pugliesi et al., 2019; Pugliesi et al., 2023). Color doppler ultrasonography allows the detection of blood perfusion in organs and structures. Different from the conventional brightness (B)-mode ultrasonography that detects pregnancy by visualizing the conceptus within the uterus, CD determines pregnancy status by estimating the corpus luteum (CL) blood perfusion as early as day 20 of gestation (Pugliesi, et al., 2014; Holton, et al., 2022). In Palhão, et al. (2020), combining a resynchronization protocol with CD examination allowed for a second FTAI service 23 days after the first FTAI and successfully increased the number of AI pregnancies in the beginning of the breeding season. Ataíde et al. (2021) exposed

Nelore females to two consecutive resynchronizations within 48 days, and the cumulative pregnancy rate was above 80%. Similarly, others reported acceptable conception rates and cumulative AI pregnancies in estradiol and progesterone-based early resynchronization strategies in *Bos indicus* cattle (Andrade, et al., 2020; Motta, et al., 2020; Silva et al., 2022). However, to our knowledge, these strategies have not been investigated using GnRH and progesterone-based protocols in *Bos taurus* beef cattle. Hence, the objective of this study is to evaluate a GnRH and progesterone-based early resynchronization strategy (**ER**) combined with CD. It was hypothesized that the use of the ER as a second service strategy results in similar pregnancy rates compared to natural service while generating a greater number of AI-pregnancies.

Materials and Methods

Animals and treatments

All animals were handled in accordance with procedures approved by the University of Georgia's Animal Care and Use Committee. (A2023 05-027-Y1-A0).

Three hundred and seventy-four postpartum *Bos taurus* beef cows (mean ± SD; body condition score = 5.41 ± 0.66; days postpartum = 64.91 ± 15.58; body weight = 1264.22 ± 180.86; age = 5.73 ± 2.66) from four locations (Georgia, Virginia 1, Virginia 2, and Mississippi) were enrolled in a completely randomized design. Within location, cows were stratified by days postpartum (**DPP**), body condition score (**BCS**), and estrus expression in the first FTAI before being randomly assigned to 1 of 2 treatments: 1) Natural Service (**NS**): Cows were exposed to the 7-d CO-Synch + CIDR protocol wherein cows were administered gonadotropin-releasing hormone (**GnRH**; 100 µg im; Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) and a controlled intravaginal drug release (**CIDR**) insert (EAZIBREED CIDR; 1.38 g progesterone; Zoetis Animal Health) on day −10. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg im; Lutalyse HighCon; dinoprost tromethamine; Zoetis Animal Health) was administered upon CIDR removal on day −3. Estrus detection patches were applied at CIDR removal and utilized to evaluate estrus expression between PG and FTAI (Estrotect Breeding Indicators; Rockway Inc., Spring Valley, WI). Cows were considered to have expressed estrus prior to FTAI when at least 75% of the rub-off coating was removed from the patch at FTAI (Fontes et al., 2019a; Oosthuizen et al., 2020). Cows were exposed to FTAI and concurrently received a second GnRH injection 60-66 h after CIDR removal. The day of FTAI was considered the day 0 of the study and considered the first FTAI service (FTAI1). Cows were exposed to natural service 15 days after FTAI1 for the remainder of the breeding season, which ranged from 65 (Tifton) to 75 d (Mississippi) from FTAI1 to cleanup bull removal. 2) Early Resynchronization (**ER**): cows received the same estrus synchronization protocol as NS cows; however, on day 15, ER cows received a CIDR that was previously used for 7 days. On day 20, CIDR was removed and CD was used to diagnose pregnancy based on the CL blood perfusion according to Holton et al., (2022 a,b). Non-pregnant cows received a 25-mg injection of PG and were FTAI concurrently with a 100-µg injection of GnRH 66 h after CIDR removal on day 23, considered as their second service strategy (FTAI2). Cows were exposed to natural service 7 days after FTAI2 for the remainder of the breeding season. All bulls utilized in the study underwent a breeding soundness evaluation and received a satisfactory potential breeder result within 45 days before the beginning of the breeding season. Bull to cow ratio was at least 1:25 across locations and treatments. Within location, the same AI sires and technicians were used in FTAI1 (both NS and ER treatments) and FTAI2 (ER treatment only). Similarly, the same natural service sires within each location were used in both NS and ER treatments. On day 53, conventional B-mode ultrasonography was performed in all cows to confirm gestation. Within location, NS and ER cows were maintained together and exposed to the same diet and environmental conditions, with the exception of the interval between days 15 to 30 of the study. On day 15, NS and ER cows were kept in a separate pen to allow NS cows to conceive via natural service. On day 30, cows were again comingled in the same pens for the reminder of the breeding season.

Color Doppler Ultrasonography and Pregnancy Diagnosis

Corpus luteum blood perfusion was evaluated 20 days after FTAI1 using CD (Sonoscape S8EXP; SonoScape Medical Corp,) as previously described (Ginther et al., 2007; Pugliesi et al., 2014; Holton et al., 2022). Cows with blood perfusion signals representing ≥30% of the CL area were considered pregnant to FTAI1. Cows without a CL and cows with a CL that had <30% of the its area with blood perfusion signals were considered not pregnant. Color doppler ultrasonography settings utilized were FPS 16, D/G 160/3, GN 255, I/P 1/40, PWR 70, PRF 1.0 *kHz,* WF 85, gain 35%, PWR 100 and frequency 7.2 *MHz.* On day 53, transrectal ultrasonography using the B-mode ultrasound was performed to confirm gestation based on the presence of a fetus or embryo within the uterus. Pregnancies were aged based on fetal morphometries to distinguish pregnancies that were generated via FTAI1 and FTAI2 in ER cows, and from pregnancies that were generated by FTAI1 and natural service in NS cows (Fontes et al., 2019b). Conception rates to FTAI1 was defined as the percentage of cows serviced in FTAI1 that became pregnant. Second service pregnancy rates was defined as the percentage of cows that failed to conceive to FTAI1 that became pregnant between 15 to 25 days after FTAI1. This included cows pregnant to FTAI2 in ER treatment and cows pregnant to natural service in the NS treatment. Cows diagnosed as pregnant based on CD on day 20 and non-pregnant based on B-mode ultrasonography were classified as receiving false-positive results in the CD examination. Conception rates to the second service for the ER treatment was defined as the percentage of cows serviced by FTAI2 that became pregnant to AI. Cows that received a false-positive diagnosis during CD examinations in the ER treatment were not included in the estimates of conception rates to the second service. For the NS treatment, conception rates to the second service was estimated using the same definition as the pregnancy rates to the second service for this treatment. Cumulative FTAI pregnancy rates was defined as the as the overall number of cows in each treatment that conceive to FTAI based on the pregnancy diagnosis performed on day 53. Final pregnancy rates was also evaluated via ultrasonography > 40 days after the end of the breeding season.

Statistical Analysis

All data were analyzed using the SAS Statistical Package (Version 9.4; SAS/STATS, SAS Inst. Inc. Cary, NC, USA) as a completely randomized design where the cow was considered the experimental unit. All continuous data (BCS, age, and DPP) were analyzed using the MIXED procedure of SAS using a Gaussian distribution, whereas all categorical data (estrus expression, conception rates to first and second service, second service pregnancy rates, cumulative pregnancy rates to FTAI and final pregnancy rates at the end of the breeding season) were analyzed using the GLIMMIX procedure and a binary distribution. Across all response variables, models utilized the fixed effects of treatment (NS and ER) and the random effect of location. Significance was declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$ was considered a statistical tendency. Data is reported as least square means and SEM unless otherwise indicated in the results.

Results

Data describing the population of cows enrolled in this study is summarized in Table 1. There were no differences between treatments in DPP (NS: 67.27 ± 2.9 and ER: 68.11 ± 2.96; $P = 0.51$), BCS (NS: 5.39 ± 0.15 and ER: 5.33 ± 0.15; $P = 0.37$), age (NS: 6.00 ± 0.34 and ER: 5.82 ± 0.34; $P = 0.58$) and percentage of cows expressing estrus prior to FTAI1 (NS: 61.42% ± 5% and ER: 56.11% ± 6%; $P = 0.30$). Data for reproductive outcomes are depicted in Table 2. Overall pregnancy rates to FTAI1 was 64.17% based on B-mode ultrasonography on day 53 and ranged from 59.89 to 74.71% between different locations. Moreover, pregnancy rates to FTAI1 based on the pregnancy diagnosis performed 20 days after AI using CD was 73.37% (ER treatment only), ranging from 68.18 to 77.14%. False-positive results were observed in 16.57% of the cows.

There was a significant effect of treatment on conception rates to FTAI1, where NS cows had greater ($P = 0.02$) conceptions rates compared with ER cows (70.44 vs

59.32%, respectively). Moreover, pregnancy rates to the second service were also greater (*P* = 0.01) in NS cows compared with ER cows (65.67 and 42.93% ± 10.47%, respectively). However, when conception rates were analyzed after the exclusion of ER cows that receive a false-positive diagnoses and did not receive FTAI2, there were no differences (*P* = 0.69) in conception rates between ER and NS cows (NS: 63.85 ± 10.47% and ER: 60.19 ± 9.4%; P = 0.01). There was also no effect of treatment (*P* = 0.40) on cumulative AI pregnancy rates during the first 23 days of the breeding season (NS: 71.47 ± 5.29% and ER: 75.42 ± 4.92%).

Discussion

One of the main limitations of conventional resynchronization strategies is the long interval between AI events, which delays the establishment of pregnancy in cows that failed to conceive to the first AI (Sa Filho et al., 1014). Cows that conceive later in the breeding season wean lighter calves and are less likely to stay in the herd (Funston et al., 2012; Stevenson et al., 2015). Moreover, a short breeding season allows for a more homogenous calf crop, better utilization of the available forage and facilitates management practices in a cow-calf operation (Vavra and Raleigh, 1976). Thus, research has aimed to establish early resynchronization strategies to decrease the interval between the first and second FTAI. The use of CD for early pregnancy diagnoses allowed the development of early resynchronization protocols starting as early as 12 (Pugliesi et al., 2019; Andrade et al., 2020), 13 (Ataide et al., 2021), and 14 (Motta et al., 2020; Vieira et al., 2021) days after the initial FTAI. These studies utilized estradiol benzoate to synchronize the emergence of a new follicular wave during the early resynchronization protocol and resulted in acceptable conception rates to the second FTAI. Hence, these protocols are gradually being adopted for early resynchronization programs in South American beef herds. Exogenous estradiol (i.e., estradiol benzoate and estradiol cypionate) products commonly utilized in South America are currently not available for estrus synchronization in the United States and resynchronization programs that combine CD with a GnRH and progesterone-based protocol have not been established.

The present study evaluated the fertility of a GnRH and progesterone-based early resynchronization strategy compared with natural service. While pregnancy rates to FTAI resulted in acceptable pregnancy rates (64.17%), the early resynchronization strategy proposed herein decreased conception rates to FTAI1. Considering that treatments were assigned after FTAI1, these results indicate a negative impact of ER on the pregnancy establishment. Early pregnancy is a characterized by remarkable changes in conceptus development and uterine function. Around 40% of pregnancy failure that occurs during the first 30 days of gestation coincides with the maternal recognition of pregnancy, a period when the conceptus secretes *interferon t*, a substance that inhibit PG synthesis and prolong the corpus luteum lifespan (Meyer, et al., 1995). Previous studies reported a decrease in pregnancy rates when cows were exposed to stressful events such as transportation or handling during the period of maternal recognition of pregnancy (Merrill et al., 2007; Geary, et al., 2010). In the present study, ER cows were brought to facilities and handled at least twice during this critical period in which onset of conceptus elongation, maternal recognition of pregnancy, and the beginning of conceptus attachment occur. Thus, decreased FTAI1 pregnancy rate observed in ER cows might be associated with a detrimental effect of handling stress during resynchronization on pregnancy establishment. Moreover, although previous literature validating CD for pregnancy diagnosis reported no false-negative results (Fontes and Oosthuizen, 2022), the subjective measurements given chute-side might have generated false-negative diagnosis. Conversely, several studies also reported no detrimental effect to the first FTAI when cows were handled during maternal recognition of pregnancy (Van Cleeff et al., 1995; Stevenson, et al., 2003; Larson, et al, 2009). Although there is considerable literature evaluating early resynchronization strategies, this is the first time an early resynchronization strategy is compared solely with natural service as the second service strategy, thus, more research is warranted to understand embryonic losses during early resynchronization protocols.

Pregnancy rate to FTAI2 was decreased for ER compared with NS. Although natural breeding methods require a considerable proportion of cows in adequate reproductive status to express estrus and, postpartum anestrus is a major limiting factor of early conception in beef herds (Yavas and Walton, 2000), studies have demonstrated greater pregnancy rates (Ferreira, et al., 2018) and decreased average conception day (lamb et al., 2008) to natural service when estrus synchronization was performed. Yet, Perry, et al. (2004) demonstrated that progestin exposure resumed cyclicity and decreased the incidence of short estrous cycle. Therefore, we believe that ES prior to the initial FTAI induced cyclicity in cows beneficiating NS. Moreover, the resynchronization protocol utilized herein was based on progestin sources only and studies that resynchronized estrus utilizing exclusively progestin sources reported different outcomes. Stevenson, et al, (2003) reported an increased percentage of inseminated non-pregnant females returning to estrus after progestin exposure from day 13 to 20. Likewise, cows exposed to CIDR between days 14 and 21 after FTAI were successfully resynchronized and returned in estrus in a short time frame (Larson, et al., 2009); however, in both studies, conception rates to the resynchronized estrus were lower compared to cows that return in estrus naturally, which was attributed to the ovulation of dominant persistent follicles induced by prolonged serum progesterone concentration (Sanchez et al., 1993; Mihm et al., 1994). Under sub-luteal progesterone concentration, the absence of luteinizing hormone (LH) suppression prevents follicular atresia. Thus, the lifespan of the dominant follicle is prolonged, forming persistent follicles that are associated with lower fertility (Savio, et al., 1993). In order to reduce the formation of persistent follicles, resynchronized cows were exposed to CIDR only for five days in this study. More recent data compared conception rates to FTAI in Nelore heifers submitted to an early resynchronization protocol with or without a long-acting progesterone (P4-LA). Heifers received either a CIDR or a CIDR and 75 mg P4-LA 12 days after the initial FTAI. At day 20, cows identified as non-pregnant via CDU received a PG injection, estradiol cypionate and equine chorionic gonadotropin and FTAI 48 h later. Heifers exposed to greater progesterone concentration had increased pregnancy rates to the second FTAI (Pugliesi, et al., 2019). Indeed, progesterone administration regress dominant follicles and recruits a follicular wave (Anderson and Day, 1994); however, only high doses of progesterone induce atresia in follicles before divergence (Cavalieri, 2018).

Another factor explaining the lower pregnancy rate in the ER group is the percentage of cows having false-positive results in the CD diagnosis. In this study, 16.57% of cows in the ER group were misdiagnosed as pregnant in the CD, therefore, they were not exposed to the second FTAI, compromising pregnancy rates. According to Siqueira et al. (2013), the proportion of false-positive results (**FP**) for CD 20 days after AI in dairy cows was 25.8%. In beef cows, test performance parameters for CD 20 days after FTAI reported 9% FP and absence of false-negative results (**FN**; Pugliesi et al., 2014). More recent data from our lab also reported no FN results, although the proportion of cows having FP results were 13% in cows (Holton et al., 2022a) and 9% in heifers (Holton et al., 2022b). According to the authors abovementioned, some of the FP results may be attributed to embryonic losses occurring between the CD diagnosis and the gold-standard B-mode ultrasonography at day 30 (Fontes and Oosthuizen, 2022). Thus, CD is an accurate method to identify non-pregnant animals with high sensibility; however, the relatively high FP results leads to lower specificity. In an early resynchronization scenario, absence of FN results allows for utilization of synchronization protocols that induce luteolysis, without compromising established pregnancies; however, the percentage of FP results decrease the proportion of non-pregnant cows exposed to the second AI, which leads to lower pregnancy rates. When conception rates for the second serviced were analyzed including only cows exposed to FTAI2 in the ER treatment, conception rates were not different between ER and NS treatments (63.85 and 60.19%, respectively). This indicates that an early resynchronization protocol utilizing only progesterone without GnRH to synchronize follicular wave emergence results in acceptable conception rates.

It was hypothesized that ER would generate greater cumulative AI pregnancy rates compared to the natural service strategy. In Pugliesi, et al. (2019), two early resynchronization strategies yielded up to 76% of pregnancy rates in the first 22 days of the breeding season. Similarly, Palhao, et al. (2020) utilized an early resynchronization protocol starting 13 days after the initial FTAI and successfully increased the number of AI pregnancies in the beginning of the breeding season, yielding 70.5% cumulative conception rate in the first 23 days. Others reported acceptable conception rates and cumulative AI pregnancies in early resynchronization strategies in *Bos indicus* cattle (Andrade, et al., 2020; Motta, et al., 2020; Ataide Junior, et al., 2021; Silva et al., 2022). In this study, although ER generated 73.4% of AI pregnancies within 23 days, the decrease in conception rates to the initial FTAI1 compared with NS treatment resulted in no differences in cumulative pregnancy rates to AI (ER: 75.46% and NS: 71.67 %) Therefore, the hypothesis that ER would generate greater AI cumulative pregnancy rates compared with NS was not supported by the results reported herein.

References

Anderson, L. H., & Day, M. L. (1994). Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melengestrol acetate. *Journal of Animal Science*, *72*(11), 2955–2961. https://doi.org/10.2527/1994.72112955x

Andrade, J. P. N., Gomez-León, V. E., Andrade, F. S., Carvalho, B. P., Lacouth, K. L., Garcia, F. Z., Jacob, J. C. F., Sales, J. N. S., Wiltbank, M. C., & Mello, M. R. B. (2020). Development of a novel 21-day reinsemination program, ReBreed21, in Bos indicus heifers. *Theriogenology*, *155*, 125–131. https://doi.org/10.1016/j.theriogenology.2020.04.021

Ataide Junior, G. A., Kloster, A., de Moraes, É. G., Motta, I. G., Claro Junior, I., Vasconcelos, J. L. M., Ferraz, P. A., de Paula Nogueira, G., & Pugliesi, G. (2021). Early resynchronization of follicular wave emergence among Nelore cattle using injectable and intravaginal progesterone for three timed artificial inseminations. *Animal Reproduction Science, 229*, 106759. https://doi.org/10.1016/j.anireprosci.2021.106759

Baruselli, P. S., Ferreira, R. M., Sá Filho, M. F., & Bó, G. A. (2018). Review: Using artificial insemination v. natural service in beef herds. *Animal*, *12,* s45–s52. https://doi.org/10.1017/S175173111800054X

Cavalieri, J. (2018). Effect of treatment of Bos indicus heifers with progesterone 0, 3 and 6 days after follicular aspiration on follicular dynamics and the timing of oestrus and ovulation. *Animal Reproduction Science*, *193*, 9–18. https://doi.org/10.1016/j.anireprosci.2018.03.026

Colazo, M. G., Kastelic, J. P., Mainar-Jaime, R. C., Gavaga, Q. A., Whittaker, P. R., Small, J. A., Martinez, M. F., Wilde, R. E., Veira, D. M., & Mapletoft, R. J. (2006). Resynchronization of previously timed-inseminated beef heifers with progestins. *Theriogenology*, *65*(3), 557–572. https://doi.org/10.1016/j.theriogenology.2005.06.001

Cushman, R. A., Kill, L. K., Funston, R. N., Mousel, E. M., & Perry, G. A. (2013). Heifer calving date positively influences calf weaning weights through six parturitions1. *Journal of Animal Science, 91*(9), 4486–4491. https://doi.org/10.2527/jas.2013-6465

Ferreira, R. M., Conti, T. L., Gonçalves, R. L., Souto, L. A., Sales, J. N. S., Sá Filho, M. F., Elliff, F. M., & Baruselli, P. S. (2018). Synchronization treatments previous to natural breeding anticipate and improve the pregnancy rate of postpartum primiparous beef cows. *Theriogenology*, *114*, 206–211. https://doi.org/10.1016/j.theriogenology.2017.11.022

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

Fontes, P. L. P., & Oosthuizen, N. (2022). Applied Use of Doppler Ultrasonography in Bovine Reproduction. *Frontiers in Animal Science*, *3*. https://doi.org/10.3389/fanim.2022.912854

Fontes, P. L. P., Oosthuizen, N., Ciriaco, F. M., Sanford, C. D., Canal, L. B., Pohler, K. G., Henry, D. D., Mercadante, V. R. G., Timlin, C. L., Ealy, A. D., Johnson, S. E., DiLorenzo, N., & Lamb, G. C. (2019). Impact of fetal vs. maternal contributions of *Bos indicus* and *Bos taurus* genetics on embryonic and fetal development1. *Journal of Animal Science*, *97*(4), 1645–1655. https://doi.org/10.1093/jas/skz044

Funston, R. N., Musgrave, J. A., Meyer, T. L., & Larson, D. M. (2012). Effect of calving distribution on beef cattle progeny performance. *Journal of Animal Science*, *90*(13), 5118–5121. https://doi.org/10.2527/jas.2012-5263

Geary, T. W., Ansotegui, R. P., MacNeil, M. D., Roberts, A. J., & Waterman, R. C. (2010). Effects of flunixin meglumine on pregnancy establishment in beef cattle1. *Journal of Animal Science*, *88*(3), 943–949. https://doi.org/10.2527/jas.2009-2087

Guimarães da Silva, A., Nishimura, T. K., Rocha, C. C., Motta, I. G., Laurindo Neto, A., Ferraz, P. A., Bruni, G. A., Orlandi, R. E., Massoneto, J. P. M., & Pugliesi, G. (2022). Comparison of estradiol benzoate doses for resynchronization of ovulation at 14 days after timed-AI in suckled beef cows. *Theriogenology*, *184*, 41–50. https://doi.org/10.1016/j.theriogenology.2022.02.025

Holton, M. P., Oosthuizen, N., Melo, G. D. de, Davis, D. B., Stewart, R. L., Pohler, K. G., Lamb, G. C., & Fontes, P. L. P. (2022). Luteal color doppler ultrasonography and pregnancy-associated glycoproteins as early pregnancy diagnostic tools and predictors of pregnancy loss in *Bos taurus* postpartum beef cows. *Journal of Animal Science*, *100*(2). https://doi.org/10.1093/jas/skac018

Lamb, G. C., Dahlen, C. R., Vonnahme, K. A., Hansen, G. R., Arseneau, J. D., Perry, G. A., Walker, R. S., Clement, J., & Arthington, J. D. (2008). Influence of a CIDR prior to bull breeding on pregnancy rates and subsequent calving distribution. *Animal Reproduction Science*, *108*(3–4), 269–278. https://doi.org/10.1016/j.anireprosci.2007.08.012

Larson, J. E., Thielen, K. N., Funnell, B. J., Stevenson, J. S., Kesler, D. J., & Lamb, G. C. (2009). Influence of a controlled internal drug release after fixed-time artificial insemination on pregnancy rates and returns to estrus of nonpregnant cows1. *Journal of Animal Science*, *87*(3), 914–921. https://doi.org/10.2527/jas.2008-1443

Merrill, M. L., Ansotegui, R. P., Burns, P. D., MacNeil, M. D., & Geary, T. W. (2007). Effects of flunixin meglumine and transportation on establishment of pregnancy in beef cows1. *Journal of Animal Science*, *85*(6), 1547–1554. https://doi.org/10.2527/jas.2006-587

Meyer, M. D., Hansen, P. J., Thatcher, W. W., Drost, M., Badinga, L., Roberts, R. M., Li, J., Ott, T. L., & Bazer, F. W. (1995). Extension of Corpus Luteum Lifespan and Reduction of Uterine Secretion of Prostaglandin F2 of Cows in Response to Recombinant Interferon-τ. *Journal of Dairy Science*, *78*(9), 1921–1931. https://doi.org/10.3168/jds.S0022-0302(95)76817-5

Mihm, M., Baguisi, A., Boland, M. P., & Roche, J. F. (1994). Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *Reproduction*, *102*(1), 123–130. https://doi.org/10.1530/jrf.0.1020123

Motta, I. G., Rocha, C. C., Bisinotto, D. Z., Melo, G. D., Ataide Júnior, G. A., Silva, A. G., Gonzaga, V. H. G., Santos, J. A., Freitas, B. G., Lemes, K. M., Madureira, E. H., & Pugliesi, G. (2020). Increased pregnancy rate in beef heifers resynchronized with estradiol at 14 days after TAI. *Theriogenology*, *147*, 62–70. https://doi.org/10.1016/j.theriogenology.2020.02.009

Oosthuizen, N., Cooke, R. F., Schubach, K. M., Fontes, Pedro. L. P., Brandão, A. P., Oliveira Filho, R. V., Colombo, E. A., Franco, G. A., Reese, S., Pohler, K. G., & Lamb, G. C. (2020). Effects of estrous expression and intensity of behavioral estrous symptoms on variables associated with fertility in beef cows treated for fixed-time artificial insemination. *Animal Reproduction Science*, *214*, 106308. https://doi.org/10.1016/j.anireprosci.2020.106308

Oosthuizen, N., Fontes, P. L. P., Sanford, C. D., Ciriaco, F. M., Henry, D. D., Canal, L. B., DiLorenzo, N., & Lamb, G. C. (2018). Estrus synchronization and fixed-time artificial insemination alter calving distribution in Bos indicus influenced beef heifers. *Theriogenology*, *106*, 210–213. https://doi.org/10.1016/j.theriogenology.2017.10.028

Palhão, M. P., Ribeiro, A. C., Martins, A. B., Guimarães, C. R. B., Alvarez, R. D., Seber, M. F., Fernandes, C. A. C., Neves, J. P., & Viana, J. H. M. (2020). Early resynchronization of non-pregnant beef cows based in corpus luteum blood flow evaluation 21 days after Timed-AI. *Theriogenology*, *146*, 26–30. https://doi.org/10.1016/j.theriogenology.2020.01.064

Perry, G. A., & Geary, T. W. (2004). *Ability of CIDRs or Melengestrol Acetate to Initiate Estrous Cycles in Early Postpartum Beef Cows*. http://openprairie.sdstate.edu/sd_beefreport_2004http://openprairie.sdstate.edu/sd_beefreport_2004/16

Pieterse, M. C., Szenci, O., Willemse, A. H., Bajcsy, C. S. A., Dieleman, S. J., & Taverne, M. A. M. (1990). Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology*, *33*(3), 697–707. https://doi.org/10.1016/0093-691X(90)90547-7

Pugliesi, G., Bisinotto, D. Z., Mello, B. P., Lahr, F. C., Ferreira, C. A., Melo, G. D., Bastos, M. R., & Madureira, E. H. (2019). A novel strategy for resynchronization of ovulation in Nelore cows using injectable progesterone (P4) and P4 releasing devices to perform two timed inseminations within 22 days. *Reproduction in Domestic Animals*, *54*(8), 1149–1154. https://doi.org/10.1111/rda.13475

Pugliesi, G., Guimarães da Silva, A., Viana, J. H. M., & Siqueira, L. G. B. (2023). Review: Current status of corpus luteum assessment by Doppler ultrasonography to diagnose non-pregnancy and select embryo recipients in cattle. *Animal*, *17*, 100752. https://doi.org/10.1016/j.animal.2023.100752

Pugliesi, G., Miagawa, B. T., Paiva, Y. N., França, M. R., Silva, L. A., & Binelli, M. (2014). Conceptus-Induced Changes in the Gene Expression of Blood Immune Cells and the Ultrasound-Accessed Luteal Function in Beef Cattle: How Early Can We Detect Pregnancy?1. *Biology of Reproduction*, *91*(4). https://doi.org/10.1095/biolreprod.114.121525

Quintela, L., Barrio, M., Peña, A., Becerra, J., Cainzos, J., Herradón, P., & Díaz, C. (2012). Use of Ultrasound in the Reproductive Management of Dairy Cattle. *Reproduction in Domestic Animals*, *47*(s3), 34–44. https://doi.org/10.1111/j.1439-0531.2012.02032.x

Rodgers, J. C., Bird, S. L., Larson, J. E., Dilorenzo, N., Dahlen, C. R., DiCostanzo, A., & Lamb, G. C. (2012). An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows1. *Journal of Animal Science*, *90*(11), 4055–4062. https://doi.org/10.2527/jas.2011-4836

Sá Filho, M. F., Marques, M. O., Girotto, R., Santos, F. A., Sala, R. V., Barbuio, J. P., & Baruselli, P. S. (2014). Resynchronization with unknown pregnancy status using progestin-based timed artificial insemination protocol in beef cattle. *Theriogenology, 81*(2), 284–290. https://doi.org/10.1016/j.theriogenology.2013.09.027

Sanchez, T., Wehrman, M. E., Bergfeld, E. G., Peters, K. E., Kojima, F. N., Cupp, A. S., Mariscal, V., Kittok, R. J., Rasby, R. J., & Kinder, J. E. (1993). Pregnancy Rate is Greater When the Corpus Luteum is Present during the Period of Progestin Treatment to Synchronize Time of Estrus in Cows and Heifers1. *Biology of Reproduction*, *49*(5), 1102–1107. https://doi.org/10.1095/biolreprod49.5.1102

Savio, J. D., Thatcher, W. W., Morris, G. R., Entwistle, K., Drost, M., & Mattiacci, M. R. (1993). Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *Reproduction*, *98*(1), 77–84. https://doi.org/10.1530/jrf.0.0980077

Siqueira, L. G. B., Areas, V. S., Ghetti, A. M., Fonseca, J. F., Palhao, M. P., Fernandes, C. A. C., & Viana, J. H. M. (2013). Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. *Journal of Dairy Science, 96*(10), 6461–6472. https://doi.org/10.3168/jds.2013-6814

Stevenson, J. S., Hill, S. L., Bridges, G. A., Larson, J. E., & Lamb, G. C. (2015). Progesterone status, parity, body condition, and days postpartum before estrus or ovulation synchronization in suckled beef cattle influence artificial insemination pregnancy outcomes1. *Journal of Animal Science*, *93*(5), 2111–2123. https://doi.org/10.2527/jas.2014-8391

Stevenson, J. S., Johnson, S. K., Medina-Britos, M. A., Richardson-Adams, A. M., & Lamb, G. C. (2003). Resynchronization of estrus in cattle of unknown pregnancy status using estrogen, progesterone, or both1. *Journal of Animal Science*, *81*(7), 1681–1692. https://doi.org/10.2527/2003.8171681x

Van Cleeff, J., Macmillan, K. L., Drost, M., Lucy, M. C., & Thatcher, W. W. (1996). Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to service. *Theriogenology*, *46*(7), 1117–1130. https://doi.org/10.1016/S0093-691X(96)00284-1

MARTIN VAVRA, & ROBERT J. RALEIGH. (1976). Coordinating Beef Cattle Management with the Range Forage Resource . *JOURNAL OF MARTIN VAVRA, & ROBERT J. RALEIGH. (1976). Coordinating Beef Cattle Management with the Range Forage Resource* . *JOURNAL OF RANGE MANAGEMENT , 29(6), 449–452.RANGE MANAGEMENT* , *29*(6), 449–452.

Vieira, C. C., Pinto, H. F., Buss, V., Gonzalez de Freitas, B., Guerreiro, B. M., Leivas, F. G., Pugliesi, G., & Mesquita, F. S. (2021). Resynchronization of follicular wave using long-acting injectable progesterone or estradiol benzoate at 14 days posttimed AI in Bos taurus x Bos indicus beef heifers. *Theriogenology*, *176*, 194–199. https://doi.org/10.1016/j.theriogenology.2021.09.017

Yavas, Y., & Walton, J. S. (2000). Postpartum acyclicity in suckled beef cows: A review. *Theriogenology*, *54*(1), 25–55. https://doi.org/10.1016/S0093-691X(00)00323-X

Tables and figures

Table 4.1. Descriptive data for cows enrolled in the study

Item	Natural Service	Early Resynch	Overall	*P*-value
Body weight[1]	1262.14 ± 155.69	1266.46 ± 207.40	1264.22 ± 180.68	0.93
DPP[2]	64.43 ± 15.08	65.39 ± 16.09	64.91 ± 15.58	0.58
BCS[3]	5.44 ± 0.65	5.38 ± 0.67	5.41 ± 0.66	0.37
Age	5.82 ± 2.56	5.64 ± 2.77	5.73 ± 2.66	0.53
Estrus expression[4]	61.42% ± 5.9%	56.11% ± 6.1%	56.14%	0.30

[1] BW: body weight (Kg) at the time of CIDR insertion. Reported as mean ± SD.

[2] DPP: day postpartum at the time of fixed-time artificial insemination. Reported as mean ± SD.

[3] BCS: body condition score (Wagner et al., 1988). Reported as mean ± SD.

[4] Estrus expression between progesterone insert withdrawal and fixed-time artificial insemination. Reported as least square means ± SEM.

Table 4.2. Impact of treatment on reproductive outcomes of beef cows exposed to natural service (NS) or early resynchronization (ER).

Item	Natural service	Early resynch	SEM	*P*-value
FTAI1 conception rate[1]	70.44	59.32	4.80	0.02
2nd service conception rate[2]	63.85	60.19	9.40	0.58
2nd service pregnancy rate[3]	65.67	42.93	10.47	0.01
Cumulative AI pregnancy rate	71.67	75.46	5.20	0.40

NS: Cows were exposed to the 7-d CO-Synch + CIDR protocol and fixed-time artificial inseminated (**FTAI**) 60-66 h after CIDR removal. The day of FTAI was the day 0 of the study and considered the first FTAI service (**FTAI1**). Cows were exposed to natural service 15 days after FTAI1 for the remainder of the breeding season.

ER: Cows received the same estrus synchronization protocol as NS cows; however, on day 15, ER cows received a CIDR that was previously used CIDR for 7 days. On day 20, CIDR was removed and color-doppler ultrasonography (CD) was used to diagnose pregnancy based on the corpus luteum (CL) blood perfusion according to Holton et al., (2022 a,b). Non-pregnant cows received a 25-mg injection of PG and were FTAI concurrently with a 100-µg injection of GnRH 66 h after CIDR removal on day 23, considered as their second service strategy (FTAI2).

[1] FTAI1 conception rate was defined as the percentage of cows serviced in FTAI1 that became pregnant.

[2] 2nd service conception rate for ER was defined as the percentage of cows serviced by FTAI2 that became pregnant to AI. Cows that received a false-positive diagnosis during examinations were not included in the estimates of conception rates to the second service. For the NS treatment, conception rates to the second service was estimated using the same definition as the pregnancy rates to the second service for this treatment.

[3] 2nd Service pregnancy rate was defined as the percentage of cows that failed to conceive to FTAI1 that became pregnant between 15 to 25 days after FTAI1.

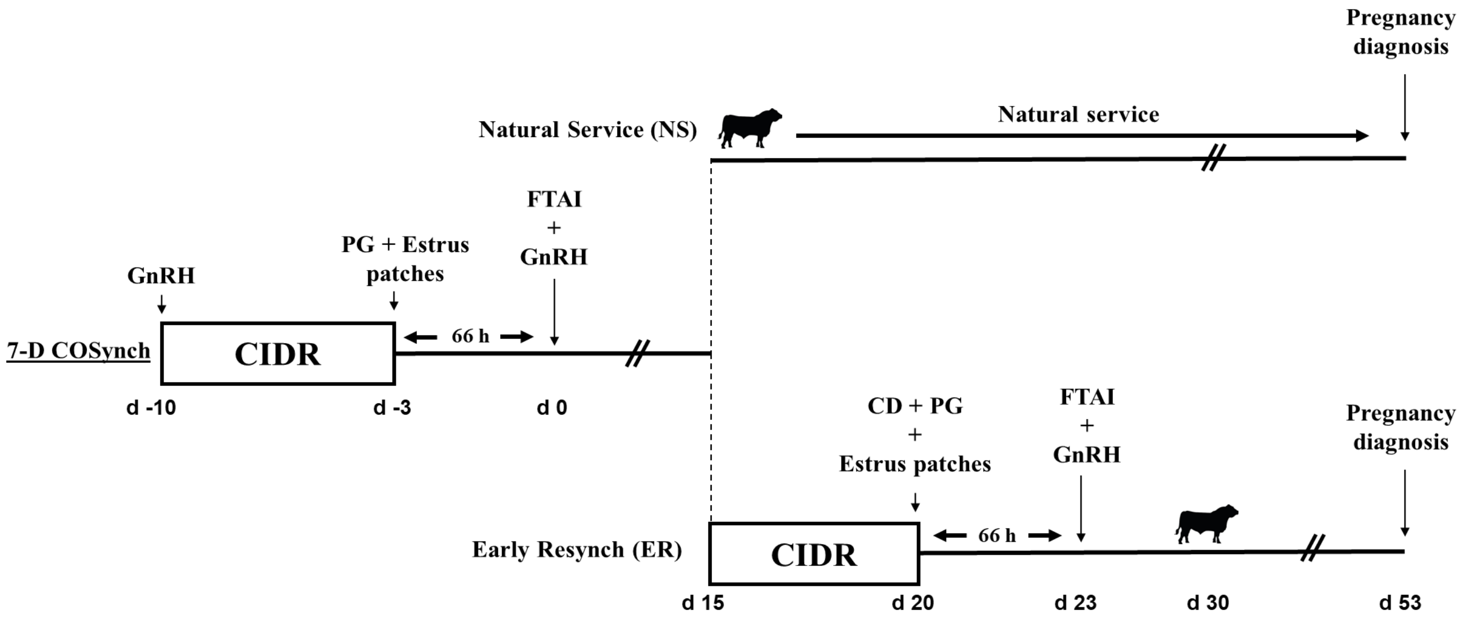

Figure 4.1. Schematics of experimental treatments. Postpartum beef cows (n = 374) from 4 locations were exposed to the 7-d CO-Synch + CIDR protocol and randomly assigned to either the Natural service (NS) or Early resynch (ER) treatments. GnRH: gonadotropin-releasing hormone. PG: Prostaglandin $F_{2\alpha}$. FTAI: fixed-time artificial insemination. CD: color Doppler ultrasonography. D: day and h: hours.

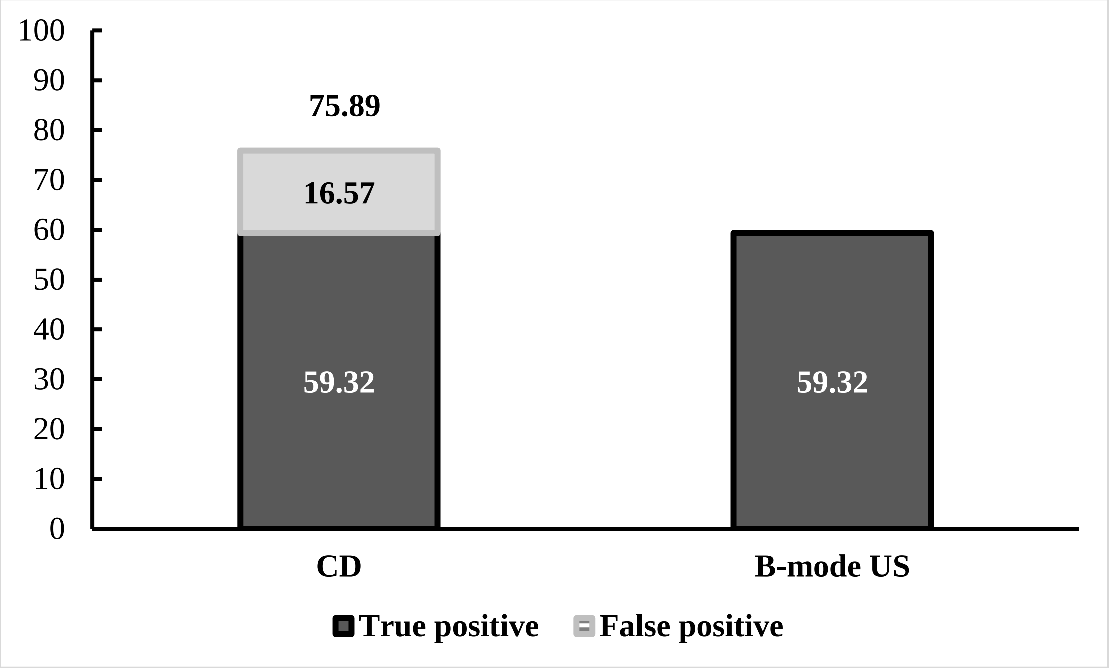

Figure 4.2. Pregnancy for cows exposed to the Early Resynch (ER) treatment based on color Doppler (CD) and conventional B-mode ultrasound (US). Cows diagnosed as pregnant based on CD on day 20 and non-pregnant based on B-mode ultrasonography on day 53 were classified as receiving false-positive diagnoses.

CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

The aim of this research was to evaluate two different strategies to increase the number of AI pregnancies in beef herds. Due to the large utilization of estrus synchronization protocols and the need for fertility improvement in beef herds, it was evaluated in two experiments different strategies to enhance pregnancy rates and overall fertility of beef cows exposed to FTAI. In the first experiment, decreasing the interval between GnRH and PG from 7 to 6 days resulted in minor changes in follicular development and failed to increase estrus expression and pregnancy rates. The lack of detrimental effect in pregnancy rates observed in the present study indicates that the 7&6 Synch treatment can be utilized as an alternative to the 7&7 Synch, providing flexibility when producers are determining breeding schedules. Yet, further research is warranted to better understand the impact of decreasing the interval between follicular wave emergence and luteolysis on the fertility of presynchronized postpartum beef cows.

The second experiment focused in enhancing the cumulative AI pregnancies through resynchronization protocols in the beginning of the breeding season. In conclusion, the proposed early resynchronization strategy using CD resulted in fewer pregnancies compared to natural service as the second service strategy. Moreover, the proposed early resynchronization decreased pregnancy rates to the initial FTAI, indicating a detrimental effect of ER on pregnancy establishment. Further research is warranted to understand how to minimize pregnancy loss in cows exposed to early resynchronization programs and how to optimize follicular synchrony in progesterone and GnRH-based early resynchronization protocols.