COMPARISON OF FOLLICULAR AND LUTEAL DYNAMICS IN PROTOCOLS DEVELOPED FOR SYNCHRONIZATION OF OVULATION IN *Bos indicus*-INFLUENCED BEEF COWS: THE ROLE OF GONADOTROPIN-RELEASING HORMONE AT TREATMENT ONSET

A Thesis

by

JOSE OSWALDO DE SOUZA SCARPA

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

MASTER OF SCIENCE

Chair of Committee,	Gary L. Williams
Co-Chair of Committee,	Rodolfo de Carvalho Cardoso
Committee Members,	Thomas H. Welsh, Jr.
	Charles R. Looney
Head of Department,	G. Cliff Lamb

August 2018

Major Subject: Physiology of Reproduction

ABSTRACT

The 5-day Bee Synch + CIDR (Bee Synch) protocol for *Bos indicus*-influenced cows utilizes CIDR, GnRH (GnRH-1) and PGF on day 0 to eliminate mature corpora lutea, with FTAI and GnRH (GnRH-2) at 66 hours after CIDR removal to yield pregnancy rates of ~ 50%. The objective was to test the hypothesis that GnRH-1 is not required to optimize follicle synchrony. Seventy-one cycling Brangus and Brahman × Hereford suckled cows were used in 2 replicates (35-36 per replicate). Cows were stratified by BW, BCS, and days postpartum, and assigned randomly in a 2 × 3 factorial arrangement involving 2 truncated (no FTAI or GnRH-2) versions of Bee Synch (BS-I and II) begun 3, 7 and 10 days post ovulation. Ovulation was pre-synchronized with PGF. Cows in BS-I received 100 µg GnRH i.m., 25 mg PGF i.m., and a CIDR on treatment onset. Cows in BS-II did not receive GnRH-1. On day 5, CIDRs were removed and all cows received 50 mg PGF i.m. Estrus was detected with Estrotect patches. Daily ultrasonography confirmed ovulation. Synchronized new follicular wave emergence (NFWE; days 1-4) was observed in 68.6 and 38.9% (BS-I vs. BS-II; $P = 0.01$) of cows and increased to 93.3 and 72.2%, respectively, if days 0-4 were considered. Size of the largest follicle at 66 hours (13.5 ± 0.47 mm) did not differ by treatment or day post ovulation. Interval from CIDR removal to ovulation was greater ($P = 0.02$) for BS-I (5.2 ± 0.2 days) than BS-II (4.4 ± 0.2 days) and greater ($P < 0.0001$) for day 3 (6.35 ± 0.3 days) than days 7 and 10 (4.31 ± 0.3 and 3.7 ± 0.3, respectively). Progesterone concentrations did not differ between treatments (P = 0.12). The LH peak at 120 minutes

after GnRH-1 was higher (4.28 ± 0.71 ng/mL, $P < 0.05$) for day 3 than days 7 and 10. A greater ($P = 0.06$) proportion (15.5%) of cows in BS-II ovulated by 72 hours than in BS-I. GnRH-1 reduced variation in day to NFWE and incidence of early ovulations.

DEDICATION

To Larissa, Mirella, Camila, Penelope and Florinda.

ACKNOWLEDGEMENTS

I would first like to thank my advisor Dr. Gary L. Williams for the opportunity and mentorship. His counsels and ideas guided my progress at Texas A&M University. Also, I would like to thank my committee members, Dr. Thomas Welsh, Dr. Charles Looney, and Dr. Rodolfo Cardoso for all the support and help.

I would also like to thank my friends Dr. Heloisa Canesin, Dr. Rui Afonso d'Orey Branco, Vinícius Papin Briani, Camila Sandoval, Jose Liberona, Catarina Stilwell, Taila Kawano, Dr. Colleen Lambo, and John Long for all the good moments.

I would also like to specially thank Meaghan M. O'Neil and Youwen Zhang for all their help, partnership, and friendship during our animal work, lab work, and social life.

A great thank goes to my friends in Brazil for all their support: Republica Pe no Brejo, Evandro, Laura, Lucas, and Babi.

Last, but certainly not least, I must thank my family for believing in me. Mom and Dad were always there to help, support, and talk to me when I needed. To my brother and sister, thanks for always encouraging me for the best. I will always be there for you.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a thesis committee consisting of Professor Dr. Gary L. Williams - advisor, Professor Dr. Rodolfo de Carvalho Cardoso, co-advisor, Dr. Charles R. Looney, of the Department of Animal Science, and Professor Dr. Thomas H. Welsh, Jr., of the Department of Veterinary and Integrative Biosciences.

All other work conducted for this thesis was completed by the student independently.

Funding Sources

Graduate study was supported by a fellowship from Texas A&M University.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

CHAPTER I

INTRODUCTION

The global human population is expected to reach 9.1 billion people by 2050, which represents a 34% increase (FAO, 2009). Developing countries will lead this increment and is a major concern of international agencies involved with food production and demand over the next 30 years.

Meat is a significant human dietary source of protein (FAO, 2009). Beef consumption represents the third highest meat consumption rate per capita (10.1 kg/year), ranking only below pork (15.3 kg/year) and chicken (13.8 kg/year; FAO, 2015). Based on the foregoing, an increase in beef production of up to 470 million tons annually will be required to meet global demands, which represents an increase of 200 million tons of actual beef production. The world's increasing beef demand is due mainly to an increase in average family incomes, which tends to alter the consumption of meat towards beef (FAO, 2015).

The world cattle population reached 1 billion animals in 2014 (USDA, 2017). More than 600 million of these cattle are in India, Brazil, and the United States, with Brazil having the largest commercial beef herd in the world (USDA, 2017). These three countries were responsible for 51% of beef exports in 2016, which involved more than 27 million tons of beef in the global market.

With large areas encompassed by tropical and subtropical climates, beef production in Brazil and India is based primarily on use of *Bos indicus* breeds of cattle

that are more adapted to this environment compared to *Bos taurus* breeds (Alvarez et al., 2000; Bó et al., 2003; Baruselli, 2004). In Brazil, approximately 80% of the total cattle population (~180 million head) is estimated to be comprised of *Bos indicus* or *Bos indicus*-influenced breeds, according to the Brazilian Zebu Breeders Association (ABCZ, 2015).

Several technologies have been developed and applied to improve beef cattle production and efficiency, including artificial insemination (AI) and fixed-time artificial insemination (FTAI). These reproductive technologies are responsible for helping to develop and improve commercial beef herds worldwide, making it possible to utilize genetically-superior sires to improve growth rate, feed efficiency, and beef quality.

During the last 15 years, numerous protocols have been developed with the objective of precisely synchronizing follicle wave emergence and the timing of ovulation in dairy and beef cows to facilitate the concept of FTAI. These procedures have usually involved the use of GnRH (Pursley et al., 1995; Geary et al., 1998; Bridges et al., 2007) or estradiol (Bó et al., 1993; Colazo et al., 2003; Baruselli et al., 2011) to synchronize new follicular wave emergence (NFWE). Applied to *Bos taurus* beef cows, these protocols have often been reported to achieve pregnancy rates ≥ 50%. (reviewed by Lamb et al., 2001). However, use of either the 7-day (Saldarriaga et al., 2007; Zuluaga et al., 2010) or 5-day (Williams et al., 2013) CO-Synch + CIDR protocols in *Bos indicus*-influenced mature beef cows has failed to achieve results consistently above 40%. Recently, a modification in the 5-day CO-Synch + CIDR protocol (STD-5-day) involving the addition of prostaglandin F2α (PGF) at treatment onset has resulted in

marked increases in FTAI pregnancy rates in *Bos indicus*-influenced (Braford, Brangus, Nelore cross) cows with average FTAI pregnancy rates in mature cows exceeding 50%. Originally referred to as 5-day Bee Synch + CIDR (Bee Synch; Williams et al., 2013), the procedure consists of the insertion of a controlled internal drug-releasing device (CIDR) and injection of both PGF (25 mg) and gonadotropin-releasing hormone (GnRH; 100 µg) on day 0 (treatment onset). This is followed by removal of the CIDR and injection of 50 mg PGF on day 5, with FTAI 66 hours later. Following additional research, the original protocol is now referred to as Bee Synch I because of additional modifications as outlined below.

Recently, Cruppe et al. (2014) tested the hypothesis that the inclusion of GnRH on day 0 does not contribute appreciably to follicle synchronization in *Bos taurus* beef heifers synchronized with the STD-5-day protocol. The hypothesis was based on the observation that the frequency of ovulation on day 0 following GnRH in cycling heifers is quite low. Efficacy for synchronizing NFWE on day 0 using GnRH appears to be optimized only when ovulation of the dominant follicle occurs. Results showed that FTAI pregnancy rates did not differ in *Bos taurus* heifers when GnRH was included or not. Thus, elimination of GnRH on day 0 in straight *Bos taurus* heifers is now recommended (Cruppe et al., 2014). Similarly, Williams et al. (2015) proposed a modification of the Bee Synch protocol that eliminates GnRH on day 0 (Bee Synch II). The premise was that GnRH treatment on day 0 using Bee Synch I in mature *Bos indicus*-influenced cows also results in a low frequency of ovulation. By eliminating GnRH on day 0, the requirement for the double dose (50 mg) of PGF is also eliminated

since no new ovulations are being induced and regression of 5-day corpus luteum (CL) is restricted to only those that, by coincidence, were formed naturally at treatment onset.

Based on results of preliminary field trials, it appears that overall pregnancy rates in Bee Synch I and Bee Synch II are similar for *Bos indicus*-influenced mature beef cows. However, significant within-trial variability has been noted that could argue against routine elimination of GnRH on day 0. Therefore, more intensive evaluations are warranted to determine factors contributing to such variability of mature cows in response to these protocols.

The objective of this study was to test the hypothesis that GnRH treatment on day 0 (GnRH-1) in the 5-day Bee Synch + CIDR protocol is not required to optimize follicle synchrony for presumptive FTAI in *Bos indicus*-influenced, mature beef cows.

CHAPTER II

LITERATURE REVIEW

World Market for Beef

In 2014, beef exports reached 10,000 million tons in the global market (USDA, 2017). India, which historically has not been considered a leading beef export country, displaced Brazil from first place in 2014, exporting 1,764 tons of beef and carabeef (from buffalo) combined (USDA, 2017).

The beef industry represents an important segment of the economy of some countries. There are around 800,000 farms in the United States dedicated to cattle and calf production and involve 922 million acres (McGrann, 2010). These farms generate employment for more than one million workers, had a retail equivalent of US$105 billion in 2015, and trade exports of US$5.6 billion (USDA, 2017). In Brazil, the vast areas of natural pastures located in tropical climates makes it an excellent environment for producing grass-fed beef. Beef exported in 2016 generated US$5.3 billion for the Brazilian economy and provided employment for 1.6 million people (ABIEC, 2017).

A significant source of protein, beef consumption is influenced by the average income of the population being considered. With an increase in purchasing power in the developing countries (FAO, 2012), specially BRICS countries (Brazil, Russia, India, China, and South Africa), the demand for higher quality protein has increased. The growing middle class is expected to reach 2.1 billion people by 2050, equivalent to 28.4% of the global population (FAO, 2009). International agencies (FAO, USDA) are

concerned because as more people enter the middle class, the greater the demand for high quality protein, such as beef.

Another reason for the global increase in total beef exports was the 2008-2009 world economic crisis, which resulted in a decline in the value of the currency in several important countries involved in the beef market, such as Brazil, Argentina, Australia, Canada, and Uruguay. With the global market more profitable than the domestic market, many animals, including a large number of females, were slaughtered. This created an instability in the beef market and resulted in an increase in the price of replacement heifers. Thus, increasing productivity and efficiency is imperative on a global scale for producers to meet the challenges associated with market instabilities. The application of advanced technologies, including estrus synchronization and AI among others, could help producers to become more competitive in a globalized market.

Bovine estrous cycle

The bovine estrous cycle is a period comprising two consecutive estruses, which involves the growth of ovarian follicles and characteristic hormone profile changes. With a length ranging from 18 to 24 days (average of 21 days), the estrous cycle is characterized by having two or three waves of follicular growth, depending on individual animal variability, age, nutrition, and breed (Remnant et al., 2015; Honig et al., 2016; Sveberg et al., 2015). The estrous cycle is comprised of four phases: proestrus, estrus, metestrus, and diestrus.

The proestrus phase, also known as the proliferative phase or follicular phase, is characterized by a marked drop in the concentrations of serum progesterone (P4). This

occurs as a result of luteolysis caused by the release of PGF secreted from the endometrium (Peters & Ball, 1995; McCracken et al., 1999).

The length of estrus in cows can range from 10-19 hours (Hafez., 1982), depending on breed and method of determination. Estradiol-17β (E2) is the predominant circulating hormone during estrus, reaching a peak around 24 hours before the onset of estrus (Glencross and Pope, 1981). The high concentration of E2 leads to a pre-ovulatory surge of luteinizing hormone (LH), resulting in ovulation (Hafez, 1982). Ovulation occurs in *Bos taurus* approximately 24 to 30 hours after the LH peak (Randel et al, 1973; Hafez, 1982). During this metestrus period, circulating concentrations of follicle-stimulating hormone (FSH), E2, and LH are relatively low, and concentrations of serum P4 serum are increasing due to formation of the CL. In straight *Bos indicus* cows, the average length of estrus is approximately 6 hours, ranging from 3 to 13 hours, with ovulation occurring around 25 hours after the LH peak (Plasse et al., 1970; Randel et al., 1973). For *Bos indicus*-crossbred cows, Mikeska & Williams (1988) reported an estrus length of 14 hours and ovulation 24-30 hours after estrus onset.

The diestrus phase of the estrous cycle represents the phase in which the CL is fully functional (Peters & Ball, 1995). Circulating concentrations of P4 $\geq$ 1 ng/ml are observed from day 3 to 17 (Hafs et al., 1968; Dobrowolski et al., 1968; Bearden et al., 2004) and prevent the surge release of LH and ovulation (Hafs et al., 1975).

Follicular Dynamics

A complete follicular wave is characterized by several phases, including recruitment, selection and growth, a static phase, and regression (Ginther et al., 1989). Briefly, a cohort of follicles is recruited to grow until a dominant follicle is selected to continue growing, while the remaining follicles become subordinate and undergo atresia (Ginther et al., 1989).

Sirois and Fortune (1988) and Ginther et al. (1989) defined the day of NFWE as the day in which a cohort of growing follicles of 4 to 5 mm can be detected by ultrasonography. This is known as the recruitment phase. It occurs on approximately the day of ovulation (day 0) and again on about the 10th day of the estrous cycle in cows with two follicular waves. Cows with three follicular waves recruit new cohorts on days 0, 9, and 15 of the estrous cycle.

The recruitment of a cohort of follicles is controlled by a peak in circulating concentrations of FSH (Adams et al., 1992). Although primordial and primary follicles do not have functional FSH receptors, these follicles and the pregranulosa cells respond to activators of the cAMP pathways with increased expression of aromatase and FSH receptors (Mayerhofer et al., 1997).

Following the recruitment stage, selection and growth of the dominant follicle occurs. The process of selection occurs under declining FSH concentrations, since 3-5 mm follicles already have the capacity to suppress FSH (Ginther et al., 2001). When the largest follicle achieves approximately 8.0 at 8.5 mm in diameter (Ginther et al., 2000) follicular deviation occurs. The process of deviation defines the period in which a

divergence in growth rate of the largest and next largest subordinate follicle can be observed (Ginther et al., 1997). At this point, the largest follicle increases its secretion of estradiol, which leads to suppression of FSH concentrations below levels that support the recruitment of smaller follicles but not the largest follicle (Ginther et al., 2000), resulting in all remaining follicles undergoing atresia via apoptosis. In addition to the decline in FSH release, it is generally accepted that LH performs a key role during the dominance phase of the large follicle following deviation. Ginther et al. (2001) reported that, in heifers, LH receptors emerge in granulosa cells of the future dominant follicle 8 hours before the beginning of deviation. This change in expression of receptors allows the dominant follicle to undergo a transition in gonadotrophin dependency from FSH to LH (Mihm et al., 2006), allowing it to survive and mature despite low circulating FSH. The increase in estradiol production is important for the determination of follicular dominance because the negative feedback effect of E2 on the secretion of FSH prevents FSH-dependent subordinate follicles from continuing growth.

After reaching its largest size, the dominant follicle remains static until ovulation following luteal regression and estrus or regression for early and mid-cycle follicles (Ginther et al., 1989; Sirois & Fortune, 1988). On average, the dominant follicle grows linearly for 6 days (growing phase), remains at the static stage for 6 days (dominant phase), and then regresses (regressing phase; Ginther et al., 1989).

During follicle dominance, circulating concentrations of E2 remain elevated. Increasing concentrations of E2 exerts a positive feedback effect on gonadotrophin pulse frequency (Wiltbank et al., 2002), resulting in elevated secretion of LH secretion (Aerts

and Bols, 2010). In the absence of a functional CL and high concentrations of P4, an LH surge is induced by E2 which results in ovulation and formation of a CL.

Estrus synchronization

Enhancing our understanding of follicular dynamics during the bovine estrous cycle has made it possible to develop protocols for manipulating NFWE and control ovulation using hormones (Lamb & Mercadante, 2016). Managing the estrous cycle and follicular waves in cows is crucial for the success of a synchronization program. Differing by timing of hormone injections, price, animal management, and targeted breed type, all protocols of estrus and ovulation synchronization have been developed to increase reproductive efficiency while optimizing cost:benefit.

Ovsynch

The development of the Ovsynch protocol (Pursley et al. 1995), which employs the use of GnRH on day 0, PGF on day 7, GnRH on day 9, and AI 24 hours later, resulting in >50% FTAI pregnancy rates in Holstein cows, encouraged other scientists around the world to develop similar protocols for use in combination with FTAI. Objectives were to create a synchronized follicular wave in order to optimize this process. The development of the Ovsynch protocol appeared to provide a significant improvement over previous procedures, allowing farmers to plan and synchronize a large number of cows in combination with FTAI.

7-day CO-Synch

The first modification in the Ovsynch protocol was to combine the timing of AI with injection of GnRH on day 9. The objective was to decrease the number of times cattle had to be handled through the chute (Geary and Whittier, 1998).

7-day CO-Synch + CIDR

The further development of progestin devices facilitated the ability to more precisely manipulate the timing of ovulation. Therefore, the 7-day CO-Synch + CIDR protocol was developed (Lamb et at., 2001). Nowadays, the 7-day CO-Synch + CIDR protocol is one of the most common procedures for use in *Bos taurus* females, particularly in dairy cattle.

5-day CO-Synch + CIDR

With the objective of prolonging the length of the follicular phase, and thus decreasing the probability of creating a persistent dominant follicle with decreased fertility, Bridges et al. (2007) reduced the period during which the CIDR was in place from 7 to 5 d. This resulted in the so-called 5-day CO-Synch + CIDR protocol. Since the CL is only fully responsive to PGF-induced regression beginning on day 6 after ovulation, the 5-day CO-Synch + CIDR protocol requires the use of a double dose of PGF at CIDR removal (day 5) to effect CL regression.

Bee Synch I

A modification of the 5-day CO-Synch + CIDR protocol involving the addition of PGF at treatment onset has resulted in marked increases in FTAI pregnancy rates in *Bos indicus*-influenced (e.g., Braford, Brangus, Nelore cross) cows with average FTAI

pregnancy rates in mature cows exceeding 50%. Objectives were to reduce mean circulating concentrations of P4 during the synchronization period by causing regressing of mature CL on day 0 of the protocol. This idea stemmed from the belief that *Bos indicus*-influenced females may be more sensitive to the negative feedback effects of P4, thus reducing the rate of dominant follicle maturation. Originally referred to as 5-day Bee Synch + CIDR (Bee Synch; Williams et al., 2013), the procedure consists of the insertion of a CIDR and injection of both PGF (25 mg) and GnRH (100 µg) on day 0 (treatment onset). This is followed by removal of the CIDR and injection of 50 mg PGF on day 5, with FTAI 66 hours later.

Bee Synch II

Recently, Cruppe et al. (2014) tested the hypothesis that the inclusion of GnRH on day 0 does not contribute appreciably to follicle synchronization in *Bos taurus* beef heifers synchronized with the STD-5-day protocol. The hypothesis was based on the observation that the frequency of ovulation on day 0 following GnRH in cycling heifers is quite low. Efficacy for synchronizing NFWE on day 0 using GnRH appears to be optimized only when ovulation of the dominant follicle occurs. Results showed that FTAI pregnancy rates did not differ in *Bos taurus* heifers when GnRH was included or not. Thus, elimination of GnRH on day 0 in straight *Bos taurus* heifers is now recommended (Cruppe et al., 2014). Similarly, Williams et al. (2015) proposed a modification of the Bee Synch protocol that eliminates GnRH on day 0 (Bee Synch II). The premise was that GnRH treatment on day 0 using Bee Synch I in mature *Bos indicus*-influenced, mature cows also results in a relatively low and highly variable

frequency of ovulation. By eliminating GnRH on day 0, the requirement for the double dose (50 mg) of PGF is also eliminated since no new ovulations are being induced and presence of 5-day CL is restricted to those that, by coincidence, were formed naturally around the time of treatment onset.

Effect of GnRH on Follicular Dynamics

The effect of GnRH in synchronizing a follicular wave was first demonstrated by Thatcher et al. (1993). Gonadotropin-releasing hormone stimulates the release of anterior pituitary FSH and LH in cattle (Zolman et al., 1974) as it does in all mammals. In bovine females, pharmacological doses of GnRH increase serum concentrations of LH and FSH immediately in a surge-like manner. Depending on blood sampling frequency, the first detectable increase in LH and FSH can be observed within a matter of minutes, reach peak concentrations at approximately 120 minutes, then decrease to basal concentrations within about 4 hours. When a surge-like release of LH occurs in the presence of a dominant, estrogen-active follicle, ovulation is observed between 24 to 30 hours later (Randel et al., 1973; Thatcher et al., 1993). Twagiramungu et al. (1995) were one of the first to demonstrate that GnRH could be employed to synchronize follicular waves through ovulation or atresia of the dominant follicle, hence inducing a NFWE within ~2.5 days from its injection.

The follicular response to GnRH injection varies according to the functional status of the follicle (growing, dominant, or regressing phase). Twagiramungu et al. (1995) reported a 60% ovulation rate for *Bos taurus* cows when GnRH is administrated at an unknown stage of the estrous cycle. Saldarriaga et al. (2007) reported that

approximately 40% of *Bos-indicus*-influenced beef cows ovulate and 39% exhibit follicle regression when treated at a random or unknown stage of the estrous cycle. Vasconcelos et al. (1999), working with Holstein cows, showed that GnRH resulted in ovulation in 23%, 96%, 54% and 77% of cows when treatment was administered on day 1 to 4, 5 to 9, 10 to 16, and 17 to 21, respectively. Sartori et al. (2001) reported a 0 and 100% rate of ovulation for days 3 and 6 of the cycle, respectively. Conversely, Martinez et al. (1999) administered GnRH on day 3, 6 and 9 of the estrous cycle in *Bos taurus* beef heifers and observed ovulation in 89%, 56% and 22%, respectively.

Vasconcelos et al. (1999) suggested that ovulation rates following GnRH in growing, dominant, and regressing phases of dominant follicles vary because of differences in ovulatory capacity related to the number of LH receptors. Sartori et al. (2001) showed that follicular ovulatory capacity is achieved immediately after deviation. In a recent study in Holstein heifers (Ginther et al., 2016), deviation was shown to occur on day 2 ± 0.2 (range from 1 to 4) for the 1st wave, and on day 10.8 ± 0.2 (range from 9 to 12) for the 2nd wave, with a follicle diameter of 8.5 mm. Martinez et al. (1999) indicated that all heifers with a dominant follicle larger than 9 mm ovulated in response to GnRH. Similarly, no ovulations were obtained in crossbred *Bos taurus* × *Bos indicus* cows with follicles smaller than 8.0 mm (Zuluaga et al., 2010). Ovulatory capacity appears to be dependent upon both the amount of LH released and diameter of the dominant follicle (Sartori et al., 2001). Collectively, data indicate that ovulation or regression induced by GnRH treatment is directly related to follicle size. Follicles in the dominant growth phase (Vasconcelos et al., 1999), or larger than 14 mm (Zuluaga et al.,

2010), exhibit ovulation rates greater than 90%, whereas follicle regression occurs when follicles are smaller than 8.5 mm. Interestingly, in the study by Zuluaga et al. (2010), GnRH had no effect at all in ~ 29% of *Bos indicus*-influenced beef cows submitted to a 7-day CO-Synch + CIDR protocol. However, the synchronization rate remained relatively high (76%). This relationship was corroborated by Saldarriaga et al. (2007) in which 21% of *Bos indicus*-influenced beef cows exhibited no follicular response to GnRH on day 0 of a 7-day CO-Synch + CIDR protocol, but which resulted in what was considered to be a relatively satisfactory synchronization of a mature follicle at FTAI.

The Effect of PGF Administration

Initial objectives of the Bee Synch I protocol for use in *Bos indicus*-influenced cattle was to eliminate mature CL at the onset of treatment and thus lower circulating concentrations of P4 during the 5-day synchronization period. Thus, mature cows submitted to the synchronization protocol received an i.m. injection of PGF (Lutalyse; 25 mg) to lyse any mature CL present on day 0 (Williams et al., 2010; 2013; 2015). As a result, cows with a mature CL would only have the CIDR as a source of P4.

Progesterone, which is produced by the large and small luteal cells of the CL, has a negative feedback effect on the hypothalamus-pituitary axis (Stock & Fortune, 1993). One of the key roles of P4 is related to maintenance of pregnancy. During the estrous cycle, P4 is responsible for regulating the length of the cycle and the follicular growth pattern (Stock & Fortune, 1993). Rahe et al. (1980) demonstrated that in early luteal phase, when circulating concentrations of P4 are dramatically lower, LH pulses occur in greater frequency than in the mid-luteal phase.

The increase in frequency of LH pulses during the follicular phase of the estrous cycle is the primary factor for development of ovarian follicles. In ewes (Karsh et al., 1987) and cows (Kinder et al., 1996), it has been demonstrated that the final stages of maturation of an ovulatory follicle are inhibited because of the suppressive effect of P4 on frequency of LH pulses during luteal phase of the estrous cycle, which in turn affects the rate of follicle maturation. Intending to lower the serum P4 concentration, Cutaia et al. (2001) has demonstrated that employing re-used CIDR for synchronization enhanced pregnancy rates in *Bos indicus* cows. The objective was to create a dominant ovulatory follicle growing in the presence of lowered P4, thus increasing LH pulse frequency and achieving dominant follicles with a larger final diameter and consequently higher pregnancy rates.

PGF Effects on Day 5 after GnRH Treatment

The STD-5-day CO-Synch + CIDR protocol was developed with the intention of prolonging the proestrus stage by removing the CIDR 2 days earlier than in the 7-day CO-Synch + CIDR protocol (Bridges et al, 2007). However, in order to achieve regression of CL on day 5 that were either spontaneously-occurring or produced in response to GnRH on day 0, a double dose of PGF was demonstrated to be required. Nascimento et al. (2014) confirmed this relationship in Holstein cows by comparing single (25 mg), double (50 mg) and double split (25 + 25 mg) doses of PGF given 8 hours apart. Results showed a dramatic superiority of either the double or double split dose compared to the single dose for regressing 5-day CL. In field trials, Rabaglino et al. (2010) observed no difference in pregnancy rate of dairy heifers synchronized using

either single or double doses of PGF at day with the STD-5-day protocol. Peterson et al. (2011) reported that pregnancy rates tended to be greater when the double dose of PGF was given 6 hours apart rather than as a single dose in *Bos taurus* beef heifers synchronized with STD 5-day protocol.

The STD-5-day and Bee Synch I protocols require the use of a double dose (50 mg) of PGF on day 5 to promote adequate lysis of any 5-day-old-CL formed. Since GnRH is not used in the Bee Synch II protocol, the double dose of PGF is not necessary on day 5.

CHAPTER III

COMPARISON OF FOLLICULAR AND LUTEAL DYNAMICS IN PROTOCOLS DEVELOPED FOR SYNCHRONIZATION OF OVULATION IN *Bos indicus* - INFLUENCED BEEF COWS: THE ROLE OF GONADOTROPIN-RELEASING HORMONE AT TREATMENT ONSET

Introduction

Numerous protocols have been developed to synchronize NFWE and timing of ovulation for FTAI in dairy and beef cows, usually involving GnRH (Pursley et al., 1995; Geary et al., 1998; Bridges et al., 2007) or estradiol (Bó et al., 1993; Colazo et al., 2003; Baruselli et al., 2011). Applied to *Bos taurus* beef cows, these protocols have often been reported to achieve pregnancy rates ≥ 50% (reviewed by Lamb et al., 2001). However, use of either the 7-day (Saldarriaga et al., 2007; Zuluaga et al., 2010) or 5-day (Williams et al., 2013) CO-Synch + CIDR protocols in *Bos indicus*-influenced mature beef cows has failed to achieve results consistently above 40%. Recently, a modification in the 5-day CO-Synch + CIDR protocol involving the addition of PGF at treatment onset has resulted in marked increases in FTAI pregnancy rates *in Bos indicus*-influenced mature cows (> 50%).

Originally referred to as 5-day Bee Synch + CIDR (Bee Synch; Williams et al., 2013), this protocol utilizes a CIDR, GnRH (GnRH-1) and PGF on day 0, with FTAI and GnRH (GnRH-2) at 66 hours after CIDR removal. However, Cruppe et at. (2014) demonstrated that the inclusion of GnRH on day 0 does not contribute appreciably to

follicle synchronization in *Bos taurus* beef heifers synchronized with the 5-day CO-Synch + CIDR protocol. Similarly, Williams et al. (2015) proposed a modification of the Bee Synch protocol for use in mature *Bos indicus*-influenced cows that eliminates GnRH on day 0 (Bee Synch II).

Based on preliminary field trials, it appears that overall pregnancy rates in Bee Synch I and Bee Synch II are similar for *Bos indicus*-influenced mature beef cows. However, significant within-trial variability has been noted that could argue against routine elimination of GnRH on day 0. Therefore, the objective of this study was to test the hypothesis that GnRH treatment on day 0 (GnRH-1) in the Bee Synch I protocol is not required to optimize follicle synchrony for presumptive FTAI in *Bos indicus*-influenced, mature beef cows.

Materials and Methods

Study Location

This research outlined in this proposal has been submitted to and approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University. The experiment was conducted in two replicates from March to June and from September to November 2016, respectively, at the Texas A&M Agricultural Research Station, Beeville, Texas.

Animals

Seventy-two mature, suckled *Bos indicus*-influenced beef cows were used in this experiment. All cattle were required to have a minimum BCS of 5 (1 to 9 scale, 1 = emaciated, and 9 = obese) and be at least 50 days postpartum. Cows were fed according

to recommendations of the National Research Council (NRC, 1996) for lactating beef cows. To be used in this experiment, cows were required to exhibit evidence of ovarian cyclicity which was defined as the presence of an ultrasonographically-definable CL. The experiment was conducted in two replicates (36 cows/replicate) during the months of March through June (Brahman × Hereford, F-1) and September through November (Brangus). Cows were placed in pens measuring 25.9 × 9.5 m (6 cow-calf pair per pen) 15 days before institution of a pre-synchronization procedure as described below.

Experiment

Pre-synchronization

The estrous cycles of all cows were pre-synchronized with two injections of 25 mg PGF, administered 11 days apart (Figure 1A). Following the second injection of PGF, the ovaries were scanned daily using transrectal ultrasonography until ovulation was confirmed. Ovulation was defined as the sudden disappearance of a large follicle followed by appearance of a CL within 2 days.

Synchronization Treatments

Following ovulation, cows were assigned randomly to receive 1 of 2 synchronization treatments (truncated versions of Bee Synch I or II as described below) on 1 of 3 days of the estrous cycle (day 3, 7 or 10 post ovulation) in a 3 × 2 factorial arrangement. Since the objectives of the study were to monitor follicular dynamics until natural ovulation, FTAI was not employed in this experiment. Thus, cows did not receive GnRH treatment at 66 hours after CIDR removal (GnRH-2) as they would under normal FTAI circumstances.

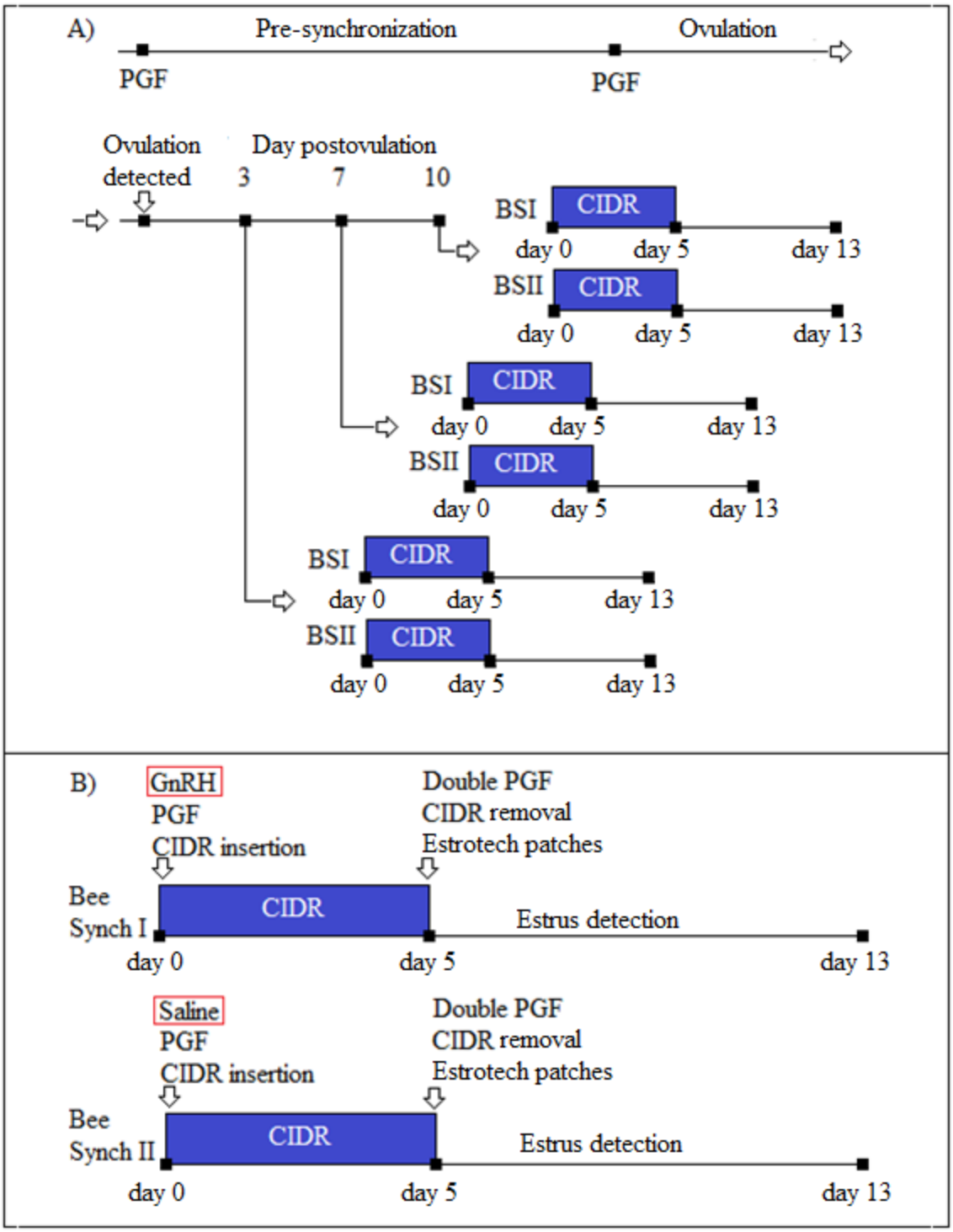

Figure 1: A) Experiment scheme. B) Bee Synch I and Bee Synch II treatments.

Bee Synch I (Fig. 1B) includes the insertion of a CIDR (Zoetis Animal Health, New York, NY), the intramuscular injection of 100 μg GnRH (Factrel; Zoetis Animal Health, New Your, NY) and 25 mg PGF (Lutalyse; Zoetis Animal Health, New York, NY) on day 0. On day 5, CIDRs are removed and cows receive 50 mg Lutalyse i.m. Bee Synch II follows the same treatment protocol as Bee Synch I except that GnRH on day 0 (GnRH-1) is omitted. However, in order to maintain experimental integrity, the double dose (50 mg) of Lutalyse was utilized for both treatments on day 5. Normally, a single dose (25 mg) is employed for Bee Synch II under a field scenario. Days of the cycle for initiation of treatments represent 3 putative stages of follicular growth. On day 3, no dominant follicle is expected to be present, P4 is low but increasing, and ovulation is not expected to occur in response to GnRH. On day 7, a large, dominant follicle in the growth phase should be present, P4 will be maximally elevated, and ovulatory response to GnRH is expected to be large (> 90%). On day 10, circulating concentrations of P4 are maximal, approximately 50% of dominant follicles will have begun to regress, and only about 50% will ovulate in response to GnRH.

Ovarian Ultrasonography

Transrectal ovarian ultrasonography was performed daily beginning on day 0 of synchronization treatment and continuing until ovulation. Ovarian follicular populations, NFWE, size of the largest and second largest (subordinate) follicle, follicle growth rate, size of the dominant follicle at deviation, and size of the dominant follicle at 48, 66, and 72 hours and at estrus/ovulation were determined.

Estrus Detection and AI

After CIDR removal, cows were observed for estrus 3 times daily beginning at the time of CIDR removal and continuing through D13 or ovulation. Estrus detection was aided by use of estrus-detection patches (Estrotect®) and an androgenized cow. Estrus was confirmed when the cow stood to be mounted and/or the Estrotect patch had become fully activated by an undetected standing mount. Upon detection of estrus, each cow was artificially inseminated to facilitate overall management of the herd but pregnancy outcome was not part of experimental variables associated with this study.

Blood Sample Collection

Blood samples were collected daily by caudal venipuncture to monitor serum concentrations of P4. Additional blood samples were obtained on day 0 of synchronization treatments at 0, 30, 60, 120, and 240 minutes after GnRH or saline injection. Samples were placed on ice immediately after collection. Before centrifugation, samples were allowed to clot at room temperature for 3 hours. Serum were stored at -20º C until hormone analysis by RIA.

Hormone Assays

Serum concentrations of P4 and LH were measured by RIA. The MP Biomedicals Kit® (Santa Ana, CA) with modifications for assay of bovine serum was validated and used to assay P4. Assay sensitivity was 0.2 ng/mL. Recovery of added mass for high, mid, and low references was 113.9, 117.7, and 104.6 %, respectively. Because of the precision of the assay and objectives of measurement, samples were assayed as singlets. The inter assay CV was 6.7%. For LH, a previously validated RIA

(McVey et al., 1991) was used. The sensitivity of the assay was 0.1 ng/mL, and intra- and inter assay CV were 8.8 and 5.8%, respectively.

Statistical Analysis

Statistical analysis of data was conducted using JMP Statistical Discovery™ (JMP Pro 12; SAS Inst. Inc., Cary, NC) and Statistical Analysis System Software (SAS Software®; SAS 9.3; SAS Inst. Inc., Cary, NC). Hormone concentrations of LH and P4 were analyzed as for a 3 × 2 factorial with repeated measures using PROC MIXED of SAS, with treatment, day post ovulation, replication, and all possible interactions included in the model. Cows were included as a random effect, and time was included as the repeated variable. Non-repeated variables, BW, BCS, DPP, size of follicle ovulating during pre-synchronization; size of the largest follicle on day 0 of treatment within day of cycle; follicle size at NFWE, at CIDR removal, and at 24, 48 and 66 hours; interval to a NFWE, interval from CIDR removal to ovulation, and follicle daily growth rate were analyzed in JMP by one-way ANOVA, accounting for interaction between treatments (Bee Synch I or II) and groups (day post ovulation cycle: 3^{rd}, 7^{th}, or 10^{th}). Post-hoc comparison of means was performed with the Tukey Procedure. The effects of treatment and groups, and their interaction, on categorical data (ovulatory responses to GnRH-1, occurrence of a synchronized follicular wave, and frequency of estrus) were analyzed in JMP using Fisher's Exact Test.

Results

Mean (±SEM) BCS, BW, and days postpartum (DPP) were 5.7 ± 0.07, 628 ± 7.8 kg, and 162 ± 6.6 days, respectively. There were no significant treatment × replicate, day

× replicate, or treatment × day × replicate interactions. For each treatment, average weight loss during the experiment was 23 ± 5.2 kg in BSI, and 26.4 ± 5.2 kg in BSII ($P = 0.64$). For DPP at start of synchronization, there was a difference ($P < 0.0001$) between replicates 1 (107.9 ± 2.88 d) and 2 (214.2 ± 2.80 d), as cows were held from the breeding herd in replicate 2 for use in this study.

Follicle data are summarized in Table 1. Follicle size data did not differ between treatments for the following variables: size of follicle ovulating during pre-synchronization; size of the largest follicle on day 0 of treatment within day of cycle; follicle size at new follicular wave emergence, at CIDR removal or 24, 48 and 66 hours ($P > 0.05$). Daily growth rate of the largest follicle was 1.3 ± 0.1 mm ($P = 0.93$). Within day of the estrous cycle, smaller follicles ($P < 0.05$) were detected on day 3 post ovulation for the variables analyzed. Size of the largest follicle at 66 hours after CIDR removal was 13.5 ± 0.3 and was not affected by treatment, day of the cycle at treatment onset or replicate ($P = 0.70$).

Table 1: Follicle diameter (mm) in Bee Synch I and II treatments (TRT) initiated on different days postovulation (DPO), including size at the time of presynchronized ovulation (Pre-Synch), time of initiation of treatments (day 0), day of emergence of the new follicle wave (NFWE), time following CIDR removal, and just prior to the synchronized ovulation (OV). Means with different superscripts within each column differ ($P < 0.05$).

TRT	DPO	Follicle size (mm)			Follicle size (mm) relative to CIDR removal (hours)				
		Pre - Synch	Day 0	NFWE	0	24	48	66	OV
Bee Synch I	3	14.6	9.5^{b}	4.8	8.0^{b}	9.3^{cd}	10.9^{bc}	11.5^{c}	15.5
	7	15.2	12.4^{a}	5.1	10.6^{a}	11.6^{bc}	12.9^{ab}	13.5^{bc}	14
	10	15.8	11.6^{a}	5.7	12.0^{a}	13.0^{ab}	14.4^{a}	15.2^{ab}	15.4
Bee Synch II	3	14.4	9.3^{b}	5.6	6.8^{b}	8.1^{d}	9.8^{c}	10.5^{d}	13.3
	7	16	12.1^{a}	5.1	11.0^{a}	12.1^{ab}	13.2^{ab}	14.1^{ab}	14.4
	10	14.4	11.8^{a}	5.6	12.9^{a}	14.1^{a}	15.1^{a}	16.1^{a}	15.1
Mean	BSI	15.2	11.1	5.2	10.2	11.3	12.7	13.4	14.9
	BSII	14.9	11.1	5.4	10.6	11.5	12.7	13.6	14.3

Ovarian and reproductive outcomes in response to GnRH or saline on day 0 are presented in Table 2. Ovulation to GnRH on day 0 was detected in only 5/35 cows (14.3 %) and was unaffected by treatment, day of the cycle or replicate. Follicle regression was detected in 63/71 (88.7%) cows, and did not differ between treatments, day of cycle

or replicate ($P > 0.52$). In 4.22% of the cows, no ovarian follicular response was observed.

The frequency of synchronized NFWE was greater in BSI regardless of whether NFWE was considered as occurring between 1 to 4 days after GnRH or saline ($P = 0.01$) or between days 0 to 4 ($P = 0.02$). Interval from the onset of treatments to NFWE averaged 1.8 ± 0.3 (BSI) and 2.2 ± 0.3 days (BSII), respectively, and did not differ ($P = 0.45$). Cows treated with BSII on day 3 post ovulation exhibited a longer ($P < 0.05$) interval to NFWE than cows treated with BSI for the same day post ovulation. Cows that did not exhibit synchronized NFWE after GnRH or saline on day 0 eventually ovulated the follicle that was present at onset of treatment following CIDR removal.

Table 2: Ovarian synchronization outcomes for Bee Synch I and II treatments starting at each day postovulation. Different superscripts represent a significant difference ($P < 0.05$)

Item	Treatments	
	BSI (n = 35)	BSII (n = 36)
Follicle response to GnRH-1 or saline, No. (%)		
Ovulation	5 (14.3)	-
Regression	29 (82.8)	34 (94.4)
No response	1 (2.9)	2 (5.6)
New Follicular Wave Emergence (NFWE), No. (%)		
Synchronized NFWE, day 1 – 4	24 (68.6)[a]	14 (38.9)[b]
No synchronized NFWE, days 1 – 4	11 (31.4)[a]	22 (61.1)[b]
Synchronized NFWE, days 0 – 4	33 (94.3)[a]	26 (72.2)[b]
No synchronized NFWE, days 0 – 4)	2 (5.7)[a]	10 (27.8)[b]
Interval to NFWE (days) with treatments initiated on different days postovulation		
Day 3	3.0[a]	5.0[b]
Day 7	1.8	1.1
Day 10	0.6	0.4
CL regression after PGF on day 5, No. (%)	35 (100)	36 (100)
Interval from CIDR Removal to ovulation (days) with treatments initiated on different days postovulation		
Day 3	6.6 ± 0.40	6.1 ± 0.36
Day 7	4.6 ± 0.38	4.0 ± 0.38
Day 10	4.3 ± 0.38	3.2 ± 0.36
Estrus (%)	30 (85.7)	34 (94.4)

All cows (n = 71) had serum concentrations of P4 below 1 ng/mL 1 day after double PGF injection on day 5. Interval from CIDR removal to ovulation did not differ by treatment but differed ($P < 0.0001$) due to day of the cycle treatments began (Table 2). Ovulations detected earlier than 72 hours after CIDR removal averaged 5.6 % (n = 4/35) in BSI and 15.5 % (n = 11/36) in BSII ($P = 0.06$) and all were observed when treatments began on day 10 of the cycle.

Frequency of estrus did not differ ($P = 0.21$) due to treatment but the interval from CIDR removal to estrus did differ by treatment ($P = 0.05$) and day of the cycle in which treatments were initiated

($P < 0.0001$; Fig. 2).

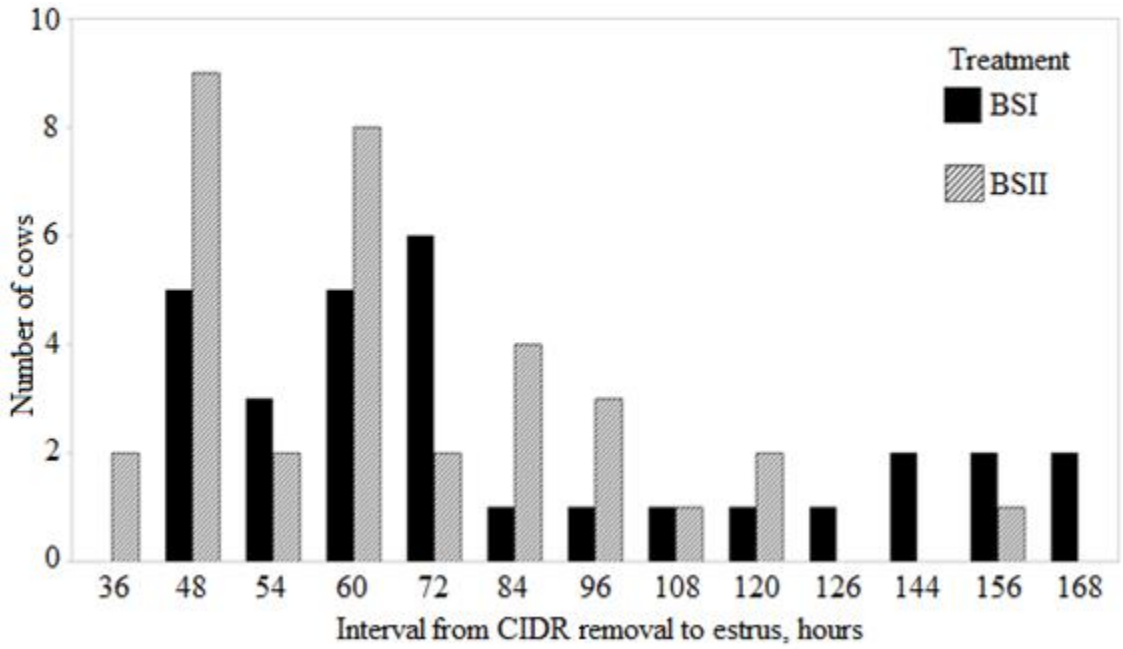

Figure 2: Interval (in hours) from controlled internal drug-releasing device (CIDR) removal to visual estrus (BS-I = 30; BS-II = 34) of postpartum, mature suckled beef cows treated with Bee Synch I or Bee Synch II protocols.

Serum concentrations of P4 are illustrated in Figure 3. Mean P4 during the synchronization period and until CIDR removal did not differ ($P = 0.12$) by treatment. An apparent incomplete lysis of the CL after double injection of PGF on day 5 resulted in a rebound in P4 concentrations above 1 ng/mL in 5 cows treated with Bee Synch I. The effect of this on variability in serum P4 can be seen in Figure 3 from day 8 to day 13 after the onset of treatment (3 days after PGF treatment).

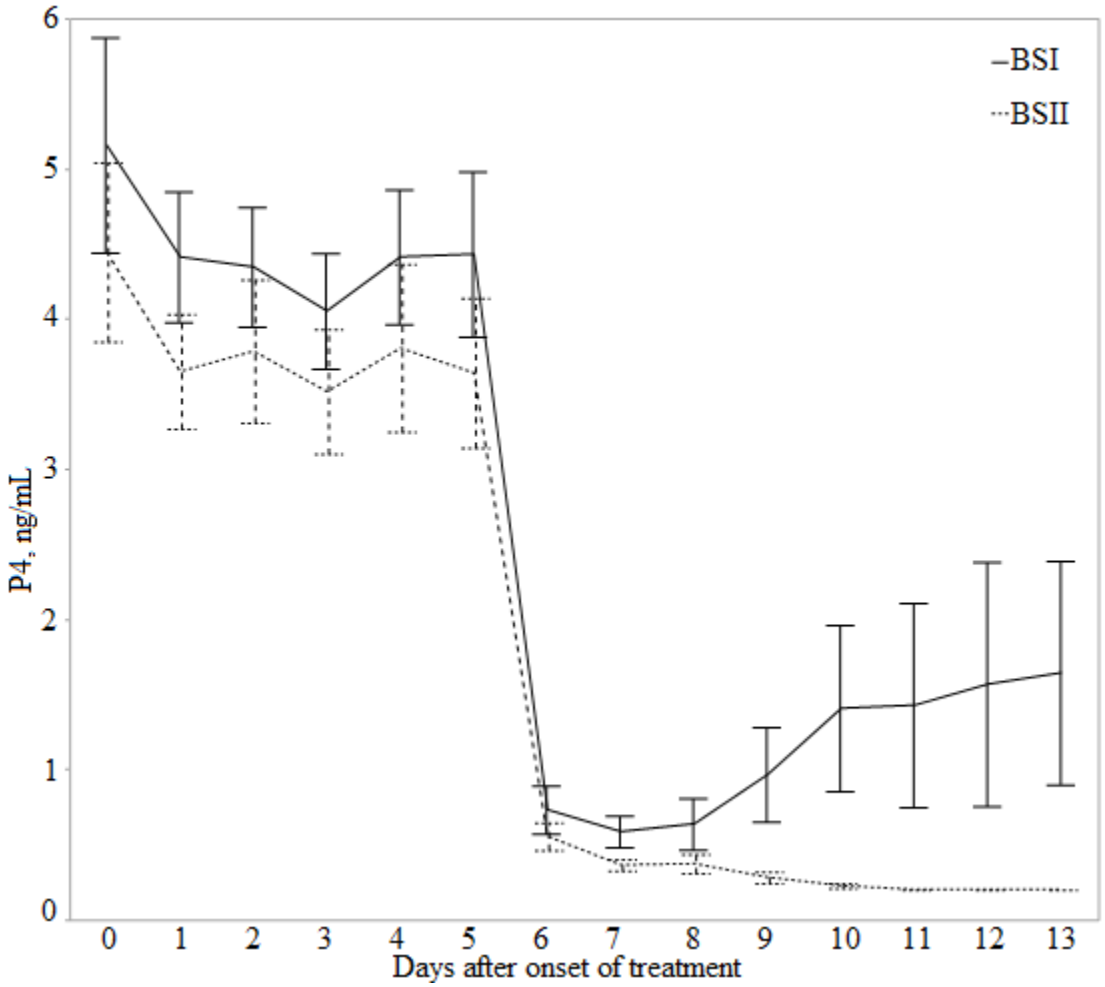

Figure 3: Serum concentration of P4 in Bee Synch I (n = 35) and II (n = 36) treatments, from treatment onset (day 0) until day 12 or ovulation ($P > 0.05$).

The timing and growth of follicles relative to follicle deviation was assessed in 49 cows (Fig. 4). Since day of deviation did not differ due to treatment, day of cycle or

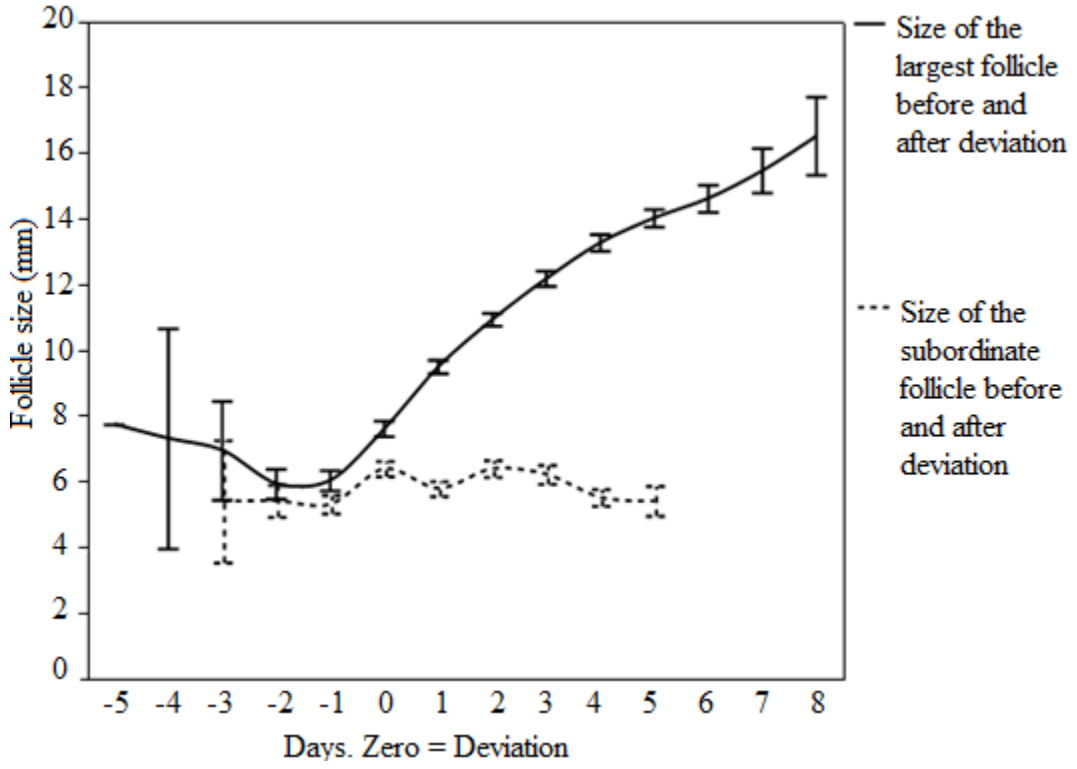

Figure 4: Large follicle diameter and largest subordinate follicle diameter normalized to the day of deviation.

replicate, data are presented as pooled means. Deviation of the dominant follicle was observed 3.3 ± 0.13 days after emergence and averaged 7.61 ± 0.23 mm the day before and 9.5 ± 0.2 mm on the day of deviation. Size of the largest subordinate follicle on the

day before deviation was 6.39 ± 0.23 mm and averaged 5.78 ± 0.22 mm on the day of deviation.

Mean serum concentration of LH following injection of GnRH or saline are illustrated in Figure 5. As expected, mean concentrations were greater ($P < 0.0001$) after GnRH in BSI (1.86 ± 0.1 ng/mL) than for BSII (0.22 ± 0.1 ng/mL) after saline. Peak serum concentrations of LH at 120 minutes after GnRH injection on day 3 were greater (4.28 ± 0.71 ng/mL; $P < 0.05$) than on days 7 (2.70 ±0.23 ng/mL) and 10 (1.86 ± 0.22 ng/mL).

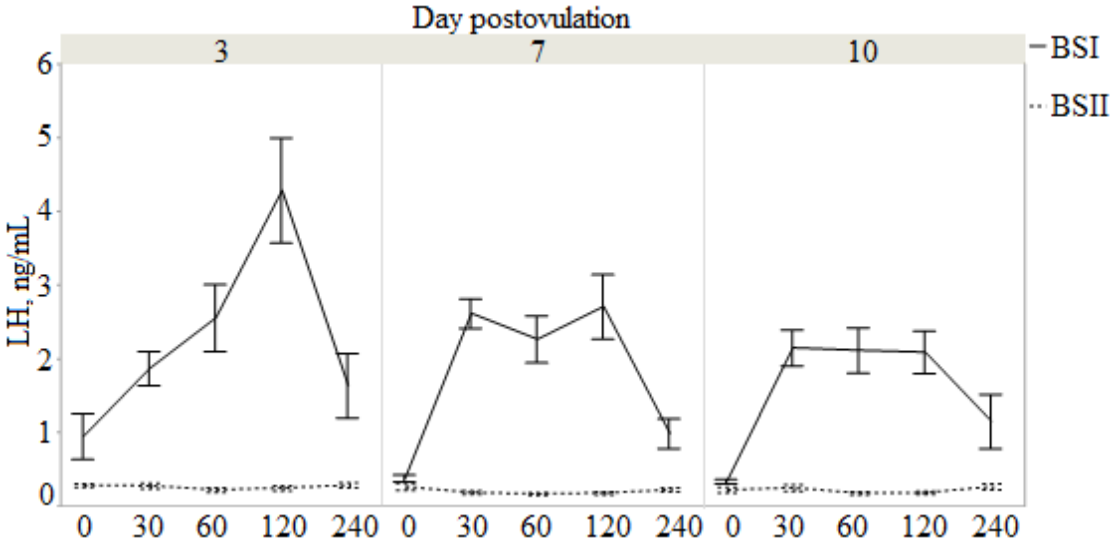

Figure 5: Serum concentration of luteinizing hormone (LH) at 0, 30, 60, 120, and 240 minutes after GnRH or saline injection in Bee Synch I (n = 35) or II (n = 36) treatments, respectively. Cows at day 3 postovulation had greater ($P < 0.05$) induced release of LH than cows at days 7 or 10 postovulation. Bee Synch I-treated cows had greater ($P < 0.0001$) induced release of LH than Bee Synch II-treated cows.

Discussion

Results of the current experiment indicate that, although GnRH on day 0 (Bee Synch 1) resulted in synchronized NFWE in 68.6% of cows between days 1 to 4 after treatment, and greater than 90% if this interval was expanded to include days 0-4 (compared to 38.9% and 61.1%, respectively for Bee Synch II), its use failed to increase size of the dominant follicle or frequency of estrus at 66 hours after CIDR removal, or to decrease mean interval to ovulation. These observations may explain why FTAI pregnancy rates in mature, *Bos indicus*-influenced cows, averaging about 50% in both Bee Synch I and II when tested in field trials, have failed to differ statistically.

Earlier reports from our group have shown that protocols such as Ovsynch (Williams et al., 2002), 7-day CO-Synch + CIDR (Saldarriaga et al., 2007; Zuluaga et al., 2010), and 5-day CO-Synch + CIDR (Williams et al., 2011; Williams et al., 2013), developed in *Bos taurus* females, fail to yield consistent and acceptable FTAI pregnancy rates in *Bos indicus*-influenced beef cows. This was in contrast to results of large field trials using these procedures in straight *Bos taurus* cows where FTAI pregnancy of 50% or greater had been reported consistently. Thus, modifications were made in the 5-day CO-Synch + CIDR regimen to address this issue and initially employed the addition of PGF on day 0 (Bee Synch I; Williams et al., 2013) to lower overall circulating P4 during the synchronization period. We had hypothesized that *Bos indicus*-influenced cattle might be more sensitive to the negative feedback effects of P4 on secretion of LH, thus delaying maturation of the dominant follicle. Employment of this strategy (Bee Synch I) resulted in FTAI pregnancy rates of 50% or greater relative consistently in these types of

cattle using a CIDR in combination with GnRH and PGF. Those studies were followed by additional field trials in which Bee Synch I was modified further by eliminating GnRH on day 0. By default, the latter obviated the need for the double dose of PGF at day 5 (Bee Synch II; Williams et al., 2015), since no new CL were induced by GnRH on day 0. The basis of this modification was our earlier observations (Saldarriaga et al., 2007; Zuluaga et al., 2010) that ovulation rates to GnRH in mature, randomly cycling *Bos indicus*-influenced cows were usually low, ranging from 30-60%. Therefore, we tested the hypothesis that FTAI pregnancy rates would be similar between Bee Synch I (full protocol) and Bee Synch II (modified to eliminate GnRH on day 0). This hypothesis followed that of Cruppe et al (2014) in which elimination of GnRH on day 0 in the standard 5-day CO-Synch + CIDR regimen using straight *Bos taurus* heifers resulted in pregnancy rates that did not differ from the original regimen that included GnRH. Although no statistically significant differences in FTAI pregnancy rates between Bee Synch I and II protocols were observed in field trials, unexpected variability in Bee Synch II in some of the individual trials suggested that GnRH on day 0 might be having beneficial effects in some females that, by coincidence, were at specific stages of the estrous cycle. Indeed, the inclusion of GnRH on day 0 in the current study resulted in measurable effects on synchrony of NFWE during the first 4 days after treatment onset. However, at 66-72 hours after CIDR removal, the time at which mature cows synchronized with both Bee Synch I and II have been inseminated, there were no differences in measurable follicle characteristics known to affect FTAI pregnancy rates.

Work of Kojima et al. (2000), DeJarnette et al. (2001), and Saldarriaga et al. (2007) inferred that synchronization of NFWE is related to ovulation response after GnRH-1. In the work of Saldarriaga et al. (2007) with *Bos indicus*-influenced beef cows, ovulation rate was 40% and synchronization rate was greater in cows ovulating after GnRH-1 (88%) compared to those not ovulating (42%). Vasconcelos et al. (1999) suggested that ovulation rates following GnRH in growing, dominant, and regressing phases of dominant follicles (days 3, 7, and 10 postovulation in our experiment) vary because of differences in ovulatory capacity related to the availability of LH receptors. In his experiment, GnRH resulted in ovulation in 23%, 96%, 54% and 77% of cows when treatment was administered on days 1 to 4, 5 to 9, 10 to 16, and 17 to 21, respectively. The findings of Sartori et al. (2001) corroborated this finding and reported a low rate of ovulation for day 3 (0%) and a high rate of ovulation for day 6 (100%) of the cycle. Conversely, in *Bos taurus* beef heifers, Martinez et al. (1999) administered GnRH on days 3, 6 and 9 of the estrous cycle and obtained ovulation in 89%, 56% and 22%, respectively. Martinez et al. (1999) indicated that all heifers with a dominant follicle larger than 9 mm ovulated in response to GnRH, which supports more recent work by Ginther et al. (2016) in which follicle deviation was shown to occur at a diameter of 8.5 mm, and by Sartori et al. (2001), where ovulatory capacity was achieved immediately after deviation. In our study, mean large follicle size on day 0 for Bee Synch I (GnRH-1 treatment) was 11.1 mm ± 0.2 and deviation of the synchronized NFWE in both treatments was achieved at 9.5 ± 0.2 mm. Therefore, ovulation after

GnRH-1 treatment was expected to occur in cows with follicles larger than 9.5 mm in diameter already present at treatment onset.

Sartori et al. (2001) have indicated that ovulatory capacity is dependent upon both the amount of LH released and diameter of the dominant follicle. The mean peak concentration of LH observed at120 minutes after GnRH-1 on day 10 in Bee Synch I-treated cows was 1.86 ng/mL, which was substantially less than reported by Saldarriaga et al. (2007). Also, when GnRH was administered on day 3 post ovulation, mean peak concentrations of LH were 4.28 ng/mL, greater than the other groups, but still lower than reported by Saldarriaga et al. (2007), which may explain the low ovulation response after GnRH-1in this study. Overall, ovulatory response to GnRH was exceptionally low. It is not known whether the repeated handling of cattle in the current study that was required for intensive data collection resulted in stress-induced release of adrenal steroids and suppression of the pituitary response to GnRH (Dobson & Smith, 1995). However, previous similar studies in this laboratory using similar types of cattle did not result in such a low ovulation rate (Saldarriaga et al., 2007; Zuluaga et al., 2010). Cows appeared to acclimate well to the experimental conditions and did not present behavioral evidence suggestive of differences from previous studies.

In the current study, the rate of largest follicle regression after GnRH or saline did not differ between Bee Synch I and Bee Synch II. Interestingly, the lack of any ovarian response to GnRH-1 was 10-fold lower (2.9%) than that reported by Zuluaga et al. (2010; ~29%). Saldarriaga et al. (2007) and Barros et al. (2000) also reported a greater rate of ‘no response’ (~21%) to GnRH-1 on day 0 of a 7-day CO-Synch + CIDR

protocol compared to the current work, all of which utilized *Bos indicus*-influenced beef cows. Nonetheless, synchronized NFWE after GnRH-1 or saline in our experiment was quite high (72% days 1-4; 94.3% days 0-4), which is in agreement with Twagiramungu et al. (1995) and Martinez et al. (2000), both of which indicated that both follicle regression and ovulation were equally effective for inducing NFWE.

Mean size of the dominant follicle at 66 hours after CIDR removal for both treatments in our study was 13.5 ± 0.3 mm and size of the dominant follicle at 66 hours after CIDR removal was always greater than 11.5 mm except for individuals starting treatment on day 3 of the cycle in the Bee Synch II treatment. In the latter, mean size of the largest follicle was 10.5 mm. Sartori et al. (2001) reported that 11.5 mm is the apparent threshold size for optimal fertility after ovulation in Holstein cows. This observation agrees with Perry et al. (2005), who indicated that follicles induced to ovulate that were smaller than 11 mm resulted in low pregnancy rates in *Bos taurus* females. Lowered fertility in smaller follicles occurs because the oocyte is physiologically-immature, resulting in increased embryonic loss. Thus, it is possible to infer that cows in our experiment could have been induced to ovulate with GnRH at 66 hours after CIDR removal without negative effects on ovulation rate or fertility, except for cows starting Bee Synch II treatment on day 3 of the cycle. It is this latter scenario where small follicle size could impact ovulation, oocyte fertility, and embryonic survival in the field using Bee Synch II.

The NFWE occurred in 91.5% of the cows in this study. From the onset of treatments, NFWE occurred at 1.8 ± 0.3 days for Bee Synch I, and 2.2 ± 0.3 days for Bee

Synch II ($P = 0.45$). However, a delay ($P < 0.05$) in NFWE when treatment began on day 3 of the cycle in Bee Synch II (NFWE on day 5) was responsible for smaller average follicle size at 66 hours after CIDR removal. Smaller follicle size in this subgroup could not be attributed to a slower rate of follicle growth since growth rate was nearly 1.3 mm per day and did not differ between or within treatments. Moreover, the interval from NFWE until ovulation was ~ 8 days for all cows. Martinez et al. (2000) reported that heifers either ovulating or regressing the largest follicle on day 0 of GnRH treatment did not differ in the day of NFWE.

The NFWE is linked closely with the stage of the estrous cycle (Geary et al., 2000). As found in this experiment, cows that regressed the largest follicle on day 0 of treatment onset tended to exhibit follicle wave characteristics as described by Sirois & Fortune (1988) and Ginther et al. (1989). Based on their observations, NFWE occurs approximately on the day of ovulation and again on about the 10th day of the estrous cycle in cows with two follicular waves and on day 0, 9, and 15 of the estrous cycle for cows with three follicular waves. Cows starting Bee Synch I or II treatments 3 days after ovulation (day 3 of the cycle) exhibited a delayed occurrence of NFWE of approximately 4.3 ± 0.3 days, which represents day 7.3 ± 0.3 of the estrous cycle. This coincides closely to timing of the second follicular wave. The same pattern was observed for cows beginning both treatments on day 7, where NFWE occurred on average at 1.4 ± 0.2 days after treatment onset. This coincides with day 8.4 ± 0.2 days of the estrous cycle. For day 10 of the cycle, average interval to NFWE was 0.5 days.

Ovulation after CIDR removal was detected in 21% of all cows (15/71) before 72 hours (average of 68 ± 4 hours), in which 80% (12/15) occurred in cows starting treatment on day 10 of the cycle and 83.3% (10/12) of these were observed in Bee Synch II-treated cows. This suggests that treatment with GnRH at treatment onset in Bee Synch I reduced the incidence of earlier ovulation after CIDR removal, which could have a negative impact on fertility by loss of oocyte viability if FTAI is conducted at 72 hours. This risk can potentially be decreased at the proposed FTAI at 66 hours after CIDR removal.

Overall mean concentrations of serum P4 during CIDR insertion was 4.15 ± 0.28 ng/mL and did not differ between treatments. Similar values have been reported by Saldarriaga et al. (2007) and Zuluaga et al. (2008). On day 6 (one day after CIDR removal and PGF), all cows exhibited a marked decline in serum P4 concentrations to below 1 ng/mL. Nascimento et al. (2014) tested single (25 mg), double (50 mg), and double-split (2 25-mg injections 8 hours apart) of PGF on day 5 in Holstein cows. Results showed a dramatic superiority in efficacy of either the double or double-split doses of PGF compared to the single dose for regressing 5-day CL. In field trials, Rabaglino et al. (2010) observed no difference in pregnancy rate of dairy heifers synchronized using either single or double doses of PGF at day 5 with the STD-5-day protocol. However, incomplete luteolysis has also been reported when the double dose (50 mg of PGF) is applied (Nascimento et al., 2014). Similar results were observed in our experiment, where the 5 cows that ovulated after GnRH-1 exhibited a resurgence in serum P4 beginning on day 8 (2 days after CIDR removal and PGF). This was likely due

to a suboptimal effect of PGF which temporarily suppressed luteal function but did not result in complete functional luteolysis of newly-formed CLs (Lauderdale et al., 1974; Macmillan et al., 1983). Nonetheless, the temporary 2 to 3-day suppression of P4 permitted final maturation of the dominant follicle and ovulation in response to GnRH at 66 hours.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Results of the experiment reported here indicate that GnRH-1 (GnRH administered at the onset of the Bee Synch I protocol) does not successfully effect synchronized development of a dominant follicle for presumptive FTAI in *Bos indicus*-influenced beef cows. However, the greater synchronization of NFWE and the reduced incidence of early ovulations could explain some of the differences in FTAI pregnancy rates observed in individual field trials comparing Bee Synch I and II. Nonetheless, these differences do not appear at the present time to justify utilizing Bee Synch I instead of II given the savings in drug costs for Bee Synch II and the overall similar FTAI pregnancy rates observed in the field.

LITERATURE CITED

ABCZ. 2015. Brazilian Cattle. http://braziliancattle.com.br/?conteudo,113 (Accessed 20 October 2017).

ABIEC. 2017. Brazilian Beef Exports. http://www.abiec.com.br/download/anual-2016.pdf (Accessed 8 November 2017).

Adams, G. P., R. L. Matteri, J. P. Kastelic, J. C. H. Ko, and O. J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fert. 94:177-188.

Aerts, J. M., and P. E. Bols. 2010. Ovarian follicular dynamics. A review with emphasis on the bovine species. Part II: Antral development, exogenous influence and future prospects. Reprod. Domest. Anim. 45:180-187.

Alvarez P., L. J. Spicer, C. C. Chase, M. E. Payton, T. D. Hamilton, R. E. Stewart, A. C. Hammond, T. A. Olson, and R. P. Wettemann, R.P., 2000. Ovarian and endocrine characteristics during and estrous cycle in Angus, Brahman, and Senepol cows in a subtropical environment. J. Anim. Sci. 78:1291-1302.

Barros, C. M., M. B. P. Moreira, H. A. Figueiredo, A. B. Teixeira, and L. A. Trinca. 2000. Synchronization of ovulation in beef cows (Bos indicus) using GnRH, PGF2 alpha and estradiol benzoate. Theriogenology 53:1121-1134.

Baruselli, P.S., E. L. Reis, M. O. Marques, L. F. Nasser, and G. A. Bo, G.A. 2004. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. Anim. Reprod. Sci. 82-83:479-486.

Baruselli, P. S., R. M. Ferreiraa, J. N. S. Sales, L. U. Gimenes, M. F. Sá Filho, C. M. Martins, C. A. Rodrigues, and G. A. Bó. 2011. Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology 76:1583-1593.

Bearden, J.H., J. W. Fuqay, and S. T. Willard. 2004. Applied Animal Reproduction, Sixth Edition. Pearson Education Inc., Upper Saddle River, New Jersey.

Bó, G.A., M. Martinez, L. F. Nasser, M. Caccia, H. Tribulo, and R. J. Mapletoft. 1993. Follicular dynamics in Bos indicus and Bos taurus beef cattle under pasture conditions in Argentina. In: Proceedings of the 10th Congresso Brasileiro de Reprodução Animal. V2. Campo Grande, p. 221 (abstract).

Bó, G.A., P. S. Baruselli, and M. F. Martinez. 2003. Pattern and manipulation of follicular development in Bos indicus cattle. Anim. Reprod. Sci. 78:307-26.

Bridges, G.A., L. A. Helser, D. E. Grum, M. L. Mussard, C. L. Gasser, and M. L. Day. 2007. Decreasing the interval between GnRH and PGF2a from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 69:843-851.

Colazo, M.G., J. P. Kastelic, and R. J. Mapletoft. 2003. Effects of estradiol cypionate (ECP) on ovarian follicular dynamics, synchrony of ovulation, and fertility in CIDR-based, fixed-time AI programs in beef heifers. Theriogenology 60:855-865.

Cruppe, L.H.; M. L. Day, F. M. Abreu, S. Kruse, S. L. Lake, M. V. Bielh, R. S. Cipriano, M. L. Mussard, and G. A. Bridges. 2014. The requirement of GnRH at

the beginning of the five-day CO-synch+controlled internal drug release protocol in beef heifers. J. Anim. Sci. 92:4198-4203.

Cutaia, L., R. Trıbulo, L. Alisio, J. C. Tegli, D. Moreno, and G. A. Bó, G.A. 2001. Efecto de los tratamientos com dispositivos DIV-B nuevos o reutilizados en los ´ındices de preñez en vacas y vaquillonas inseminadas a tempo fijo (IATF). M. Caccia (Ed.) Resúmenes Cuarto Simposio Internacional de Reproducción Animal, Huerta Grande, Córdoba, pp. 244.

DeJarnette, J. M., M. L. Day, R. B. House, R. A. Wallace, and C. E. Marshall. 2001. Effect of GnRH pretreatment on reproductive performance of postpartum suckled beef cows following synchronization of estrus using GnRH and $PGF_2\alpha$. J. Anim. Sci. 79:1675-1682.

Dobrowolski, W., E. Stupnicka, and E. Domanski. 1968. Progesterone levels in ovarian venous blood during the oestrous cycle of the cow. J Reprod Fertil. 15(3):409-414.

Dobson, H., and R. F. Smith. 1995. Stress and reproduction in farm animals. J Reprod. Fertil. Suppl. 49:451-461.

FAO. 2009. How to feed the world in 2050. http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (Accessed 15 August 2017).

FAO. 2012. Global trends and future challenges for the work of the organization. http://www.fao.org/docrep/meeting/025/md883E.pdf (Accessed 20 October 2017).

FAO. 2015. World agriculture: towards 2015/2030. An FAO perspective. http://www.fao.org/3/a-y4252e.pdf (Accessed 20 October 2017).

Geary, T.W. and J. C. Whittier Pas. 1998. Effect of a timed insemination following synchronization of ovulation using ovsynch or CO-Synch protocol in beef cows. Prof Animal Sci. 14:217-220.

Ginther, O.J., J. M. Baldrighi, M. A. R. Siddiqui, and E. R. Araujo, E. R. 2016. Complexities of follicle deviation during selection of a dominant follicle in Bos taurus heifers. Theriogenology 86:2012-2019.

Ginther, O. J., M. A. Beg, D. R. Bergfelt, F. X. Donadeu, and K. Kot. 2001. Follicle selection in monovular species. Biol. Reprod. 65(3):638-647.

Ginther, O. J., D. R. Bergfelt, L. J. Kulick, and K. Kot. 2000. Selection of the dominant follicle in cattle: role of estradiol. Biol. Reprod. 63(2):383-389.

Ginther, O.J., K. Kot, L. J. Kulick, and M. C. Wiltbank, M.C. 1997. Emergence and deviation of follicles during the development of follicular waves in cattle. Theriogenology 48:75-87.

Ginther, O.J., L. Knopf, and J. P. Kastelic, J.P. 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J. Reprod. Fertil. 87:223-230.

Glencross, R.G., and Pope, G.S., 1981. Concentrations of oestradiol-17β and progesterone in the plasma of dairy heifers before and after cloprostenol-induced and natural leutolysis and during early pregnancy. Anim. Repro. Sci. 4:93-106.

Hafez, E.S.E. 1982. Reprodução Animal. 4º edition. Ed.Manole. (in Portuguese).

Hafs, H.D.; and D. T. Armstrong. 1968. Corpus luteum growth and progesterone synthesis during the bovine estrous cycle. J. Anim. Sci. 27:134-141.

Hafs, H.D., T. M. Louis, J. N. Stellflung, E. M. Convey, and J. H. Britt, J.H., 1975. Blood LH after $PGF_{2\alpha}$ in diestrous and ovariectomized cattle. Prostaglandins 10:1001-1009.

Honig, H., L. Ofer, M. Kaim, S. Jacobi, D. Shinder, and E. Gershon. 2016. The effect of cooling management on blood flow to the dominant follicle and estrous cycle length at heat stress. Theriogenology 86:626-634.

Kinder, J. E., F. N. Kojima, E. G. Bergfeld, M. E. Wehrman, and K. E. Fike. 1996. Progestin and Estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. J. Anim. Sci. 74:1424-1440.

Kojima, F. N., S. L. Wood, M. F. Smith, and D. J. Patterson. 2000. Does pretreatment with GnRH prior to a GnRH-$PGF_{2}\alpha$ (PG) protocol improve synchronization of estrus in beef cattle? J. Anim. Sci. 78(Suppl. 1):210.

Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2a for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253-2259.

Lamb, G. C., and V. R. Mercadante. 2016. Synchronization and artificial insemination strategies in beef cattle. Vet. Clin. North Am. Food Anim. Pract. 32(2):335-347.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of cattle following PGF2 alpha injection. J. Anim. Sci. 38(5):964-967.

Macmillan, K. L. 1983. Prostaglandin responses in dairy herd breeding programmes. N. Z. Vet. J. 31(7):110-113.

Mayerhofer, A., G. A. Dissen, M. E. Costa, and S. R. Ojeda. 1997. A Role for Neurotransmitters in Early Follicular Development: Induction of Functional Follicle-Stimulating Hormone Receptors in Newly Formed Follicles of the Rat Ovary. Endocrinology 138(8):3320-3329.

Martinez, M.F., G. P. Adams, D. R. Bergfelt, J. P. Kastelic, and R. J. Mapletoft. 1999. Effects of LH or GnRH on the dominant follicle of the first follicular wave in beef heifers. Anim. Reprod. Sci. 57:23-33.

Martinez, M. F., G. P. Adams, J. P. Kastelic, D. R. Bergfelt, and R. J. Mapletoft. 2000. Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. Theriogenology 54:757-769.

McCracken, J.A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: A neuroendocrine-mediated event. Physiol. Rev. 79:263-323.

McGrann, J. 2010. The United States Beef Cattle Industry: Production, Structure and Trends. Leister K. 1–21.

Mihm, M., P. J. Baker, J. L. H. Ireland, G. W. Smith, P. M. Coussens, A. C. O. Evans, and J. J. Ireland. 2006. Molecular Evidence That Growth of Dominant Follicles Involves a Reduction in Follicle-Stimulating Hormone Dependence and an

Increase in Luteinizing Hormone Dependence in Cattle. Biol. Reprod. 74(6):1051-1059.

Mikeska, J. C., and G. L. Williams. 1988. Timing of preovulatory endocrine events, estrus and ovulation in Brahman x Hereford females synchronized with norgestomet and estradiol valerate. J. Anim. Sci. 66(4):939-946.

Nascimento, A.B.; A. H. Souza, A. Keskin, R. Sartori, and M. C. Wiltbank. 2014. Lack of complete regression of the day 5 corpus luteum after one or two doses of PGF2a in nonlactating Holstein cows. Theriogenology 81:389-395.

Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Natl. Acnd. Sci. USA. 102:5268-5273.

Peters, A.R., and Ball, P.J.H., 1995. Reproduction in Cattle, Second Edition, Blackwell Science Inc, Cambridge, Maine.

Peterson, C.; A. Alkar, S. Smith, S. Kerr, J. B. Hall, D. Moore, and R. Kasimanickam, R. 2011. Effects of one versus two doses of prostaglandin F2α on AI pregnancy rates in a 5-day, progesterone-based, CO-Synch protocol in crossbred beef heifers. Theriogenology 75:1536–1542.

Plasse, D., A. C. Wranick, and M. Koger. 1970. Reproductive behavior of Bos indicus females in a subtropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. J Anim Sci. 30:63-72.

Pursley, J. R.; M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2a and GnRH. Theriogenology 44: 915-923.

Rabaglino, M.B., C. A. Risco, M. J. Thatcher, I. H. Kim, J. E. P. Santos, and W. W. Thatcher. 2010. Application of one injection of prostaglandin F2a in the five-day Co-synch plus CIDR protocol for estrous synchronization and resynchronization of dairy heifers. J. Dairy Sci. 93:1050-1058.

Rahe, C.H., R. E. Owens, J. L. Fleeger, J. H. Newton, and P. G. Harms, P.G. 1980. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. Endocrinology 07:498-503.

Randel, R. D., R. E. Short, D. S. Christensen, and R. A. Bellows. 1973. Effect of various mating stimuli on the LH surge and ovulation time following synchronization of estrus in the bovine. J. Anim. Sci. 37:128-130.

Remnant, J. G., M. J. Green, J. N. Huxley, and C. D Hudson. 2015. Variation in the interservice intervals of dairy cows in the United Kingdom. J. Dairy Sci. 98:889-897.

Saldarriaga, J.P., D. A. Cooper, J. A. Cartmill, J. F. Zuluaga, R. L. Stanko, and G. L. Williams. 2007. Ovarian, hormonal and reproductive events associated with synchronization of ovulation and timed appointment breeding of Bos indicus influenced cattle using intravaginal progesterone. J. Anim. Sci. 85:151-162

Sartori, R., P. M. Fricke, J. P. C. Ferreira, O. J. Ginther, and M. C. Wiltbank. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol. Reprod. 65:1403- 1409.

Sirois, J., and J. E. Fortune. 1988 Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol. Reprod. 38:308-317.

Stock, A.E., and J. E. Fortune. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 132:1108-1114.

Sveberg, G., G. W. Rogers, J. Cooper, A. O. Refsdal, H. W. Erhard, E. Kommisrud, F. Buckley, A. Waldmann, and E. Ropstad. 2015. Comparison of Holstein-Friesian and Norwegian Red dairy cattle for estrus length and estrous signs. J. Dairy Sci. 98:2450-2461.

Thatcher, W.W., M. Drost, J. D. Savio, K. L. Macmillan, K. W. Entwistle, E. J. Schmitt, R. L. de la Sota, and G. R. Morris. 1993. New clinical uses of GnRH and its analogues in cattle. Anim. Reprod. Sci. 33:27-49.

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

USDA. 2017. World agricultural supply and demand estimates. http://usda.mannlib.cornell.edu/usda/current/wasde/wasde-10-12-2017.pdf (Accessed 8 November 2017).

Vasconcelos, J.L.M., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 55:1067-1078.

Williams, S. W., R. L. Stanko, M. Amstalden, and G. L. Williams. 2002. Comparision of three approaches for synchronization of ovulation for timed artificial insemination in Bos indicus-influenced cattle managed on the Texas gulf coast. J. Anim. Sci. 80:1173-1178.

Williams, G. L., R. L. Stanko, C. C. Allen, R. C. Cardoso, L. D. Presotto, J. F. Thorson. and M. Amstalden. 2011. Evidence that luteal regression at the onset of a 5-Day CO-Synch + CIDR synchronization protocol markedly improves fixed-time AI pregnancy rates in Bos indicus-influenced cattle. J. Anim. Sci. 89:251 (abstract).

Williams, G.L. R. L. Stanko, and M. Amstalden. 2013. Bee Synch for successful fixed-time AI of Brahman-influenced cattle. Proc. 59th Ann. Texas A&M Beef Cattle Shortcourse, pp G-6 – G-9.

Williams, G.L., R. L. Stanko, and M. Amstalden. 2015. Envolving concepts in the development and application of synchronization protocols for fixed-time AI in Bos indicus-influenced cattle: Bee Synch I and II. Proc. 61th Ann. Texas A&M Beef Cattle Shortcourse.

Wiltbank, M. C., A. Gümen, and R. Sartori. 2002. Physiological classification of anovulatory conditions in cattle. Theriogenology 57:21-52.

Zolman, J., E. M. Convey, and J. H. Britt. 1974. Relationship between the luteinizing hormone response to gonadotropin releasing hormone and endogenous steroids. J. Anim. Sci. 39:355-359.

Zuluaga, J.F., J. P. Saldarriaga, D. A. Cooper, J. A. Cartmill, and G. L. Williams. 2010. Presynchronization with gonadotropin-releasing hormone increases the

proportion of Bos indicus-influenced females ovulating at initiation of synchronization but fails to improve synchronized new follicular wave emergence or fixed-time artificial insemination conception rates using intravaginal progesterone, gonadotropin-releasing hormone, and prostaglandin F2a. J. Anim. Sci. 88:1663-1671.

Zuluaga, J. F., and G. L. Williams. 2008. High-pressure steam sterilization of previous used CIDR inserts enhances the magnitude of the acute increase in circulating progesterone after insertion in cows. Anim. Reprod. Sci. 107:30-35.

APPENDIX

LABORATORY PROCEDURES

Luteinizing Hormone RIA

1. Iodination: Iodination grade bLH (AFP11743B; NHPP)
   Reaction: 25 µg of hormone, 1 mCi of 125I, 90 µg chloramine T, 2 min

2. Antibody: Anti-ovine LH (rabbit anti-oLH – TEA #35; obtained from Dr. Jerry reeves) Dilution: 1:100,000

3. Standards: bLH (AFP11743B; NHPP) Range: 0.1 -30 ng/ml

4. Reference preparation: bLH added to ovariectomized cow serum

5. RIA procedure
(Williams & Ray 1980)

a) Label assay sheets and borosilicate glass tubes 4 NSB, 6 TC, 3 "0", standards in triplicate, references in duplicate, and unknown samples in duplicate
b) Day 1: Pipette the following into each tube:
   NSB: 500 µl PBS-1% EW
   0 std.: 500 µl PBS-1% EW
   Stds.: 200 µl std + 300 µl PBS-1% EW
   Ref.: 200 µl reference + 300 µl PBS-1% EW
   Unknowns: 200 µl sample + 300 µl PBS-1% EW
   Refridgerate at 4°C until next step

   Pipette 200 µl PBS-EDTA + 1:400 NRS without 1st Ab into NSB tubes
   Pipette 200 µl anti-oLH (diluted in PBS-EDTA + 1:400 NRS) into all tubes except NSB and TC tubes
   Vortex briefly and incubate for 2 hours at 4° C
   Pipette 100 µl I^{125}-bLH (20,000 cpm/tube diluted in PBS-1% EW) into all tubes, vortex briefly, and incubate for 24 hours at 4o C

c) Day 2: Pipette 200 µl of sheep-anti-rabbit gamma globulin (SARGG) diluted in PBS-EDTA into all tubes except TC
   Vortex and incubate 48-72 hours at 4° C
d) Day 4: Add 3 ml ice-cold 0.01M PBS into all tubes except TC
   Centrifuge tubes for 1 hour at 3600 rpm at 4° C
   Decant supernatant
   Count radioactivity with gamma counter

Progesterone RIA

Progesterone Coated Tube RIA Kit, MP Biomedicals, Santa Ana, CA

References:
Jones et al., 1991. J. Anim. Sci. 69:1607
Simpson et al., 1992. J. Anim. Sci. 70:1478.

1. Iodinated Product: Iodination grade hP4.
2. Antibody: Anti-human P4 coated tubes.
3. Standards: Cow serum with added P4. Range: 0.1 – 20.0 ng/ml.
4. Reference: Cow standard preparation added to bovine stripped serum.
5. RIA Procedure:
   A. Conduct assay
      1) Pipette in non-coated polypropylene tubes
         NSB – 100 µl of 0 std
      2) Pipette in antibody coated tubes
         0 Std – 100 µl
         Std – 100 µl
         Ref – 100 µl
         Unknowns – 100 µl
      3) Pipette 1 ml of I^{125}-P4 provided in the kit into all tubes including three Total Count non-coated polypropylene tubes.
      4) Vortex tubes briefly and incubate in water bath for 2 h.
      5) Pour off supernatant.
      6) Count radioactivity using gamma counter.