OVARIAN AND HORMONAL EVENTS DURING SYNCHRONIZATION OF

OVULATION AND TIMED APPOINTMENT BREEDING OF *Bos indicus*-

INFLUENCED CATTLE USING INTRAVAGINAL PROGESTERONE, GnRH

AND PROSTAGLANDIN F$_{2\alpha}$

A Thesis

by

JUAN PABLO SALDARRIAGA LOPEZ

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

MASTER OF SCIENCE

December 2005

Major Subject: Physiology of Reproduction

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Approved by:

Chair of Committee,   Gary L. Williams
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                              Thomas H. Welsh, Jr.
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ABSTRACT

Ovarian and Hormonal Events during Synchronization of Ovulation and Timed

Appointment Breeding of *Bos indicus*-Influenced Cattle Using Intravaginal

Progesterone, GnRH and Prostaglandin $F_{2\alpha}$.

(December 2005)

Juan Pablo Saldarriaga López,

D.V.M., Universidad de Antioquia, Medellín, Colombia.

Chair of Advisory Committee: Dr. Gary L. Williams

Objectives were to 1) evaluate the use of the CO-Synch + CIDR (COS-C) protocol for synchronization of ovulation and timed AI (TAI) in *Bos indicus*-influenced cattle, 2) compare cumulative pregnancy rates after COS-C synchronization and TAI to those in a traditional management (TM) scheme, and 3) evaluate specific ovarian, hormonal, and estrual events associated with COS-C. The COS-C regimen included insertion of a controlled internal drug release device (CIDR) containing progesterone and injection of GnRH (GnRH-1) on day 0, removal of the CIDR and injection of prostaglandin $F_{2\alpha}$ (PGF on d 7, and injection of GnRH (GnRH-2) and TAI 48 h later. In experiment 1 (Exp. 1), 335 females were stratified by BCS, parity and d postpartum before random assignment to COS-C or TM. An additional 96 females in which TM controls were not available for comparison also received COS-C. Conception rates to TAI averaged 39% (n = 266). Cumulative pregnancy rates were greater ($P < 0.05$) after 30 and 60 d of the breeding season in COS-C than in TM (n = 170 and 165 females

respectively). In experiment 2 (Exp. 2), 100 postpartum (F_1) females were stratified as in Exp. 1 within four replicates (25 each) and assigned randomly to receive either COS-C or COS (no CIDR) treatment. No differences were observed between treatments and all data were pooled. Percentages of cows ovulating after GnRH-1, developing a synchronized follicular wave, exhibiting luteal regression to PGF, and ovulating to GnRH-2 were 40, 60, 93, and 72%, respectively. In experiment 3 (Exp. 3), primiparous (F_1) heifers (n = 32) and pluriparous cows (n = 18) received the Select Synch + CIDR synchronization regimen (no GnRH-2 or TAI). Mean intervals from CIDR removal to estrus and ovulation, and from estrus to ovulation were 70 ± 2.9, 99 ± 2.8, and 29 ± 2.2 h, respectively. Relatively low TAI conception rates (< 50%) were attributed to failure of 40% of cattle to develop a synchronized follicular wave after GnRH-1 and to inappropriate timing of TAI/GnRH-2. It may be possible to improve TAI conception rates by delaying TAI/GnRH-2 to between 66 and 72 h, and by developing methods to increase the number of ovulations after GnRH-1.

DEDICATION

This manuscript and the work behind it is dedicated to my family. Their support has been fundamental in accomplishing this goal.

ACKNOWLEDGMENTS

Lastly, I would like to express my love to my parents, Sergio and Ana Cristina, and to my siblings, Daniel and Natalia. They are fundamental parts of my life. I also thank Diana for her patience, strength, commitment, encouragement and love. Together, they provided the emotional support to accomplish my goals throughout this extraordinary experience.

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CHAPTER I

INTRODUCTION

The beef industry is the largest single segment of the U.S. agricultural economy and the world's largest producer of beef (NASS, 2002). Similar to many other segments of the economy, the beef industry has had to implement important changes in order to maintain its leading position in the world. The lowest beef cattle inventory of the last 25 years was registered in 2004 (NASS, 2004). Due to this reduction, beef production declined about 7% compared with 2003. On the other hand, productivity has enjoyed an opposite tendency, with the number of pounds of beef produced per cow increasing steadily during the last 30 years (Brester and Marsh, 2002). The fact that total beef production has been maintained even with a reduced inventory of cattle reflects the tendency of the industry towards a more efficient production system.

In beef cattle, pounds of beef produced and quality are the major sources of profit. In the cow-calf industry, this is accomplished by the number of calves produced per year and their genetic value. Unfortunately, the generation time of cattle is one of the longest of any food-producing species, and the potential rate of genetic gain is fairly low. Therefore, under the best of circumstances, cow-calf operations can produce one annual calf per year with the goal of continuously attempting to increase their genetic merit.

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This thesis follows the style of Journal of Animal Science.

One reproductive tool that has been recognized to be extremely helpful for increasing the rate of genetic gain, increasing pounds of beef, and improving quality is artificial insemination (AI). However, only about 13 % of U.S. producers are using AI in their herds (NAHMS, 1997). There are numerous reasons for the reluctance to incorporate AI into commercial beef cattle enterprises. One of the most important reasons is the lack of biologically and economically optimized tools for the synchronization of ovulation. Several new methods of synchronization have been developed recently and made available commercially in the U.S. The main focus has been to obtain a system which allows the use of timed artificial insemination (TAI) efficiently thus eliminating the need to detect estrus. Reports involving the use of the CO-Synch + CIDR (COS-C) protocol from studies conducted in the Midwestern U.S., indicates that it might provide the characteristics necessary to make TAI feasible and consistently successful in commercial beef herds (Larson et al., 2004 a, b). This approach has been tested primarily in *Bos taurus* cattle, including pubertal heifers, first-calf heifers, and lactating cows, with pregnancy rates of about 55% achieved consistently (Johnson, 2005).

In the southern regions of the U.S., where the environment is predominantly subtropical, the common use of *Bos indicus*-influenced cattle can create additional challenges for the successful use of protocols such as COS-C. Important differences in efficiency have been reported when synchronization protocols and TAI are employed in these types of cattle (Lemaster et al., 2001; Williams et al., 2002), and newer protocols involving the COS-C have not been reported extensively. Therefore, additional work is

needed to determine whether relatively complex, high cost procedures such as COS-C, in conjunction with TAI, can provide the level of performance needed for economical incorporation into commercial beef cattle herds in the southern U.S.

Objectives

Objectives of these experiments were to 1) evaluate the use of the COS-C protocol for synchronization of ovulation and TAI in *Bos indicus*-influenced cattle, 2) compare cumulative pregnancy rates after COS-C synchronization and TAI to those in a traditional management (TM) scheme 3) evaluate specific ovarian, hormonal, and estrual events associated with the use of COS-C and related protocols to identify aspects of the system that may contribute to the relative reduced efficiency of the system.

CHAPTER II

LITERATURE REVIEW

United States Beef Industry

The beef industry is an important value-added enterprise in U.S. agriculture. The beef industry is the largest single segment of the U.S. agricultural economy, with cattle representing about 22.5 % of total farm sales, according to the 2002 U.S. census of agriculture (NASS, 2002). The U.S cattle inventory totaled 103.6 million of head as of July, 2004, and beef cows totaled 33.5 million for the same period (NASS, 2004). Although this number of animals continues to make the U.S. beef industry the largest beef producer in the world, the inventory is the lowest registered in the last 25 yr (Figure 1; NASS, 2004), with beef production declining about 7% compared with 2003 (ERS, 2004c). This negative trend has been created mainly by the end of a cattle cycle that has lasted for about 14 yr (ERS, 2004a). Other issues such as human dietary health concerns, market bans and weather conditions have also contributed to decrease both cattle inventory and beef production (ERS, 2004b).

As noted above, although the U.S. beef industry still leads world production, it has had to develop new and different market niches. Beef produced in the U.S. is recognized around the world for its quality and flavor. Strong efforts have been made to position U.S. beef in a competitive advantage against other world beef producers such as Brazil and Australia that could potentially threaten the U.S market. In parallel with

quality, health, safety and consumer friendliness of products are key attributes that make

U.S beef the most preferred in the world.
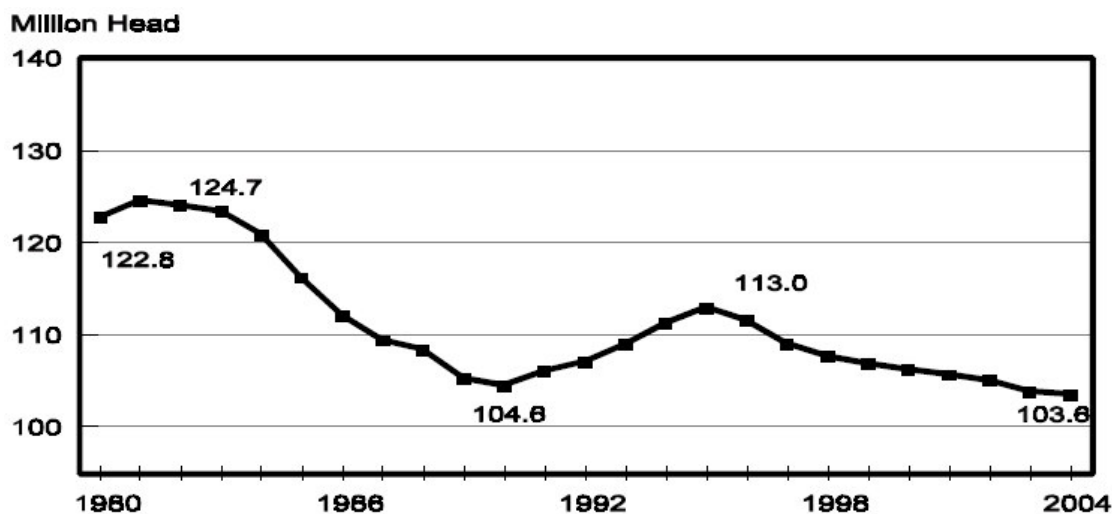
Figure 1. U.S. beef cattle inventory trends from 1980 through July 1, 2004. The inventory has declined slightly from 2003, and is the lowest in the last 25 yr. (Adapted from NASS, 2004).

Although beef inventory and beef production decreased for 2004 in the U.S., a

positive tendency has been observed in beef productivity. In fact, the beef industry has

experienced a dramatic increase in productivity over the last 30 yr (Figure 2, Brester and

Marsh, 2002). This increase in productivity has been influenced by several factors, but

one of the most important has been an increase in beef production per cow as a result of

an increase in genetic merit, which has been reflected as an increase in pounds and

quality of beef produced (Figure 3. Brester and Marsh, 2002; Schroeder et al., 1995).
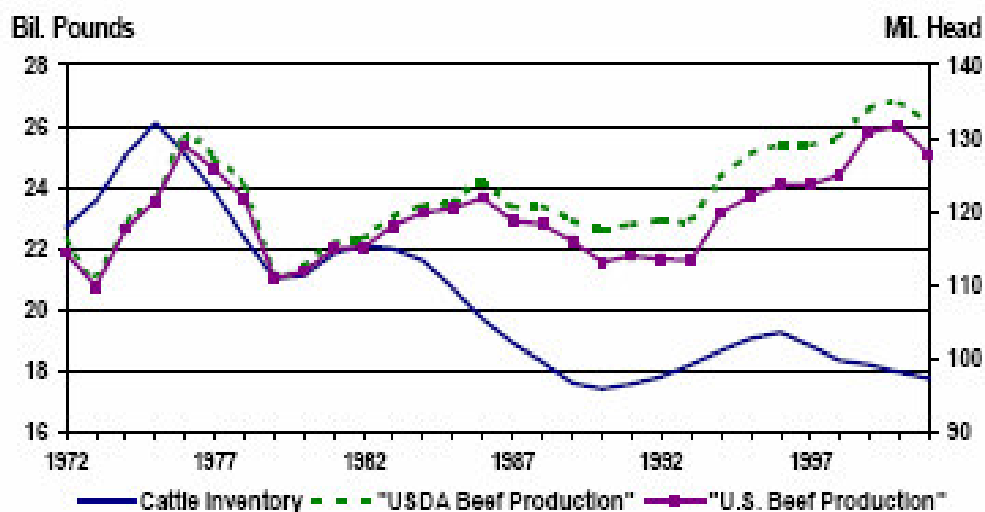
Figure. 2. Relationship between U.S. beef cattle inventories and two different measures of U.S. beef production from 1972 to 2001. USDA Beef production relates to the total of animals slaughtered within U.S. including domestic and imported; U.S. beef production relates to the total of animals slaughtered within U.S. excluding imported animals. Although total production has been maintained, cattle inventory has decreased about 25 million head. (Source: Brester and Marsh, 2002).

Importance of Reproductive Efficiency

In beef cattle, the number of pounds of beef produced continues to be the most important source of profit. This is regulated by two variables: number of calves produced per cow year and pounds weaned per calf (De Rose and Wilton, 1991). However, the goal of producing one calf per year from each cow does not necessarily guarantee long-term profitability in a beef cattle enterprise. The use of genetic selection to improve weaning weights and improve meat quality using sires with desirable expected progeny differences (EPD) in these traits should also be a goal (Harris and Newman, 1994). Rate of genetic improvement in cattle is relatively slow compared with other farm species because of the long generation interval. The use of reproductive

technologies, especially AI (Willham, 1982) is an important key that allows the widespread use of improved genetics (Harris, 1998), and when it is coupled with synchronization of estrus/ovulation, both genetic value and uniformity can be enhanced (Dziuk and Bellows, 1983).
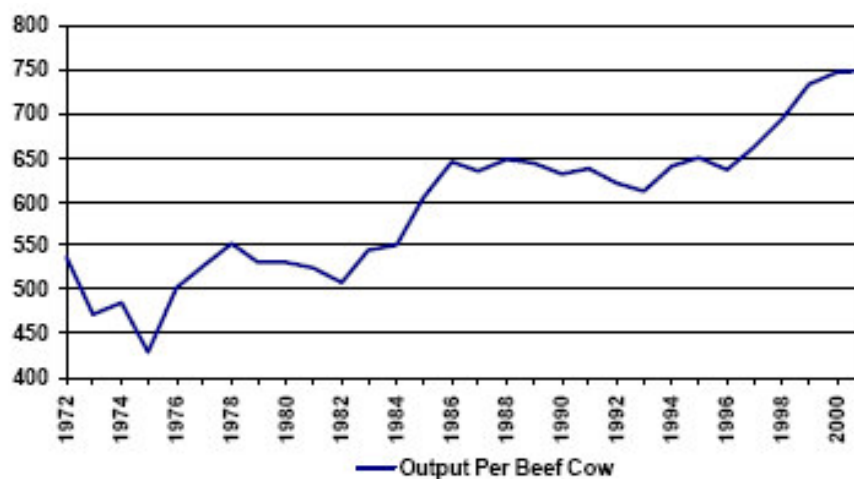
Figure 3. Productivity of U.S. beef cow breeding herd (carcass weight pounds per beef cow, annual). Beef output per U.S. beef breeding cow on a carcass weight basis has increased 40 percent over the past 28 years. (Source: Brester and Marsh, 2002).

Status of Artificial Insemination in Beef Production

There are several reproductive technologies that have been identified to be applicable and useful to increase the efficiency of beef production. Of these, breeding soundness evaluation of bulls and pregnancy determination in the cow herd are the most frequently used at 39.9% and 34.5%, respectively (NAHMS, 1997). Artificial insemination has been recognized as one of the most useful reproductive tools to increase genetic gain in cow-calf operations (Vishwanath, 2003), and estrous

synchronization has been recognized as one important tool to make it feasible (Johnson, 2005); however, neither AI nor estrous synchronization are being used by a significant number of beef producers. Only 13.3% of producers are using AI regularly, and just 11.9% of these use estrous synchronization programs in their operations (NAHMS, 1997). The principal reasons for this low rate of adoption are the time and labor required, complexity of the procedure, and costs (related to poor results) (NAHMS, 1997). These are problems that none of the commercially-available methods for synchronization of estrus/ovulation have been able to completely solve. Therefore additional work to optimize synchronization methodology is necessary.

The Bovine Estrous Cycle

The main purpose of an estrous synchronization/ovulation protocol is to place a relatively large population of cows and/or heifers into a specific physiological stage of the estrous cycle, and therefore allow mass breeding within a short period of time. This can not be accomplished if the physiology of the estrous cycle is not understood.

The bovine female is polyestrous, with a relatively uniform distribution of estrous cycles throughout the year that are not influenced remarkably by season (Kilen and Schwartz, 1998). The bovine estrous cycle averages 21 d in length. There are two major phases of the cycle. These, are differentiated primarily by the dominant structures present on the ovary within each stage (Senger, 2003). The follicular phase is the period from regression of the corpus luteum to ovulation. It encompasses about 20 % of the estrous cycle. The dominant ovarian structures are growing dominant follicles, and the

female is primarily under the hormonal influence of estradiol (E2), which is responsible for preparing the cow for mating (Senger, 2003). The luteal phase is the period from ovulation until corpus luteum (CL) regression. This phase encompasses about 80 % of the cycle, the dominant structure is the CL, and the physiologically-dominant hormone is progesterone, which prepares the uterus of the cow for receiving a potential conceptus (Senger, 2003).

Follicular Dynamics

Although the dominant, estrogen-active, follicle is the primary ovarian structure during the follicular phase, the luteal phase is also characterized by marked follicular activity and turnover (Fortune, 1994; Fortune et al., 1998). Two hypotheses were proposed regarding follicular dynamics during the bovine estrous cycle. Rajakoski (1960) initially proposed a model in which follicular growth occurred in two waves; the first starting a few days after estrus and the second starting around d 12-14. A second hypothesis proposed that follicular development was continuous and independent of the stage of the cycle (reviewed by, Sirois and Fortune, 1988). In the 1980s, development of a new technology, transrectal ultrasonography, provided a useful way to repeatedly measure and predict events occurring on the ovaries during the estrous cycle, thus helping to solve some of these contradictory theories (Pierson and Ginther, 1984; Sirois and Fortune, 1988; Ginther et al., 1989b). It is now well known that there can be two (Ginther et al., 1989a), three (Savio et al., 1988) and sometimes four (Sirois and Fortune, 1988) follicular waves within a single cycle. The number of follicular waves is influenced by many factors such as nutrition, parity and lactational status (Lucy et al.,

1992). There are several hormones that regulate follicular development, but the gonadotropic hormones, FSH and LH, are the most important. These gonadotropins are glycoproteins produced in the anterior lobe of the pituitary, and their principal function is stimulation of the gonads (Senger, 2003). The primary function of FSH in the female is to stimulate the growth of antral follicles, whereas LH causes ovulation and formation of the CL (Senger, 2003).

Follicular waves are composed of various stages. Follicular recruitment is the first step observed in each wave and is associated with an FSH surge (Turzillo and Fortune, 1990; Adams et al., 1992) in which a cohort of new follicles emerge and grow from a size of approximately 4 to 6 mm (Ginther et al., 1996). After follicular recruitment is achieved, selection or deviation of a follicle occurs. At this stage, a reduced number of follicles grow at a higher rate than the subordinates, allowing one of these to become the next dominant follicle of the cohort (Ginther et al., 1996). The last stage of the wave phenomenon occurs with the individualization of the dominant follicle. It acquires special characteristics that suppress the development of subordinate follicles. The dominant follicle subsequently grows to its maximum size and either ovulates or regresses depending upon stage of the cycle (i.e., presence or absence of a CL). The ability of this dominant follicle to growth larger than all the others is based upon its ability to secrete inhibin, a small peptide that suppresses FSH secretion. The dominant follicle also acquires more receptors for FSH, which promotes the ability to grow in a lower FSH-containing environment than its subordinates, and acquire LH driven growth capability (Ginther, 2000; Mihm and Austin. 2002). Whether dominant follicles ovulate

or not is dependent upon stage of the cycle; however, the fertility potential of all follicles selected for dominance during each follicular wave has been clearly demonstrated (Driancourt, 2001).

Hormones as Pharmacological Products

Several hormones are used as pharmacological products in order to synchronize estrus and/or ovulation in cattle. Three main groups of hormones are used in this process: prostaglandins (PG) and its analogues, steroids (mainly progesterone and E2), small peptides (GnRH) and glycoproteins (human chorionic gonadotropin; HCG and equine chorionic gonadotropin; eCG).

Prostaglandin $F_{2\alpha}$ and Analogues

Prostaglandins are lipids comprised of 20-carbon unsaturated hydroxyl fatty acids that are derived from arachidonic acid (Senger, 2003). Although there are several types of prostaglandins, the most important in relation to reproduction are prostaglandin $F_{2\alpha}$ (PGF) and prostaglandin E-2 (PGE-2) (McCracken, 1998). Prostaglandin $F_{2\alpha}$ is synthesized in the uterus, and is the most important luteolytic factor of the reproductive system (McCracken, 1998). There are numerous mechanisms by which PGF induces CL regression, but the most notable is its vascular action. After release, PGF reduces blood flow to the corpus luteum, thus depriving the gland of nutrients, substrates for steroid hormone production, and luteotropic support (Niswender et al., 2000). Other effects include action within the apoptotic cascade (Niswender et al., 2000; Milvae et al., 1996).

Exogenous PGF (Dinoprost tromethamine) is a potent luteolytic agent (Inskeep, 1973) that reduces the size of the CL (Thatcher and Chenault, 1976), reduces serum concentrations of progesterone (Lamond et al., 1973; Hansel and Fortune, 1978), and induces estrus within about 3 d (Liher et al., 1972; Welch et al., 1975), depending upon stage of the estrous cycle relative to onset of a new follicular wave. Similar to PGF, several synthetic analogues have been proven to be equally effective for inducing CL regression and synchronization of estrus/ovulation, including alfaprostol (Schams and Karr, 1982; Tolleson and Randel, 1998), cloprostenol (Schams and Karr, 1982), fenprostalene (Armstrong et al., 1989), luprostiol (Schams and Karr, 1982; Godfrey et al., 1989) and tiaprost (Schams and Karr, 1982; Peters, 1984).

Steroid Hormones

The sex steroids function as hormones to control or influence every aspect of reproduction. They are synthesized from cholesterol in the ovaries and testes where they promote oogenesis and spermatogenesis, respectively, by local action. They are also secreted into the peripheral circulation where they influence the reproductive tract, accessory sex organs, the sexual phenotype, and the secondary sexual characteristics of both males and females (Brown, 1998). Both progesterone and E2 or their bioequivalents can be used to manipulate female reproduction.

Progesterone. After CL formation, circulating concentrations of progesterone increase, reaching average peak concentrations of approximately 10 ng/mL on d 10 of the cycle (Hansel et al., 1973; Wettemann et al., 1972). The major function of progesterone is to prepare the uterus for pregnancy, converting it to an enriched

environment specifically suited for the developing embryo and for the maintenance of the pregnant state (Funk and DeMayo, 1998). Progesterone also acts at the level of the brain to modulate behavior, principally to suppress estrus and mating behavior, and to enhance maternal behavior (Etgen, 1998). High levels of progesterone also exert negative feedback effects on gonadotropin secretion at the hypothalamic level, suppressing frequency and increasing the amplitude of the GnRH pulses (Blake, 1998; Karsch et al., 1987; Goodman and Karsch, 1980), thus suppressing the frequency of LH pulses (Ulberg et al., 1951; Roche and Ireland, 1981; Karsch, 1987).

A number of different methods for administering progesterone have been developed for estrous synchronization systems in cattle. The earliest approach involved daily injections (Christian and Casida, 1948). This was followed later by intravaginal sponges impregnated with progesterone (Carrik and Shelton, 1967), progesterone-releasing intravaginal devices (PRID; Roche, 1974), controlled internal drug release devices (CIDR; Macmillan et al., 1991; Macmillan and Peterson, 1993), and intravaginal bovine device (DIB; Balla et al., 2004) all composed of Silastic silicone rubber (Dow Corning Co., MI., USA). Synthetic progestogen analogues have also been used, including medroxyacetate progesterone (MAP; Dhindsa et al., 1967), chlormadione acetate (CAP; Fulton et al., 1978), melengestrol acetate (MGA; Hill et al., 1971; Randel et al., 1972), administered either orally or injected, and norgestomet (Spitzer et al., 1978; Kazmer et al., 1981) administered by subcutaneous ear implants constructed of Silastic silicone rubber.

Estrogens. Serum concentrations of estradiol-17β (E_2) fluctuate during the estrous cycle in parallel with follicular growth and regression. Estradiol concentrations increase with the emergence of the first follicular wave to a peak of about 2 pg/ml (Evans et al., 1997). When the first follicular wave regresses, E_2 concentrations decline to a baseline concentration of about 0.2-0.5 pg/ml (Evans et al., 1997). Estradiol concentrations reach their maximum (6-9 pg/ml) approximately 24 h before ovulation, then decline rapidly after ovulation (Hansel and Convey, 1983). One of the primary targets of E_2 action is the reproductive tract. Estrogens act at this level to promote vascular, glandular and epithelial growth to prepare the female for sexual receptivity (Liundzey and Korach, 1998). High concentrations of E_2 also influence female behavior, triggering sexual receptivity and copulatory events (Uphouse and Maswood, 1998).

Fluctuating patterns of estradiol during the estrous cycle play a very important role in gonadotropin dynamics, and therefore in follicular activity. An inverse relationship between E_2 concentrations and both FSH and LH occurs during the estrous cycle (Ginther et al., 2000; Gibbons et al., 1999). Evans et al. (1997) presented a model relating ovarian follicular dynamics, gonadotropin secretion, and ovarian steroid hormones that summarized and explained these complex interactions. The usefulness of E_2 treatments within treatment schemes for synchronization of estrus and ovulation is related to its ability to induce a preovulatory surge of LH (Lammoglia et al., 1998), luteolysis in the follicular phase (Wiltbank et al., 1961) and follicular atresia (Bo et al., 1993; Burke et al., 2000). Follicular atresia is caused by a marked FSH suppression

after E_2 treatment (Kesner and Convey, 1982; Bolt et al., 1990). Suppression of FSH occurs within 6 h after E_2 (5mg) treatment, followed by a reappearance 36 to 72 h after treatment (Bo et al., 1994). Development of a new follicular wave begins an average of 4 d after treatment with E_2 and is dependant upon FSH reappearance (Bo et al., 1995).

Different E_2 esters have been used for estrous synchronization. Most of them are used in injectable solutions such as estradiol valerate (Wiltbank et al., 1971), estradiol benzoate (Bo et al., 1995; Hanlon et al., 1997), estradiol cypionate (Thundathil et al., 1998; Borman et al., 2003), and E2 (Bo et al., 1994; Murray et al., 1998). The major difference among these compounds is the half-life after injection; for example, a single injection of estradiol valerate (5 mg) results in elevated plasma estradiol concentration for a period of 5 to 7 d (Bo et al., 1993) whereas a single injection of E_2 (5 mg) results in elevated plasma estradiol concentrations for only 42h (Bo et al., 1994). These differences, consequently, result in differences in timing of events occurring within the synchronization program (Colazo et al., 2003).

Hypothalamic Peptides and Glycoproteins

Both the neuropeptides and glycoproteins influence gonadotropic activity by either inducing gonadotropin release or by mimicking their effects. The hypothalamic peptide, GnRH, and the glycoproteins HCG and eCG, have been used extensively for the pharmacological control of reproduction.

GnRH. Gonadotropin-releasing hormone is a decapeptide synthesized and stored in the preoptic area and medial basal hypothalamus. In response to neural signals, pulses of GnRH are released into the hypophyseal portal system and transported to the anterior

pituitary where they stimulate release of LH and to a lesser extent, FSH (Conn et al., 1998). Expression of GnRH mRNA and GnRH receptors (Kakar et al., 1993) has been demonstrated in several reproductive tissues such as the ovary (Oikawa et al., 1990), testicle (Bahk et al., 1995) and uterus (Ikeda et al., 1997), and cell lines such as the oocyte (Ny et al., 1987) granulosa (Harwood et al., 1980), luteal (Minaretzis et al., 1995), leydig (Clayton et al., 1980) and endometrial cells (Raga et al., 1999); however, its pharmacological use has been mainly focused to manipulate the reproductive function through gonadotropin regulation.

After exogenous administration of a pharmacological dose of GnRH, circulating concentrations of LH and FSH increase within 30 min, reach peak concentrations at 120-150 min, then decrease to basal levels between 4 and 5 h after injection (Zolman et al., 1974; Ford and Stormshak, 1978; Williams et al., 1982; Cupp et al., 1995).

Gonadorelin diacetate tetrahydrate (Zolman et al., 1974) is an analogue that is chemically synthesized but structurally equivalent to natural GnRH. Some other GnRH analogues are available commercially. Analogues differ as a result of slight chemical alterations of the native structure. These changes are intended to yield more stable molecules, better enzymatic resistance, increased binding capacity and better receptor affinity (Karten and Rivier, 1986; Thatcher et al., 1993). Such molecules include buserelin acetate (Cavestany and Foote, 1985), deslorelin acetate (Bergfeld et al., 1996), and fertirelin acetate (Chenault, 1990).

Several studies have reported on the ability of these compounds to release both LH and FSH in domestic species (Nawito et al., 1977; Ford and Stormshak, 1978;

Rettmer et al., 1992). The magnitude of release is dependant on many factors such as type of product (Rajamahendran et al., 1998) dose (Zolman et al., 1974), type of animal and physiological status (Williams et al., 1982). Few studies have been performed, under controlled conditions, to directly compare the differences on magnitude of release of LH and FSH after GnRH injection. Chenault et al. (1990) compared in a controlled study the magnitude of LH and FSH response to different compounds and doses of GnRH, and found that buserelin and fertirelin were 50 and 10 times more potent than gonadorelin respectively when used at recommended doses. Likewise, Martinez et al. (2003) also found differences among different GnRH products in Holstein cows. Nonetheless, recommended doses are designed to result in maximal release of releasable pools of LH to produce a surge capable of causing ovulation.

The first clinical use of GnRH was for the treatment of ovarian follicular cysts (Kittok et al., 1973), with 70-90% of cows with ovarian follicular cysts responding to treatment with ovulation. GnRH-induced LH release exerts numerous changes in ovarian follicles, but also affects the CL (Macmillan et al., 1985a,b). The effect generated by GnRH is highly dependant on the stage of follicular development at the time of the treatment. After GnRH treatment, ovulation and CL formation are expected when a healthy, mature or growing follicle is present at the time of the treatment (Vasconcelos et al., 1999). Follicular regression is expected in all medium-sized follicles and large follicles that are in a state of regression (Macmillan and Thatcher, 1991). Another major effect of GnRH treatment is the stimulation of a new follicular wave within 2-4 d after treatment (Twagiramungu et al., 1995). New follicular growth is

due either to increased secretion of FSH or by the subsequent FSH release caused by the disappearance of the dominant follicle (Twagiramungu et al., 1994). Although less important, the effect of GnRH treatment on CL function has been demonstrated, and is associated with structural modifications of the CL and lengthening of the luteal phase (Thatcher et al., 1993).

HCG. Human chorionic gonadotropin is a glycoprotein produced by the human conceptus whose primary function is to allow implantation and maintenance of pregnancy. This hormone has LH-like activity, thus acting at the level of the CL to prevent against luteolysis (Senger, 2003). Use of HCG treatments were initially investigated for their ability to increase the lifespan of the CL, hence increasing progesterone levels and reducing embryonic mortality (Morris et al., 1976). Not only can HCG increase the lifespan of existing CL, but also can induce ovulation and formation of accessory CL (Rajamahendran and Sianangama, 1992).

eCG. Equine chorionic gonadotropin is produced by the endometrial cups of the placenta of the mare and, similar to HCG, acts as a luteotropin providing a stimulus for maintenance of the primary corpus luteum. Additionally, eCG is responsible for controlling the formation of supplementary corpora lutea in mares (Senger, 2003). Exogenous treatment of the cow with eCG results in a FSH-like effect (Mulvehill and Sreenan, 1977); therefore, depending on the dose, it can be used to stimulate growth of single or multiple follicles (Anderson and Bondurant, 1982). Equine chorionic gonadotropin has been used mainly in cattle for superovulation (Bevers and Dieleman,

1987), and more recently has been used as an aid to stimulate follicular development in anestrous cattle for estrous synchronization protocols (Barusselli et al., 2003, 2004).

Legal Aspects of Use of Hormones

All of the foregoing compounds described are subject to government regulations; hence their availability is restricted in some countries. In the U.S., the Food and Drug Administration (FDA) regulates the use of drugs in both humans and animals. In the case of estrous synchronization protocols, with the exception of the CIDR device and oral MGA, all steroid compounds have not been licensed for use in food-producing animals. Drug companies involved in research and development of drugs for use in reproductive management of cattle and other animals have been able to obtain approval from the FDA to market GnRH, certain GnRH analogues, prostaglandin and certain prostaglandin analogues, some glycoprotein hormones, and the progestogens mentioned above for use in cattle. Current commercially-available protocols for estrous synchronization are restricted to and tend to focus on the use of these products for synchronization of ovulation and TAI protocols in the U.S.

Overview of Estrous Synchronization and Synchronization of Ovulation

Understanding the physiology of the estrous cycle, follicular wave phenomena, and use of hormones form the basis for development of tools to manipulate reproductive events in the cow. However, not all the knowledge in these areas has become available

simultaneously; thus, different protocols and pharmacological combinations have been developed as science has evolved.

Initial attempts to manipulate the estrous cycle included the use of progesterone, estradiol and PGF. Treatments using PGF to control the life span of the CL were studied intensively in the 1970's (Peters et al., 1977; Burfening et al., 1978). Initial experiments demonstrated that 70-90 % of cows injected with 30 mg native PGF would exhibit estrus within 7 d after administration (Lauderdale et al., 1974), with pregnancy rates after AI following detected estrus of about 50% (Lauderdale et al., 1974). These studies also demonstrated the ineffectiveness of PGF to cause regression of the CL between d 0 to 5 of the cycle (Lauderdale et al., 1980). The final dose approved by FDA is 25 mg of native PGF; however it has been demonstrated that the minimal effective dose may vary depending upon stage of the estrous cycle and dose. Berardinelli and Adair (1989) demonstrated that low dosages of PGF, (< 25 mg) resulted in increasing proportions of heifers exhibiting estrus as stage of cycle advanced while higher dosages yielded higher proportions earlier in the luteal phase and maximum proportions later in the luteal phase.

Use of progesterone or its analogues in different timing strategies was also pursued. Long-term treatment with progesterone failed to induce estrus and pregnancy rates high enough to allow breeding at a pre-set time; therefore, the method never achieved favor over natural breeding (Hansel and Fortune, 1978). Combination treatments using both PGF and progesterone were also developed. Induced estrus rates 5 to 7 d after treatment and first-service pregnancy rates were higher compared to the previous protocols and similar to naturally occurring estrus (Beal and Good, 1986;

Whittier et al., 1986; Odde, 1990).   Better reproductive outcomes observed with combinations of progesterone and PGF were due mainly to the ability of treatments to induce a fertile estrus in anestrous cows, prepubertal heifers, and cows with subnormal CL (Beal and Good, 1986; King et al., 1988); however, the use of this combination required in most cases the detection of estrus for AI, and timed-breeding was not recommended (Whittier et al., 1986).

The addition of estradiol (e.g., Syncro-Mate-B) to protocols using progesterone and PGF yielded better synchrony of estrus, thus allowing inclusion of TAI (Wiltbank et al., 1971; Spitzer et al., 1981; Mikeska and Williams, 1988).   First-service pregnancy rates to TAI resulted in some cases in excess of 55% (Odde, 1990); nevertheless, such results were not typical and wide variation in results contributed to poor acceptance among producers. In addition, the product was removed from the market by the FDA in 2000.

Control of the Corpus Luteum and Follicular Waves

A modern estrous synchronization method is considered efficient if it has certain basic characteristics, including the ability to effectively control the CL, follicular development, and estrus/ovulation (Twagiramungu, et al., 1995).

As reviewed previously, the control of the CL is fundamental for any synchronization protocol, and the use of PGF and its analogues is widely accepted as an effective pharmacological method to achieve it. However, these products alone are unable to regulate follicular growth or to effectively synchronize ovulation (reviewed in

Odde, 1990). Therefore, to synchronize follicular wave emergence, is necessary the use of GnRH or estradiol.

Protocols that use estradiol to re-program follicular growth in combination with a source of progesterone appear to provide more consistent results than methods that incorporate GnRH when they are compared in TAI programs. For example, in *Bos taurus* beef females, TAI pregnancy rates have been reported in the range of 60-75% (Martinez et al., 2002a,b), and in *Bos indicus*-influenced cattle, pregnancy rates have been reported between 60 and 70% (Colazo et al., 1999; Bo et al., 2003). These numbers are generally greater than those reported for protocols in which GnRH is used, as will be discussed later. Neverteless, it is important to note that strict regulations are imposed to E_2 within U.S. and its use is limited.

Estrous Synchronization and TAI in the U.S.

In the early 1990s, a new synchronization protocol was developed and evaluated. It included the use of GnRH or its analogues to induce ovulation and/or atresia of follicles present on the ovary, and thus allow emergence of a new follicular wave. An injection of PGF was administered 7 d later to induce CL regression. Collectively, the protocol objectives were to synchronize the development of a preovulatory follicle and estrus. Initial results reported induction of a new follicular wave and a preovulatory follicle within 3-4 d after the first injection of GnRH (Twagiramungu et al., 1995), and synchronized ovulation in 70-80% of cyclic cattle within a period of 4 d after PGF (Pursley et al., 1994). The new protocol was termed, Ovsynch (Fig 4, Pursley et al.,

1995). Ovsynch consists of one injection of GnRH (100µg) on d 0, an injection of PGF

(25 mg Lutalyse) on d 7, and a second injection of GnRH (100µg) 48 h later. Timed AI

is then employed 24 h after the second injection of GnRH.

```
          GnRH              PGF            GnRH       AI
            |                |              |         |
            ↓                ↓              ↓         ↓
            |_____|
Day         0                7              9        10
```
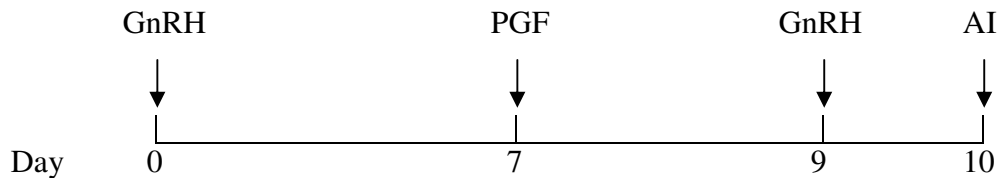
Figure 4. The Ovsynch synchronization protocol consists of one injection of GnRH (100 µg) on d 0, an injection of PGF on d 7, and a second injection of GnRH 48 h later. Timed AI is then employed 24 h after the second injection of GnRH. (adapted from Pursley et al., 1995).

Following the development of Ovsynch, other similar protocols involving GnRH

and PGF in combination with TAI or AI at estrus were developed. These included CO-

Synch (Fig 5a, Geary and Whittier, 1998), Select-Synch (Fig 5b, Lamb et al., 2004), and

Hybrid-Synch (Fig 5c, Lemaster et al., 2001).   However, the general physiological

rationale for all of these methods was the same as for Ovsynch.
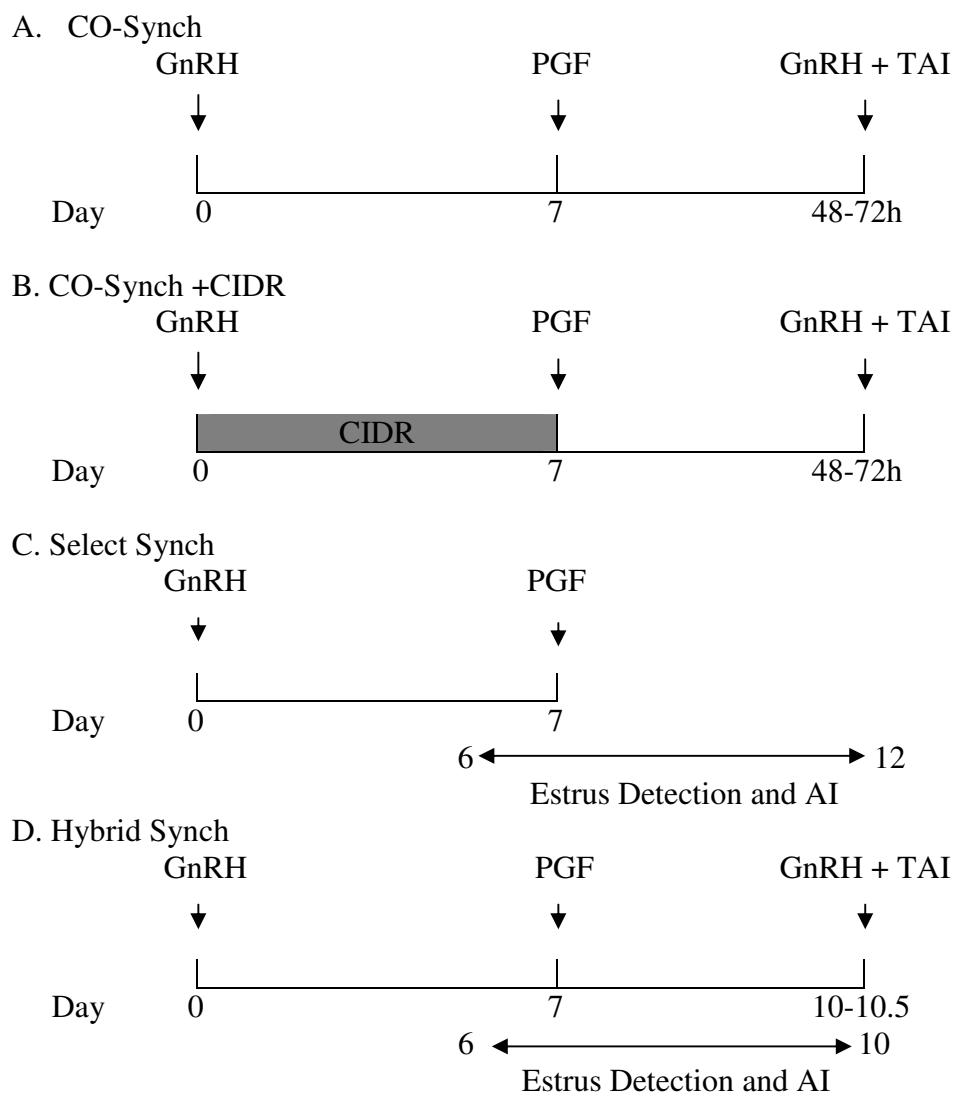
Figure 5. Different synchronization schemes using GnRH and PGF. A. CO-Synch: second GnRH injection is given 48-72 h after PGF in conjunction with TAI; B. CO-Synch + CIDR: same protocol as CO-Synch but a source of progesterone (CIDR) is added from d 0 to 7; C. Select Synch: heat detection is employed from d 6 to 12; AI is performed based upon estrus detection; D. Hybrid Synch: cows are detected in estrus as with Select Synch; cows not detected in estrus a second GnRH and TAI is given on d 10.

Pregnancy rates obtained with the Ovsynch protocol, which was initially developed and tested in dairy cows, ranged from 35% to 50%, with lower results in heifers than in cows (Pursley et al., 1997). Ovsynch was also applied in suckled *Bos taurus* beef cows, with pregnancy rates ranging from 33 to 60% (Geary et al., 1998; Thompson et al., 1999).

Weaknesses in Protocols Utilizing GnRH plus PGF

Pregnancy rates in cows using protocols that involve combinations of GnRH and PGF are greatly influenced by BCS (Herd and Sprott, 1998) and d postpartum. As a general rule the best results are obtained in cattle with a BCS of at least 5 on a scale of 1-9 , and in individuals that are at least 50 d postpartum (Lamb et al., 2001; Williams et al., 2002). In most cases when these requirements are fulfilled, TAI pregnancy rates can be increased to over 50% (Lamb et al., 2001; Williams et al., 2002). Similar to findings in dairy cattle, pregnancy rates in beef cattle have been lower in heifers than in cows (Lemaster et al., 2001; Williams et al., 2002).

Some weaknesses have been recognized in these types of protocols. Those weaknesses become apparent in animals that, at the time of the treatment, do not have a functional CL or significant follicular development. This can occur in both anestrous cows and heifers. In addition, it has been reported that only 60-80% of the animals with a large follicle present at the time of the first GnRH injection ovulate in response to that injection (Pursley et al., 1995). Vasconcelos et al. (1999) reported that ovulation rates exceeded 70 % when GnRH was given between d 5-9 and 17-21 of the estrous cycle, but were less than 50 % when the treatment was given at d 1-4 or 10-16 of the cycle.

Coincidently, Moreira et al. (2000) observed that follicles on d 2 and 10 of the estrous cycle are less likely to ovulate after injections of GnRH than follicles on d 5, 15 and 18 of the estrous cycle. Failure of follicles to respond to GnRH has been linked to the presence of high serum concentrations of progesterone that reduce LH pulse frequency and/or decrease the number of LH receptors in follicles (Macmillan and Thatcher, 1991).

Failure of CL to regress on d 7 after PGF has also been discussed as a possible contributor to synchronization failure, and is associated with the delay or inhibition of ovulation at the second GnRH injection 2 d after PGF in the Ovsynch protocol. Twagiramungu et al. (1995) reported that when luteolysis was complete, estrus and ovulation occurred, but when it was incomplete, ovulation did not occur and the dominant follicle became persistent. Lemaster et al. (2001) has reported similar findings and suggested that some differences in responsiveness to PGF may occur between *Bos indicus* and *Bos taurus* cattle. Williams et al. (1999) reported that in *Bos indicus*-influenced heifers, CL regression differed among d 6, 10, and 14 of the estrous cycle with 54.2, 63.3 and 91.7 % regression observed on each day, respectively. In contrast, Thompson et al. (1999) reported that luteal regression in *Bos taurus* beef females on d 7 occurred in 100% of the animals treated in an Ovsynch protocol and 91.7% of the animals treated in a GnRH + norgestomet protocol.

Martinez et al. (2002b), using lactating *Bos taurus* beef cows, compared pregnancy rates when PGF was given on either d 6 or 7. It was not observed any difference in pregnancy rates (52.4 and 52.0% respectively), suggesting that luteal regression was similar for the two groups injected on different days. In addition, Hiers

et al. (2003), using *Bos indicus*-influenced cows, evaluated the effect of using two different luteolytic compounds, dinoprost tromethamine and cloprostenol sodium, as well as the effect of two injections of dinoprost tromethamine administered at 12-h intervals, on TAI conception rates. Timed AI conception rates did not differ among treatments (36, 41, and 39% respectively); also suggesting that on d 7 CL regression occurs consistently. Therefore, the preponderance of data suggest that in *Bos taurus* cattle, PGF consistently regresses CL on d 7 of GnRH/PGF protocols, but this may not be true in *Bos indicus*-influenced breeds and could contribute to synchronization failure and reduced fertility. More work in this area is warranted.

As noted earlier, synchronization protocols involving the use of GnRH, while capable of producing reasonable pregnancy rates in cyclic cows, have not been as successful in heifers and in anestrous cows. In heifers, there is a relatively high incidence of estrus (6 to 28%) before d 7 (d of PGF injection) and these animals are unlikely to conceive to a fixed time AI (Roy and Twagiramungu, 1999; Martinez et al., 2002b; Williams et al., 2002). In beef cows, the incidence of premature estrus during the Ovsynch and similar protocols has also been reported to be approximately 8-10% (Geary et al., 2000; DeJarnette et al., 2001).

Addition of Progestins to GnRH plus PGF Protocols

Recent studies suggest that the use of progestins in GnRH/PGF protocols can increase conception rates above those obtained with GnRH/PGF alone. Objectives are to increase conception rates in those cattle in which TAI conception rates are not acceptable when only GnRH/PGF are utilized. Three different types of progestogens

have been used including norgestomet ear implants (Stevenson et al., 1997; Thompson et al., 1999), MGA (Martinez et al., 1998; Funston et al., 2002), and CIDR inserts (Lamb et al., 2001; Martinez et al., 2002b). In general, progestogens are included during the first 7 d of treatment, between the first injection of GnRH and injection of PGF (Stevenson et al., 1997) to induce cyclicity in anestrous females and to impede premature estrus. In several studies, pregnancy rates were increased by at least 10% compared to the GnRH/PGF treatment alone. Pregnancy rates in *Bos taurus* beef cattle have been reported as high as 60% (Thompson et al., 1999; Lamb et al., 2001), and some studies have reported a marked increase in pregnancy rates in heifers of up to 65% (Stevenson et al., 1997; Martinez et al., 2002b; Martinez et al., 2001). Pregnancy rates in Japanese Black beef cows were even greater, with values as high as 72% reported (Kawate et al., 2004). These results indicate that the inclusion of a progestogen in GnRH/PGF protocols could optimize TAI conception rates in all types of cattle, including *Bos indicus*-influenced cattle.

Subtropical Regions in the U.S. and *Bos indicus* Influence

Southern regions of the U.S. have environmental characteristics, such as high humidity and temperature and extreme degrees of solar radiation, in which the incorporation of *Bos indicus* breeding into beef cattle herds is necessary in order to optimize production efficiencies (Turner, 1980). Williams et al., (1987) reported the use of Syncro-Mate-B synchronization in Brahman-influenced cattle in south Texas. In that study, TAI pregnancy rates varied between 33.8 and 44.1 %. Ovsynch has also been applied in *Bos indicus*-influenced cattle, with pregnancy rates ranging from 31 to 42% (Lemaster et al., 2001; Williams et al., 2002). There have been few reports describing the efficiency of GnRH/PGF/progestogen treatments in *Bos indicus*-influenced cattle. Hiers et al. (2003) reported pregnancy rates in *Bos indicus*-influenced cattle between 36% and 41% when MGA was combined with the Ovsynch protocol. Although previous studies did not compare directly *Bos taurus* and *Bos indicus* cattle, it appears that pregnancy outcomes resulting from TAI after synchronization of ovulation are lower in *Bos indicus*-influenced cattle. Hence, the approach of combining GnRH, PGF, and progesterone might offer a promising alternative in regions where *Bos indicus*-influenced cattle predominate.

CHAPTER III

OVARIAN AND HORMONAL EVENTS DURING SYNCHRONIZATION OF

OVULATION AND TIMED APPOINTMENT BREEDING OF *Bos indicus*-

INFLUENCED CATTLE USING INTRAVAGINAL PROGESTERONE, GnRH

AND PROSTAGLANDIN F$_{2\alpha}$

Introduction

Several pharmacological protocols, developed primarily using *Bos taurus*
(English and European) females, are currently available for synchronization of estrus
and ovulation in beef cattle (Stevenson et al., 2003a; Patterson et al., 2003). Recently
the CO-Synch protocol (Geary and Whittier, 1998), which involves the combined use of
GnRH and PGF, has been coupled with an exogenous source of progesterone (CIDR).
This combination (COS-C) appears capable of producing TAI conception rates
averaging greater than 50% (Lamb et al., 2001, Larson et al., 2004 a, b) in *Bos taurus*
females. These TAI conception rates are consistently greater than those reported
previously using other traditional methods (Stevenson et al., 2003a). Improved
outcomes have been linked in part to the ability of exogenous progesterone to induce
ovulation in a high proportion of anestrous cows (Stevenson et al., 2000) and to reduce
the occurrence of estrus before TAI (DeJarnette et al., 2001; Martinez et al., 2002b).
However, in environments that are predominantly subtropical to tropical, the need to
utilize *Bos indicus*-influenced females may reduce the efficiency of synchronization and

TAI conception rates compared to *Bos taurus* females (Lemaster et al., 2001; Hiers et al., 2003). Although not well-characterized, this may occur due to increased excitability and stress in *Bos indicus*-influenced cattle when subjected to intense management and (or) differences in timing of ovarian events. Reports specifically evaluating the COS-C for TAI in *Bos indicus*-influenced cattle are limited.

Objectives of studies reported herein were to 1) evaluate the use of the COS-C protocol for synchronization of ovulation and TAI in *Bos indicus*-influenced cattle, 2) compare cumulative pregnancy rates after COS-C synchronization and TAI to those in a TM scheme and 3) evaluate specific ovarian, hormonal, and estrual events associated with the use of COS-C and related protocols to identify aspects of the system that may contribute to reductions or improvements in efficiency of the protocol in *Bos indicus*-influenced cattle.

Materials and Methods

The Institutional Agricultural Animal Care and Use Committee of the Texas A&M University approved in advance all procedures used in these studies.

Experiment 1. Field Trials

Specific objectives of this experiment were to determine reproductive outcomes, represented as conception rates, after TAI for synchronized females and to compare cumulative pregnancy rates obtained at early (30 d), and late (60 d) stage of the breeding season between synchronized and untreated controls (TM).

All cattle (n = 431) in this experiment were preferred to have a minimum BCS of 5 (1-9 scale, 1= emaciated, and 9= obese; Herd and Sprott, 1998) and if suckled, be at least 50 d postpartum. Predominantly Brahman x Hereford (F_1) and Brangus females (n = 335) were stratified by BCS, parity and d postpartum (primiparous and pluriparous only) at each location and assigned randomly in groups of not less than 25 to COS-C or TM groups. An additional 96 similar females in which TM controls were not available for comparison also received COS-C and TAI at 48 h. The COS-C regimen included the insertion of a controlled internal drug release device (CIDR; Pfizer Animal Health, New York, NY) which is a T-shaped silicone device containing 1.38g of progesterone, and an injection of GnRH (GnRH-1; 100 µg i.m. Cystorelin, Athens, GA) on d 0, removal of the CIDR and injection of PGF (25 mg i. m. Lutalyse; Pfizer Animal Health, New York, NY) on d 7, and injection of GnRH (GnRH-2; 100 µg i.m. Cystorelin) and TAI on d 9 (Fig 5b). Timing of AI after CIDR removal/PGF injection in this study was based on previous reports indicating that 48 h after CIDR removal was the most appropriate time for TAI (Geary and Whittier, 1998; Lamb et al., 2001; Stevenson et al., 2003a). Synchronized cows were placed with fertile bulls for 90 d beginning 5 to 7 d after TAI whereas TM cows were placed with bulls on d 0 (TAI on synchronized females). Bulls used for natural service had successfully completed a standard breeding soundness examination approximately 2 wk before the onset of breeding. Conception rates to TAI were determined by transrectal ultrasonography (Dynamic Imaging, Concept/MCV, equipped with a 7.5 MHz linear probe; Livingston, UK) 30 d after TAI in the COS-C group. Presence of both uterine fluid accumulation and embryonic vesicle determined

females diagnosed as pregnant, also embryonic heart beat was used to confirm the viability of the embryo. Final pregnancy rates were assessed by palpation per rectum 45 d after the end of the breeding season in both synchronized and TM groups to retrospectively estimate cumulative pregnancy rate at both 30 and 60 d of the breeding season.

Experiment 2. Follicular, Luteal and Hormonal Characteristics of COS and COS-C Synchronization Protocols

Specific objectives of this experiment were to a) perform a full characterization of ovarian events occurring within each treatment; b) describe the pattern of progesterone secretion and pituitary LH release in response to GnRH; c) identify endocrine, follicular, and luteal profiles associated with TAI outcomes.

Pluriparous, postpartum Brahman x Hereford (F_1) cows (n = 100) were divided into four replicate groups of 25 females each. Criteria for inclusion in the study and stratification procedures were similar to Experiment 1. Cattle were placed in pens measuring 25.6 x 9.6 m 8 d before the onset of treatments, with five cow-calf pairs per pen, and fed according to National Research Council (NRC, 1996) recommendations for lactating beef cows. Half of the cows within each replicate (n=12-13) were allocated randomly to received the COS-C treatment (Fig. 5b), as in Exp. 1, and half of the cows received the COS treatment (Fig. 5a). Cows were placed with bulls after TAI as described for Exp. 1.

Transrectal ultrasonography was performed every other day from d -8 to d 0, and then daily from d 0 until ovulation or d 12, whichever occurred first. All ultrasound

examinations were performed by the same operator. Follicles greater than 6 mm as well as luteal structures were measured and a picture of the dorsal and lateral view of each ovary was then obtained for further analysis. The dominant follicle was defined as the follicle that reached the largest diameter (Sirois and Fortune, 1980). Ovulation was defined as the sudden disappearance of a follicle within two consecutive ultrasound examinations and confirmation by the subsequent CL appearance. Follicular regression was defined as the gradual reduction of follicular size until disappearance (Ginther et al., 1989b). Emergence of a follicular wave was determined, retrospectively, as the day the dominant follicle reached 4 to 5 mm; if the follicle was not detected until it was 6 to 7 mm, then the day before was considered as the day of emergence (Ginther et al., 1989a). A synchronized follicular wave was considered to have occurred if it emerged between d 1 to 4 after GnRH-1. Follicular wave emergence occurring outside of this period was considered to be spontaneous.   Luteal regression was defined as the progressive reduction in size of the CL until disappearance (Ginther, 1998).

Blood sampling intervals followed the same time course as for transrectal ultrasonography. Samples were placed on ice immediately after collection until arrival to the laboratory. After arrival, samples were removed from the ice and allowed to stand at room temperature for approximately 1 h before centrifugation.   Samples were centrifuged at 1854 x g for 30 min within the first 4 h of collection (Lesniewski et al., 1985). Serum was collected and stored at -20°C until hormone analyses. Concentrations of progesterone in serum were determined with a solid phase RIA using the Coat-A-Count assay kit (Diagnostic Products Corporation, Los Angeles, CA) as reported

previously from this laboratory (Fajerson et al., 1999). Intra- and interassay coefficient of variation was 10.55 and 10.84% respectively (n = 7 assays). Concentrations of LH were also determined as reported previously by McVey and Williams (1991), in blood samples collected during the first replicate at 0, 30, 60 and 120 min relative to GnRH injections on d 0 (GnRH-1) and d 9 (GnRH-2). Intra- and interassay coefficient of variation was 4.28 and 6.51% respectively (n = 2 assays). All hormone determinations for a particular animal were performed within the same assay.

Cows were observed for estrus 3x daily from d 0 until ovulation or d 12, whichever occurred first, with the aid of androgenized cows. On d 12, all cows were returned to their pasture with clean-up bulls for a 90-d breeding period. Pregnancy determination was performed by transrectal ultrasonography at 30-32 d post-AI, and re-confirmed by palpation per rectum 45 d after bulls were removed.

Experiment 3. Distribution of Estrus and Ovulation in Cows Programmed with the Select Synch + CIDR Synchronization Protocol

Specific objectives of this experiment were to a) perform a full characterization of estrus and ovulation events occurring after synchronization with Select Synch + CIDR protocol; b) compare ovarian and follicular events obtained with those obtained in Exp. 2 to determine possible differences between naturally-occurring and induced ovulation; c) based on results of this and previous experiment, propose modifications that can contribute to better outcomes in *Bos indicus*-influenced cattle.

Fifty postpartum, suckled Brahman x Hereford (F-1) females were used. Criteria for inclusion were the same as for Experiments 1 and 2. Cows in the study were

primiparous heifers (n = 32), and pluriparous cows (n = 18). Females were placed in pens as in Experiment 2, with 8 cow-calf pairs per pen, and fed according to NRC recommendations for lactating beef cows. All cows received the Select Synch + CIDR synchronization regimen (Fig. 5c). This regimen follows the same procedure as COS-C, as described in Experiment 1 but the second GnRH injection (GnRH-2) is not administered.

Transrectal ultrasonography to assess ovarian morphology was conducted at the time of CIDR removal and every 12 h until ovulation or d 12, whichever occurred first. The technique of ultrasonography was the same as in experiment 2, but the main focus was on the development of the preovulatory follicle and CL regression. Observations for estrus were performed by visual observation every 3 h from CIDR removal on d 7 through d 12 based upon homosexual behavior among herdmates and followed by AI 12 h after detected estrus. Three different categories for estrus detection were distinguished. Standing estrus corresponded to cows that stood for at least 4 s while a herdmate mounted her and this action was repeated at least three times in a 6 h period. Non-standing estrus behavior corresponded to females that had all other signs of estrus but did not stand when a herdmate attempted to do so. No estrous behavior corresponded to females that did not show any sign of estrus at all.

Blood samples were collected on d -21, -11, 0 (CIDR insertion), 7, 8 and 9 following the same procedures described in experiment 2. Serum was assayed for progesterone in all samples by RIA as described in experiment 2 to retrospectively estimate cyclicity and luteal regression. Cattle were considered to be cyclic at the onset

of the study if they exhibited serum concentrations of progesterone $\geq$ 1ng/ml for two consecutive samples and a visible CL at ultrasound within the 10 d previous to d 0.

Statistical Analyses

    Experiment 1. Categorical variables (TAI conception rates and cumulative pregnancy rates after 30 and 60 d of the breeding season) were analyzed by Chi square analysis using the Proc Freq of the Statistical Analysis System (SAS Inst. Inc., Cary, NC, 1985). Analysis of variance for categorical data using the CatMod procedure of SAS was used to determine the effects of parity, BCS, and d postpartum, year, location, and respective interactions on TAI conception rates in a model that included year, location, treatment, and their respective interactions. For these and following procedures a 95% confidence level or greater was chosen to determine significative differences

    Experiment 2. Analysis of variance (Proc GLM of SAS) was used to determine effects of parity, BCS and d postpartum; however, in this case the model included replicate, treatment, cyclic status and their interaction. When a significant F-value was identified, the LSD test was used to contrast means.

    Ovulatory response to GnRH-1, new follicular wave emergence, CL regression, ovulatory response to GnRH-2, and TAI conception rates were evaluated by Chi square analysis (Proc Freq of SAS). Day of follicular wave emergence, mean follicular size at d 7, 9, and 10, as well as follicular growth rate was evaluated using Proc GLM to examine effects of treatment, cyclic status and their interaction. Treatment effects on mean concentrations of progesterone and LH were also analyzed by repeated measures

analysis of variance (Proc GLM) with treatment, cow within treatment, cyclic status and day included in the model.

Experiment 3. Differences in parity, BCS and d postpartum were evaluated by analysis of variance (Proc GLM of SAS) in a model including cyclic status, and estrus and ovulation presentation. Mean follicular size was evaluated on day 7, 9 and before ovulation by analysis of variance (Proc GLM) in a model that included cyclic status, occurrence of estrus presentation and their interaction. Mean intervals from CIDR removal to standing estrus and ovulation, and from standing estrus to ovulation, were evaluated by the GLM procedure for cyclic and non-cyclic cows.

Results

Experiment 1

Mean age, BCS, BW, and d postpartum averaged (± SEM) 4.7 ± 0.2 yr, 5.1 ± 0.03 (range 3-8), 468 ± 7.1 Kg, and 70 ± 1.1 d, respectively. Timed AI conception rates in all females synchronized with COS-C are summarized in Table 1. Conception rates to TAI averaged about 39 ± 3% overall and did not vary by location (n = 4, P = 0.47), year (n = 2, P = 0.53), BCS (P = 0.94), d postpartum (P = 0.81), parity (P = 0.88), sire (n = 6, P = 0. 95) or AI technician (n = 3, P = 0.74). Table 2 summarizes cumulative pregnancy rates at 30 and 60 d of breeding season for COS-C (TAI and/or natural service) and TM (natural service). Cumulative pregnancy rates were greater (P < 0.05) in COS-C at both 30 and 60 d of the breeding season compared to the TM group.

Table 1. Timed AI (TAI) pregnancy rates in nulliparous heifers, primiparous heifers, and pluriparous cows synchronized with CO-Synch + CIDR (COS-C)

Source	Treatment	N	TAI Pregnancy Rate, %
Nulliparous	COS-C	89	39.3
Primiparous	COS-C	34	35.3
Pluriparous	COS-C	143	39.9
Total	COS-C	266	39.1

Table 2. Cumulative pregnancy rates after 30 and 60 d of breeding in nulliparous heifers, primiparous heifers, and pluriparous cows synchronized with CO-Synch + CIDR (COS-C) followed by timed AI (TAI) or managed using traditional methods (TM)

Source	Treatment	N	Cumulative Pregnancy Rate[a], %	
			30 Days	60 Days
Nulliparous	COS-C	62	75.8	95.2
	TM	71	71.8	88.7
Primiparous	COS-C	34	67.6	100.0
	TM	28	60.7	89.3
Pluriparous	COS-C	74	75.7[b]	94.6
	TM	66	51.5[c]	90.9
Total	COS-C	170	74.1[b]	95.9[b]
	TM	165	61.8[c]	89.7[c]

[a] Cumulative pregnancy rate for COS-C females included TAI and/or natural service, for TM included natural service only. Cumulative pregnancy rate was retrospectively estimated for both groups using days of gestation obtained 45 days after the end of the breeding season by rectal palpation

[b,c] Percentages in columns with uncommon superscripts differ $P < 0.05$.

Experiment 2

Ovarian and reproductive variables are summarized in Table 3. Mean age, BCS, BW, and d postpartum averaged ($\pm$ SEM) 8.8 $\pm$ 0.3 yr, 5.3 $\pm$ 0.07 (range 4-8), 543 $\pm$ 7.4 kg, and 77$\pm$0.66 d, respectively. No differences in the major ovarian and reproductive

endpoints were observed between COS-C and COS. Therefore, data for both treatments are presented as pooled means (Table 3). Data are also presented relative to cyclic status at the onset of treatments. The number of non-cyclic cows ovulating after GnRH-1 was greater ($P < 0.01$) than for cyclic cows. The number ovulating in response to GnRH-2 also differed between cyclic and non-cyclic cows; however, in this case, cyclic cows had the greater ($P < 0.05$) response. Mean follicular diameters are presented in Table 4. Non-cyclic cows had greater ($P < 0.05$) mean follicular size at PGF than cyclic cows, and therefore a greater ($P < 0.05$) follicular growth rate. Follicular sizes were not different at the subsequent stages.

Data were also summarized relative to presence or absence of ovulation after GnRH-1 to evaluate their effects on subsequent ovarian responses (Table 5). More ($P < 0.01$) cows that ovulated after GnRH-1 developed a synchronized follicular wave compared to cows that did not ovulate. Moreover, there was a trend ($P = 0.15$) for ovulation rates after GnRH-2 to be greater in cows that ovulated in response to GnRH-1 than cows that did not. Also, ovulation and TAI pregnancy rates after GnRH-2 were increased ($P < 0.01$) in cows that developed a synchronized follicular wave after GnRH-1 compared to cows that did not develop a new wave (Table 6).

Table 3. Ovarian and reproductive outcomes in postpartum suckled cows synchronized with CO-Synch + CIDR (COS-C) or CO-Synch (COS), and for cyclic and non-cyclic cows in Exp. 2

Variable	COS-C and COS Combined	Ovarian Status	
		Cyclic	Non-cyclic
No. Cows	100	78	22
Estrous cyclic, %	78	-	-
Response to GnRH-1, %			
Ovulating	40	33[d]	64[e]
Follicle regression	39	40	36
Not responding	21	27[d]	0[e]
New follicular wave after GnRH-1, %			
Synchronized[a]	60	56	73
Not synchronized[b]	31	35	18
No emergence	9	9	9
Day of emergence	2.5 ± 0.12	2.4 ± 0.15	2.75 ± 0.23
CL regression, % (No.)	92 (75/81)	91(61/67)	100(14/14)[c]
Ovulatory Response to GnRH-2, %			
0-24 h after TAI	15	14.1	18.2
24-48 h after TAI	57	62.8	36.3
Total	72	76.9[d]	54.5[e]
TAI pregnancy, %			
Ovulation 0-24 h after AI	9	10.3	4.5
Ovulation 24-48 h after AI	24	23	27.3
Total	33	33.3	31.8

[a] Cows that developed a follicular wave from d 1 to d 4 after GnRH-1.

[b] Cows that developed a follicular wave before d 1 and after d 4.

[c] Non-cyclic cows with observed luteal regression correspond to females that developed a CL after GnRH-1

[d,e] Percentages within row with uncommon superscripts letters differ (P < 0.01).

Table 4. Mean follicular diameters in postpartum suckled cows synchronized with CO-Synch + CIDR (COS-C) or CO-Synch (COS), measured at different stages of the experiment (Exp. 2)

Variable	COS-C and COS Combined	Ovarian Status	
		Cyclic	Anestrous
Diameter of the largest Follicle, mm (range)			
GnRH-1	9.6 ± 0.2	9.4 ± 0.2	10.2 ± 0.3
	(4.0 - 12.95)	(4.0 - 12.95)	(6.8 - 12.3)
PGF	9.8 ± 0.2	9.6 ± 0.2^a	10.5 ± 0.2^b
	(6.3 – 15.4)	(6.3 – 13.9)	(7.0 - 15.4)
GnRH-2	11.1 ± 0.2	11 ± 0.3	11.4 ± 0.5
	(6.0 – 15.4)	(6.0 – 15.4)	(7.5 – 14.5)
Before ovulation	11.6 ± 0.2	11.4 ± 0.2	12.2 ± 0.5
	(8.1-15.4)	(8.1 – 15.4)	(9.1 – 14.7)
Follicular growth rate, mm/day	1.4 ± 0.06	1.3 ±0.07^a	1.7 ± 0.1^b

^{a,b} Percentages within row with uncommon superscripts letters differ (P < 0.05).

Table 5. Effects of the response to the first GnRH injection (GnRH-1) on subsequent ovarian and reproductive outcomes in cows synchronized with CO-Synch + CIDR (COS-C) or CO-Synch (COS) in Exp. 2

Variable	Ovulatory Response to GnRH-1	
	Ovulating No. (%)	Not Ovulating No. (%)
No of cows	40	60
Synchronized follicular wave		
Yes	35 (88)^a	25 (42)^b
No	5 (12)	35 (58)
Ovulated after GnRH-2		
Yes	32 (80)	40 (67)
No	8 (20)	20 (33)
TAI pregnancy	15 (37)	18 (30)

^{a,b} Percentages within rows with uncommon superscripts differ (P < 0.01).

Table 6. Effects of synchronized follicular wave emergence after GnRH-1 on subsequent ovarian and reproductive outcomes in cows synchronized with CO-Synch + CIDR (COS-C) or CO-Synch (COS) in Exp. 2

| | Occurrence of Synchronized Follicular Wave after GnRH-1 | |
| | Yes | No |
Variable	No. (%)	No. (%)
No of cows	60	40
Ovulation after GnRH-2		
Yes	51 (85)[a]	21 (52)[b]
No	9 (15)	19 (48)
TAI pregnancy	26 (43)[a]	7 (17)[b]

[a,b] Percentage within row with uncommon superscripts letters differ ($P < 0.01$).

Mean serum concentrations of progesterone are illustrated in Figure 6. As expected, concentrations of progesterone from d -8 to 0 relative to GnRH-1 differed between cyclic and non cyclic cows. After CIDR insertion (d 0), serum progesterone increased ($P < 0.001$) acutely for both cyclic and non-cyclic cows that received the COS-C treatment. Serum concentrations of progesterone on day 1 were highest ($P < 0.05$) for cyclic cows receiving COS-C compared to all other groups. Mean concentrations of progesterone did not differ between cyclic cows treated with COS and non-cyclic cows treated with COS-C.

Mean concentrations of progesterone were lowest ($P < 0.01$) for the non-cyclic COS-treated group compared to all others, and mean serum concentrations of progesterone never exceeded 1 ng/ml during the treatment period. After injection of PGF and CIDR removal (d 7), progesterone decreased below 1 ng/ml within 24 h in all

groups and remained low until d 12 when mean progesterone exhibited a slight increase (P = 0.09) in cyclic, COS-treated cows. The latter was caused by two cows that ovulated asynchronously before d 9.
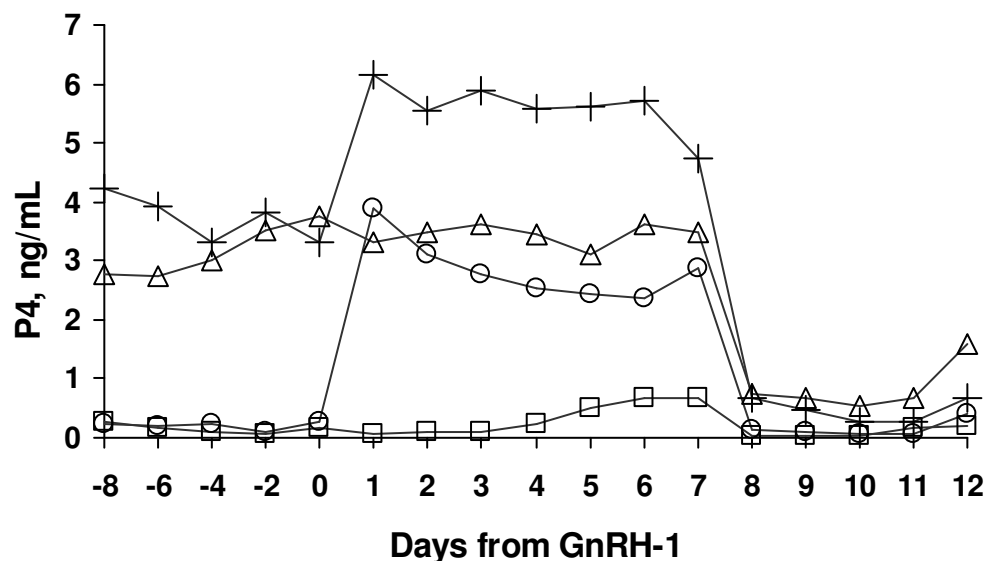
Figure 6. Concentrations of progesterone (P4) in serum of cyclic (+; n = 39) and non-cyclic (○; n = 11) cows treated with COS-C, and cyclic (Δ; n = 39) and non-cyclic (□; n = 11) cows treated with COS only (Exp. 2).

Release of LH induced by GnRH was considered to have occurred when an increment in the concentration of LH of at least 2 SD above the baseline was observed. Two cows had an endogenous LH surge before GnRH-2 and were excluded from further analysis in relation to this variable. The latter conclusion was based on the fact that concentrations of LH during the sampling period were in a declining mode. All other cows (n = 23) in replicate 1 exhibited increases (P < 0.01; Figure 7) in LH after both GnRH-1 and 2. Magnitude of release did not differ between treatments (COS-C vs

COS). Non-cyclic cows had an induced LH release greater ($P < 0.05$; Fig 3) than cyclic cows after GnRH-1, but concentrations of LH did not differ between cyclic and non-cyclic cows after GnRH-2. A time x cyclic status interaction ($P < 0.05$) associated with GnRH-induced LH release was observed after GnRH-2. Also, overall mean concentrations of LH were greater ($P < 0.01$) after GnRH-2 than after GnRH-1 (7.2 ± 0.71 and 4.3 ± 1.1, respectively).
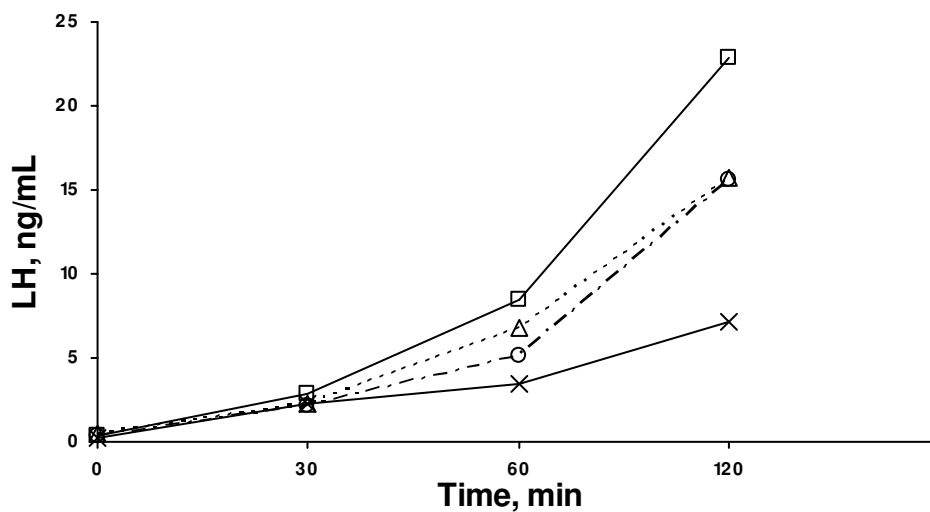
Figure 7. Mean serum concentrations of LH after GnRH-1 in cows that were cyclic (×; n = 15) and non-cyclic (○; n = 10) before treatment onset, and in cyclic (Δ; n = 14) and non-cyclic (□; n = 9) cows after GnRH-2. Cows not cyclic before treatment onset had greater ($P < 0.05$) induced release of LH after GnRH-1 than cyclic cows, but not after GnRH-2 (cyclic status x time, $P < 0.05$) in Exp. 2.

Experiment 3

Neither ovarian cyclic status (cyclic 60%, non-cyclic 40%) nor parity affected the number of cows exhibiting estrus or ovulating. Mean age (± SEM), BCS, BW, and d postpartum were 5.81 ± 0.5y, 5.6 ± 0.1 (range 4-8), 565 ± 10.2 Kg and 60 ± 1.1 d, respectively. On d 7, cows (72%) had a visible CL at ultrasound, and of those (97%) exhibited CL regression after PGF, as evidenced by a reduction in ultrasonographic size and morphology of the CL and a reduction in serum concentrations of progesterone to less than 1 ng/mL. No cows were observed in estrus during the first 48 h after CIDR removal. The majority (75 %) of estrual events was observed between 60 and 82 h after CIDR removal (Figure 8). Mean size of the largest follicle at CIDR removal and 48 h after removal were 9.45 ± 0.26 and 11.65 ± 0.26 mm, respectively (Table 7). Follicular diameter was greater for cows showing standing estrus than for cows showing only non-standing estrous behavior or no estrous behavior at both CIDR removal ($P < 0.05$) and 48 h after removal ($P < 0.01$). Cows that showed standing estrus had more ($P < 0.01$) ovulations than cows not standing. Timing from standing estrus to ovulation is shown in table 7.

Table 7. Estrual, follicular, and ovulatory characteristics of postpartum, suckled cows programmed with Select Synch + CIDR

Variable	All Cows	Estrus		
		Standing	Non-Standing	None
No.	50	27	14	9
Mean follicle size, mm				
At CIDR removal		10.1 ± 0.4^a	8.8 ± 0.6^b	8.62 ± 0.3^b
(range)		(6.1 - 13.5)	(6.0 - 12.8)	(7.2 - 10.2)
48 h after CIDR		12.6 ± 0.4^c	10.4 ± 0.4^d	10.83 ± 0.4^d
removal (range)		(9.6 - 14.7)	(8.3 - 13.8)	(9.7 - 13.9)
Ovulating, %	56	93^c	21^d	0^d
Mean ovulatory follicle size, mm (range)	12.9 ± 0.3 (9.4 - 15.1)	12.9 ± 0.3 (9.4 - 15.1)	13.5 ± 0.5 (12.5 -14.1)	-
Mean interval from CIDR removal to:				
Standing estrus, h (range)		70 ± 2.9 (49 - 108)	-	-
Ovulation, h (range)	99 ± 2.8 (68 - 127)	99 ± 3 (68-127)	104 ± 11 (82 - 117)	-
Mean interval from estrus to ovulation, h (range)		29 ± 2.2 (5 - 55)	-	-

[a,b] Percentage within row with uncommon superscripts letters differ (P < 0.05).
[c,d] Percentage within row with uncommon superscripts letters differ (P < 0.01).
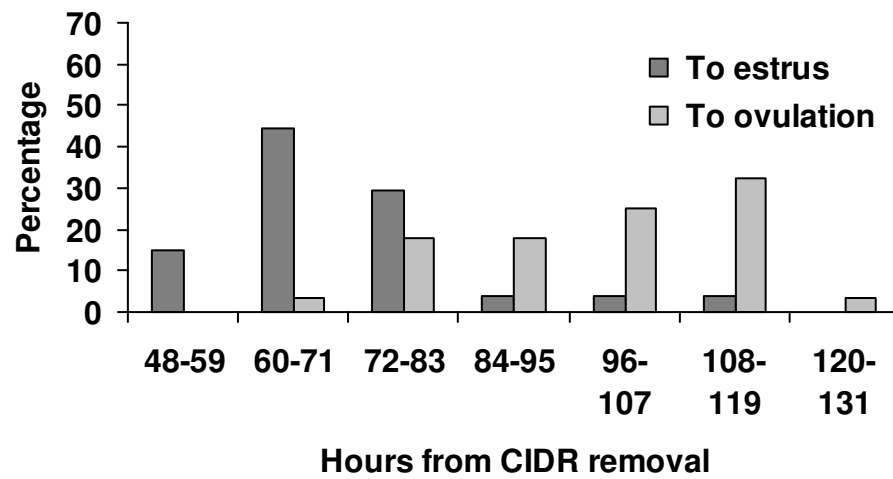
Figure 8. Interval from CIDR removal to visual estrus (n = 27) and ovulation (n=28) of early postpartum suckled cows treated with Select Synch + CIDR.

Discussion

Two keys essential for increasing the use of AI in commercial beef operations are the development of a synchronization system that is easily applicable and the ability to consistently achieve TAI conception rates over 55% (NAHMS, 1997). The COS-C synchronization regimen appears to have the potential to achieve these two goals, particularly in relation to its use in *Bos taurus* females (Larson et al., 2004 a, b; Lamb et al., 2001). Therefore, we were encouraged to test whether the COS-C regimen could be economically employed in south Texas commercial beef operations in which *Bos indicus*-influenced cattle are commonly used due to their sub-tropical and tropical adaptation. In a recent study conducted in our laboratory, we obtained and overall TAI conception rate of 42.2% using the Ovsynch synchronization procedure (Williams et al., 2002), which is less complex than COS-C regimen. Thus, initial goals were to compare management systems involving COS-C with traditional management under the assumption that TAI conception rates over 50% could be achieved consistently following synchronization with COS-C. Overall TAI conception rates in our studies (39%) were substantially lower than the 50% level observed in *Bos taurus* females (Larson et al., 2004 a, b; Lamb et al., 2001). For that reason, it is not likely that the COS-C regimen, as used in Exp. 1, could be economically employed for incorporation into commercial beef operations in south Texas. Efforts to examine economic feasibilities of COS-C as employed in Exp. 1 were abandoned in favor of additional studies designed to determine the cause of suboptimal outcomes associated with the regimen in *Bos indicus*-influenced cattle in this region. Attempts to account for the

lower pregnancy rates in *Bos indicus*-influenced vs straight *Bos taurus* cattle would be to question differences in overall fertility of these two breed types. However the fertility of Braford and Brangus cattle in Exp. 1 was high ($\geq$ 90 % pregnancy rates in 60 d) and therefore cannot be used to account for the poor TAI conception rates obtained.

The proportion of cows that are anovulatory at the time of synchronization is an important contributor to reduced rates of success in synchronization regimens and TAI (Stevenson et al., 2003a). Anovulatory conditions are greatly influenced by BCS (Short et al., 1990; Yavas and Walton, 2000) and d postpartum (Williams, 1990). Therefore a general approach for most investigators to minimize the effect of anestrus has been to require a BCS of at least 5, with cows a minimum of 50 d postpartum at the time of TAI (Lamb et al., 2001; Williams et al., 2002). In the current studies, these conditions were fulfilled; moreover TAI conception rates were not influenced by BCS or d postpartum in Exp. 1 or 2. Even though anestrous females can negatively influence outcomes of a synchronization program, an increase in TAI conception rates in anestrous females has been observed with the addition of an exogenous source of progesterone (Thompson et al., 1999; Stevenson et al., 2003b). Lamb et al. (2001) increased TAI conception rates in postpartum anestrous females by 25% with the addition of progesterone to the COS synchronization regimen. Low-level increases in progesterone occur during the natural resumption of ovulatory cycles postpartum (Arije et al., 1974; Williams et al., 1983) and the use of exogenous sources of progesterone can increase the frequency of LH pulses in postpartum cows to hasten first ovulation (Williams et al., 1983). Although some differences were observed in follicular dynamics between cyclic and non-cyclic cows in

Exp. 2, TAI conception rates did not differ between the two groups when treated with COS-C and, therefore, anestrous did not account for reduced efficiency.

Follicular events were examined in Exp. 2 and 3 as sources of variation that could contribute to reduced TAI conception rates. Hypotheses were that COS-C, as utilized in these experiments, failed in one or more areas to control ovarian physiological events necessary to optimize TAI conception rates. These could include failure to 1) optimize the frequency of ovulation or regression of follicles after GnRH-1 on d 0; 2) cause optimally-timed emergence of a new follicular wave between d 1 to 4; 3) efficiently regress the CL at the time of PGF; 4) produce an optimally-receptive preovulatory follicle at the time of the second GnRH injection.

Frequency of ovulation after GnRH-1 (40%) clearly accounted for a significant proportion of synchronization failure in Exp. 1 and 2. Timing of administration of GnRH-1 is random relative to different follicular stages. Pursley et al. (1995) reported that only 60 to 80% of females with a large follicle present at the time of the first GnRH injection ovulate in response to that injection. Vasconcelos et al. (1999) reported that ovulation rates exceeded 70 % when GnRH was given between d 5 to 9 and 17 to 21 of the estrous cycle, but were less than 50 % when the treatment was given on d 1-4 or 10-16 of the cycle. In beef heifers, Martinez et al. (1999) administered GnRH on d 3, 6, and 9 of the cycle and obtained ovulation rates of 67, 100, and 67% respectively. Overall results obtained in the current experiments were lower than those in the foregoing reports.

After exogenous administration of GnRH, circulating concentrations of LH and FSH increase within 30 min, reach peak concentrations at 120-150 min, then decrease to basal levels between 4 and 5 h after injection (Zolman et al., 1974; Ford and Stormshak, 1978). Results of Exp. 2 confirmed the expected pattern of LH release. However, a few (n = 4) individual females exhibited unexplained, relatively small increases in LH after GnRH-1 and -2 in which peak concentrations of LH did not exceed 2 ng/mL. Increased hypothalamo–pituitary–adrenal activity is involved in the suppression of gonadotropin secretion by stressors (Dobson and Smith, 1995). Treatment with ACTH delays or abolishes estradiol-induced LH surges in anestrous sheep (Dobson et al., 1988) and also suppresses and delays LH responses to exogenous GnRH in vivo and in vitro (Matteri et al., 1986; Phogat et al., 1997). Whether the failure to detect more robust increases in GnRH-induced release of LH in some cows was caused by a physiological alteration such stress or experimental error is uncertain; however, with the exception of one cow, the low response was observed in only one (GnRH-1 or 2) of the two sampling periods, but not in both. Also, ovulation was not observed in cows with low LH release after GnRH-1, whereas cows with a low response ovulated after GnRH-2. This indicates that an endogenous LH release probably occurred after the sampling period in the latter group.

Even though a complete replenishment of pituitary LH stores occurs between d 15 and 20 after parturition in cows (Lamming et al., 1981; Moss et al., 1985), we observed a greater release of LH after GnRH-1 in non-cyclic females than in cyclic females. In accordance with this observation, Williams et al. (1982) also observed that

suckled cows had a greater magnitude of LH release after GnRH injection than non-suckled cows. It has been postulated that pituitary gonadotropin stores in non-cyclic cows may continue to build as the postpartum period progress, with larger amounts of LH and FSH secreted when an exogenous stimulus is applied (Williams et al., 1982). The greater amount of LH released overall after GnRH-2 compared to GnRH-1 is likely attributable to the increased sensitivity of the anterior pituitary to GnRH caused by endogenous estradiol in the low progesterone environment at the time of GnRH-2 (Padmanabhan et al., 1978; Kesner et al., 1981). These observations allow us to assume that GnRH-induced LH release is not a source of variation that could potentially affect the final outcome of the system.

The stimulation of new follicle growth after GnRH has been attributed to either the acute release of FSH directly associated with the GnRH injection (Chenault et al., 1990; Roche et al., 1999) or by the subsequent endogenous increase in release of FSH caused by the disappearance of the dominant follicle (Twagiramungu et al., 1994). Bodensteiner et al. (1996) demonstrated in dairy cows that new follicular wave emergence after GnRH is initiated 21.3 ± 1.7 h after treatment, and occurs immediately after the FSH peak. In the current study, induction of a synchronized follicular wave between d 1 and 4 after GnRH-1 occurred in 60 % of the cows, with 31% developing a follicular wave outside of this range. Kim et al. (2005) demonstrated in lactating dairy cows that GnRH induced a new follicular wave in 95 % of cows within 7 d; however, follicular waves occurring after d 4 occur too late to represent true follicular wave synchrony. The proportion of cows that developed a synchronized follicular wave in the

present work was inadequate to ensure a high rate of success for a TAI protocol. The importance of developing a synchronized follicular wave after GnRH-1 is accentuated when one examines ovulation and TAI conception rates in the current experiments (Table 6).

Induction of a new follicular wave was closely related to ovulation after GnRH-1. A greater proportion of cows that ovulated after GnRH-1 were induced to develop a new follicular wave compared with cows that only exhibited follicular regression or that did not respond in any manner (Table 5).   This is in contrast to a report by Twagiramungu et al. (1994) in which both ovulation and follicular regression after GnRH-1 were equally effective for inducing new follicular wave emergence. However, in support of the current findings, Martinez et al. (2000) demonstrated that the synchrony of an induced follicular wave was less variable in heifers that ovulated after GnRH-1 than in heifers that did not ovulate.

As described earlier, a relationship between stage of the cycle and response to GnRH injection has been demonstrated; hence presynchronization schemes have been developed (Peters and Pursley 2002; DeJarnette et al., 2003) aiming to place a larger number of females into a predetermined stage of the cycle just before the initiation of the synchronization procedure, thus increasing synchronization rates. These presynchronization schemes have not been extensively tested in *Bos indicus*-influenced cattle, but should be evaluated in the future. Alternatively, E_2 and its esters have been effectively used to synchronize follicular wave emergence. Both E_2 and GnRH are equally efficient for inducing a synchronized follicular wave; however less variability

has been demonstrated on timing of emergence of the synchronized follicular wave in females treated with E_2 than in females treated with GnRH (Martinez et al., 1999). The mechanism by which E_2 synchronizes follicular wave emergence has been primarily attributed to its ability to suppress FSH (Kesner et al., 1982; Bolt et al., 1990) and consequently to induce follicular atresia (Bo et al., 1993; Burke et al., 2000). Several reports are available that demonstrate the value of this alternative in *Bos indicus* (Bo et al., 2003) and *Bos taurus* cattle (Martinez et al., 2005); nevertheless it is important to note that strict regulations are imposed on the use of E_2 within the U.S. and its extra-label use for synchronization of cattle is illegal (Johnson, 2005).

Incomplete luteolysis has been demonstrated to reduce the rates of efficiency in GnRH based regimens. Twagiramungu et al. (1995) reported that incomplete luteolysis alters estrus rates and induces the formation of persistent follicles. Lemaster et al. (2001) suggested that a possible cause of the low rate of estrus in the Select Synch synchronization protocol in *Bos indicus*-influenced cows was due to an inadequate regression of the CL; however, Hiers et al. (2003) found no differences in pregnancy rates when different PGF treatments were employed in *Bos indicus*-influenced females. Results of Exp. 2 and 3 of the current work indicate that the rate of luteal regression after PGF on d 7 was relatively high (93%). Concentrations of progesterone exhibit a marked decrease after PGF on d 7 (Twagiramungu et al., 1994; Thompson et al., 1999). Studies herein supported this observation, even in females with a recently induced CL in which only a small increase in progesterone was observed before PGF injection. Therefore,

failure of luteal regression did not account for a significant proportion of synchronization failure in the current studies.

Even though progesterone concentrations decreased after PGF injection below 1 ng/ml within 24 h in all groups and remained low until d 12, a few (4%) animals in the COS group exhibited premature estrus and ovulation which caused a small increase in overall mean concentrations of progesterone in this group after d 12. In heifers, there is a relatively high incidence of estrus (6 to 28 %) before d 7 (d of PGF injection) in GnRH/PGF protocols and these animals are unlikely to conceive to a fixed time AI (Roy and Twagiramungu, 1999; Martinez et al., 2002b; Williams et al., 2002). In beef cows, the incidence of premature estrus during the Ovsynch and similar protocols has been reported to be approximately 8-10% (Geary et al., 2000; DeJarnette et al., 2001). Premature estrus was observed only in the COS group in which a source of progesterone was not present. Progesterone eliminates the occurrence of premature estrus in GnRH/PGF protocols (Thompson et al., 1999; Stevenson et al., 2000).

Ovulation rate after GnRH-2 (72%) in the current studies also accounted for reduced efficiency. Vasconcelos et al. (1999, 2001) reported ovulation rates after GnRH-2 in lactating dairy cows of 87% and 91.3% respectively. Similarly, Pursley et al. (1995) obtained ovulation rates of 100% within 32 h after GnRH-2 in dairy cows, and in beef cows, Thompson et al. (1999) reported an ovulation rate of 84.6%. Even though the overall ovulation rate in our experiment was lower than expected, cows that developed a synchronized follicular wave after GnRH-1 exhibited ovulation rates after GnRH-2 (85%) similarly to previous reports. Therefore, the ability to increase ovulation

rates after GnRH-1 will increase ovulation rates after GnRH-2, and attainment of this objective should undoubtedly increase conception rates.

At the time of GnRH-2, mean diameter of the largest follicle was 11.1 ± 0.2 mm (Range 6.0 - 15.4). In *Bos taurus* cattle, mean follicular diameter has been reported as greater than 13 mm at the time of GnRH-2 (Thompson et al., 1999; Perry et al., 2005). Follicles acquire ovulatory capacity immediately after deviation (Sartori et al., 2001) which occurs around 8.5 mm (Ginther et al., 1996); however ovulatory capacity is also dependant upon the amount of LH released and follicular diameter. In Holstein cows treated with GnRH on d 0 to synchronize a follicular wave, 4 mg of an exogenous LH preparation on d 7 was insufficient to cause ovulation of 10-mm follicles, although many of these follicles had undergone physiological deviation. However, all growing follicles greater than 12 mm and 17% of all 11-mm follicles ovulated to the same LH treatment (Sartori et al.; 2001). In the same experiment, a greater dose of LH (24 or 40 mg) resulted in ovulation of 70–80% of 10 mm follicles, and those 10-mm follicles that failed to ovulate had generally not yet undergone physiological deviation at the time of treatment. Thus, follicles that had undergone deviation and had reached a diameter of 10 mm had acquired ovulatory capacity and ovulated in response to a high dose of LH, but not to a low dose. This suggests that some of the follicles present at the time of GnRH-2 injection in our studies had not yet reached ovulatory capacity, thus reducing the proportion of cows ovulating after GnRH-2. Additionally, although we obtained an 85% ovulation rate within 48 of h after GnRH-2 and TAI in cows that developed a synchronized follicular wave, only 45 % became pregnant (table 6). This suggests that a

significant proportion of the follicles that ovulated were immature and infertile. In support of this assumption, Perry et al. (2005) reported that in beef cows synchronized with CO-Synch, follicles ovulating at sizes of 12.1 mm or less in diameter are less likely to support a pregnancy to d 25 after insemination compared with cows that ovulate follicles of 14.7 mm. In our experiment, mean size of the ovulatory follicle was 11.6 ± 0.2 mm (range 8.1-15.4) which, based on the report by Perry, would substantially reduce the likelihood of a viable pregnancy.

In Exp. 3, we examined the distribution of estrus and ovulation in cows treated with the Select-Synch protocol (observe for estrus; no GnRH-2) to determine whether TAI and GnRH at 48 h in COS-C were too early to optimize fertile ovulations and pregnancy. Mean size of the ovulatory follicle in Exp. 3 was 12.9 ± 0.3 mm (range 9.4 - 15.1), which was higher than observed in Exp.2 at the time of GnRH-2 and TAI. The larger follicle size would increase the likelihood that oocytes from those follicles would yield a viable pregnancy (Perry et al., 2005). Lemaster et al. (1999) reported a mean size of the ovulatory follicle of 14.4 ± 0.9 (range 14-17) in *Bos indicus*-influenced females synchronized with a CIDR-PGF system, demonstrating that *Bos indicus*-influenced females achieve ovulatory sizes comparable to *Bos taurus* females. In support of the assumption that follicular maturity and timing of GnRH administration is key for the success of these protocols, Peters and Pursley (2003) evaluated the effect of administering the second GnRH injection at different times after PGF injection in the Ovsynch protocol in dairy cows. Follicle diameter was measured and correlated with TAI conception rates. A linear relationship was observed between the time of GnRH

injection, follicular size and TAI conception rate. The greatest TAI conception rate was obtained at 36 h after PGF with a mean follicle size of 14.6 ± 0.4. In a similar experiment, Vasconcelos et al. (2001), using dairy cows and the Ovsynch synchronization protocol, aspirated all follicles greater than 4 mm on d 4 after GnRH injection to initiate a delayed follicular wave. The aim was to reduce the size of the preovulatory follicle at the time of second GnRH injection so as to test whether a reduction in follicular size would reduce subsequent luteal size, progesterone levels and thus conception rates. It was concluded that ovulation of follicles smaller than 11.5 mm have reduced fertility, possibly because of development of smaller CL and decreased circulating concentrations of progesterone.

In Exp. 3, the interval from CIDR removal to standing estrus was 70 ± 2.9 h and the earliest ovulation detected was at 69 h after CIDR removal (99 ± 2.8 h), which is in accordance with results obtained by Lemaster et al. (1999, 2001) using *Bos indicus*-influenced cattle. In *Bos taurus* beef females, the mean interval from progesterone removal/PGF injection to estrus has been reported to be 55 ± 4 h (Geary et al., 1998a; Stevenson et al., 2000; Martinez et al., 2000; Lamb et al., 2004), indicating that *Bos taurus* females are more likely to have a fertile induced ovulation with GnRH-2 and TAI at 48 h compared to *Bos indicus*–influenced females. Initial reports in *Bos taurus* females suggested that TAI at 48 h using the CO-Synch regimen would optimize pregnancy rates (Pursley et al., 1995), and studies comparing the timing of insemination revealed no statistical differences in conception rates when TAI at 48 h was compared to later times (Stevenson et al., 2003a; Bremer et al., 2004). However more recent studies

indicate that increasing the time of GnRH-2 and TAI to 66 h improves conception rates in *Bos taurus* females compared to 48 h (Schafer et al, 2004; Walker et al., 2005). There are no data to determine whether the same assumption holds for *Bos indicus*-influenced females. As noted earlier, *Bos taurus* females appear to exhibit a shorter interval from CIDR removal to estrus than *Bos ind*icus-influenced females. Therefore, changing the timing of GnRH-2 from 48 to 66 or 72h should increase conception rates, but this hypothesis remains to be tested.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Synchronization of ovulation using COS-C in *Bos-indicus* crossbred cattle did not yield TAI conception rates of ≥ 50% in the current studies. The low TAI conception rates observed in Exp. 1 and 2 were not be related to factors such as subfertility, low BCS or a high incidence of anestrus. The relatively low rates of synchronization and TAI conception in the *Bos indicus*-influenced cattle used in these studies were primarily attributable to failure of at least 40% of cattle to develop a synchronized follicular wave after GnRH-1 and to inappropriate timing of TAI/GnRH-2. The COS-C is a relatively convenient and systematically functional synchronization protocol that is designed to maximize the ability to achieve high TAI conception rates. However, in order to successfully utilize this or similar methodology in *Bos indicus*-influenced cattle, further modifications will be required. Adjustments in the timing of insemination need to be evaluated extensively. Other approaches for inducing a greater proportion of ovulations after GnRH-1, and thereby increasing the frequency of a synchronized follicular wave, should also be examined.

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APPENDIX

LABORATORY PROCEDURES

Luteinizing Hormone RIA

1. Iodination: Iodination grade bLH (USDA–bLH-I-1; AFP 6000)
Reaction: 5 μg of hormone, 0.5 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: Biological grade bLH (NIH bLH-B-10) Range: 0.25 -100ng/ml

4. Reference preparation: bLH added to cow serum

5. RIA procedure
(Davis et al. 1971; Biol Reprod 4:415 and Williams and Ray 1980; J. Anim Sci 50: 906)

   a) Label assay sheets and polypropylene or borosilicate glass tubes 4 NSB, 9 TC, 3 "0", standards in triplicate, 2 X references in duplicate, and unknown samples in duplicate
   b) <u>Day 1</u>: 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
           Store at 4^oC until next step

           Pipette 200 μl PBS-EDTA + 1:400 NRS without 1st Ab into the 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 h at 4° C
           Pipette 100 μl 125I-bLH (20,000 cpm/tube diluted in PBS-1% EW) into All tubes, vortex briefly, and incubate for 24 h at 4° C
   c) <u>Day 2</u>: Pipette 200 μl of sheep-anti-rabbit gamma globulin (SARGG) diluted in PBS-EDTA into all tubes except TC
           Vortex and incubate 48-72 h at 4° C
   d) <u>Day 4</u>: Add (per spin basis) 3 ml ice-cold 0.01M PBS into all tubes except TC
           Centrifuge tubes for 1 h at 3600 rpm at 4° C
           Decant supernatant
           Count radioactivity associated with the pellet in gamma counter

Progesterone RIA

Single Antibody RIA Kit, Diagnostic Products Corporation, Los Angeles, 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: Human serum with added P4. Range: 0.1 – 20.0 ng/ml.
4. Reference: Human standard preparation added to bovine serum.
5. RIA Procedure:
    A. Begin and complete 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 ^{125}I-P4 provided in the kit into all tubes including two Total Count non-coated polypropylene tubes.
        4) 4. Vortex tubes briefly and incubate at room temperature for 3 h.
        5) Pour off supernatant.
        6) Count radioactivity of each tube using a gamma counter.

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Selected Publications

Saldarriaga, J. P., D. A. Cooper, J. A. Cartmill, R. L. Stanko and G. L. Williams. 2005. Performance of CIDR-based synchronization programs for timed AI in Brahman-influenced cattle. Proceedings Ninth Course on Advances in Production and Reproduction of Cattle, IRAC, Uberlandia, Brazil, pp. 233-242

Saldarriaga, J., D. Cooper, J. Cartmill, R. Stanko, G. Williams. Synchronization of ovulation for timed AI (TAI) in Bos indicus-influenced cattle using CIDR-based, GnRH-prostaglandin combinations I: ovarian follicular, luteal and hormonal events associated with suboptimal reproductive outcomes. Proceedings American Society of Animal Science, Annual Meeting, 2005 (accepted)

Saldarriaga, J., J. Zuluaga, J. Cartmill, D. Cooper, G. Williams. Synchronization of ovulation for timed AI (TAI) in Bos indicus-influenced cattle using CIDR-based, GnRH-prostaglandin combinations II: assessment of estrual and ovulatory distributions with Select Synch + CIDR to optimize TAI with Co-Synch + CIDR. Proceedings American Society of Animal Science, Annual Meeting, 2005 (accepted)