CONTROL OF NEW FOLLICULAR WAVE EMERGENCE AND RATE OF FOLLICULAR MATURATION IN *BOS INDICUS*-INFLUENCED CATTLE WITH ESTRADIOL BENZOATE, TEMPORARY CALF REMOVAL AND PROGESTERONE

A Thesis

by

JULIE DIANE PACK

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 2008

Major Subject: Physiology of Reproduction

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Approved by:

Chair of Committee,	Gary L. Williams
Committee Members,	Steven P. Brinsko
	David W. Forrest
Head of Department,	Gary R. Acuff

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ABSTRACT

Control of New Follicular Wave Emergence and Rate of Follicular Maturation in *Bos indicus*-influenced Cattle with Estradiol Benzoate, Temporary Calf Removal and Progesterone. (December 2008)

Julie Diane Pack, B.S., Tarleton State University

Chair of Advisory Committee: Dr. Gary L. Williams

Objectives were to determine: 1) whether estradiol benzoate (EB) provides a superior alternative to GnRH for synchronizing emergence, growth and maturation of a new follicular wave for fixed timed AI (TAI) in *Bos indicus*-influenced cattle using CIDR-based protocols, 2) the effect of 48 h calf removal at CIDR removal on the rate of maturational synchrony of the dominant follicle and 3) the effect of varying the magnitude of peak plasma progesterone (P4) concentrations following CIDR insertion on the suppression of FSH and LH secretion in a CIDR-based protocol using EB. In experiment 1, sixty-four Braford (F-1) females were stratified by BCS, parity and days postpartum and assigned randomly to one of four groups in a 2 x 2 factorial arrangement of treatments: 1) Select-Synch + CIDR, 2) Select-Synch + CIDR with 48 h calf removal, 3) E-Synch + CIDR or 4) E-Synch + CIDR with 48 h calf removal. A greater number of cattle in the EB treated group exhibited NFWE compared to the GnRH group, 29 vs 17 cows for EB and GnRH respectively, ($P<0.0006$). Intervals to NFWE were also greater in EB treated cattle than in GnRH treated cattle, 4.2 vs 2.7 d for EB and GnRH treated

cattle respectively, ($P<0.0001$). Proportions of GnRH- and EB-treated cows ovulating after CIDR removal did not differ. Post-CIDR suckling status did not affect ovulation frequency or interval to ovulation. In experiment 2, eight pubertal (F-1) heifers were used in a Latin Square design with four treatment levels of P4: 1) EB only, 2) EB and new CIDR, 3) EB and new autoclaved CIDR, 4) EB, new autoclaved CIDR and P4 injection at CIDR insertion. Treatments 2 through 4 increased ($P < 0.01$) mean plasma P4 concentrations compared to treatment 1, with treatment 4 creating the greatest increase in P4 with the longest duration. Suppression of plasma FSH was greatest in group 4 ($P<0.08$), with mean 60 h concentrations less than in all other groups. Mean concentrations of LH were lesser in group 4 than groups 1 and 2. Frequencies of occurrence of NFWE and ovulation and intervals to NFWE did not differ among treatments. Results indicate that the use of EB and CIDR to synchronize Brahman x Hereford females may provide better synchronization for TAI compared to GnRH and CIDR based protocols.

DEDICATION

To my Me-Maw

Requiem æternam dona eis, Domine, et lux perpetua luceat eis.

ACKNOWLEDGEMENTS

This work would not have come to pass without the knowledge, assistance and support of a plethora of individuals and to thank every one of them would be another work in and of itself. However, there are a few individuals without whose key assistance this work would have been impossible. First and foremost, I must thank Dr. Gary L. Williams for his knowledge, assistance and most of all patience. I have acquired so much more than simple knowledge while under his direction. I cannot imagine anywhere else during my two short years at A&M that I could have acquired any lessons more valuable than the ones I have gained from working with him and his laboratory. I must also extend my gratitude to the members of my committee Dr. Steven P. Brinsko and Dr. David W. Forrest. For your additional guidance and investment in my life and education I will forever be in your debt. I would also like to thank Dr. Paul G. Harms in addition to Dr. Forrest for allowing me to assist in teaching for them. I thoroughly enjoyed my time in your classrooms and I consider the hours spent teaching some of the most rewarding during the last two years.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

The sale of beef cattle and calves in the United States generated $49.7 billion in revenue in the year 2007 (NASS, 2008b). Given the magnitude of the economic influence of beef cattle production on agriculture in the United Sates, it is not surprising that large amounts of time, money and effort have gone into developing ways to improve the efficiency of production.

One goal of the beef producer should be to improve the genetic value of the herd over time. Perhaps the fastest route for achieving genetic improvement is through the use of artificial insemination (AI). Use of this technology provides the opportunity to improve the genetic quality of herd replacements more expediently and more economically than with natural service by bulls whose genetic values are usually much less than that of AI sires. However, according to recent surveys from the top 23 beef cattle-producing states in the US, only 13.3% of producers are taking advantage of this technology (NAHMS, 1997).

The most common reasons given for not adopting new production technologies are variability in success rate and the cost of implementation. One way to help overcome these obstacles in an AI program is to implement a program to synchronize

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This thesis follows the style of Animal Reproduction Science.

ovulation in conjunction with a single timed-AI (TAI). One of the more recently developed synchronization methods is the CO-Synch protocol, which involves the combined use of GnRH and prostaglandin $F_{2\alpha}$ (PGF) to synchronize new follicular wave emergence (NFWE) and regress the corpus luteum. When this protocol is used in conjunction with TAI, pregnancy rates as great as 49% have been achieved in *Bos taurus* cattle (Geary and Whittier, 1998). With the addition of an exogenous source of progesterone (P4) to the protocol, such as the controlled internal drug releasing (CIDR-B) insert, conception rates have improved to nearly 60% in some studies (Lamb et al., 2001). Both Geary and Whittier (1998) and Lamb et al. (2001) conducted their research using *Bos taurus* cattle in the more temperate Midwestern climate of the United States. Such results are greatly encouraging for the industry as a whole relative to the potential for widespread application of AI. However, beef cattle producers in the Gulf coast states face an additional challenge in their attempts to synchronize ovulation and employ TAI because of the common use of *Bos indicus*-influenced cattle. Although breed types with *Bos indicus* influence are more adapted to the subtropical environments of the gulf coast states (Alvarez et al., 2000; Riley et al., 2001; Bo et al., 2003) they have consistently exhibited markedly lower TAI pregnancy rates when subjected to synchronization and TAI protocols similar to those used in cattle without *Bos indicus* influence (Hardin et al., 1980; Lemaster et al., 2001; Saldarriaga et al., 2007). For example, TAI pregnancy rates of only 39% were obtained in Brahman x Hereford and Brangus cattle subjected to the CO-Sync + CIDR protocol in south Texas (Saldarriaga et al., 2007). One of the primary

conclusions of that study was that the first administration of GnRH in the CO-Synch + CIDR protocol failed to optimally synchronize NFWE. Therefore, modifications that can lead to more effective synchronization of NFWE and subsequent maturation could improve TAI pregnancy rates in these types of cattle.

One alternative to GnRH for synchronization of follicular waves is the use of an estrogen such as estradiol benzoate (EB) (Bo et al., 1993, 1994; Lane et al., 2003; Ando et al., 2004; Martinez et al., 2007). Administration of exogenous estradiol causes atresia of the dominant follicle (Clark et al., 1981; Bo et al., 1993; Dierschke et al., 1994; Ando et al., 2004). Estradiol has the ability to suppress secretion of LH (Edgerton and Baile, 1977) and FSH (Colazo et al., 2003). Treatment of cattle with estradiol-17β or one of its conjugates (e.g., EB) first suppresses circulating concentrations of these hormones and then results in their resurgence. The initial suppression of gonadotropins results in atresia of the dominant follicle and the subsequent resurgence of FSH that follows clearance of estradiol from the circulation results in NFWE within 3 to 4 d (Ko et al., 1991). Since estradiol only induces atresia, whereas GnRH can cause ovulation or atresia, the range over which new NFWE occurs has been postulated to be less variable with estradiol than with GnRH (Clark et al., 1981; Bo et al., 1994, Twagiramungu et al., 1994, 1995a; Thompson et al., 1999;). Some evidence suggests that the magnitude of the acute increase in P4 at onset of synchronization also positively affects NFWE (Bo et al., 1995a). This is accomplished because an acute increase in P4 causes suppression of the frequency of LH pulses. The greater the magnitude of the increase in P4, the more time is required for the animal to resume normal secretion patterns (Fike et al., 2004).

Anderson and Day (1994) showed that administration of 200 mg of P4 induced atresia in persistent dominant follicles.

A second option for improved synchronization is the addition of temporary calf removal. Temporarily removing calves from cows has been shown to induce cyclicity in a proportion of anestrous suckled females by removing the effects of suckling on basal secretion of LH and by ultimately initiating a preovulatory-like LH surge (Walters et al., 1982; Williams, 1990). Efficacy depends on length of weaning (Shively and Williams, 1989). In synchronization protocols with Syncro-Mate-B (SMB), temporary calf removal enhanced estrus and ovulation synchrony, improved TAI pregnancy rates and the percent of females remaining pregnant/continuing to cycle after 21 d (Smith et al., 1979; Walters et al., 1982; Williams, 1990). Recent studies showed that conception rates could also be increased using the more modern CO-Sync and Ovsync protocols if a 48 h calf removal component was added (Geary et al., 2001).

Objectives of this study were to determine 1) whether EB provides a superior alternative to GnRH for synchronizing emergence, growth and maturation of a new follicular wave for TAI in *Bos indicus*-influenced cattle using a CIDR-based protocol , 2) the effect of 48 h calf removal at CIDR removal on the rate of maturational synchrony of the dominant follicle and 3) whether the magnitude of the acute increase in serum P4, in combination with EB at onset of synchronization, affects degree of suppression of FSH and LH secretion, magnitude and timing of FSH resurgence and synchrony of new follicular wave emergence (NFWE).

CHAPTER II

LITERATURE REVIEW

Beef Cattle Production in the United States

The United States is the largest producer of beef in the world (ERS, 2007a). As of January 2008, the USDA reported an estimated 32.6 million beef cows, 5.67 million beef replacement heifers and 2.21 million bulls in the U.S. inventory. Approximately 14.3 million head of feeder cattle are on feed in feedlots across the nation (NASS, 2008a). While impressive, the U.S. cattle inventory has declined due to factors such as drought, higher grain prices and the discovery of a Bovine Spongiform Encephalopathy (BSE) infected cow in 2003. Prior to the discovery of BSE in U.S. herds exports had reached 2.5 billion pounds annually. Most of this exported beef was high quality, grain-finished beef for Japanese markets. After the discovery of a BSE positive animal, there was a drastic and immediate drop in the export of U.S. beef to overseas markets (ERS, 2007b; Figure 1; TAMU, 2008). Some countries have reopened their markets to U.S. beef, but most are restricting imports to animals under 20 mo of age (ERS, 2007b). This has caused some U.S. beef producers to investigate niche markets and alternative marketing strategies.

With the uncertainty and volatility characteristic of today's beef and cattle markets, it is now more critical than ever for U.S. beef producers to optimize the production potential of their cattle. Alternative or additional markets might include lean

or grass-fed beef, organic beef or natural beef. Such markets promote products that may be advertised as antibiotic-free, locally produced or produced by a family farm (Fanatico, 2006). Regardless of how the eventual beef product is marketed, it is essential to have the genetic quality capable of meeting the requirements of each market. Whether those genetics are for forage efficiency, marbling ability, or simply frame score, having the appropriate genetic base is essential.

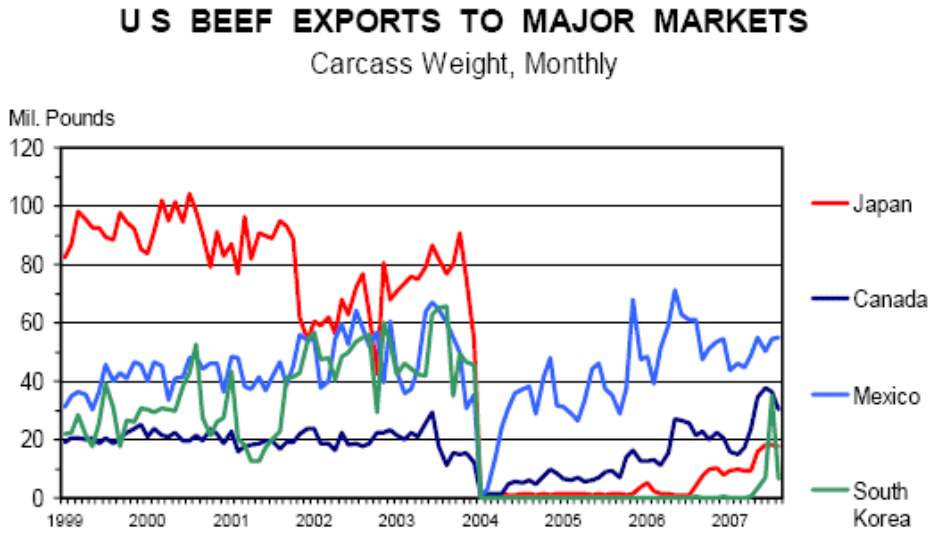

Figure 1. U.S. beef cattle exports from January 1999 through August 2007 to the top four beef-importing countries, before and after the discovery of BSE in the U.S. (Adapted from Anderson, 2008).

Production Strategies of United States Beef Producers

American beef producers have numerous options for developing strategies for efficiently managing reproduction in the cow herd. The traditional scheme for most producers is to use bulls for natural service of the female herd; however, other alternatives such as AI and embryo transfer are technologies that may be selected under specific circumstances.

Natural Service

Approximately 92% of all beef cattle in the U.S. are bred by natural service, with 6% bred by a combination of natural service and AI (NAHMS, 1997). According to Rhoad (1944), the proportion of cattle in a herd with no known reproductive problems that become pregnant on their first estrous cycle of the breeding season in a natural service system is 52%. The combined percent of cattle that become pregnant on either the first or second cycle is 80% and by their third cycle 91% of the cattle will have become pregnant. The main advantage to this type of management system is its relatively low labor cost. Assuming the bulls have been determined to be fertile and no injuries or illnesses are detected, there is little or no additional management required from a reproductive standpoint until the herd is checked for pregnancy at the end of the breeding season. Disadvantages to this system include the spread of any reproductive diseases present in the herd, the cost of purchasing and maintaining bulls during the non-breeding season, lesser quality genetics from the sire and the hazards of handling aggressive bulls.

Artificial Insemination

The most popular alternative to a strictly natural service system is the use of AI. Advantages to this system are numerous and include prevention of the spread of disease, increased genetic impact of superior sires, eliminating the need to maintain a potentially dangerous and destructive animal and the ability to spread higher quality genetics over a wider geographical area (Davis, 1938; Vishwanath, 2003). The first recorded successful

scientific AI of a mammal was performed by Spallanzani in the late 1700's on a bitch of the Barbet breed (Heape, 1897; Senger, 2003; Bearden et al., 2004). However, this area of research did not progress significantly until the late 1800's and early 1900's when the first artificial vaginas (AV) were invented and used for semen collection. Prior to the invention of the AV, semen collection techniques were somewhat crude. Heape (1897) was the first to describe the collection of a stallion by allowing him to breed a mare and then collecting the ejaculate in a bowl as retrograde flow from the vagina. However, the most widespread method for collection was to simply retrieve the ejaculate directly from the vagina of the female (Heape, 1897; Foote, 2002). It was in 1914 that the Italian scientist, Gieseppe Amantea, fabricated and used the first AV (Bearden et al., 2004). Amantea's work was with the dog, but subsequent artificial vaginas for the major farm species were patterned after his original work (Herman, 1981; Foote, 2002; Bearden et al., 2004). Some forty years after Heape's description, Davis (1938) listed and described six ways in which bulls were commonly collected, noting that collection of ejaculate from the vagina of a female in estrus was the most desirable method of collection. One year later, Cole and Winters (1939) reported three methods for collecting bulls, the most successful of which in their experience was the AV. This remains the method of choice for most collections today.

The first research in AI of cattle was conducted by Ivanow in Russia in the late 1800's and early 1900's (Milovanov, 1964; Herman, 1981; Foote, 2002). Milovanov (1964) also reported that Soviet Scientists had perfected the use of AI in cattle in the years 1927-1932, and that their method was adopted as the most popular in many foreign

countries. This assertion was perhaps a little biased, as the method in question was the vaginal speculum method that had been proven to be less efficient than the intrauterine insemination being practiced in North America in the early 1940's, some twenty years before Miolvanov's publication (Weeth and Herman, 1951; Salisbury et al., 1978). Research in the area of AI in cattle proceeded in numerous laboratories around the world in the years following Ivanow's initial research (Foote, 2002).

Embryo Transfer

The third main option for yielding pregnancies in cattle is the use of embryo transfer (ET). With this technology, selected donors can be used to provide embryos distributed to females across the entire herd in order to produce the most economically desirable calves. Embryo transfer technology dates back to the 1800's when Walter Heape completed the first successful ET. Heape proved with rabbits that it was possible for a female to carry to term and deliver the young of another female (Betteridge, 2003). It was not until the early 1930's, however, that the first recorded ET of a farm animal took place. Warwick and Berry (1949), at the Agricultural and Mechanical College of Texas later known as Texas A&M University, successfully transferred both ovine and caprine embryos during their studies concerning the failure of hybrid matings. Twenty years later, Willet et al. (1951) would announce the birth of the first known successful bovine ET calf.

Until the 1960's, all ET research involved surgical recovery and transfer of embryos. The first report of a successful transcervical ET in cattle was in 1964 (Mutter et al., 1964). However, this did not usher in an era of widespread use of non-surgical

transfers due to a lower success rate when compared to the surgical techniques of the day (Rowson et al., 1969). Later, in the mid 1970's, non-surgical methods were again reinvestigated and lead to the eventual shift from surgical methods to non-surgical methods (Betteridge, 2003). The final boost for this technology in the latter half of the 21st century, and indeed for AI as well, was the discovery that PGF had no luteolytic effects when administered in the early stages, d 1 to 4 of the estrous cycle (Rowson et al., 1972), but was effective at inducing Luteolysis from d 5 to 16 (Lauderdale, 1972; Rowson et al., 1972).

While both AI and ET have made significant contributions to cattle production, they are not used widely or equally throughout the industry. Artificial insemination is used universally in purebred and grade dairy herds as well as in seed stock and commercial beef cattle production. However, ET is used most often in purebred or seed stock production. This is not to say that there is no use for ET in a commercial herd. To the contrary, valuable commercial cattle can be used as donors for ET in specialty herds. Nonetheless, the return on investment for ET is generally much greater in registered cattle and is utilized most often for these purposes.

The Bovine Estrous Cycle

The bovine female is polyestrous with an average estrous cycle length of approximately 21 d (Salisbury et al., 1978; Peters and Ball, 1995; Bearden et al., 2004). There are four stages to the estrous cycle (Figure 2), each with unique hormonal and ovarian characteristics (Bearden et al., 2004; Ibrahim and Olaloku, 2000).

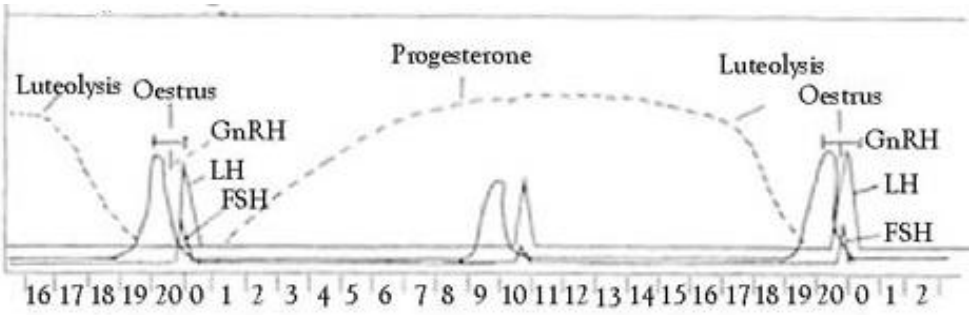

Figure 2. Hormones of the bovine estrous cycle. (Adapted from Ibrahim and Olaloku, 2000).

Stages of the Estrous Cycle

Estrus is the time period during which a cow or heifer displays sexual behavior and allows mounting by herd mates or a bull. This period can last from a few hours to as long as 24 h, with 10 to 12 h being the normal range. Other sexual behaviors such as head butting, mounting herd mates or the bull, vocalization, mucus discharge and general hyperactivity may last for 16 to 24 h (Salisbury et al., 1978; Roelofs, 2005). Hormonally, the female is under the influence of increased circulating concentrations of the ovarian steroid, estradiol-17β, which peaks approximately 24 h before the onset of estrus (Glencross and Pope, 1981). The onset of estrus is associated with increased serum concentrations of FSH and a dramatic pre-ovulatory peak, or surge, of LH (Akbar et al., 1974; Chenault et al., 1975; Bernard et al, 1984).

The period from approximately d 1 to 5 following estrus is termed metestrus. This stage is associated with a sharp decline in estradiol, LH and FSH concurrent with a steady increase in P4 secreted from the developing CL (Chenault et al., 1975; Salisbury et al., 1978; Bearden et al., 2004). Intervals from onset of estrus to ovulation in *Bos*

taurus cattle have been reported as 30 h (Roelofs et al., 2005) and 27 h (Bernard et al., 1984), or approximately 14 h after the end of estrus (Brewster and Cole, 1941). In a study conducted on the Gulf coast of Florida, Plasse et al. (1970) showed that Brahman females had an average estrus duration of 6 h, with a range of 3 to 13 h. The average interval to ovulation from the onset of estrus was 25 h with a range of 13 to 34 h. Pinheiro et al. (1998), conducting experiments in Brazil, found a similar interval to ovulation from the onset of estrus for Nelore cattle (*Bos indicus*), but a slightly greater duration of estrus of 11 h. Lemaster et al. (1980) reported that *Bos indicus* x *Bos taurus* crosses had an interval from estrus to ovulation of 26 h and a duration of estrous of 14 h, while Mikeska and Williams (1988) reported an interval from estrus to ovulation of 23 h. Following ovulation, the animal is no longer sexually receptive, but may appear mildly restless and show a slight bloody discharge due to the rupture of uterine capillaries (Salisbury et al., 1978; Bearden et al., 2004).

The third stage of the bovine estrus cycle is termed diestrus and corresponds to the luteal phase (Peters and Ball, 1995; Senger, 2003). During this stage, from approximately d 5 of the cycle to d 17 (Bearden et al., 2004), the bovine female has increasing concentrations of circulating P4 produced by the CL, with maximal concentrations reached around d 17 (Vaca et al., 1983). During diestrus, serum LH is suppressed by the increased circulating concentrations of P4 (Hafs et al., 1975). It is during this stage that maternal recognition of pregnancy occurs. The bovine conceptus, similar to ovine and caprine conceptuses, secrete interferon tau which is the signal for maternal recognition of pregnancy (Roberts et al., 1992; Bazer et al., 1994). The critical

time for maternal recognition in the cow is between d 15 and 16 of the cycle (Bazer et al., 1994).

The final stage of the estrous cycle is proestrus, more commonly referred to as the follicular phase. The follicular phase is characterized by sharply declining serum concentrations of P4 due to lysis of the CL by PGF released from the endometrium of the uterus (Peters and Ball, 1995; McCracken et al., 1999; Curtis-Prior, 2004). From approximately d 17 to ovulation the animal experiences rising serum concentrations of FSH, LH and estradiol in preparation for ovulation (Ginther et al., 1997; Senger, 2003).

Synchronization of Estrus

Since the advent of ultrasound and radioimmunoassay technologies, science has sought to elucidate the biological events regulating the estrous cycle in order to manipulate it to greatest advantage. Synchronization programs can be an effective way to efficiently and economically implement a large-scale AI program in a cow-calf operation. Early protocols focused on the use of P4 and PGF to manipulate the estrous cycle. Today, there are numerous synchronization protocols available; however, there are large variations in cost, labor requirements and effectiveness among the choices. Currently, all synchronization programs function by their ability to regulate new follicular wave emergence (NFWE), the lifespan of the CL and (or) their combination.

Follicular Waves

Follicular dynamics associated with the bovine ovary include ongoing cycles of selection, growth and atresia of smaller follicles, eventual dominance and ovulation of a single follicle and subsequent resumption of the cycle. Follicular development in cattle

(Figure 3) occurs in waves (Pierson and Ginther, 1984; Lucy et al., 1992; Viana et al., 2000).

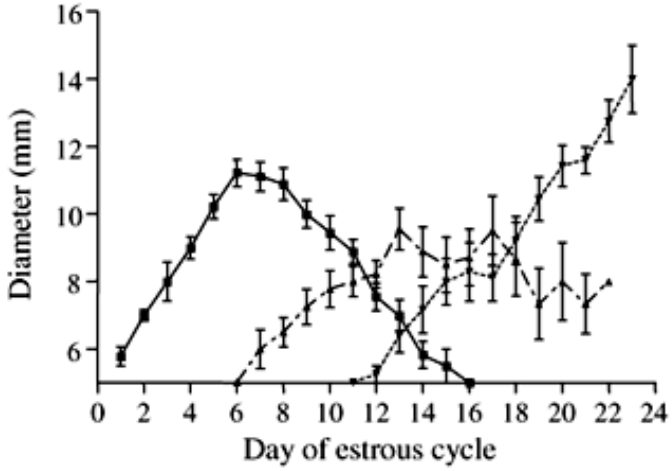

Figure 3. Growth of the dominant follicle during a three wave estrous cycle. (Adapted from Viana et al., 2000).

A follicular wave consists of several follicles that begin to grow as a cohort. The number of waves per cycle ranges from two to four (Savio et al., 1988; Sirois and Fortune, 1988), with three waves being the most prominent (Savio et al., 1988). Follicular wave patterns were first confirmed by ultrasonographic imaging in studies conducted by Pierson and Ginther (1984). However, in those studies, only two follicular waves per cycle were observed. This finding concurred with the work of Rajamahendran and Taylor (1990), but contrasted with that of Savio et al. (1988). Bo et al. (2003) found that follicular wave dynamics of *Bos indicus* cattle were similar to those of *Bos taurus* cattle with the exception that more four wave cycles were sometimes observed in *Bos indicus* cattle. Variation in these characteristics may be due to breed, parity or season. Usually, only one follicle out of the selected cohort of follicles will

achieve dominance and the remaining smaller follicles of the cohort will regress (Pierson and Ginther, 1984). Findings by Thatcher et al. (1989) that cattle ovulate a single follicle ≥ 95% of the time agree with those of Pierson and Ginther (1984).

Before each new wave of follicular growth, circulating concentrations of FSH rise (Ginther et al., 1997). Initially, growth of the cohort is slow, but after the recruitment stage proceeds at a more rapid pace (Fortune, 1994). As the follicle continues to grow, its granulosa cells begin to produce estradiol (Hansel and Convey, 1983), this estradiol acts to suppress both LH and FSH (Charlesworth and Schwartz, 1986). The dominant follicle continues to grow under the influence of reduced concentrations FSH due to up regulation of FSH and LH receptors as well as an increase in the number of granulosa cells (Bodensteiner et al., 1996). Large follicles also secrete inhibin, which in turn inhibits FSH secretion, thereby insuring the concentrations of FSH remain low enough to be inhibitory to the growth of additional follicles while the dominant follicle is maturing (Echternkamp and Schanbacher, 1990; Ginther, 2000). Ovulation is accomplished by a surge release of LH caused by positive feedback of estrogen produced by the dominant follicle on the hypothalamus which results in surge release of GnRH causing a LH surge (Peters and Ball, 1995)

Manipulation of follicular waves is one of the key principals of a synchronization protocol, along with the manipulation of the CL. Early work on controlling follicular waves included hormonal treatments (Adams et al., 1992b; Thatcher et al., 1989), as well as direct ablation of the dominant follicle (Bergfelt et al., 1994) to reset the follicular wave by either ovulation or atresia. Hormonal control of the follicular wave is

most commonly accomplished by use of either GnRH or an estrogen (Bo et al., 1995a; Baruselli et al., 2004; Kim et al., 2005).

Corpus Luteum

The bovine is generally a single-ovulating species and thus usually has a single CL after ovulation. Luteinizing hormone is the luteotropic agent involved in CL formation and is released from the anterior pituitary in response to stimulation by GnRH (Hansel et al., 1973; Reviewed by Schams and Berisha, 2004). Progesterone and estradiol both have inhibitory effects on LH release from the pituitary during the majority of the estrous cycle. This is accomplished by negative feedback to the pituitary and hypothalamus inhibiting both LH and GnRH (Milvae et al., 1996). Once the inhibitory effects of P4 are removed, LH can cause luteinization by triggering the transformation of follicular cells into luteal cells (Reviewed by Schams and Berisha, 2004). Mature luteal cells produce P4 throughout the luteal phase of the estrous cycle or pregnancy if the female conceives (Milvae et al., 1996).

Luteolysis is the process by which the CL undergoes functional and physical regression (Milvae et al., 1996). Exogenous PGF can also be given to regress the CL (Thatcher and Chenault, 1976). This discovery lead to the first synchronization protocols, which involved intrauterine administration of PGF into the uterus ipsilateral to the CL (Rowson et al., 1972). The same year, Lauderdale (1972) showed that administration of PGF i.v. or s.c. achieved the same results as the intrauterine administration. Another important conclusion from that study was that administration of PGF before d 6 of the estrous cycle did not result in regression of the CL. Chipepa et al.

(1977) published findings from their research indicating that a 2 dose i.m. PGF treatment could be used to synchronize females. They concluded that conception and subsequent calving rates for treated animals were no different than control animals that were bred on a natural heat.

Hormones of the Estrous Cycle and Their Use as Pharmacological Agents

As our understanding of the bovine cycle has improved, so too has our ability to manipulate it. While research is still ongoing, great strides have been made in the area of synchronization with the goal in many minds being the elimination of the need to detect estrus. The use of reproductive hormones and in some case their more potent analogues have provided many opportunities for research as well as improved productivity at the farm level.

GnRH

GnRH is a polypeptide hormone originating in the hypothalamus which acts on the anterior pituitary to cause the release of both LH and FSH (Schally et al., 1971). As early as the late 1970's, investigators were studying the use of GnRH as a component of a synchronization protocol (Chipepa, 1977); however, it was not until the 1990's that the Ovsynch (Pursley et al., 1995) and Co-Synch protocols became the first examples of GnRH and progesterone based protocols with widespread use for synchronization (Figure 4: Geary et al., 2001a).

Gonadotropin-releasing hormone acts to synchronize a follicular wave by stimulating a surge release of LH, causing large follicles (usually ≥ 10 mm) to ovulate and smaller follicles to become atretic, regardless of stage of the estrous cycle

(Reviewed by Twagiramungu et al., 1995; Pursley et al., 1995). Emergence of the new follicular wave occurs between d 2 to 4 after GnRH treatment (Bo et al., 1995b; Kesler, 2005; Kim et al., 2005; Reviewed by Twagiramungu et al., 1995). Early studies conducted by Thatcher et al. (1989) showed that administration of GnRH agonists followed by PGF enhanced estrous synchrony and were later supported by the work of others (Twagiramungu et al.,1995).

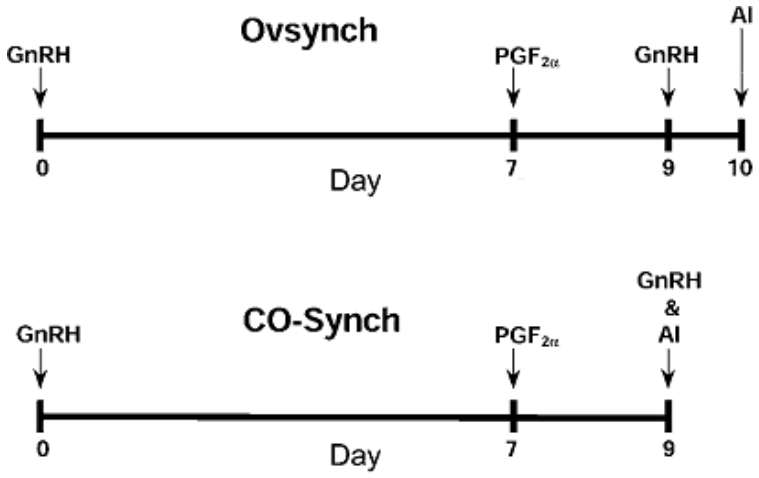

Figure 4. Timeline of the protocol for the GnRH containing synchronization protocols Ovsynch and CO-Synch. (Adapted from Geary et al., 2001).

When administered to randomly cycling cows, Pursley et al. (1995) reported that GnRH causes ovulation and subsequent resetting of the follicular wave in a large proportion, 90%, of mature, randomly-cycling *Bos taurus* females. In contrast to Pursley et al. (1995), Saldarriaga (2006) observed only 40% of randomly-cycling *Bos indicus* x *Bos taurus* underwent ovulation and subsequent NWFE. Zuluaga (2006) also conducted studies involving *Bos indicus* x *Bos taurus* females and reported an ovulation rate to a single injection of GnRH of 27 and 50%. When cattle were presynchronized

with an additional treatment of GnRH 7 d before initiation of the CO-Synch + CIDR protocol ovulation rate to GnRH-1 of CO-Synch + CIDR was 58%. These results indicate that the degree of synchrony of NFWE produced in *Bos indicus*-influenced cattle by a single injection or two injections of GnRH at the start of commonly-used synchronization programs is less than in *Bos taurus* cattle. Factors affecting the ability of the follicle to ovulate are its maturational stage (Sartori et al., 2001) and P4 levels (Twagiramungu et al., 1994) which are both a function of the phase of the estrous cycle.

There are four popular protocols utilizing GnRH to reset the follicular wave: Select Synch, CO-Synch, Hybrid Synch (Reviewed by Kesler, 2005) and 7-11 Synch (Kojima et al., 2000). All four protocols involve GnRH to reset the follicular wave and PGF to regress the newly-formed CL. Of the four protocols, only the CO-Synch protocol uses TAI and has resulted in conception rates of > 50% in *Bos taurus* cattle (reviewed by Kesler, 2005). Stutts et al. (2007) found that when using the CO-Synch + CIDR protocol in *Bos indicus* cattle, straight-bred Brahman, TAI conception rates were only 23%.

Estrogens and Progestogens/Progestins

An alternative to GnRH for resetting the follicular wave is use of an estrogen such as estradiol benzoate. Early work with estrogens led to evidence that estradiol caused luteal regression in cattle. Although later proven to be inefficient for this purpose, this early observation led to its inclusion in P4-based synchronization schemes in the 1960's (reviewed by Bo et al., 2003). Almost 30 yr later, Bo et al. (1991) found that estradiol valerate caused atresia of follicles in heifers synchronized with the SMB

protocol. Further work by Bo et al. (1994) showed that heifers treated with estradiol-17β and norgestomet resulted in regression of the dominant follicle, leading to a more synchronous emergence of the next follicular wave. They concluded that the suppressive effects of estradiol-17β were greater when used in conjunction with a progestin. Bo et al. (1995a) also observed that synchrony of NFWE was greater in estradiol-17β + norgestomet-implanted heifers than in heifers receiving norgestomet alone. These concepts were affirmed in studies in which estrous cycles of cattle treated with the combination of estradiol-17β and a CIDR were more efficiently synchronized than cattle treated with two doses of PGF or a CIDR alone (Bo et al, 1995b). Stutts et al. (2007) reported TAI conception rate of 47% when using estradiol-17β and a CIDR, further indicating the advantages of the addition of a progestin to an estrogen based synchronization protocol.

Methods for acutely increasing circulating concentrations of P4 or a progestin in cattle include the now defunct SMB protocol (i.e., injection of norgestomet at the time of norgestomet implant insertion and injection of estradiol valerate), injection of P4 at the time of CIDR insertion (Colazo et al., 2003, 2004; Martinez et al., 2000), and may potentially include the application of re-used CIDR inserts after pressurized steam sterilization (Zuluaga and Williams, 2007). The latter treatment appears to markedly increase serum concentrations of P4 during the first 8 h after insertion compared to a non-autoclaved, re-used CIDR.

New waves of follicular growth are preceded by a surge in FSH (Adams et al., 1992a). Estradiol has been shown to suppress FSH (Bo et al., 1993; O'Rourke et al.,

2000; Colazo et al., 2003, 2005). Therefore, when the inhibitory effect of estradiol is removed, FSH concentrations in the circulation rise, precipitating NFWE (Adams et al., 1992a). A new follicular wave is initiated approximately 1 d after the onset of FSH resurgence (Bo et al., 1993, 1994; Martinez et al., 2007).

Several estradiol conjugates have been reported in the literature for use in estrous synchronization protocols, with EB being one of the most commonly used forms. Estradiol benzoate, a conjugate of estradiol-17β and benzoic acid, is metabolized to estradiol-17β, the most potent naturally-occurring form of estradiol. Estradiol benzoate is metabolized rapidly upon entering the circulation and is similar in potency to native estradiol-17β (reviewed in O'Rourke et al., 2000).

Temporary Calf Removal

Scientific attempts to understand the role of suckling in regulating length of the postpartum anestrous/anovulatory period in cattle date back as far as the 1930's (reviewed by Short et al., 1990; Williams, 1990). It has been well-documented that the postpartum anestrous interval is greater in suckled than in nonsuckled (Lusby et al., 1981; Williams et al., 1982; Williams et al., 1987; Shively and Williams, 1989) or restricted suckling cows (Randel, 1981). Suckling inhibits cyclicity by suppressing hypothalamic secretion of GnRH (Gazal et al., 1998), leading to decreased circulating concentrations of LH (Walters et al., 1982; Williams, 1990). The effects of varying durations of calf removal on secretion of LH were investigated by Shively and Williams (1989). They observed that weaning for 96 h or longer maximized weaning-induced increases in LH. More recently, studies conducted by Soto Belloso et al. (2002) reported

similar conclusions to those of Shively and Williams (1989) in that calf removal for 96 h resulted in significantly shorter intervals to estrus and conception in Zebu x Holstein cattle. Although temporarily weaning of calves for up to 96 h can optimize reproductive responses of the suckled cow, it is managerially infeasible. Weaning calves for more than 48 to 72 h results in detrimental impacts on their nutrition and health, subsequent decreases in milk production by dams and disruption of the cow-calf bound leading to an increased occurrence of orphan calves (Williams and Griffith, 1992; Griffith and Williams, 1996). Shively and Williams (1989) proposed that a 48 to 72 h temporary weaning period was adequate to achieve the desired response if it was performed concurrently with a steroid-based synchronization scheme. Several other studies conducted in the 1980's indicated that weaning for 48 h was more effective than 24 h weaning for increasing first-service pregnancy rates when used in conjunction with an estrous synchronization protocol such as SMB (Kiser et al., 1980; Fogwell et al., 1986).

Bos indicus-Influenced Cattle

Bos indicus-influenced breeds of cattle are more adapted to tropical and subtropical environments (Figueiredo et al., 1997; Alvarez et al., 2000; Bo et al., 2003; Baruselli et al., 2004). For this reason, many ranchers on the Texas Gulf coast utilize breed crosses with up to 50% *Bos indicus* influence. Unfortunately, these breeds of cattle often show a marked reduction in fertility when subjected to artificial breeding protocols compared to their straight-bred *Bos taurus* counterparts (Bo et al., 2003; Hiers et al., 2003; Baruselli, 2004). Reasons for these observations can include mistakes in heat detection and a greater proportion of animals being anestrus at the start of the

breeding season under some conditions, such as those in South America where straight *Bos indicu*s breeds (e.g., Nelore and Brahman) are utilized (Baruselli, 2004). One way to overcome these problems is to implement a synchronization program with TAI. This can allow the potential elimination of heat detection as well as yield the added benefit, in some cases, of inducing cyclicity in anestrous animals (Lamb et al., 2001; Saldarriaga et al., 2007). However, pregnancy rates following synchronization and TAI in *Bos indicus*-influenced cattle have generally been less than impressive. Saldarriaga et al. (2007) reported 39% TAI conception rates in Braford and Brangus cattle using the Co-Synch + CIDR protocol. The latter involves the use of GnRH and PGF with the addition of a CIDR insert for 7 d. Such levels of success are well below those generally observed in *Bos taurus* cattle treated similarly (Geary and Whittier, 1998). In a separate study, Geary et al. (2001a) examined the effects of temporary calf removal on pregnancy rates using two different protocols, Ovsynch and CO-Synch. They found that 48 h calf removal increased (P=0.09) TAI conception rates compared to those without calf removal (62% vs 53%). They also reported that the use of these protocols resulted in conception rates of 58% for anestrous animals in the calf removal group verses 48% for those in the non calf removal group. Based on these reports, the use of the CO-Synch + CIDR synchronization protocol in conjunction with 48 h calf removal may offer a practical AI scheme to producers of *Bos indicus*-influenced cattle.

Other Factors Affecting TAI Conception Rates

Body Condition Score

Both Zuluaga (2006) and Souza et al. (2008) found that BCS had an effect on pregnancy rates to TAI. Zuluaga (2006) compared TAI pregnancy rates in all cattle in one experiment to only those that maintained a BCS of ≥5 throughout the synchronization and breeding period. They observed an average pregnancy rate for all cattle synchronized with the CO-Synch + CIDR protocol of 48.5% compared to 54% when only those animals with a BCS of ≥5 were considered. This agrees with the findings of Souza et al. (2008) who reported that pregnancy rates for cattle with low BCS was 28.3% while animals with a high BCS had a pregnancy rates of 48.3% within the same treatment group. Similarly, data collected by Rae et al. (1993) from eight herds of beef cattle showed that animals having a BCS of ≤3 had a mean pregnancy rate at the end of the breeding season of 30.9% vs. 60.4% and 89% for those with BCS of 4 and ≥5, respectively. Based on these studies, it is obvious that nutritional management is an important component of a successful synchronization protocol.

Pre-synchronization

Pre-synchronization is the process of grouping animals into a lose synchrony before initiating a synchronization program. This serves to avoid having cattle at random stages of the estrous cycle at the onset of the desired synchronization program. Many pre-synchronization programs have been investigated using PGF, GnRH, P4 or a combination of these hormones. However, results have been contradictory, with some investigators reporting a marked increase in synchronized conception rates following

pre-synchronization while others observed no benefit from this approach. LeBlanc and Leslie (2003) found that when a single injection of PGF was given to dairy cattle 10 d before the implementation of the Ovsynch protocol, 37.3% became pregnant vs. 36.6% of cattle receiving Ovsynch without pre-synchronization. These findings are in agreement with those of Meyer et al. (2007) who investigated four different synchronization methods including presynchronization with one injection of PGF before Ovsynch. Presynchronization did not improve conception rate compared to Ovsynch alone. Rivera et al. (2006) found similar results, but used GnRH as a presynchronization agent. They administered one injection of GnRH 7 d before the onset of an Ovsynch protocol and obtained conception rates of 51% and 45%, respectively, for presynchronized and non-presynchronized cattle. Similar to Rivera et al. (2006), Zuluaga et al. (2006) used a single injection of GnRH 7 d before onset of the CO-Synch + CIDR protocol in Braford cattle. Conception rates to TAI at 66 h were not different than those obtained with CO-Synch + CIDR alone ((37.3 ± 6% for presynch vs 48.5 ± 6.1% for CO-Synch). Peters and Pursley (2002) used a combination of PGF and GnRH, with timing similar to LeBlanc and Leslie (2003) and Rivera et al. (2006) preceding an Ovsynch. Although presynchronization did not yield greater pregnancy rates, they did find that a larger proportion of animals were in the mid luteal phase at the onset of synchronization. Navanukraw et al. (2004) opted to use a two- injection PGF protocol (PGF on d -28 and -14 from GnRH-1 of Ovsynch) followed by Ovsynch. Using this approach, they were able to achieve pregnancy rates of 49% vs. 37% for those animals receiving Ovsynch alone. One of the most involved presynchronization protocols is that

advocated by Souza et al. (2008). They found that by using a double Ovsynch protocol (Ovsynch without AI followed by Ovsynch with AI), they could create a slight increase in pregnancy rates compared to animals receiving a two injection PGF presynchronization before Ovsynch (49.7% for Double-Ovsynch vs 41.9% for PGF presynchronization). The degree of synchrony of NFWE produced in *Bos indicus*-influenced cattle by a single injection of GnRH at the start of commonly-used U.S.-based synchronization programs is less than in *Bos taurus* cattle. This appears to be a major contributor to the poorer TAI pregnancy rates obtained in *Bos indicus*-influenced cattle using protocols such as CO-Synch + CIDR (Saldarriaga, 2006). Therefore, it was assumed that presynchronization could improve TAI pregnancy rates in *Bos indicus*-influenced cattle, but results to date have been disappointing (Zuluaga, 2006).

Timing of AI after PGF

The timing of AI in a TAI protocol is critical to the success of the procedure. The standard time for insemination in the CO-Synch and CO-Synch + CIDR protocols is 48 h after PGF or PGF and CIDR removal (Geary and Whitter, 1998; Geary et al., 2001a; Martinez et al., 2002). An additional injection of GnRH, either 24 h before insemination (Ovsynch) or at the time of insemination (Co-Synch and Co-Synch + CIDR), to initiate the LH surge is a common feature of currently-used protocols (Pursley et al., 1995; Walker et al., 2005). Earlier studies indicated that adjusting the timing of AI in the CO-Synch + CIDR protocol from 48 to 60 h post-PGF failed to improve conception rates (Stevenson et al., 2003). However, more recent research by Busch et al. (2008) has demonstrated that an increase in conception rates occurs when AI is

performed at 66 h when compared to AI at 48 to 54 h. This agrees with the results of Walker et al. (2005) who found that pregnancy rates to TAI at 72 h were greater than those at 48 h. The aforementioned studies were conducted using *Bos taurus* cattle. A basis for delaying insemination well-beyond 48 to 54 h in *Bos indicus*-influenced cattle using Co-Synch + CIDR may be found in the work of Saldarriaga et al. (2007), who reported that the interval to ovulation from PGF and CIDR removal was 99 ± 2.8 h, approximately 15 h earlier than *Bos taurus* using the same protocol (Geary et al., 1998; Stevenson et al., 2003). Based on the average time of ovulation observed by Saldarriaga (2006), insemination at approximately 75 to 87 h would theoretically increase the probability of conception in *Bos indicus*-influenced females. However, this does not take into account the wide range (68 to 127 h) over which ovulation still occurs in this system. Thus, while delaying insemination would potentially increase conception rates in those cattle ovulating at 72 h or later, any pregnancies that would have resulted from a TAI at 48 to 54 would not occur.

Vaginal Electrical Resistance

The use of vaginal electrical resistance (VER) has been investigated for over 30 yr as a means to determine the optimum time for conception, i.e. just prior to the LH surge when follicular size is maximal and estradiol concentrations are high (Leidl and Stolla, 1976). The sampling method involves the insertion of a probe into the anterior vagina of the cow to measure the resistance of the vaginal mucus in ohms (Leidl and Stolla, 1976; Gartland et al., 1976). In a study by McCaughey and Patterson (1981), measurements taken at the anterior vagina were the most reproducible; confirming that

the best placement of the probe was at the anterior vagina as opposed to the posterior vagina or the mid regions of the vagina. The VER of ovariectomized cattle does not fluctuate; however, when those same cattle are given exogenous estradiol their VER readings drop, indicating that estradiol concentrations have an effect on VER readings (Leidl and Stolla, 1976). Based on this information, several investigators examined the use of VER as a tool to monitor estrus and ovulation for AI. In the late 1970's, both Heckman et al. (1979) and Foote et al. (1979) reported that conception rates for cattle bred based on VER readings and those bred based on a visual estrus were not different. Thus, it was proposed that there could be a use for VER as a way to detect those cattle that do not exhibit behavioral estrus. Aboul-Ela et al. (1983) demonstrated that VER values were inversely related to serum concentrations of LH such that the lowest VER reading corresponded to the pre-ovulatory LH peak. These findings concurred with those of Canfield and Butler (1989) who also found a correlation between LH concentrations and VER values. Scipioni and Foote (1999) postulated that combining milk P4 and VER values could be an economical way to assist in estrus detection. More recently Zuluaga (2006) compared the results of VER and ultrasonography when monitoring cattle subjected to a CO-Synch + CIDR regimen. The intent of that study was to determine if ultrasound or VER readings could indicate an animal's follicular maturity/readiness to breed. Results from that study showed that an inverse relationship exists between VER and size of the dominant follicle as the animal approaches ovulation following synchronization. As expected, cattle that had larger follicles had a greater probability of becoming pregnant to TAI; however, there was no relationship detected

between VER and pregnancy outcome. It was concluded that ultrasonography at the time of TAI could be a useful tool for eliminating females that are less likely to conceive to TAI. However, VER would likely be of use only if cattle were examined more frequently than once daily. Unfortunately, added examinations would require additional handling and processing, subjecting cattle to additional stress, which would likely have negative impacts on fertility.

CHAPTER III

CONTROL OF NEW FOLLICULAR WAVE EMERGENCE AND RATE OF FOLLICULAR MATURATION IN *BOS INDICUS*-INFLUENCED CATTLE WITH ESTRADIOL BENZOATE, TEMPORARY CALF REMOVAL AND PROGESTERONE

Introduction

Bos indicus and *Bos indicus*-influenced cattle are the predominant types of cattle used for beef production in sub-tropical regions of the world (Figueiredo et al., 1997; Alvarez et al., 2000). These regions are characterized by high heat and humidity, an environment that *Bos indicus*-influenced cattle are better adapted to than straight *Bos taurus* due to their natural heat tolerance (Svotwa et al., 2007) and parasite resistance (Utech et al., 1978; Wambura et al., 1998).

Regardless of breed type, the use of AI is the most efficient method for creating genetic improvement in most beef cattle production systems. However, because estrus detection is often both labor-intensive and inefficient, research has long focused on developing successful methods for employing TAI in order to eliminate the need for estrus detection. Unfortunately, TAI pregnancy rates in *Bos indicus* and *Bos indicus* crossbred cattle are typically much less than those observed for *Bos taurus* breeds when subjected to similar synchronization of ovulation protocols (Lemaster et al., 2001; Saldarriaga, 2006). This occurs even though the natural fertility and productivity of the

crossbreds are often equal to or superior to straight-bred *Bos taurus* cattle (Saldarriaga, 2006).

Currently, the most successful, commercially available synchronization method for use with TAI in the U.S. is the CO-Synch + CIDR protocol (Geary et al., 2001b; Kesler, 2005). Use of this protocol in *Bos taurus* breed types has been reported to result in TAI conception rates consistently ranging between 52 and 66 % (Larson et al., 2004; Larson et al., 2006; Schafer et al., 2007; Kasimanickam et al., 2008). However, such results are not typical when CO-Synch + CIDR is used in *Bos indicus*–influenced cattle (Saldarriaga, 2006; Zuluaga, 2006). Thus, modifications to existing methods or development of other alternatives are needed in order to improve TAI pregnancy rates in these types of cattle. Based on recent reports, there is evidence to suggest that at least three previously reported strategies could improve synchrony of new follicular wave emergence (NFWE), and thereby conception rates, in modifications of the CO-Synch + CIDR or similar protocols. These include, substituting EB for GnRH at CIDR insertion (Bo et al., 1994, 1995b), 48 h calf removal at the time of CIDR withdrawal (Kiser et al., 1980; Geary et al., 2001b) and magnitude of the acute increase in circulating P4 at the onset of the synchronization protocol (Cassia and Bo, 1998; Moreno et al., 2001).

Objectives of this study were to determine 1) whether EB provides a superior alternative to GnRH for synchronizing emergence, growth and maturation of a new follicular wave for TAI in *Bos indicus*-influenced cattle using a CIDR-based protocol , 2) the effect of 48 h calf removal at CIDR removal on the rate of maturational synchrony of the dominant follicle and 3) whether the magnitude of the acute increase

in serum P4, in combination with EB at onset of synchronization, affects degree of suppression of FSH and LH secretion, magnitude and timing of FSH resurgence and synchrony of new follicular wave emergence (NFWE).

Materials and Methods

Study Locations and Animal Protocols

Experiments were conducted at the Texas AgriLife Research Center, Beeville, Texas. The Institutional Agricultural Animal Care and Use Committee of the Texas A&M University system approved all procedures used in these studies in advance of each experiment.

Experiment 1: Control of NFWE with GnRH or EB and Progesterone

Animals

Sixty-four crossbred Brahman x Hereford cows from the TAES Beeville spring herd were used in a 2 x 2 factorial arrangement of treatments to compare method of follicle wave synchronization (EB vs. GnRH) and suckling status (suckled vs. 48 h calf removal). The experiment was conducted in two concurrent replicates (32 cows/replicate) based on calving date. Replicates were also segregated by parity (primiparous vs. pluriparous) and placed with calves in pens measuring 25.6 x 9.5 m (5 to 6 cow-calf pairs per pen) beginning 10 d before the onset of treatments. All cattle were required to have a minimum body condition score of 5 on a 1 to 9 scale (Herd and Sprott, 1998), and cows were required to be at least 50 d post partum (DPP). Each group was fed forage-based diets with a concentrate supplement formulated collectively to meet National Research Council (NRC, 1996) recommendations for lactating beef cows.

Experimental Procedures and Synchronization Protocols

During the 10 d pre-experimental period, blood samples were collected every 2 to 3 d for measurement of P4 by RIA to determine cyclicity status. Blood samples were collected via coccygeal tail vessel using evacuated 10-ml tubes and 20 g x 1.5 in. bleeding needles and placed on ice immediately after collection. Samples remained on ice until transported to the laboratory where they were allowed to clot at room temperature for approximately 1 h before centrifugation at 1800 x g for 30 min. Serum was collected and stored at -20 $^{\circ}$C until hormone analyses.

Females were stratified by parity (primiparous vs. pluriparous), DPP and BCS and assigned randomly to one of four treatment groups: 1) Select-Synch + CIDR , 2) Select-Synch + CIDR with 48 h calf removal at CIDR removal, 3) E-Synch + CIDR or 4) E-Synch + CIDR with 48 h calf removal (Figure 5). Beginning on d 0, all animals received a once-used, autoclaved CIDR (Pfizer Animal Health, New York, NY). Females in groups 1 and 2 received a single IM injection of GnRH (100 µg; Cystorelin, Merial, Inc., Iselin, NJ, USA), whereas groups 3 and 4 received a single IM injection of EB (2.5 mg, compounded on site in oil). On d 7, CIDRs were removed and a single IM injection of PGF (25 mg; Lutalyse; Pharmacia & Upjohn Co., Kalamazoo, MI, USA) was administered to all cattle. Females in groups 2 and 4 had their calves removed to a separate facility, located approximately one quarter of a mile away, for 48 h at the time of CIDR removal. Calves were provided fresh water and free-choice alfalfa hay with high-energy creep feed while separated from their dams. Calves were reintroduced to their dams on d 2 after CIDR removal. Transrectal ultrasonography (Dynamic Imaging,

Concept/MCV, equipped with a dual 5/7.5 MHz linear array probe; Livingston, UK) of ovaries to identify follicular structures was performed daily, beginning on d 0 and continuing through ovulation or a maximum of 144 h after CIDR removal. Interval to NWFE was determined retrospectively as the interval from treatment to appearance of the 5mm follicle that subsequently became the dominant follicle. Synchronized NFWE was characterized as occurring between d 1 - 4 for GnRH treated animals and d 2 - 6 for EB treated animals.

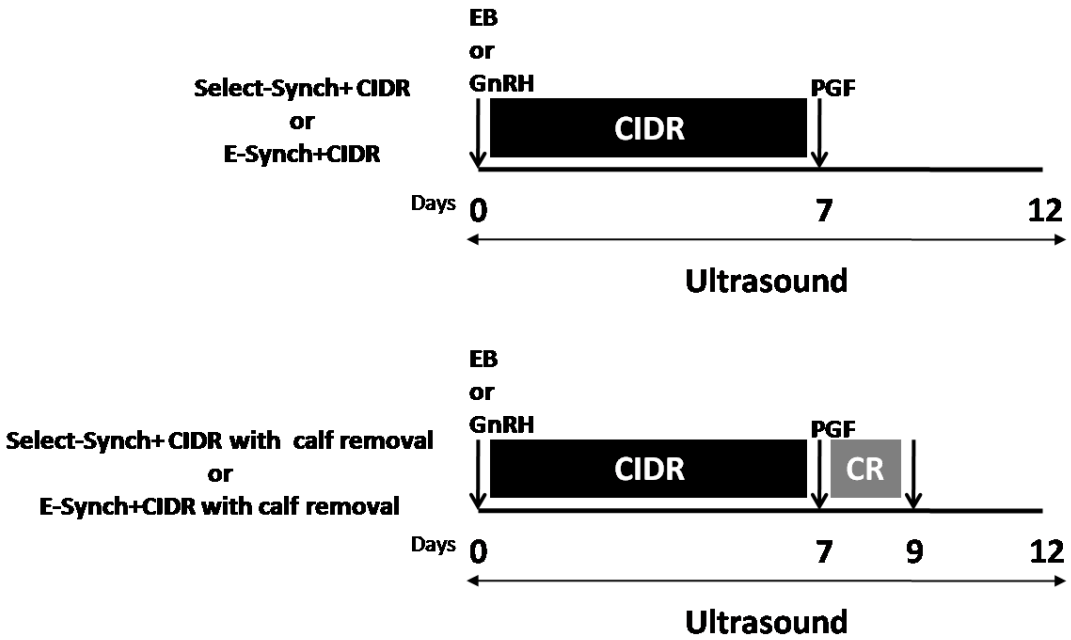

Figure 5. Experimental protocols for synchronization of ovulation in Experiment 1. Cattle in groups 1 and 2 received a CIDR insert plus a GnRH injection, 100 µg IM, on d 0 (GnRH). Groups 3 and 4 received a CIDR insert plus an EB injection, 2.5 mg IM, on d 0 (EB). All cattle had their CIDR removed on d 7 concurrent with an injection of PGF, 25 mg IM. Females in groups 2 and 4 also had their calves removed (CR) to a remote location for 48 h following CIDR removal.

RIA

Serum was assayed for P4 using a commercial direct RIA, Coat-A-Count (DPC, Los Angeles, CA), as reported previously from this laboratory.

Statistical Analysis

All data were analyzed using the Statistical Analysis System (SAS) program (SAS Inst. Inc., Cary, NC). Frequency data were analyzed using CATMOD and PROC FREQ procedures. The CATMOD procedure was used to determine the effects of treatment, replicate and suckling status on the number of cattle ovulating at 72, 96, 120 and 144 h after CIDR removal. The model included treatment, replicate and suckling status effects on each post-CIDR removal time interval. Ovulation frequency was evaluated using the FREQ procedure. Interval data were analyzed using the PROC MIXED procedures where treatment, replicate, cyclicity, suckling status and their two and three way interactions were used to determine the effect of treatment on the interval to new follicular wave emergence (INFWE) and interval to ovulation after CIDR removal (IOV). Repeated measures data (follicle size) were analyzed using the PROC MIXED procedure where treatment, suckling status, time, replicate, cyclicity and their two and three way interactions were used to examine the treatment effects on follicular size. All reported means are least squared means.

Experiment 2: Effect of the Acute Increase in Progesterone on Synchronization of NFWE Animals

Eight pubertal, Brahman x Hereford heifers from the Texas AgriLife Research-Beeville herd were used in a replicated Latin square design. All heifers were required to have a body condition score of >5 on a 1 to 9 scale (Herd and Sprott, 1998). Heifers were placed in individual pens for the intensive sampling period and in groups of four thereafter in pens measuring 25.6 x 9.5 m. They were fed forage-based, concentrate-supplemented diets formulated to meet National Research Council (NRC, 1996) recommendation for growing beef heifers. Between experimental periods, heifers had access to pasture and were fed a concentrate supplement to maintain body condition.

Experimental Procedures

All heifers were pre-synchronized before the start of the experiment with a single injection of PGF, 25 mg IM, on d -19 and two injections of PGF, 25 mg IM, 12 h apart on d -9. The Latin square design with four treatments, two replicates and four heifers per replicate consisted of the following treatments beginning on d 0: 1) EB; 2.5 mg EB i.m. in oil on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0; 3) EB + AC-CIDR; EB as in 1 and new autoclaved CIDR on d 0, 4) EB + AC-CIDR + P4; EB as in 1 plus new autoclaved CIDR + 500 mg P4 in oil i.m. at CIDR insertion. Heifers with CIDRs (groups 2 through 4) had them removed on d 7, and were administered an i.m. injection of PGF. A 3 wk washout period followed each successive treatment period in the Latin square. Transrectal ultrasonography (Dynamic Imaging, Concept/MCV, equipped with a dual 5/7.5 MHz linear array probe; Livingston, UK) was

performed on d 9 through ovulation or 120 h after CIDR removal, whichever came first, during each experimental period. Interval to NWFE was determined retrospectively as the interval from treatment to appearance of the 5 mm follicle that subsequently became the dominant follicle. Synchronized NFWE was characterized as occurring between d 2 to 6.

Blood samples were collected via indwelling jugular catheters inserted aseptically on d -1 of each experimental series and maintained patent with heparinized saline locks. Samples were collected at 0, 30, 60, 120, 240 min and 360 min after CIDR insertion or EB (control) and at 6 h intervals thereafter until 60 h in each replicate (Figure 6). Blood samples were placed on ice immediately after collection and remained on ice until transported to the laboratory where they were centrifuged at 1800 x g for 30 min. Plasma was collected and stored at -20 °C until hormone analyses were performed.

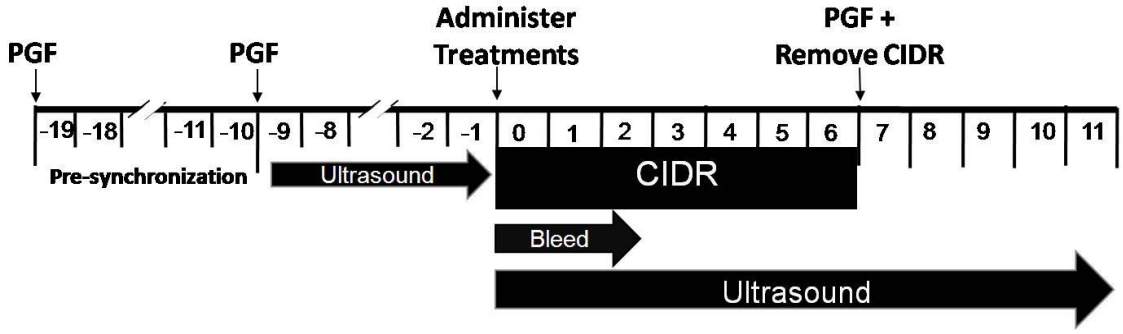

Figure 6. Experimental timeline for experiment 2. Treatments administered on day 0 were 1) EB; 2.5 mg EB i.m. on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0; 3) EB + AC-CIDR; EB as in 1 and new autoclaved CIDR on d 0, 4) EB + AC-CIDR + P4; EB as in 1 plus new autoclaved CIDR + 500 mg P4 i.m. at CIDR insertion. Heifers receiving CIDRs (groups 2 through 4) had the CIDR removed on d 7. Heifers in all groups received an i.m. injection of PGF at the time of CIDR removal. Heifers were examined by ultrasonography daily beginning on d 9 and continuing through ovulation following PGF administration or 120 h.

RIA

Concentrations of hormones were determined by previously validated RIAs. Concentrations of LH were determined using rabbit anti-ovine LH (TEA #35; McVey and Williams, 1989) and bovine LH (AFP11743B; NHPP) as labeled tracer and reference preparation. Concentrations of FSH were determined using rabbit anti-ovine FSH (AFP7711690; Amstalden et al., 2004) and bovine FSH as labeled tracer (AFP5332B; NHPP) and reference preparation (bFSH.ig.1; Tucker Endocrine Research). Concentrations of P4 were determined using a commercial direct RIA, Coat-A-Count (DPC, Los Angeles, CA).

Statistical Analysis

The PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used to evaluate the main effects of treatment on hormone concentrations, interval to NFWE from CIDR insertion and interval to ovulation from CIDR removal.

Results

Experiment 1

Mean (± SEM) BCS, DPP and percent of females cycling at onset of treatments for cattle in reps 1 and 2 were 5.6 ± 0.16 and 6.1 ± 0.12, 70.3 ± 1.26 and 71.5 ± 1.93 d, and 78% and 81%, respectively. Responses of the dominant follicle to EB or GnRH were characterized as ovulated, regressed or other (e.g., cystic, persistent dominant follicle or no dominant follicle at the time of treatment) and are shown in Table 1.

Data were considered in two ways comparing: 1) GnRH- and EB-treated groups using published definitions for synchronized follicle waves associated with GnRH and EB treatments, respectively, and 2) GnRH- and EB-treated groups without regard to

those definitions and including all NFWE up to the time of CIDR removal. There was no replicate x treatment interaction so data from both replicates were pooled. Percentages of GnRH and EB-treated cows exhibiting synchronized NFWE, by dominant follicle response to initial treatment, are shown in Table 2. Synchronized NFWE for GnRH treated animals was characterized as occurring between d 1 to 4. Based on this definition, 53% (17/32) of GnRH-treated females had a synchronized NFWE. Synchronized NFWE for EB-treated cattle was defined as occurring between d 2 to 6. Percentage of females with a synchronized NFWE in the EB treated group was 91% (29/32). Mean intervals (± SEM) to NFWE, frequencies of ovulation after CIDR removal and intervals to ovulation for GnRH- and EB-treated females with a synchronized NFWE were 2.7 ± 0.3 and 4.2 ± 0.2 d, 14/17 and 24/29 and 89.1 ± 6.9 and 102.3 ± 5.5 h, respectively. Distribution of ovulation after CIDR removal was slightly prolonged in EB-treated compared to GnRH treated cattle. None of the females in the EB treated group ovulated before 48 h after CIDR removal (Figure 7).

Mean (± SEM) intervals to NFWE for all GnRH and EB treated females up to the time of CIDR removal (without regard to synchrony definitions) were 3.9 ± 0.33 and 4.2 ± 0.21 d. Percentage of females with a NFWE (synchronized and non-synchronized combined) in the GnRH and EB treated groups were 91% (29/32) for both. Mean intervals (± SEM) to NFWE, frequencies of ovulation after CIDR removal, and intervals to ovulation for all GnRH- and EB-treated females were 3.9 ± 0.3 and 4.2 ± 0.2 d, 23/29 and 26/29 and 95.0 ± 5.9 and 99.8 ± 5.3 h, respectively.

Table 1 Effect of synchronization treatment on distribution of dominant follicle responses

Treatment	Dominant follicle response (n)		
	Ovulated[1] (%)	Regressed[2] (%)	Other[3] (%)
GnRH[4]	15 (46.9)	13 (40.6)	4 (12.5)
EB[5]	0 (0)	23 (71.9)	9 (28.1)

[1] Acute disappearance of the dominant follicle
[2] Serial reduction in diameter of the dominant follicle
[3] Animals with cystic ovaries, persistent dominant follicles, or lacking a dominant follicle at the time of treatment
[4]100 µg GnRH i.m. at insertion of CIDR
[5]2.5 mg EB in oil i.m. at insertion of CIDR

Table 2 Synchronized new follicular wave emergence (NWFE) after treatment with GnRH or EB relative to dominant follicle response category

Treatment	Dominant follicle response category (n)		
	Ovulated[1]	Regressed[2]	Other[3]
	New follicular wave emergence		
	(%)	(%)	(%)
GnRH[4]	10 (66.7)	7 (53.8)	0 (0)
EB[5]	0 (0)	23 (100)	6 (66.7)

[1] Acute disappearance of the dominant follicle
[2] Serial reduction in diameter of the dominant follicle
[3] Animals with cystic ovaries, persistent dominant follicles, or lacking a dominant follicle at the time of treatment
[4]100 µg GnRH i.m. at insertion of CIDR
[5]2.5 mg EB in oil i.m. at insertion of CIDR

Similarly, post-CIDR ovulation frequencies of weaned and suckled cows did not differ due to suckling status (Weaned, 69.2%; Suckled; 85.7%). Intervals to ovulation for GnRH- and EB-treated (95.0 ± 5.9 and 99.8 ± 5.3 h) and for suckled and weaned cows (86 ± 6.9 vs. 96 ± 6.6 h) were similar. Size of the dominant follicle did not differ between GnRH- and EB-treated or suckled and weaned females at the time of CIDR removal, 24 h after CIDR removal, or 48 h after CIDR removal (Figure 8). Mean diameter at ovulation for GnRH, EB, suckled and weaned cattle were 12.6 ± 0.48, 12.2 ± 0.48, 12.6 ± 0.43 and 12.1 ± 0.33 mm, respectively, and did not differ.

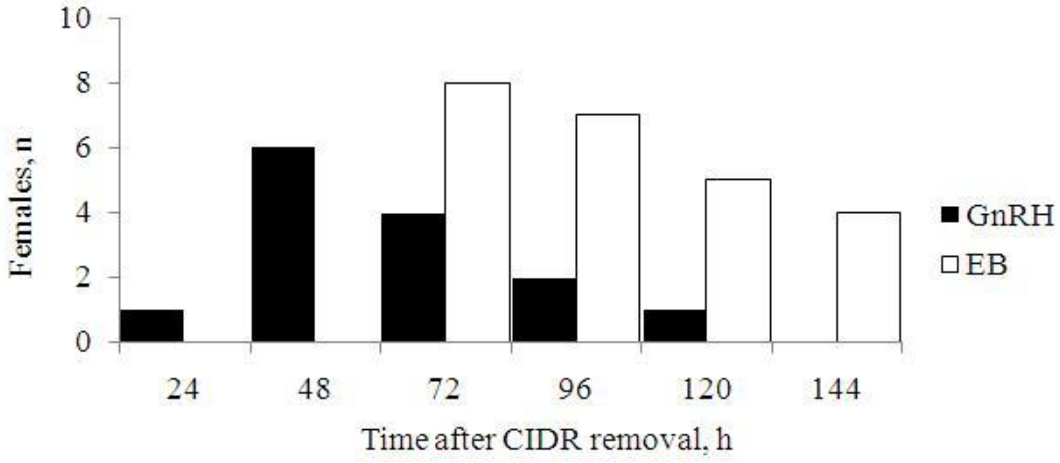

Figure 7. Distribution of synchronized females ovulating in each hormonal treatment group at 24-h intervals after CIDR removal.

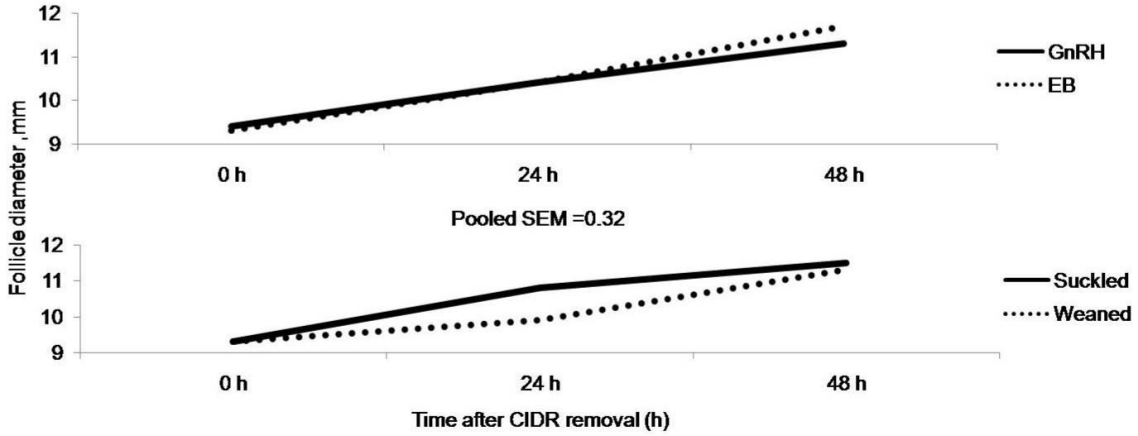

Figure 8. Mean daily follicle diameter of synchronized GnRH vs. EB and suckled vs. weaned cattle for 48 h following CIDR removal.

Experiment 2

Average BCS for heifers was 5.9 ± .13 (range 5.5-6.5). Number of females exhibiting a synchronized NFWE was 8/8, 6/8, 8/8 and 5/8 for groups 1 through 4, respectively, with mean intervals to NFWE for each of the groups of 4.8 ± 0.33, 5.2 ± 0.38, 4.6 ± 0.33, 5.2 ± 0.42 d which did not differ. Number of females in groups 1 through 4 with synchronized NFWE that ovulated after CIDR removal and PGF or PGF only, was 7/8, 6/6, 6/8, and 3/5, respectively. Mean intervals to ovulation were similar for all groups (91 ± 7, 104 ± 7, 109 ± 7 and 110 ± 9 h).

Overall mean serum concentrations of P4 (ng/ml) for the first 60 h of the experiment for the four treatment groups are presented in Figure 9. Differential effects ($P<0.0001$) were observed, with treatment 4 (CIDR + 500 mg P4) creating the greatest increase of longest duration ($P<0.0001$) compared to all others. Treatments 2 and 3 did

not differ and were intermediate to 1 (EB only) and 4, with 1 having concentrations less than all other groups throughout the sampling period.

Overall mean serum LH for the first 60 h of the experiment are depicted in Figure 10. Mean (± SEM) serum concentrations of LH (ng/ml) between 12 and 24 h after onset of treatments and sampling (when LH was expected to be maximally suppressed) were 0.38 ± 0.05, 0.41± 0.05, 0.35 ± 0.05 and 0.32 ± 0.05, for treatments 1 through 4, respectively. A time effect, reflecting suppression of LH, was observed in all treatment groups ($P<0.0005$). Treatment 4 had the greatest suppressive effect compared to treatments 1 ($P<0.01$) and 2 ($P<0.0007$), but this difference was not as consistent ($P<0.2$) relative to treatment 3.

Overall mean serum concentrations of FSH for the first 60 h after onset of treatments is shown in Figure 11. Mean (± SEM) serum concentrations of FSH (ng/ml) for the period between 24 and 48 h (when suppression of FSH was expected to be greatest) were 1.8 ± 0.2, 1.8 ± 0.2, 1.6 ± 0.2 and 1.4 ± 0.2 for treatments 1 through 4, respectively. Treatment 4 caused the greatest suppression of FSH during this time period compared to all other groups ($P<0.08$)

Mean (± SEM) sizes (mm) of ovulatory follicles for treatments 1 through 4 were 10.9 ± 0.63, 10.1 ± 0.62, 10.6 ± 0.90 and 11.1 ± .71 mm, respectively. These means include data from heifers with synchronized NWFE, as well as those whose NFWE fell outside of the synchronized range, but which ovulated before the end of the experimental period. Mean (± SEM) sizes of the dominant follicle from time of CIDR removal through d 12 for treatments 3 and 4, or d 11 for treatments 1 and 2 are shown in Figure

12. Mean (± SEM) diameters (mm), of the ovulatory follicle for treatments 1 – 4 were 10.9 ± 0.63, 10.1 ± 0.62, 10.6 ± 0.90 and 11.1 ± .71 mm respectively.

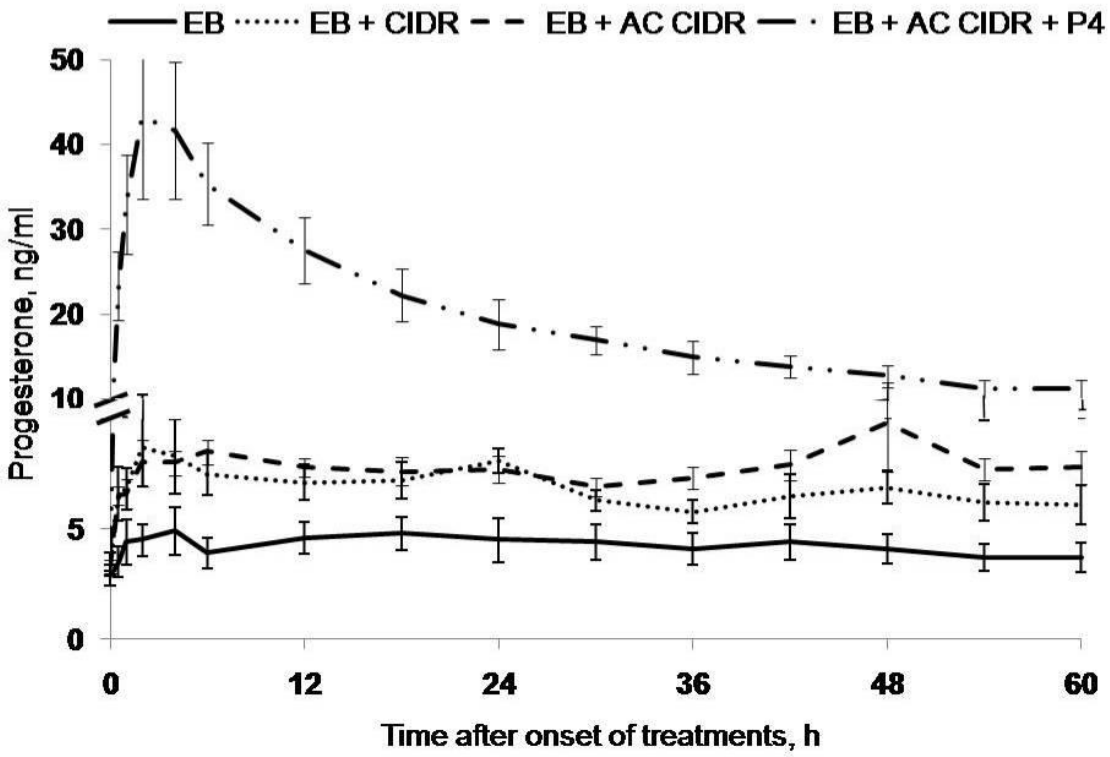

Figure 9. Mean (± SEM) concentrations of serum P4 from 0 to 60 h after the onset of treatments. Overall means for treatments 1 through 4 (1) EB; 2.5 mg EB i.m. on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0; 3) EB + AC-CIDR; EB as in 1 and new autoclaved CIDR on d 0, 4) EB + AC-CIDR + P4) were 4.1 ± 1.1, 6.8± 1.1, 7.4 ± 1.1 and 21.9 ± 1.1 ng/ml, respectively.

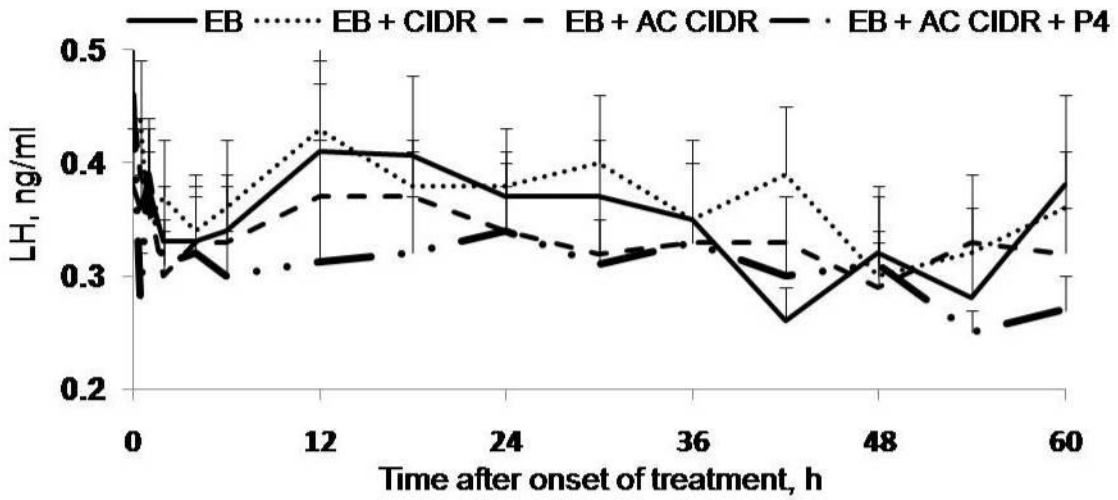

Figure 10. Mean concentrations of serum LH from 0 to 60 h after the onset of treatments. Overall means for treatments 1 through 4 (1) EB; 2.5 mg EB i.m. on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0; 3) EB + AC-CIDR; EB as in 1 and new autoclaved CIDR on d 0, 4) EB + AC-CIDR + P4) were 0.35 ± .05, 0.37 ± 0.05, 0.35 ± .05 and 0.32 ± 0.05 ng/ml, respectively.

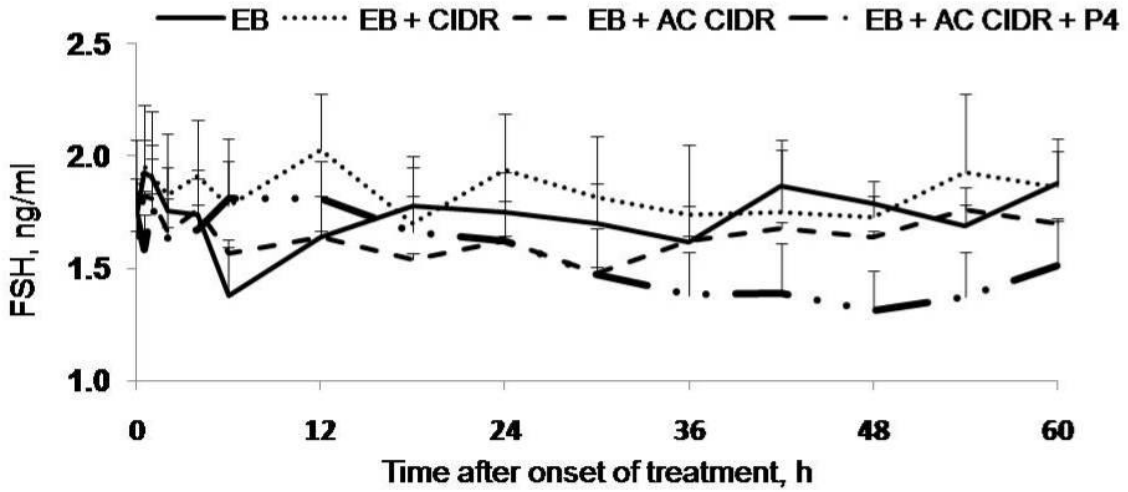

Figure 11. Mean concentrations of serum FSH from 0 to 60 h after the onset of treatments. Overall mean serum FSH for treatments 1 through 4 (1) EB; 2.5 mg EB i.m. on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0; 3) EB + AC-CIDR; EB as in 1 and new autoclaved CIDR on d 0, 4) EB + AC-CIDR + P4) were 1.75 ± 0.24, 1.85 ± 0.24, 1.67 ± 0.24 and 1.58 ± 0.24 ng/ml, respectively.

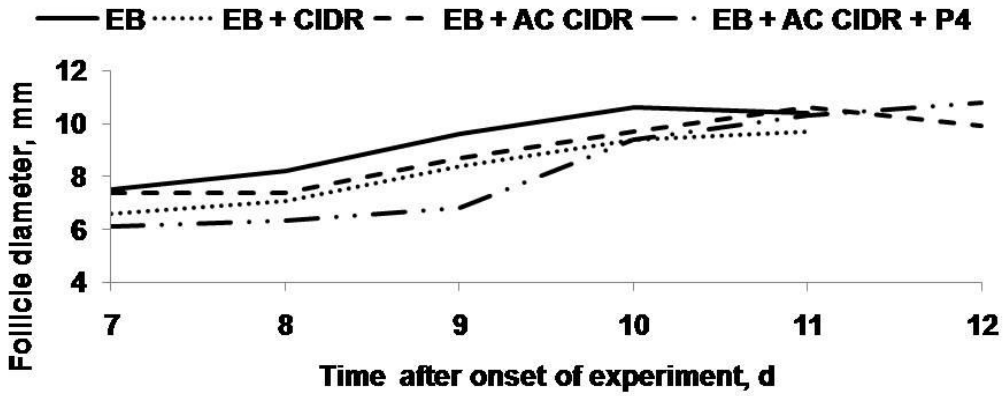

Figure 12. Mean diameter of the dominant follicle from CIDR removal through d 11 or 12 for treatments 1 through 4. (1) EB; 2.5 mg EB i.m. on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0; 3) EB + AC-CIDR; EB as in 1 and new autoclaved CIDR on d 0, 4) EB + AC-CIDR + P4). Data is reported through d 11 for heifers in treatments 1 and 2 (1) EB; 2.5 mg EB i.m. on d 0 and 25 mg PGF i.m. on d 7; 2) EB + CIDR; EB as in 1 and new CIDR on d 0) due to all but one heifer from each group ovulating on or before d 12.

Discussion

Objectives of the studies presented here were to test the effectiveness of three modifications designed to either increase control of NFWE or rate of follicle maturation in a CIDR-based estrus synchronization protocol. Control of NFWE is one of the most important factors determining success of synchronization in conjunction with regulating lifespan of the CL.

One modification compared the efficacy of EB vs. GnRH in a Co-Synch + CIDR protocol for controlling the synchrony of the NFWE (Exp. 1). Based upon historically-defined definitions of synchronized follicle waves induced by GnRH and EBG, respectively (Kesler, 2005; Kim et al., 2005; Saldarriaga, 2006), EB improved synchrony of NFWE compared to GnRH, as overall, 91 and 53% of animals respectively exhibited synchronized NFWE. In the current study, 46.9% of randomly-cycling cattle ovulated the dominant follicle in response to GnRH treatment. In studies involving *Bos indicus*-influenced cattle (Saldarriaga, 2006; Zuluaga et al., 2007), the percentages of randomly-cycling cattle at this same location that ovulated after GnRH treatment were 40 and 43%, respectively. In contrast, Pursley et al. (1995) reported that as few as 60% and as great as 80% of randomly-cycling *Bos taurus* females ovulate in response to a single injection of GnRH.

In the current study for those cattle treated with GnRH in which the dominant follicle responded by either ovulation or regression 66.7 and 53.8% had a synchronized NFW. These results tend to agree with those from Saldarriaga (2006) which indicate that a greater proportion of cows that ovulate after GnRH, as opposed to regression or

other response, will develop a synchronized NFW. This is in contrast to Twagiramungu et al. (1994) who reported that both ovulation and follicular regression to GnRH were equally effective for inducing synchronized NFWE. Of females treated with GnRH responding by ovulation, 87% had a subsequent synchronized NFWE. These results are similar to those obtained by Saldarriaga (2006) and Zuluaga (2006) who, using similar cattle at the same location, observed 86% and 88% of females, respectively, exhibiting NFWE after GnRH-induced ovulation.

Ability of a follicle to ovulate depends upon several factors, including day of the estrous cycle (Vasconselos et al., 1999; Moreira et al., 2000) and peripheral P4 concentrations (Moreira et al., 2000) at the time of GnRH administration. Vasconselos et al. (1999) administered GnRH to *Bos taurus* cows on d 1 to 4, 5 to 9, 10 to 16, or 17 to 21. They found that only 23% of cattle ovulated to GnRH administered on d 1 to 4 of the cycle, whereas 96% ovulated at d 5 to 9, 54% ovulated at 10 to 16 and 77% ovulated at d 17 to 21. Of cattle with synchronized NFWE in that study, 68% had ovulated in response to GnRH. In the current study, the percentage of females with NFWE after ovulating to GnRH was slightly higher, at 86.7%, than the overall rate reported by Vasconselos et al. (1999). Moreira et al. (2000) conducted an experiment similar to Vasconcelos et al. (1999) in which *Bos taurus* heifers were treated with GnRH. Ovulation rates for that experiment were 0, 100, 25, 60 and 100% for d 2, 5, 10, 15 and 18, respectively. Overall ovulation rate to GnRH was reported as 58.3%, which is less than the overall rate observed in both the current experiment and Vasconselos et al. (1999). A rationale for explaining these results is the size of the dominant follicle at the

time of GnRH administration. In both Vasconselos et al. (1999) and Moreira et al. (2000), cattle treated on d 1 to 4 and 10 to 16 had lower ovulation rates than did cattle treated on d 5 to 9 or 17 to 21. High serum concentrations of P4 decrease the frequency of LH pulses (reviewed by Karsch, 1987). An LH pulse frequency typical of the follicular phase of the estrous cycle is required for final maturation of the follicle for ovulation. This may explain the lower ovulation in response to GnRH during d 5 to 9 of the estrous cycle.

Cattle responding to GnRH by regression of the dominant follicle in Experiment 1 exhibited a synchronized NFWE at a rate of 62%. This is slightly less than results from the work of Zuluaga (2006), who found that 96% of *Bos indicus*-influenced females at this location exhibiting follicle regression to GnRH subsequently developed a synchronized NFW. Dominant follicle selection begins again after NFWE and the selected follicle eventually becomes the ovulatory follicle after PGF administration (Twagiramungu et al., 1995). Interval to NFWE for GnRH- treated cattle was 2.7 ± 0.2d in this experiment which falls within the expected range based on published data (Bo et al., 1995b; Kesler, 2005; Kim et al., 2005;).

Estradiol benzoate treatment resulted in regression of the dominant follicle in 72% of cattle in Experiment 1. Of those responding by regression, 100% had a synchronized NFWE, defined for this treatment as occurring between d 2 and 6 d after treatment onset. Bo et al. (1994) also reported that 100% of cattle treated with estradiol valerate had a synchronized NFW. Estradiol causes follicular regression through the suppression of FSH (Bo et al., 1993; O'Rourke et al., 2000; Colazo et al., 2005). When

the inhibitory effect of estradiol is removed, FSH concentrations in the circulation rise, precipitating NFWE (Adams et al., 1992a) approximately 1 d later (Bo et al., 1993, 1994). In studies conducted using *Bos taurus* females, Bo et al. (1995a) found that the interval to NFWE from the time of administration of 5 mg estradiol-17β averaged 4.1 to 4.7 d depending on the day of the cycle on which the estradiol-17β was administered. Colazo et al. (2005), also using *Bos taurus* females, found that the interval to NFWE ranged from 2 to 5 d for heifers treated with 2 mg of estradiol valerate and 3 to 7 d when given 5 mg. This demonstrated that interval to NFWE can be affected by the dose of estradiol administered. Estradiol benzoate was administered at a rate of 2.5 mg in this experiment, so the criteria of 2 to 6 d for NFWE and the mean NWFE of 4.2 d agrees with the data of both Bo et al. (1995a) and Colazo et al. (2005).

Of equal importance to synchronous NFWE in a TAI program is a synchronous ovulation after PGF. Temporary calf removal stimulates an increase in frequency of LH pulses (reviewed by Williams, 1990) leading to final maturation of the ovulatory follicle. We had anticipated that there would be an increase in the size of the dominant follicle in weaned vs. suckled cattle in both hormonal treatment groups, which in turn would allow for a more synchronous ovulation. In Experiment 1, the size of the dominant follicle at 48 h after calf removal averaged 11.3 ± .32 and 11.5 ± .32 mm in temporarily weaned and. suckled females, respectively. From these data, it would appear that temporary calf removal, while capable of increasing synchrony of follicle development/maturation in post-partum anestrous cattle, may not afford a measurable and consistent influence beyond that created earlier as a result of pharmacological interventions with GnRH and

EB. These results concur with those of Geary et al. (2001b) who, using *Bos taurus* cattle, reported that when using the Co-Synch protocol conception rates to AI were not improved with 48 h calf removal. Contrary to Geary et al. (2001b), in a separate study Geary et al. (2001) found that 48 h calf removal improved conception rates to TAI by 9 percentage points in both the CO-Synch and Ovsynch protocols. These studies were not conducted using additional progesterone to synchronize females. Results similar to ours were found by Rivera et al. (1998) who reported no difference in ovulation rate or maximum follicle size in cattle treated with calf removal only, CIDR and calf removal or CIDR with calf removal and E-17β. It can be surmised from this information that while calf removal has been shown to improve synchrony in some cases, overall it does not provide a consistent improvement in synchrony. It is therefore not a highly effective option for most producers, especially when the addition of progesterone to a synchronization protocol, a much less labor intensive practice, has demonstrated the ability to increase TAI conception rates to over 50% in *Bos taurus* cattle.

Size of the dominant follicle in Experiment 1 remained similar through the time of ovulation in both hormonal treatment groups, as well as in suckled and weaned females. The mean ovulatory follicle diameter 12.9 ± 0.3 mm reported by Saldarriaga (2006) was similar to those observed in the current study (12.6 ± 0.48, 12.2 ± 0.48, 12.6 ± 0.43 and 12.1 ± 0.33 mm for GnRH, EB, suckled and weaned cattle, respectively). Saldarriaga (2006) reported an interval to ovulation of 99 ± 2.8 h in cows synchronized with SelectSynch + CIDR which was slightly greater than the interval observed in Experiment 1 for GnRH treated cattle (89.1 ± 6.9).

For Experiment 2, we tested the effect of incremental, acute increases in P4 on degree of suppression of FSH and LH secretion, magnitude and timing of FSH resurgence, and synchrony of NFWE in heifers synchronized with EB and CIDR. Zuluaga et al. (2007) showed that autoclaving and re-using CIDRs resulted in greater peak circulating concentrations of P4 in ovariectomized cows than did new or re-used disinfected CIDRs. We therefore assumed that autoclaving new CIDRs would produce similar results. New autoclaved CIDRs did not result in greater P4 concentrations than un-treated new CIDRs. As a result, we only created two functionally different circulating concentrations of P4 compared to EB alone rather than three.

Treatment 4 (EB + AC CIDR + P4) created the greatest increase in P4 which remained elevated for the longest duration, as expected. Suppression of FSH was greatest for this treatment, with greatest suppression for all treatments occurring between 24 to 48 h. Interval from treatment to NFWE ranged between 4.6 and 5.2 d. New follicular wave emergence is initiated approximately 1 d after the onset of the FSH resurgence (Bo et al., 1993, 1994; Martinez et al., 2007). Since plasma FSH concentrations in this experiment were suppressed maximally between 24 and 48 h after treatment, a resurgence of FSH would be expected to begin approximately on d 2 to 3, with NFWE occurring on d 3 to 4. Our results agreed with this expectation.

Related studies have investigated both dose and type of estrogen in regards to timing of FSH resurgence. Colazo et al. (2005) found that cattle treated with 2 or 5 mg of estradiol valerate, an estrogen conjugate with longer half-life than EB, had mean intervals to NFWE of 3.4 and 4.8 d, respectively, when administered in conjunction with

a CIDR. In an earlier study, Colazo et al. (2003) treated cattle with 1 mg estradiol cypionate and 50 mg progesterone at the time of CIDR insertion, with a resulting interval to NFWE of 4 d. However, treatment with 5mg estradiol-17β, with no additional progesterone, resulted in a shorter interval of 3.4 d (Martinez et al., 2000) which is similar to results from Colazo et al. (2005) using a low dose of long acting estradiol valerate. These results confirm that estradiol given in conjunction with progesterone delays the NFWE when compared to estradiol alone, and suggest that similar NFWE results can be obtained by a variety of estradiol conjugates.

The number of heifers in Experiment 2 with a synchronized NFWE, based on our definition, were 8/8, 6/8, 8/8 and 5/8 animals in groups 1 through 4 respectively. However, one heifer in group 2 and 3 heifers in group 4 presented with NFWE between d 6 and 9. The group with the fewest number of synchronized NFWE was treatment 4 (EB + AC CIDR + P4). As expected, this group had the greatest circulating concentrations of P4, maximally suppressing both FSH and LH, and delaying NFWE in 3/8 heifers beyond d 6. If a synchronized NFW for this group was defined as occurring between d 2 and 7 as done by Colazo et al. (2003), then two additional heifers would have been considered as having synchronized NFWE. Although heifers receiving a CIDR ovulated later than those receiving EB alone, intervals to ovulation among groups receiving a CIDR did not differ when defining a synchronized NFWE as occurring between d 2 and 6. Thus, the addition of an injection of 500 mg P4 in the current study appeared to delay NFWE well beyond the time desired and created greater variation, based on a limited number of observations. In groups 1 through 3, which resulted in

circulating P4 closer to the conventional ranges typically produced, growth of the new follicle wave remained similar, including size of the dominant follicle at CIDR removal. In contrast, heifers in group 4 had a smaller dominant follicle at CIDR removal than did those in groups 1 or 3. The latter observation would be expected due to the enhanced suppression of FSH created by this treatment. However, ovulatory follicle sizes did not differ between any of the groups. Similarly, Colazo et al. (2003) found that ovulatory follicle sizes did not differ between cattle receiving a CIDR and estradiol cypionate or a CIDR and estradiol cypionate plus 50 mg P4.

CHAPTER IV

SUMMARY AND CONCLUSIONS

From this series of experiments, it can be postulated that EB may be superior to GnRH for creating a synchronized NFW in CIDR-based protocols in Brahman-influenced females, based on traditional definitions. However, overall intervals to NFWE and variation association with maturation and potential ovulation of NFW did not differ appreciably from a practical perspective. The addition of 48 h calf removal at the time of CIDR withdrawal, although shown to be effective in some previous experiments, did not improve synchrony of follicular maturation or increase the proportion of females ovulating. While is has been shown that autoclaving once-used CIDRs results in greater initial concentrations of P4, autoclaving new CIDRs does not result in circulating concentrations of P4 greater than new CIDRs without autoclaving. Acutely increasing circulating P4 to > 40 ng/mL (treatment 4; EB + AC CIDR + P4, experiment 2), suppressed both LH and FSH to a greater extent than all other treatments; however, this degree of suppression likely exceeded that which is desirable because frequency and synchrony of NFWE in this study were not improved. All CIDR-based treatments delayed ovulation compared to EB in females exhibiting a synchronized NFWE between d 2 and 6 (experiment 2).

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APPENDIX

LABORATORY PROCEDURES

Luteinizing Hormone RIA

1. Iodination: Iodination grade bLH (AFP11743B; NHPP)
   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: bLH (AFP11743B; NHPP) Range: 0.1 -30 ng/ml

4. Reference preparation: bLH added to cow serum

5. RIA procedure
(Williams and Ray 1980)

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

   Pipette 200 µl PBS-EDTA + 1:400 NRS without 1st Ab into NSB tubes
   Pipette 200 µl anti-oLH (diluted in PBS-EDTA + 1:400 NRS) into all tubes except NSB and TC tubes
   Vortex briefly and incubate for 2 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) Day 2: Pipette 200 µl of sheep-anti-rabbit gamma globulin (SARGG) diluted in PBS-EDTA into all tubes except TC
   Vortex and incubate 48-72 h at 4° C
d) Day 4: Add 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 with gamma counter

Follicle Stimulating Hormone RIA

1. Iodination: Iodination grade oFSH (oFSH-I-1; AFP-5679C)
   Reaction: 5 µg of hormone, 0.5 mCi of 125I, 18 µg chloramine T, 1 min

2. Antibody: Anti-ovine FSH (rabbit anti-oFSH – AFPc528813; NHPP)
   Dilution: 1:12,000

3. Standards: bFSH (bFSH.ig.1; Tucker Endocrine Research)
   Range: 1.0 -500 ng/ml

4. Reference preparation: bFSH (bFSH.ig.1; Tucker Endocrine Research) added to cow serum

5. RIA procedure

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

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

   c) Day 2: Pipette 200 µl of goat-anti-rabbit gamma globulin (GARGG) diluted in PBS-EDTA into all tubes except TC
      Vortex and incubate 48-72 h at 4° C
   d) Day 4: Add 1 ml ice-cold 0.01M PBS into all tubes except TC
      Add 200 µl of PEG to all tubes except TC
      Centrifuge tubes for 1 h at 3600 rpm at 4°C
      Aspirate supernatant
      Count radioactivity with 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. Conduct assay
      1) Pipette in non-coated polypropylene tubes
         NSB – 100 µl of 0 std
      2) Pipette in antibody coated tubes
         0 Std – 100 µl
         Std – 100 µl
         Ref – 100 µl
         Unknowns – 100 µl
      3) Pipette 1 ml of ^{125}I-P4 provided in the kit into all tubes including three Total Count non-coated polypropylene tubes.
      4) Vortex tubes briefly and incubate at room temperature for 3 h.
      5) Pour off supernatant.
      6) Count radioactivity using gamma counter.

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Selected Publications

Pack, J.D., Velez, I.C., Amstalden, M., Williams, G.L., 2008. Synchronizing new follicular wave emergence in Bos indicus-influenced heifers with estradiol benzoate: Role of the magnitude of the acute increase in progesterone. Proceedings American Society of Animal Science, Annual Meeting.

Pack, J.D., Velez, I.C., Amstalden, M., Williams, G.L., 2008. Substitution of estradiol benzoate for GnRH in the Select Synch + CIDR protocol with or without temporary calf removal in Bos indicus-influenced cattle. Proceedings American Society of Animal Science, Annual Meeting.