PRE- AND POST-SYNCHRONIZATION METHODOLOGIES TO ENHANCE THE EFFICIENCY OF FIXED TIMED ARTIFICIAL INSEMINATION IN PHARMACOLOGICALLY-CONTROLLED BREEDING SYSTEMS WITH *BOS INDICUS*-INFLUENCED CATTLE

A Thesis

by

JUAN FEDERICO ZULUAGA VELEZ

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 2006

Major Subject: Physiology of Reproduction

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Approved by:

Chair of Committee, Gary L. Williams
Committee Members, Steven P. Brinsko
Thomas H. Welsh, Jr.
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ABSTRACT

Pre- and Post-Synchronization Methodologies to Enhance the Efficiency of Fixed Timed Artificial Insemination in Pharmacologically-Controlled Breeding Systems with *Bos indicus*-Influenced Cattle. (December 2006)

Juan Federico Zuluaga Velez

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Chair of Advisory Committee: Dr. Gary L. Williams

Objectives were to: 1) Evaluate the effectiveness of presynchronization with GnRH before the CO-Synch + CIDR protocol with timed AI (TAI) at 66 h in *Bos indicus*-influenced cattle; 2) Characterize ovarian events associated with the presynchronization; 3) Evaluate the efficacy of measuring vaginal electrical resistance (VER) to assess follicular maturity at TAI; and 4) Compare serum concentrations of progesterone (P4) in ovariectomized cows bearing new or previously used CIDR devices with or without autoclaving. In Exp. 1 and 2, cattle received either GnRH or saline on day -7. The CO-Synch + CIDR protocol included a CIDR insert and GnRH (GnRH-1; day 0), removal of CIDR and PGF2α on day 7, and GnRH (GnRH-2) and TAI 66 h after CIDR removal. In Exp. 1, pregnancy rate of females with BCS ≥ 5 tended to differ (P=0.085) between

Presynch (38%) and CO-Synch + CIDR (54%). In Exp. 2, ovulatory response to GnRH-1 was greater (P<0.01) in the Presynchronization (58%) than in the CO-Synch + CIDR (27.1%) group. Emergence of a follicular wave after GnRH-1 and ovulation rate after GnRH-2 did not differ between groups. More (P<0.01) females that developed a follicular wave after GnRH-1 ovulated (82%) after GnRH-2, compared to those that did not (29%). Mean VER (ohms) was greatest (101.4±0.8) on day 0 and declined (P<0.01) to 95.2±0.8 and 82±0.8, respectively, on days 7 and 10. We observed a low negative but significant relationship (r=0.38; P<0.001) between VER and follicular size on day 0, 7, and 10. VER difference (day 10 minus day 7) did not differ between females with small and large follicles at TAI. Mean concentrations of P4 during the 7-day insertion period were greater (P<0.03) for new (3.7 ng/ml) and re-used autoclaved (3.4 ng/ml) than for re-used disinfected CIDRs (2.8 ng/ml). In summary, Presynch improved ovulation rate after GnRH-1, but did not improve pregnancy rates compared to CO-Synch + CIDR. Follicular maturity estimation was not feasible using VER as applied in this study. Autoclaving may be the best option when re-using CIDR inserts because it creates greater concentrations of P4 during the first 48 h.

Dedicated to my parents and grandparents

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CHAPTER I

INTRODUCTION

Artificial insemination (AI) is the main vehicle for rapid dispersal of valuable genes and genetic advancement in cattle populations (Vishwanath, 2003). Currently, only 5-7% of the beef cows in United States are inseminated artificially (NAHMS, 1997). Nonetheless, it is predicted that the practice of AI in pure-bred and commercial beef cattle operations will increase with the introduction of sexed semen (Hohenboken, 1999; Seidel, 2003). The main reasons for the low rate of adoption of AI among beef producers are the problems of accurate detection of estrus and the poor and inconsistent results of the protocols used to synchronize the onset of estrus and/or ovulation.

The rationale of any estrus and ovulation synchronization protocol for beef or dairy cattle is to induce ovulations at a predictable time so that all of the females can be inseminated en masse at a chosen time, and referred to as timed artificial insemination (TAI).

This thesis follows the style and form of Animal Reproduction Science.

Many different estrus synchronization protocols provide good synchronization of estrus (Odde, 1990). However, only a few provide effective synchronization of ovulation or a high fertilization rate to TAI (Martinez et al., 2002a; Perry et al, 2002). A variation of the Ovsynch protocol (Pursley et al., 1994; Schmitt et al. 1996a), which includes the utilization of prostaglandin F2α (PGF) and gonadotropin-releasing hormone (GnRH), was reported by Geary and Whittier (1998). Termed CO-Synch, this approach requires the administration of GnRH concurrently with TAI. The CO-Synch protocol required one less handling of animals but resulted in lower pregnancy rates than the Ovsynch protocol in beef cows (Geary and Whittier, 1998). Later the addition of a progesterone source (controlled internal drug release device; CIDR®) to the CO-Synch protocol yielded a treatment (CO-Synch + CIDR) that produced higher pregnancy rates than CO-Synch alone in *Bos taurus* cows (Lamb et al., 2001) and heifers (Martinez et al., 2002b). After the 7-day insertion period, CIDR inserts still contain adequate progesterone to suppress estrus. No difference was reported in the pregnancy rate to fixed-time AI between cattle synchronized with a new or once-used CIDR insert (Colazo et al., 2004a). Therefore, methodologies for exploiting re-used CIDRs in the most effective

manner should be examined, including the effects of sterilization or disinfection methods.

Previous studies in our laboratory have demonstrated that the effectiveness of CO-Synch + CIDR in *Bos indicus* influenced cows and heifers is lower (< 40%) than reported for *Bos taurus* females (> 50%; Johnson, 2005). Our long-term objective is to achieve a conception rate > 50% consistently. The basis of the lower results in *Bos indicus*-influenced cattle is that the CO-Synch treatment fails to induce a synchronized follicular wave in a high proportion of females after the first injection of GnRH (Saldarriaga et al., 2006). Additional attempts to improve the effectiveness of this protocol have been made using PGF (El-Zarkouny et al., 2004) or GnRH (Kojima et al., 2000a; DeJarnette et al., 2001) as a presynchronization treatment, with the intent of improving the responsiveness to GnRH at the beginning of the synchronization treatment. However, there are no reports on use or efficacy of a GnRH presynchronization treatment with CO-Synch + CIDR in beef cattle with *Bos indicus* genetic influence.

Given the limited effectiveness of currently available protocols for synchronization of ovulation and TAI in *Bos indicus*-influenced cattle, an additional strategy might be to develop methods for indirectly assessing

synchronization success before TAI. If this were possible, then females that were not synchronized would not be inseminated, thus saving the semen investment costs for cattle unlikely to conceive. Several electronic devices have been developed to alleviate the need for visual estrus detection in cattle and to provide a precise determination of the onset of estrus. Vaginal electrical resistance (VER) has been measured and used as an aid in estrus detection and for timing of insemination in cattle (Schams et al., 1977). The VER fluctuates with stage of the estrous cycle, is highest during the luteal phase, and declines during the follicular phase. The lowest VER values are correlated with the preovulatory LH peak (Canfield and Butler, 1989). Therefore, use of VER as a decision tool for suitability for TAI needs to be investigated. Collectively, the use of multiple strategies for improving the economic efficiency of the synchronization/TAI process should be investigated. These might include improvements in the synchronization process, methods for maximizing the value of re-used CIDRs, and detecting synchronization failure could contribute to improved economic efficiencies for cattlemen managing *Bos indicus*-influenced females.

Objectives of studies reported here were to 1) Evaluate the effectiveness of presynchronization with GnRH before the CO-Synch + CIDR protocol with

TAI at 66 h in crossbred cattle in south Texas, 2) Characterize ovarian events associated with the presynchronization and CO-Synch + CIDR protocol, 3) Evaluate the efficacy of measuring VER to identify cows that do not have an ovulatory follicle at TAI, and 4) Compare serum concentrations of progesterone in ovariectomized cows bearing new or previously used CIDR devices with or without autoclaving.

CHAPTER II

LITERATURE REVIEW

The Estrous Cycle

The bovine estrous cycle is regulated by endocrine and neuroendocrine mechanisms that encompass orchestrated secretions from the hypothalamic-pituitary-gonadal axis and the uterus. The estrous cycle is divided into luteal (metestrus – diestrus) and follicular phases (proestrus – estrus). During each of these phases gonadotropins influence folliculogenesis, follicular dynamics, ovulation and ovarian steroidogenesis.

Ovarian follicular dynamics is the process of incessant growing and atresia of follicles that leads to the development of an ovulatory follicle derived from the last follicular wave at luteal regression. In the bovine, follicular growth during the estrous cycle is characterized by the development of two, three or four follicular waves (Savio et al. 1988; Sirois and Fortune, 1988).

The terms recruitment, selection, dominance, and deviation are used to describe different phases of each follicular wave during the estrous cycle (Ireland, 1987; Lucy et al., 1992; Fortune, 1994). Recruitment is a process

whereby a cohort of follicles begins to mature in a milieu of sufficient pituitary gonadotropic stimulation to permit progress toward ovulation. Selection is the process by which a single follicle is chosen and avoids atresia with the potential competence to achieve ovulation. Dominance is the means by which the selected follicle dominates through inhibition of recruitment of a new cohort of follicles (Lucy et al., 1992). Deviation is defined as the beginning of the greatest difference in growth rates between the two (dominant and subordinate) largest follicles (Ginther et al., 1996b; Ireland et al., 2000).

Follicular Growth

Follicular growth begins during fetal life when primordial follicles are established on the ovary. There are approximately 156.000 primordial follicles in the bovine ovary at birth, but this number is reduced to approximately 3,000 by the age of 20 (Erickson, 1996). The precise mechanisms controlling the initiation and the number of primordial follicles that start to grow are still not known. However, their growth probably depends on the presence of oocyte/granulosa cell interactions and the secretion of a range of local growth factors (GDF-9, BMP, EGF), activins and inhibins (reviewed in Webb et al., 2003; 2004). The primordial follicles develop through primary and secondary stages before acquiring an antral cavity. At the antral stage, most follicles undergo atretic

degeneration, whereas a few follicles from the original store of follicles will ovulate. During follicular activation (primordial to primary), granulosa cells change in shape from flattened to cuboidal and the zona pellucida forms (Van Wezel and Rodgers, 1996). The transition from primary to secondary follicle is characterized by granulosa cell proliferation and a rapid increase in the size of the oocyte (Webb et al., 2003). A critical point for the initiation of oocyte growth has been estimated to occur in bovine follicles when they contain approximately 40 granulosa cells (McNatty et al., 1999). Transition to the antral stage is characterized by the development of fluid-filled spaces that gradually coalesce to form a single antral cavity (Knight and Glister, 2006). In the bovine, the antrum formation occurs when the follicle is 0.14 to 0.28 mm, and it takes approximately 41.5 days (two estrous cycles) to grow from 0.13 to 8.56 mm (Lussier et al., 1987). Follicular growth of preantral follicles is thought to be independent of gonadotropins, although studies in vivo and in vitro have demonstrated that FSH can accelerate the rate of preantral follicle development (Webb et al., 2003). Antral follicle growth is, beginning at approximately 2 mm in diameter, under gonadotropic control and the action of numerous locally-produced growth factors that enhance the actions of the gonadotropins (Webb et al., 2004).

Follicular Waves

The wave-like pattern of follicular growth during the bovine estrous cycle was first proposed by Rajakoski (1960), based on histological studies. Pierson and Ginther (1984) were the first to use ultrasonography to monitor follicular diameters during the estrous cycle of heifers and provided data that supported the postulate of two waves of follicular growth during the estrous cycle (Pierson and Ginther, 1987).

Reports of the number of follicular waves during the estrous cycle have differed between studies, but indicate a high incidence of either two-wave (Rajamahendran and Taylor, 1990) or three-wave cycles (Sirois and Fortune, 1988). Follicular waves begin around day 2 and 11 of the estrus cycle in heifers with two waves and around day 2, 9 and 16 in heifers with 3 waves (Sirois and Fortune, 1988). Each follicular wave is preceded by a surge in circulating FSH 1 or 2 days earlier (Adams et al., 1992; Ginther et al., 1996a). The corpus luteum (CL) begins to regress earlier in two-wave cycles (day 16) than in three-wave cycles (day 19), resulting in a correspondingly shorter estrous cycle (Adams, 1999).

The *Bos Indicus* Female

Traditionally, a majority of studies in ovarian follicular dynamics has used the *Bos taurus* female, with a great emphasis on the Holstein breed, as a model (Pierson and Ginther, 1987; Sirois and Fortune, 1988; Rajamahendran and Taylor, 1990; Adams et al., 1992). Beef breeds have also been used (Savio et al., 1988; Sunderland et al., 1994; Martinez et al., 1999). In the last 10 years, a number of studies have characterized follicular dynamics in *Bos indicus* cattle (Rodhes et al., 1995; Zeitoun et al., 1996; Figueiredo et al., 1997; Ruiz-Cortez and Olivera-Angel, 1999; Alvarez et al., 2000; Henao et al., 2000; Viana et al., 2000; Borges et al., 2001, 2004; Sartorelli et al., 2005; Castilho et al. 2006). In general, the dynamics of follicular waves are similar to those of *Bos taurus*. However, dominant follicle (Rodhes et al., 1995; Figueiredo et al., 1997; Viana et al., 2000; Borges et al., 2004) and CL diameters (Rhodes et al., 1995; Figueiredo et al., 1997) have consistently been found to be smaller when compared with those of *Bos taurus*. Similar to *Bos taurus* breeds, the number of follicular waves per cycle in *Bos indicus* varies. Figuerido et al. (1997) found estrous cycles characterized by 2 and 3 follicular waves in Nelore cows and heifers, respectively. Moreover, Borges et al. (2004) found a greater frequency of estrous cycles with three follicular waves in Gyr (68%) and Nelore (67%) cows. Follicular dynamics in Gyr

cattle are characterized by a greater incidence of cycles with three or four waves and are associated with low persistence of the dominant follicle (Viana et al., 2000). Some studies with Brahman cattle have reported a majority of estrous cycles characterized by two (55%, Alvarez et al., 2000) or three (54%, Zeitoun et al., 1996) waves per cycle.

Although follicular dynamics in *Bos taurus* and *Bos indicus* breeds are similar, there appear to be some differences in the physiology of *Bos indicus* and *Bos taurus* females relative to estrus and ovulation (reviewed in Galina and Arthur 1990; Bo et al., 2003). The duration of the estrus has been reported to be reduced in straightbred *Bos indicus* cattle (Plasse et al., 1970; Randel, 1976; Orihuela et al., 1983; Pinheiro et al., 1998; Rae et al., 1999) compared to *Bos taurus*. Across studies, mean duration of estrus using visual observation ranged from 6.7 (Plasse et al., 1970) to 11.3 h (Orihuela et al., 1983). Most recent studies have used electronic devices (HeatWatch® system) to detect the onset and duration of a synchronized estrus in Brahman females, with variations between 6.65 (Rae et al., 1999; Flores et al., 2006) and 17 h (Landaeta-Hernandez et al., 2002). In straightbred *Bos indicus* females, ovulation occurs approximately 26 h after the onset of estrus (Donaldson et al., 1968; Plasse et al., 1970; Lamothe-Zavaleta et al., 1991; Cavalieri et al., 1997; Pinheiro et al., 1998), with reports of

this interval in *Bos taurus* females ranging between 27 and 38 h (Walker et al., 1996; White et al., 2002; Stegner et al., 2004; Saumande and Humblot, 2005). In a study with crossbred Brahman heifers in Florida, the average interval from estrus to ovulation was 32.9 h using the HeatWatch® system to determine precisely the onset of estrus and ultrasonography every 4 h to determine ovulation, while the interval was 26.1 h when using visual observation (Lemaster et al., 1999). Mikeska and Williams (1988) found that ovulation occurred at a mean of 23.3 h after onset of a synchronized estrus in *Bos indicus* x *Bos taurus* heifers. Estrus occurred approximately 30 h after norgestomet implant removal in Brahman crossbred females (Williams, 1986, 1988; Mikeska and Williams, 1988).

In a recent study, we observed a mean interval from estrus to ovulation of 29 h in F1 Brahman x Hereford cows in south Texas (Saldarriaga et al., 2006). Randel (1976) reported a shorter interval from the LH surge to ovulation in Brahman (18.5 h) than F1 Brahman x Hereford (22.2 h) or Hereford (23.3 h) heifers. Mikeska and Williams (1988) found a similar interval (23.1 h) in F1 Brahman x Hereford heifers synchronized with Syncro-Mate-B® (SMB). However, in a more recent study, the interval between the LH surge and ovulation was 25.9 h in Brahman cows after four different synchronization

protocols (Cavalieri et al., 1997). A precise understanding of the timing of these events is important to the successful use of reproductive technologies in *Bos indicus* cattle.

Synchronization of Estrus and Ovulation

The ultimate goal of any estrus and ovulation synchronization protocol is to synchronize estrus and to induce ovulations effectively at a predictable time so that all the females can be inseminated en masse at a predetermined time. Several commercial protocols have been developed to facilitate appointment breeding and enhance efficiency (Reviewed in Odde, 1990; Patterson et al., 2003).

Early work in estrus synchronization generally involved the use of two approaches in cattle. The first involved the use of exogenous progesterone or progestogens to suppress estrus and to inhibit ovulation. The second approach was to induce regression of the CL with injections of PGF or its analogs.

Progesterone and Progestogens

The first reports of pharmacological intervention of the estrous cycle were published in the 1950s with the discovery that progesterone inhibited ovulation (Ulberg et al., 1951) and preovulatory follicular development (Nellor and Cole, 1956). The progestogen, melengestrol acetate (MGA), was the first progestogen

used orally to suppress estrus (Zimbelman and Smith, 1966; Randel et al., 1972). Zimbelman et al. (1970) reviewed the effectiveness of several studies using MGA to synchronize estrus in cows and heifers. He noted a reduction in conception rates at first synchronized estrus compared with controls. After it was reported that estrogens caused luteolysis (Wiltbank et al., 1961), they began to be used in combination with progestogens in an effort to gain better control of estrus and ovulation (Smith and Zibelman, 1968; Wiltbank et al., 1971; Beal et al., 1984) and to reduce the length of treatment, thus avoiding long-term exposure to progestogens that appeared to reduce fertility (Kojima et al., 1992).

An extensively-used treatment to synchronize estrus based on a progestogen and an estrogen is SMB (Miksch et al., 1978; Spitzer et al., 1981; Mikeska and Williams, 1988; Colazo et al., 2005) and includes the insertion for 9 days of an implant containing 6 mg of norgestomet and the injection of 5 mg of estradiol valerate (EV) and 3 mg of norgestomet at the time of implant insertion. The SMB regimen was approved by the Food and Drug Administration (FDA) in 1982 and then removed from the U.S. market in 2000. Odde (1990) reviewed several studies that used SMB to synchronize estrus in cattle. The range in proportion of females showing estrus after SMB treatment was 77 to 100% and the first-service conception rate ranged from 33 to 68%. Nonetheless, the high

estrus response after SMB treatment is not necessarily indicative of the presence of an ovulatory follicle, as demonstrated in a study by McGuire et al. (1990) where more than 50% of ovariectomized cows and heifers showed estrus after a SMB treatment. The EV injection elevates the concentration of estrogens in serum and it remains sufficiently high to induce estrus behavior and an LH surge after implant removal (Larson and Kiracofe, 1995).

Prostaglandins

With the demonstration of the luteolytic effects of PGF in the cow (Lauderdale, 1972; Rowson et al., 1972), intervention within the estrous cycle was simplified and PGF or its analogs became the most commonly-used treatment to synchronize estrus in cattle. A common procedure to synchronize estrus using only PGF is the application of two injections 10 to 12 days apart; which in some cases resulted in pregnancy rates over 40% after 5 days of estrus detection. Approximately 70% of estrous cycling females should show estrus after the first injection of PGF, with these and the rest of cycling females expected to respond to the second injection as well (Reviewed in Odde, 1990). A single injection of PGF has also been used in cycling *Bos taurus* (Lauderdale et al., 1974) and *Bos indicus* (Rekwot et al., 1999) females. This system can be employed by either observing for estrus for 5 days before and after PGF

injection or by utilizing TAI at 72 and 96 h post PGF. Prostaglandin $F_{2\alpha}$ or its analogs have been used in combination with progestogens to induce regression of the CL at the end of the progesterone/progestogen treatment (Beal et al., 1984; Kojima et al., 2000b; Lamb et al., 2001) or 17 days after MGA withdrawal (Brown et al., 1988; Patterson and Corah, 1992; Larson et al., 1996).

Control of Follicular Wave Emergence and Ovulation

With the advent of transrectal ultrasonography (Pierson and Ginther, 1984), our understanding of the bovine estrous cycle was expanded. Based on information generated using this technology, it is now accepted that to precisely control the estrous cycle both the lifespan of the CL and follicular wave emergence must be controlled..

GnRH

Different approaches have been used to synchronize follicular wave emergence in cattle, including GnRH or its analogs (Twagiramungu et al., 1992), estradiol (Bo et al., 1995) and dominant follicle ablation (Bergfelt et al., 1994). Earlier studies indicated that treatment with GnRH agonists alters ovarian follicular dynamics and luteal function and improves precision of the estrous response (Macmillan et al., 1985; Thatcher et al., 1989; Macmillan and Thatcher, 1991; Twagiramungu et al., 1995). Gonadotropin-releasing hormone has been

used successfully in combination with PGF for synchronization of estrus (Twagiramungu et al., 1992; Wolfenson et al., 1994). Injection of GnRH or its analogs causes follicles to undergo atresia or to ovulate, depending upon their stage of development, thus reducing concentrations of estradiol. Formation of a new CL inhibits recurring estrus. Regardless of ovarian status at the time of GnRH treatment, a new follicular wave emerges within 3 to 4 days and a dominant follicle is selected and becomes the ovulatory follicle after the PGF injection (Fig. 1; Twagiramungu et al., 1995). Ovulatory responses to GnRH treatment at random stages of the estrous cycle are highly variable, and may range from 40 to 90% (Pursley et al. 1995; Saldarriaga et al., 2006). The ability of a follicle to ovulate depends upon its developmental stage (Sartori et al., 2001), P4 concentration (Twagiramungu et al., 1994) and the phase of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000) at the time of treatment. Ovulation rate has been reported to be higher when GnRH is administered on day 5 to 9 (96%) or 17 to 21 (77%) than on day 1 to 4 (23%) or 10 to 16 (54%) of the estrous cycle (Vasconcelos et al., 1999). In a similar study, Martinez et al. (1999) administered GnRH on day 3, 6 and 9 of the cycle and obtained ovulation rates of 89, 56, and 22% respectively.

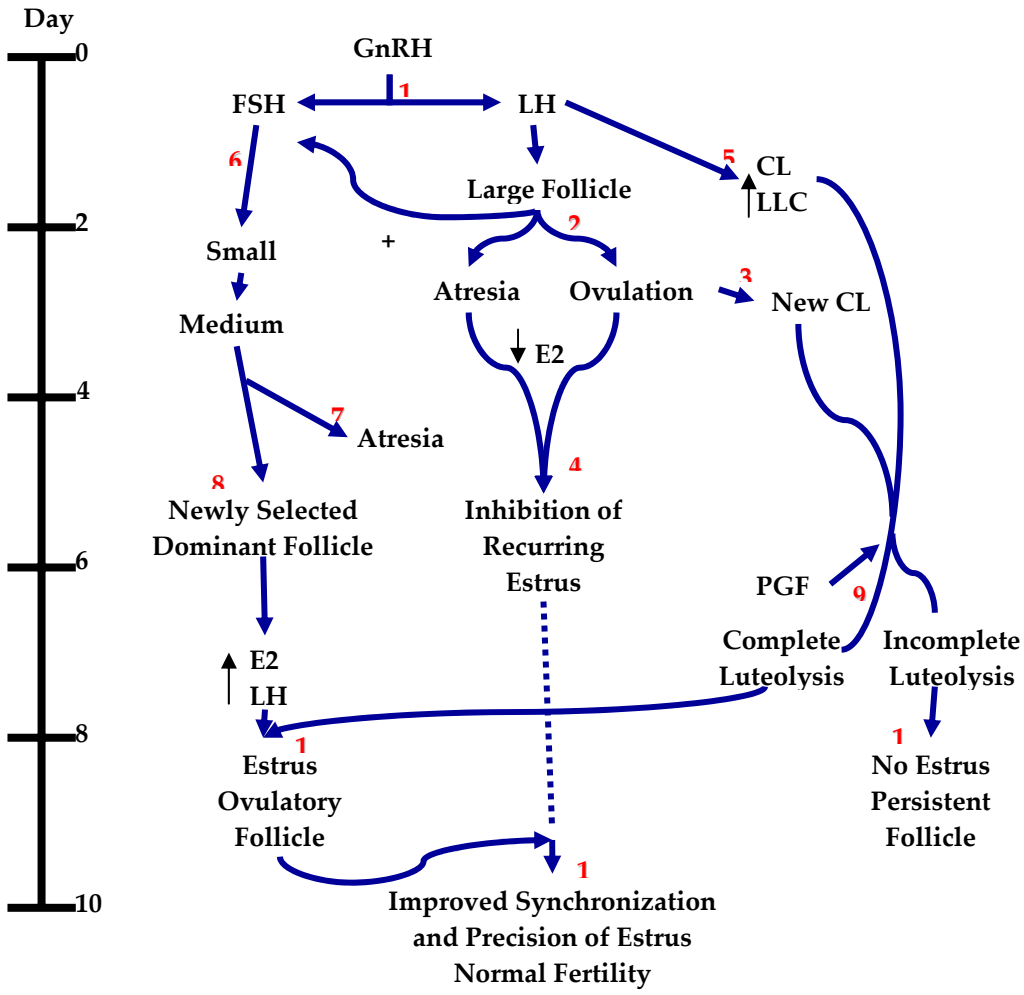

Fig. 1. Proposed model for the effect of a GnRH agonist. The model is based on a 10-day program for cattle. Treatment with a GnRH agonist on day 0 causes release of gonadotrophins (1). Depending on their stage of development, large follicles, either become atretic or ovulate (2) in response in to the induced release of LH. If ovulation occurs a new CL is form (3). With the disappearance of the large follicle estradiol (E2) concentrations decrease, and recurring estrus in inhibited between day 0 and 6 (4). Large luteal cells (LLC) increase in number on the CL present at the time of GnRH (5). FSH stimulates turnover of small (1.58 to 3.67 mm) and medium (3.68 to 8.56 mm) follicles (6). Increased atresia of medium follicles limits further growth of follicles (7). A new dominant follicle is selected from the new follicular wave 3 to 4 days after treatment (8). Complete luteolysis occurs after injection of PGF on day 6-7 (9). E2 levels and LH pulse frequency increase, estrus and LH surge occur, and the selected dominant follicle becomes the ovulatory follicle (10). Between day 7 and 10 synchronization rate and precision of estrus are improved and results in normal fertility (11). In a small proportion of females, estrus is inhibited due to incomplete luteolysis, and the selected dominant follicle becomes persistent (12). (Modified from Twagiramungu et al., 1995).

GnRH-PGF-Based Protocols

In the early 1990s, a new protocol to synchronize ovulation in dairy cows was developed and termed Ovsynch (Pursley et al., 1995). The new protocol consisted of an injection of GnRH followed by PGF 7 days later, a second injection of GnRH 48 h after the PGF treatment and TAI 24 h after the second GnRH injection. The addition of the second injection of GnRH in the Ovsynch protocol provides a surge of LH to increase the synchrony of ovulation.

Variations of the Ovsynch protocol are being used extensively in beef and dairy cattle. The CO-Synch protocol (Geary and Whittier, 1998) follows a similar sequence of injections as for Ovsynch, and differs only in that animals are time inseminated when the second injection of GnRH is administered at 48-72 h. Cattle treated with the Select Synch protocol (Geary et al., 2000) also receive the same injections as in the Ovsynch protocol; however, cows do not receive the second GnRH injection and are not time inseminated; estrus is detected during a 6-day period starting 24 h before the PGF treatment and cows are AI 12 h after detected estrus. In the Hybrid Synch protocol, cattle receive the same series of injections as in the Ovsynch protocol, estrus is detected during 4 day starting 24 h before the PGF treatment, and those females not showing estrus receive GnRH + TAI on day 10 after the onset of treatment (Lemaster et al., 2001).

Estrogens

Another approach for synchronizing follicular wave emergence in cattle is the use of estradiol. The latter was first used in combination with progesterone/progestogens to synchronize estrus in cattle when it was found that estradiol tended to cause luteolysis (Wiltbank et al., 1961) and subsequently formed the basis for reducing the period of implantation with norgestomet. It was not until the last decade that the effects of estradiol on follicular dynamics were elucidated. Bo et al. (1991) found that EV induced atresia of antral follicles in SMB-implanted cows. It was later demonstrated that an injection of estradiol-17β (E-17β) caused the synchronous emergence of a new follicular wave in cattle (Bo et al., 1994). The emergence of a new follicular wave depends on a transient increase in FSH concentrations (Adams et al., 1992). It has been demonstrated that an estradiol injection decreases FSH concentrations (Bo et al., 1993, 1994; Martinez et al., 2005) and emergence of a new follicular wave is initiated 1 day after the resurgence of FSH (Bo et al., 1993, 1994; O'Rourke et al., 2000; Martinez et al., 2005). The administration of 5 mg of E-17β in SMB-implanted *Bos taurus* beef females resulted in the consistent emergence of a new follicular wave 4.3 ± 0.2 days after treatment, regardless of the stage of development of the dominant follicle at the time of treatment (Bo et al., 1995).

Recently, Martinez et al. (2005) investigated the effects of two doses (1 or 5 mg) of E-17β and estradiol benzoate (EB) on the synchrony of ovarian follicular wave emergence in CIDR-treated beef cows. They found that cows given 5 mg of E-17β had the least variable interval to wave emergence (3.6 ± 0.2 days) while the longest interval was in those given 5 mg of EB. A recent study comparing two different estradiol preparations and three different doses of EV in CIDR-treated beef cattle reported that the interval from treatment to follicular wave emergence was longer and more variable following treatment with 5 mg of EV (5.7 ± 1.5 d) than with 5 mg of E-17β (3.6 ± 0.5 d). They found also that follicular wave emergence of cows given reduced doses of EV (1 mg, 3.2 ± 0.9 days; 2 mg, 3.4 ± 0.8 days) were similar to those given E-17β (Colazo et al., 2005). Lower doses of the long-acting EB (Martinez et al., 2005) and EV (Colazo et al., 2005) were as efficacious in synchronizing follicular wave emergence as the shorter-acting E-17β; therefore, the interval from treatment to the emergence of a new follicular wave depended on the clearance of estradiol from the circulation (Martinez et al., 2005).

Progesterone Intravaginal Insert

Recently the introduction of a progesterone/progestogen source with the GnRH-PGF-based protocols have shown benefits that are reflected in higher

pregnancy rates, using either progestogen ear implants (Stevenson et al., 1997; Thompson et al., 1999), MGA (Reviewed in Patterson et al., 2003), or intravaginal devices (Martinez et al., 2002a,b; Lamb et al., 2001; Mapletoft et al., 2003). Intravaginal devices containing progestogens/progesterone have been use for more than 30 years. In the late 1960s, sponges impregnated with progesterone were used in cattle and in the early 1970s the Progesterone Releasing Intravaginal Device (PRID®) was developed. The latter consisted of a stainless steel spiral coated with silicone rubber and impregnated with progesterone. In the late 1980s a new intravaginal device was developed and named CIDR-B® (Reviewed in Rathbone et al., 1997, 2001). The CIDR is a T-shaped vaginal insert containing 1.9 g of P4 (Canada) or 1.38 g of P4 (United States) in silicone molded over a nylon spine (Mapletoft et al., 2003). The original CIDR insert (1.9 g) was re-engineered to reduce the initial drug load to 1.38 g, decreasing the residual P4 remaining in the silicon after use, while maintaining the biological performance of the insert (Rathbone et al., 2002). Following CIDR (1.9 g) insertion in ovariectomized heifers, plasma progesterone concentrations were reported to increase to approximately 8.7 ng/ml by 6 h and then decreased to 6.8 ng/ml and 2.5 ± 0.2 ng/ml on day 1 and 12 after insertion, respectively (Macmillan et al., 1991). Serum progesterone concentrations have

been shown to be similar in cows receiving a CIDR device with 1.38 g or 1.9 g of progesterone (Rathbone et al., 2002), but peak concentrations occurring within 1 h after insertion are higher in cows receiving the 1.38 g device (Santos et al., 2004).

An increase in pregnancy rates of > 20% compared with controls was reported after the addition of a CIDR insert to the Ovsynch protocol in dairy (El-Zarkouny et al., 2004) and beef (Kawate et al., 2004) cows. Treatment of suckled beef cows with the CO-Synch protocol yielded acceptable pregnancy rates, but addition of a CIDR improved pregnancy rates in non-cycling cows (Lamb et al., 2001). Similar results were reported by Stevenson et al. (2003a) where the addition of a CIDR device to the CO-Synch protocol improved pregnancy rate in anestrus (42.9 Vs. 60.0%) but not in cycling (66.2 Vs. 68%) cows. Likewise, a significant improvement in pregnancy rate in heifers but not in cows was found when a CIDR device was added to the CO-Synch protocol (Martinez et al., 2002b). Nonetheless, in a large trial involving postpartum beef females (*Bos taurus*) in which CO-Synch protocols with or without a CIDR were compared, a significant increase (10%) in pregnancy rates was observed when the CIDR was used (Larson et al., 2006). Flores et al. (2006) concluded that treatment of Brahman-influenced cows with a CIDR for 7 days, along with administration of

PGF at CIDR removal, increases the number of mounts received, improves synchronization and first service conception rates, and may be effective inducing estrous cycles in anestrous females. Recently, Saldarriaga et al. (2006) found no statistical difference in pregnancy rate in *Bos indicus*-influenced females receiving the CO-Synch or the CO-Synch + CIDR protocol, but results were based on a small number of cows.

The residual P4 content of the 1.38 g-CIDR after a 7-day insertion period is 0.72 g (Rathbone et al., 2002), thus having the potential for reutilization. A single use is recommended by the manufacturer to prevent the potential transmission of venereal and blood borne diseases, but re-utilization of CIDR inserts has been reported (Schmitt et al., 1996b; Beal et al., 2003; Martinez et al., 2003; Stevenson et al., 2003c; Colazo et al., 2004a). Previously used CIDR inserts (containing 1.38 or 1.9 g of P4 when new) were able to suppress estrus during a 7-day insertion period in dairy and beef females (Richardson et al., 2002; Beal et al., 2003). Furthermore, no differences were observed in pregnancy rate to fixed-time AI between cattle synchronized with a new or once-used CIDR insert (Colazo et al., 2004a). Cerri et al., (2005) found no differences in concentrations of plasma P4 in lactating dairy cows receiving a new (1.38 g of P4) or an autoclaved insert used previously for 7 days. Different approaches have been

used to clean, disinfect or sterilize inserts in studies reporting CIDR reuse. There are apparently no reports comparing serum concentrations of P4 produced by washed and autoclaved used CIDRs.

Increasing Pregnancy Rates Using the Newest Protocols

Strategies to improve the performance of the Ovsynch and CO-Synch protocols such as modifying the timing of events, calf removal and presynchronization treatments have been investigated. The timing of AI and GnRH treatment in the CO-Synch protocol have been conventionally accepted to be 48 h after the PGF administration (Lamb et al. 2001). An early study comparing different intervals to TAI in *Bos taurus* beef females found no difference in conception rates between TAI at 48 or 60 h post PGF (Stevenson et al., 2003b). However, recent studies indicate that pregnancy rates in *Bos taurus* females are optimized when TAI is performed at 66-72 h after the PGF administration (Bremer et al., 2004; Schafer and Patterson, 2005; Schafer et al., 2005; Walker et al., 2005; Hesler et al., 2006; Larson et al., 2006). Furthermore, the CO-Synch + CIDR with TAI at 64 h after CIDR removal yielded similar pregnancy rates compared to the Ovsynch + CIDR with TAI 16 h after the second GnRH injection in crossbred Angus females (Kasimanickam et al., 2006). Dobbins et al. (2006) compared the CO-Synch + CIDR with TAI between 48 to 72

h in beef cows and concluded that pregnancy rate was maximized when inseminations occurred at 56 to 64 h. Larson et al. (2006) found similar pregnancy rates in beef females synchronized using the Select Synch + CIDR with estrus detection and TAI at 84 h (58%) or the CO-Synch + CIDR with TAI at 60 h (54%). In a recent study, we determined the time of ovulation of *Bos indicus* -influenced females after a GnRH-CIDR-PGF protocol (Select Synch) to be 99 ± 2.8 h relative to the time of PGF injection (Saldarriaga et al., 2006). Based on these results, delaying TAI-GnRH to 66 h should theoretically increase TAI pregnancy rates.

Calf removal has also been used with estrus synchronization protocols in beef cattle to increase pregnancy rates at TAI. Calf removal for 48 h improved the pregnancy rates of cycling and anestrous beef females synchronized with the CO-Synch protocol (Geary et al., 2001a). Surprisingly, the same author reported no effects of 48-h calf removal on pregnancy rates using the CO-Synch protocol in another study (Geary et al., 2001b). Pregnancy rates were optimized when 48-h calf removal was performed along with SMB (Kiser et al., 1980), MGA-PGF (Yelich et al., 1995) or Ovsynch (Geary et al., 2001a).

Attempts to improve the effectiveness of the newest protocols have been made using PGF (Colazo et al., 2004b; El-Zarkouny et al., 2004) or GnRH

(Kojima et al., 2000a; DeJarnette et al., 2001; DeJarnette and Marshall, 2003) as presynchronization treatments, with the intent of improving responsiveness to GnRH at the beginning of the estrus synchronization treatment. This approach is based on the ability of GnRH to induce ovulation or luteinization of dominant follicles which is dependent on stage of follicular development at the time of injection (Vasconcelos et al., 1999; Moreira et al., 2000; Sartori et al., 2001). Presynchronization with GnRH 7 days before the initiation of a GnRH-PGF protocol in *Bos taurus* beef cows tended to increase (P = 0.06) synchronized pregnancy rates (53%) compared to those without presynchronization (47%; DeJarnette et al., 2001). However, the addition of a GnRH injection administered 7 days before a GnRH-PGF protocol did not affect pregnancy rates in lactating dairy cows (DeJarnette and Marshall, 2003) and *Bos taurus* beef cows (Kojima et al., 2000a). Furthermore, Rivera and Fricke (2004) concluded that presynchronization with GnRH 7 days before initiation of synchronization of ovulation using GnRH and PGF failed to decrease the proportion of heifers expressing estrus during the protocol and did not improve synchronization response or fertility to the protocol. Recently, Bello et al. (2006) and Stevenson et al. (2006), reported that a presynchronization with GnRH 6 days before the

initiation of the Ovsynch protocol resulted in a greater ovulatory response after the first GnRH in lactating dairy cows and heifers respectively.

There are no reports on the implementation of a GnRH presynchronization treatment in beef cattle with *Bos indicus* influence, nor is the amount of *Bos indicus* genetics required to affect the outcome of presynchronization known. However, it is possible to theorize that a presynchronization treatment used in conjunction with the newest protocols (CO-Synch + CIDR) may result in a highly synchronized follicular wave emergence and increase TAI conception rates in *Bos indicus* influenced females.

Vaginal Electrical Resistance

Several electronic devices have been developed to alleviate the need for visual estrus detection in cattle and to provide a precise determination of the onset of estrus. Commercially-available electronic technologies for estrus detection are based on changes in physical activity (pedometers), changes in electrical and chemical properties of the reproductive tract (intravaginal resistance probes), or mounting activity (mount detectors) (Reviewed in Rorie et al., 2002).

Vaginal electrical resistance (VER) has been measured and used as an aid in estrus detection and for timing of insemination in cattle (Schams et al., 1977;

Foote et al., 1979; Canfield and Butler, 1989). The electrical resistance of reproductive tissues and their secretions has been studied not only in cattle but in buffaloes (Gupta and Purohit, 2001), sheep (Bartlewiski et al., 1999), goats (Rezac et al., 2001), mares (Ley et al., 1981), pigs (Rezac et al., 2003), foxes (Boue et al., 2000), rhinoceros (Bowers et al., 2005), rats (Singletary et al., 2005), guinea pigs (Bartos and Sedlacek, 1977), macaques (Fischer et al., 1990) and women (Brown et al., 2000). The VER fluctuates with stage of the estrous cycle, is highest during the luteal phase, and declines during the follicular phase. The lowest VER values are correlated with the preovulatory LH peak (Schams et al., 1977; Canfield and Butler, 1989).

Leidl and Stolla (1976) found a correlation between low VER readings and higher pregnancy rates. However, Foote et al. (1979) compared the pregnancy rates of dairy cows inseminated when estrus was detected using VER readings or visual detection of estrus and found no difference. Likewise, a recent report using a commercially-available probe (Ovatec®), indicates that readings obtained with the device accurately reflect estrous activity, with readings obtained every 12 h more reliable than 12-h visual observations (Wehner et al., 1997). Scipione and Foote (1999) found VER less accurate than milk progesterone values in designating which cows were suitable or unsuitable for

insemination, but both low milk progesterone and low VER values 21 to 23 days after insemination provided an early and accurate indication of a need for re-insemination. Measuring VER every 2 or 3 days was not considered effective because of the large individual variation (Gartland et al., 1976). Depth of probe insertion in the vagina (McCaughey and Patterson, 1981; Aboul-Ela et al., 1983; Kitwood et al., 1993), position of the probe within the vagina (dorsal or ventral; Foote el at., 1979; Heckman et al., 1979; Cavestany and Foote, 1985), pressure against the mucous membrane (Leidl and Stolla, 1976), pathological conditions of the reproductive tract (Leidl and Stolla, 1976) and technician (Foote el at., 1979) also influences the results.

CHAPTER III

EFFECTS OF PRESYNCHRONIZATION WITH GnRH ON CONCEPTION RATES AND OVARIAN EVENTS IN *BOS INDICUS*-INFLUENCED FEMALES SYNCHRONIZED WITH CO-SYNCH + CIDR

Introduction

Artificial insemination (AI) is the main vehicle for rapid dispersal of valuable genes and genetic advancement in cattle populations (Vishwanath, 2003). Currently, only 5 to 7% of beef cows in the United States are inseminated artificially (NAHMS, 1997). The main reasons for the low rate of adoption of AI among beef producers are the problem of accurate detection of estrus and the poor and inconsistent results of the protocols used to synchronize the onset of estrus and/or ovulation.

The rationale of any estrus and ovulation synchronization protocol for beef or dairy cattle is to induce ovulations at a predictable time so that all of the females can be inseminated en masse at a chosen time, referred to as timed artificial insemination (TAI). A variation of the Ovsynch protocol (Pursley et al.,

1994), which includes the utilization of prostaglandin F2α (PGF) and gonadotropin-releasing hormone (GnRH), was reported by Geary and Whittier (1998). Termed CO-Synch, this approach requires the administration of GnRH concurrently with TAI. The CO-Synch protocol required one less handling of animals but resulted in lower pregnancy rates than the Ovsynch protocol in beef cows (Geary and Whittier, 1998). Later the addition of a progesterone source (controlled internal drug release device; CIDR®) to the CO-Synch protocol yielded a treatment (CO-Synch + CIDR) that produced higher pregnancy rates than CO-Synch alone in *Bos taurus* cows (Lamb et al., 2001) and heifers (Martinez et al., 2002b).

Previous studies in our laboratory have demonstrated that the effectiveness of CO-Synch + CIDR in cattle with *Bos indicus* genetic influence is lower (< 40%) than reported for *Bos taurus* females (> 50%; Johnson, 2005). The basis of the lower results are that the CO-Synch treatment failed to induce a synchronized follicular wave in a high proportion of females after the first injection of GnRH and that the time of AI (48 h) might not have been optimal (Saldarriaga et al., 2006). Recent studies indicate that pregnancy rates in *Bos taurus* females are optimized when TAI is performed at 66 to 72 h after the PGF administration (Walker et al., 2005; Larson et al., 2006). Additional attempts to

improve the effectiveness of this protocol have been made using PGF (El-Zarkouny et al., 2004) or GnRH (Kojima et al., 2000a; DeJarnette et al., 2001) as a presynchronization treatment, with the intent of improving the responsiveness to GnRH at the beginning of the synchronization treatment. This approach is based on the ability of GnRH to induce ovulation or luteinization of dominant follicles, which is dependent on stage of follicular development at the time of injection (Vasconcelos et al., 1999; Moreira et al., 2000; Sartori et al., 2001). However, there are no reports on use or efficacy of a GnRH presynchronization treatment with CO-Synch + CIDR in beef cattle having *Bos indicus* influence.

Objectives of this experiment were to evaluate conception rates in *Bos indicus*-influenced cows and heifers following presynchronization with GnRH seven days before the CO-Synch + CIDR protocol and TAI at 66 h, and to characterize intensively ovarian events associated with the CO-Synch + CIDR protocol, with and without presynchronization.

Materials and Methods

Study Locations and Animal Protocols

Experiments were conducted at the Texas Agricultural Experiment Station, Beeville, Texas. The Institutional Agricultural Animal Care and Use

Committee of the Texas A&M University approved in advance all procedures used in these studies.

Experiment 1: Effects of Presynchronization with GnRH on Conception Rates in Bos indicus-Influenced Females Synchronized with the CO-Synch + CIDR Protocol and TAI at 66 h.

Cattle

One hundred and thirty five Brahman x Hereford (F1) females (nulli-, primi-, and multi-parous) were maintained on Coastal Bermuda and Kleingrass pastures. All cattle were required to have a minimum BCS of 5 (1-9 scale, 1 = emaciated, and 9 = obese) and if suckled be at least 50 days postpartum (DPP) at TAI. Females were stratified by BCS, age and DPP and assigned to one of two treatments. An additional 77 females in which the Presynch treatment were not given were synchronized with the CO-Synch + CIDR with TAI at 66 h.

Synchronization Procedure

Treatments were: 1) Presynchronization followed by CO-Synch + CIDR protocol (Presynch) or 2) CO-Synch + CIDR protocol without presynchronization (CO-Synch + CIDR; Fig. 2). On day -7, females assigned to the Presynch treatment received a single injection of GnRH (GnRH-P; 100 μg; Cystorelin®, Merial, Inc., Iselin, NJ) and those not presynchronized (CO-Synch +

CIDR only) received an injection of physiological saline (2 ml). Seven days later, all cattle received the CO-Synch + CIDR treatment for synchronization of ovulation. The regimen included a CIDR insert (1.38 g of P4; Pfizer Animal Health, New York, NY) plus a GnRH (GnRH-1; 100 μg; Cystorelin®) injection (day 0), removal of the CIDR 7 days later (day 7) coincident with an injection of PGF (25 mg; Lutalyse®; Pharmacia & Upjohn Co., Kalamazoo, MI). Sixty-six hours following CIDR removal and PGF injection (day 10), all cattle were inseminated and received a second injection of GnRH (GnRH-2; 100 μg; Cystorelin®). All females were turned with bulls 5 days after TAI for a 90-day breeding season. Pregnancy status was determined by transrectal ultrasonography (Dynamic Imaging, Concept/MCV, equipped with a dual 5/7.5 MHz linear array probe; Livingston, UK) 30 days after TAI.

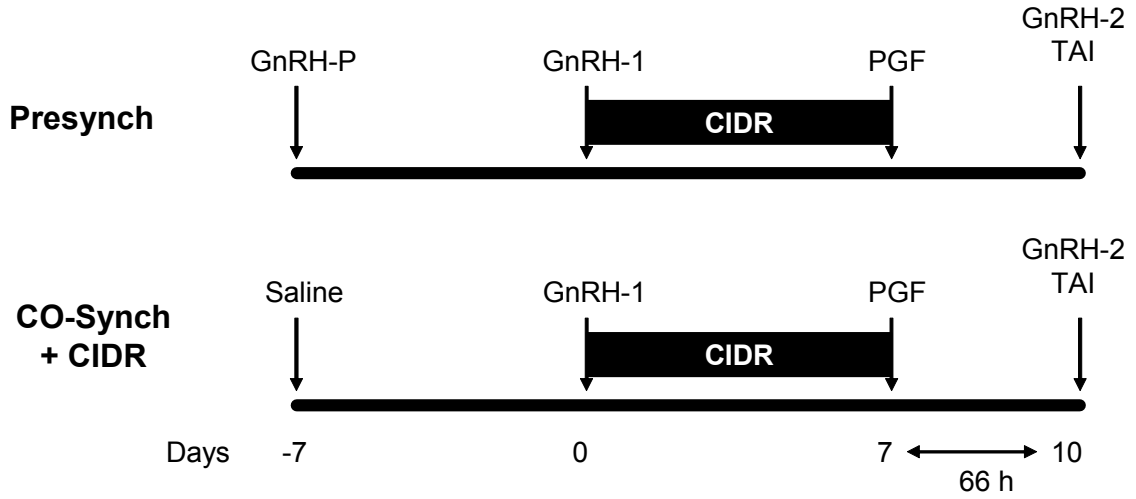

Fig. 2. Experimental protocols for synchronization of ovulation. All cattle received the CO-Synch + CIDR treatment for synchronization of ovulation. The regimen included a CIDR insert plus a GnRH injection on day 0 (GnRH-1), removal of the CIDR 7 days later coincident with an injection of Prostaglandin F2α (PGF). On day 10 (66 h after CIDR removal and PGF injection), all cattle were timed AI (TAI) and received a second injection of GnRH (GnRH-2). Females assigned to the Presynch treatment received a GnRH injection on day -7 (GnRH-P) and those assigned to the CO-Synch + CIDR treatment received an injection of saline

Experiment 2: Effects of Presynchronization with GnRH on Ovarian Events in Bos indicus-Influenced Females Synchronized with the CO-Synch + CIDR Protocol with TAI at 66 h.

Cattle

Ninety-eight Brahman x Hereford (F1) females were divided into three replicates based on date of calving. Criteria for inclusion and stratification were similar to Experiment 1. Cattle were placed in pens measuring 25.6 x 9.5 m 5

days before the onset of treatments with 5 cow-calf pairs per pen and fed a forage-concentrate diet formulated to meet National Research Council (NRC, 1996) recommendation for lactating beef cows. This included coastal Bermuda grass hay ad libitum and 2.3 to 3.2 kg/head daily of a ground corn-cottonseed (82 : 18) mixture containing a vitamin/mineral pre-mix.

Synchronization Procedure

Females received one of two treatments as described for Experiment 1 (Presynch, n = 50; CO-Synch + CIDR, n = 48). Transrectal ultrasonography of ovaries to evaluate follicular and luteal structures was performed on selected days as shown in Fig. 3. All ultrasound examinations were performed by the same operator. Follicular and luteal structures were measured and an image of the dorsal and lateral view of each ovary was then obtained. The dominant follicle was defined as the follicle that reached the largest diameter (Sirois and Fortune, 1988). Ovulation was defined as the disappearance of a follicle within two consecutive ultrasound examinations, and follicular regression was defined as the gradual reduction of follicular size until disappearance (Ginther et al., 1989a). Emergence of a follicular wave was determined, retrospectively, as the day the dominant follicle reached 4 to 5 mm; if the follicle was not detected until it was 6 to 7 mm, then the day before was considered as the day of emergence

(Ginther et al., 1989b). A synchronized follicular wave was considered to have occurred if it emerged between 1 and 4 days after GnRH-1. Follicular wave emergence occurring outside of this period was considered to be spontaneous. Luteal regression was defined as the progressive reduction in size of the CL until disappearance (Ginther, 1998).

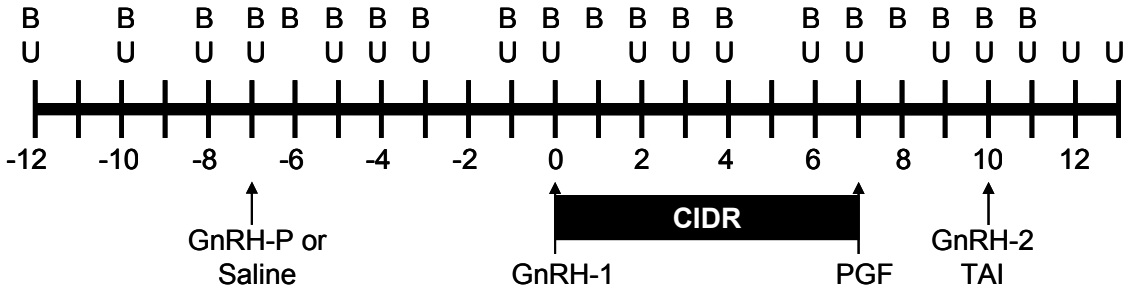

Fig. 3. Timeline of events for Exp. 2. Females received one of two treatments as described for Exp. 1 (see Fig. 2). Ultrasound (U) examination and blood sampling (B) were performed on specific days

Blood samples were collected via coccygeal tail vessel using evacuated 10-ml tubes and 20-g bleeding needles following the schedule shown in Fig. 3. Blood samples were placed on ice immediately after collection and remained on ice until transported to the laboratory. Samples were allowed to clot at room temperature for approximately 1 h before centrifugation at 3000 x g for 30 m Serum was collected and stored at -20°C until hormone analyses. Serum was

assayed by RIA for progesterone using the Coat-A-Count assay kit (Diagnostic Products Corporation, Los Angeles, CA). Intra- and interassay CV were 5.0 and 10.4% respectively and sensitivity was 0.05 ng/mL. Pregnancy rates were determined by transrectal ultrasonography 30 days after TAI.

Statistical Analyses

Experiment 1. The CATMOD procedure (SAS Inst. Inc., Cary, NC) was utilized to determine the effects of BCS, days postpartum (DPP), parity, replicate, AI sire, AI technician and appropriate interactions on TAI conception rates. A model that included treatment, replicate and its interaction was used to examine treatment effects on TAI conception rates. Pregnancy rates were compared using chi square analysis (PROC FREQ of SAS).

Experiment 2. Chi Square analysis (PROC FREQ of SAS) was used to evaluate the effects of treatment on categorical variables. Effect of treatment and replicate and its interaction on follicular sizes was evaluated using the GLM procedure.

Results

Experiment 1

Mean (± SEM) BCS and BW for nulliparous heifers (n = 46) were 5.7 ± 0.1 and 352 ± 5.2 kg, respectively. Mean (± SEM) BCS, BW and DPP were; 5.1 ± 0.1,

470 ± 10.2 kg, and 90 ± 6 days for primiparous (n = 25), and 5.2 ± 0.1, 555 ± 5.8 kg, and 71 ± 1.1 days for multiparous (n = 141) females, respectively (Table 1). Timed AI pregnancy rates are summarized in Fig. 4. Timed AI pregnancy rates did not differ (P = 0.18) between Presynch (37.3 ± 6%; n = 67) and CO-Synch + CIDR (48.5 ± 6.1%; n = 68) groups. However, due to extreme drought conditions, some (n = 18) cows (13.3%) fell below the targeted BCS of 5. When these individuals were removed from the analysis, pregnancy rates of those animals remaining with a BCS ≥ 5 (n = 117) tended to differ (P = 0.085) between Presynch (38 ± 6.3%) and CO-Synch + CIDR (54 ± 6.7%) groups. The additional 77 females that were synchronized with the CO-Synch + CIDR but were not involved in the comparison with Presynch had a TAI pregnancy rate of 36 ± 0.06%. Thus, total cumulative pregnancy rate for females with BCS ≥ 5 receiving the CO-Synch + CIDR (n = 110) was 45 ± 0.05%. Pregnancy rates were similar (P = 0.5) for nulliparous (37 ± 7.2%), primiparous (38 ± 10%), and multiparous (46 ± 5%) females (Table 2).

Table 1
Mean (± SEM) BCS, DPP, BW, age and puberty in nulli-, primi- and multiparous *Bos indicus*-influenced females used in Exp. 1 and 2

Parity	n	BCS	DPP (d)	BW (kg)	Age (yr)	Puberty (%)
Nulliparous	46	5.7 ± 0.1	-	352 ± 5	1	74 ± 7
Primiparous	32	5.4 ± 0.1	86 ± 5	458 ± 10	2	-
Multiparous	232	5.1 ± 0.1	74 ± 1	556 ± 6	7.9 ± 0.2	-

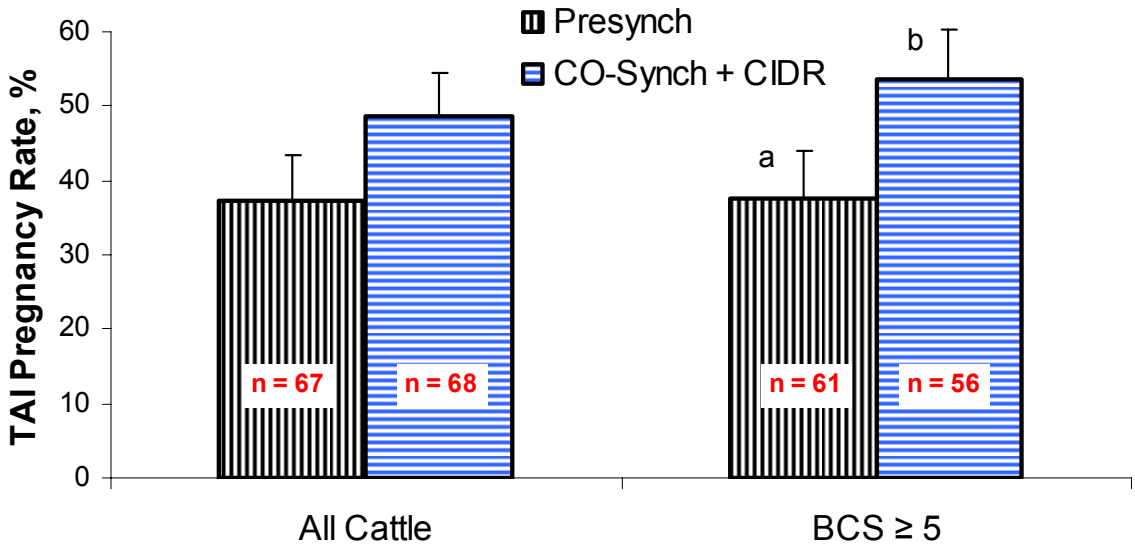

Fig. 4. Timed AI (TAI) conception rates in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR with or without Presynchronization in Exp. 1. [a,b] differ (P = 0.085)

Table 2
Timed AI (TAI) pregnancy rates in *Bos indicus*-influenced females with BCS ≥ 5 synchronized with CO-Synch + CIDR with and without presynchronization in Exp. 1

Source	n	TAI Pregnancy Rate, %
Nulliparous	46	37
Primiparous	24	38
Pluriparous	101	46
Total	171	42

Experiment 2

Mean (± SEM) BCS, BW, and DPP were 5 ± 0.07, 540.5 ± 8.2 Kg and 77.7 ± 1.3 days, respectively (Table 1). Table 3 summarizes information related to ovarian cyclicity. Before onset of treatments (GnRH-P; day -7), 52% and 54% of females on the Presynch and CO-Synch + CIDR groups, respectively, had ovulated based on serum progesterone and observation of a morphologically-identifiable CL. On day 0 (onset of CO-Synch + CIDR treatment), 68% of females in the Presynch group had ovulated, while the proportion of cyclic females in the CO-Synch + CIDR group (54%) did not change. Presynch treatment induced cyclicity in 33% of anovulatory females (8/24).

Table 3
Cyclic status before onset of treatments in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR with or without presynchronization in Exp. 2

Treatment	n	Cycling before Presynch, n (%)	Cycling before CO-Synch, n (%)
Presynch	50	26 (52)	34 (68)
CO-Synch + CIDR	48	26 (54)	26 (54)
Total	98	52 (53)	60 (61)

Table 4 summarizes ovarian events of all females synchronized. For females in the Presynch group ovulation and dominant follicle regression rate after GnRH-P treatment was 50% and 32% respectively. Ovulatory response to GnRH-1 was greater ($P < 0.01$) in the Presynch (58%) than in the CO-Synch + CIDR (27.1%) group. The proportion of females that did not ovulate or regress a follicle was greater ($P < 0.01$) in those receiving the CO-Synch + CIDR (42 ± 7.2%) compared to those in the Presynch group (18 ± 5.5%). Emergence of a synchronized follicular wave after GnRH-1 (88 vs 83.3%) and ovulation rate after GnRH-2 (78 vs 70.8%) did not differ between groups ($P = 0.4$). All CL that developed (n = 71) before or during the synchronization protocol regressed after PGF treatment.

Table 4
Ovarian events in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR and treated with GnRH (Presynchronization) or saline (CO-Synch + CIDR) in Exp. 2

Item	Treatment	
	Presynch No. (%)	CO-Synch + CIDR No. (%)
No. Females	50	48
Response to GnRH-P or Saline		
Ovulation	25 (50)	0
Regression	16 (32)	0
No Response	9 (18)	48
Response to GnRH-1		
Ovulation	29 (58)[a]	13 (27)[b]
Regression	12 (24)	15 (31)
No Response	9 (18)[a]	20 (42)[b]
Synchronized Follicular Wave Emergence	44 (88)	40 (83.3)
Ovulation after GnRH-2	39 (78)	34 (70.8)
CL Regression, %	100	100

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

Follicular sizes were similar for both treatments at every point during the synchronization period ($P > 0.2$). Mean follicular diameters are illustrated in Fig.

5. Ovulation rate after GnRH-2 differed ($P < 0.01$) by follicular size at time of treatment (Table 5). Ovulation rates by follicular size were 0% (< 8 mm), 53% (8 to 10 mm), 79% (10 to 12 mm), 82% (12 to 14 mm) and 91% (> 14 mm).

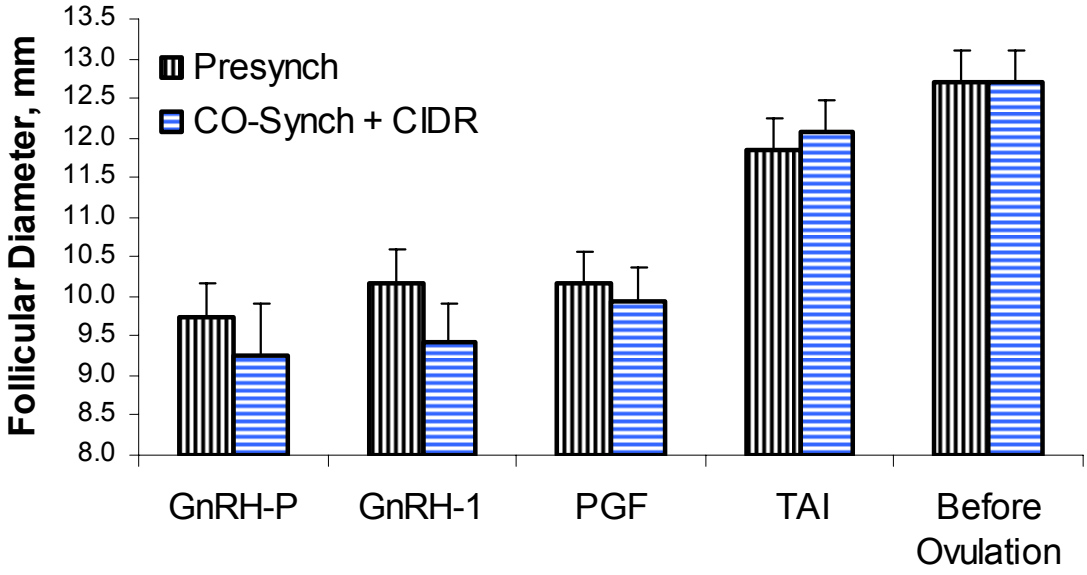

Fig. 5. Mean follicular diameters in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR with or without Presynchronization, measured at different times during Exp. 2. (Presynch, n = 50; CO-Synch + CIDR, n = 48)

Table 5
Ovulation rate in relation to follicular diameter at timed AI (TAI) in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR with and without Presynchronization in Exp. 2

Follicular Diameter at TAI, mm	n	Ovulation Rate, %
< 8	4	0[a]
8 - 10	19	53[b]
10 – 12	24	79[c]
12 – 14	22	82[c]
> 14	22	91[c]
Total	91	74

[a,b,c] Percentages within column with uncommon superscripts letters differ (P < 0.05)

Data were also summarized relative to occurrence or failure of ovulation after GnRH-1 to evaluate their effects on subsequent ovarian responses (Table 6). A greater (P < 0.05) proportion of females that ovulated or regressed a follicle in response to GnRH-1 developed a synchronized follicular wave compared to females that did not ovulate. Ovulatory response to GnRH-2 was greater (P < 0.01) in animals that ovulated (83.3 ± 6.4%) or regressed (85.2 ± 8%) the dominant follicle after GnRH-1 compared to those that did not respond (51.7 ± 7.7%) to the treatment.

Table 6
Effects of the response to GnRH-1 treatment on subsequent ovarian events in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR with and without Presynchronization in Exp. 2

Item	Ovulatory Response to GnRH-1		
	Ovulating	Regressing	No Response
	No. (%)	No. (%)	No. (%)
No. of Females	42	27	29
Synchronized Follicular Wave Emergence			
Yes	36 (86)[a,b]	26 (96)[a]*	22 (76)[b]
No	6 (14)	1 (4)	7 (50)
Ovulation after GnRH-2			
Yes	35 (83)[a]	23 (85)[a]	15 (52)[b]**
No	7 (17)	14 (15)	14 (48)

[a,b] Percentages within row with uncommon superscripts letters differ
* $P < 0.05$
** $P < 0.01$

Effects of emergence of a synchronized follicular wave on subsequent ovarian events are summarized in Table 7. More ($P < 0.01$) females that developed a synchronized follicular wave after GnRH-1 ovulated (82 ± 4.2%) after GnRH-2, compared to those that did not develop a synchronized follicular wave (29 ± 12.5%). In addition, mean follicular diameters at PGF and GnRH-2 treatment were increased ($P < 0.01$) in females that developed a synchronized follicular wave after GnRH-1 compared to those that did not developed a new follicular wave. Moreover, females that became pregnant had larger follicles (13

± 0.5 mm) at GnRH-2 than those that did not become pregnant (11.5 ± 0.3 mm; P < 0.05).

Table 7
Effects of synchronized follicular wave emergence on subsequent ovarian events, mean (±SEM) follicular diameter, and reproductive outcome in *Bos indicus*-influenced females synchronized with CO-Synch + CIDR with and without Presynchronization in Exp. 2

Variable	Occurrence of Synchronized Follicular Wave after GnRH-1	
	Yes	No
	No. (%)	No. (%)
No. of Females	84	14
Ovulation after GnRH-2		
Yes	69 (82)[a]	4 (29)[b]
No	15 (18)	10 (71)
Follicular Diameter, mm		
PGF	10.4 ± 0.3[a]	8.2 ± 0.8[b]
GnRH-2	12.3 ± 0.3[a]	9.3 ± 0.7[b]
Timed AI Pregnancy	28 (34)	2 (14)

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

Discussion

Strategies to improve the performance of the CO-Synch+ CIDR protocol such as modifying the timing of events and presynchronization treatments have been investigated (Walker et al., 2005; DeJarnette and Marshall, 2003). These

presynchronization schemes are intended to place a large proportion of females into the follicular phase of the estrous cycle at the onset of the synchronization protocol such that ovulatory responses to GnRH-1 and synchronization of a new follicular wave are optimized. In the current study, effects of presynchronization with GnRH on pregnancy rates and ovarian events in *Bos indicus*-influenced females were evaluated. Results of Experiment 1 indicate that a presynchronization strategy utilizing GnRH failed to increase pregnancy rates after TAI compared to non-presynchronization. Recent studies involving lactating dairy cows (DeJarnette and Marshall, 2003) and *Bos taurus* beef cows (Kojima et al., 2000a) have reported similar findings.

Testing a different interval from CIDR removal/PGF to TAI (66 h) was not a direct aim of this study. However, subjective comparison of pregnancy rates in the current study (45%) in which TAI was at 66 h with those of another recent report (39%) from our laboratory in which TAI was at 48 h (Saldarriaga et al., 2006), indicates the possibility that TAI at 66 h is preferred. Moreover, in Saldarriaga et al. (2006), we also determined that the time of ovulation of *Bos indicus*-influenced females after a GnRH-CIDR-PGF protocol (Select Synch) averaged 99 ± 2.8 h from the time of CIDR removal/PGF treatment. Ovulation of the dominant follicle in a GnRH-PGF-GnRH protocol occurs approximately 30 h

after the second treatment of GnRH (Pursley et al., 1995; Twagiramungu et al., 1995), which is comparable to a spontaneous ovulation that occurs between 27 to 38 h after onset of standing estrus in *Bos taurus* (Walker et al., 1996; White et al., 2002; Stegner et al., 2004; Saumande and Humblot, 2005) and *Bos indicus*-influenced females (Lemaster et al., 1999; Saldarriaga et al., 2006). Based on these results, delaying TAI-GnRH to 66 h should theoretically increase TAI pregnancy rates.

Unfortunately, due to drought conditions during the conduct of the current experiment, adequate numbers of females in BCS ≥ 5 were not available for study. Delaying the TAI-GnRH to 66-72 h post PGF has been reported to optimize pregnancy rates in *Bos taurus* females (Bremer et al., 2004; Schafer and Patterson, 2005; Schafer et al., 2005; Walker et al., 2005; Hesler et al., 2006; Larson et al., 2006). Moreover, TAI at 64 h with the CO-Synch + CIDR protocol yielded pregnancy rates similar to those obtained with the Ovsynch + CIDR protocol and TAI 16 h after the second GnRH injection (Kasimanickam et al., 2006). Dobbins et al. (2006) compared pregnancy rates in cows synchronized with CO-Synch + CIDR and TAI at different intervals between 48 and 72 h in beef cows. It was concluded that pregnancy rates were maximized when inseminations occurred at 56 to 64 h. Larson et al. (2006) found similar pregnancy rates in beef

females synchronized using the Select Synch + CIDR with estrus detection and TAI at 84 h (58%) or the CO-Synch + CIDR with TAI at 60 h (54%).

The main factors affecting postpartum anovulation are nutritional status, measured as BCS (Randel, 1990; Short et al., 1990), DPP and suckling (Williams, 1990), therefore to minimize these effects during synchronization studies, investigators have often required minimum BCS and DPP at TAI of 5 and 50 days, respectively (Williams et al., 2002; Saldarriaga et al., 2006). In the current study, TAI pregnancy rates were not influenced statistically by BCS or DPP, perhaps due to the small numbers of females involved, even though some fell below BCS 5. Nearly 50% of females in the current study had evidence of luteal activity at the onset of treatments, which is somewhat lower than that reported in previous studies in our laboratory (78%; Saldarriaga et al., 2006) and was likely a function of the extreme drought conditions experienced during the experimental timeline.

Treatment with GnRH can induce estrous cycles in some anestrous beef cows (Geary et al. 1998; Thompson et al., 1999). In the current study, presynchronization treatment induced cyclicity in a modest proportion (> 30%) of anovulatory females. The effects of a low proportion of cyclic females may be

counteracted to a large extent with the utilization of an exogenous source of P4 (Stevenson et al., 2003a; 2003b).

In experiment 2, we hypothesized that GnRH treatment 7 days before the onset of the CO-Synch + CIDR protocol could increase the proportion of females with a responsive dominant follicle on day 0, improving the ovulation rate after GnRH-1 and the emergence of a new follicular wave. Previously, we observed that a low ovulation rate after GnRH-1 accounted for a significant proportion of the synchronization failure using CO-Synch + CIDR, and that the emergence of a synchronized follicular wave was closely related to ovulation after GnRH-1 (Saldarriaga et al., 2006). In the current study, ovulation rate after GnRH-1 was greater in females that were presynchronized (58%) than those that were not (27%), and was also greater than that observed in previous studies (40%) using *Bos indicus* x *Bos taurus* (Saldarriaga et al., 2006) or straightbred *Bos indicus* females (Barros et al., 2000). However, surprisingly, this did not influence TAI pregnancy rates. The proportion of follicular structures regressing after GnRH-1 in both treatments was similar to that observed in the earlier studies (Barros et al., 2000; Saldarriaga et al., 2006), but the proportion of unresponsive (not ovulating or regressing a follicle) females receiving the CO-Synch + CIDR without presynchronization was greater in the current study. However, the

proportion of unresponsive females in the Presynch treatment (18%) was comparable to that observed for CO-Synch + CIDR without presynchronization in the previous study (21%; Saldarriaga et al., 2006). Ovulation rate after GnRH treatment is highly variable. Pursley et al. (1995) reported that 90% of random cycling cows and 54% of heifers ovulated after a single GnRH treatment. Geary et al. (2000) reported an ovulation rate of 66% in suckled *Bos taurus* cows and found that ovarian responses to GnRH were dependent on day of the estrous cycle when the treatment was given. However, ovulation rate is greater when GnRH is administered on day 5 to 9 (96%) or 17 to 21 (77%) than on day 1 to 4 (23%) or 10 to 16 (54%) of the estrous cycle (Vasconcelos et al., 1999). In a similar study, Martinez et al. (1999) established that ovulation rate was greater when GnRH was administered on day 3 or 6, than on day 9.

Emergence of a synchronized follicular wave after GnRH-1 was achieved effectively in both treatment groups (> 80%) in the current experiment. Independent of treatment group, emergence of a synchronized follicular wave was comparable in females that ovulated (86%) or regressed a follicle (96%). This is in agreement with a report by Twagiramungu et al. (1995) in which both ovulation and follicle regression after GnRH-1 were equally effective for inducing the emergence of a new follicular wave. Emergence of a new follicular

wave after GnRH treatment can be coincidental with the spontaneous emergence of a new follicular wave (Geary et al., 2000). Martinez et al. (2000) examined the interval between GnRH treatment and emergence of a new follicular wave. There was no difference in interval to a new follicular wave between heifers that ovulated and those that did not; however variability of this interval was less in heifers that responded to GnRH by ovulation. Barros et al. (2000) reported that the emergence of a new follicular wave occurred 1.8 ± 0.3 days after GnRH treatment in 19 of 24 (79%) *Bos indicus* cows.

Incomplete luteolysis has been demonstrated to affect the effectiveness of estrus synchronization protocols (Burke et al., 1996; Moreira et al., 2000b; Kim et al., 2003). Lemaster et al. (2001) suggested that a low rate of estrus in *Bos indicus*-influenced females synchronized with the Select Synch protocol was due to an inadequate regression of the CL. This is in contrast to results of Experiment 2 of the current study where all the females that developed a CL during the synchronization protocol either regressed it naturally before day 7 or after PGF treatment. This was confirmed ultrasonographically by visual reductions in size and concomitant decreases in serum P4 concentrations (< 0.5 ng/mL) 48 h after PGF treatment. When PGF-induced luteolysis is incomplete, estrus does not

occur, relatively high concentrations of estradiol are maintained and the dominant follicle becomes persistent (Twagiramungu et al., 1994).

Ovulation rate after GnRH-2 is another factor influencing the effectiveness of PGF-GnRH-based protocols. In the current study ovulation rate did not differ between treatment groups (78 vs 70.8%) and was similar to ovulation rates obtained in previous studies (72%) using the same type of cattle (Saldarriaga et al., 2006). Ovulatory response to GnRH-2 varies depending on the effectiveness of previous hormonal treatments to synchronize the emergence of a follicular wave and the ability of the female to develop a large, estrogen-active follicle with the capacity to respond to GnRH-induced LH release. Pursley et al. (1995) reported an ovulation rate of 100% by 32 h after GnRH-2 in dairy cows synchronized using the Ovsynch protocol. Similarly, Cartmill et al. (2001) reported that 94% of dairy cows ovulated by 40 h after GnRH-2. In general, ovulation rate after GnRH-2 in dairy cows synchronized with the Ovsynch protocol ranges from 80 to 90% (Fricke et al., 1998; Vasconcelos et al., 1999; Kim et al., 2003). Ovulation rate in *Bos taurus* beef cows (84%) is similar to that of dairy cattle (Thompson et al., 1999). Ovulation rate in lactating *Bos indicus* cows (76%) seems to be lower compared to that of *Bos taurus* females (Barros et al., 2000) and is in accordance with ovulation rates obtain in the current study.

Ovulation rates after GnRH-2 are improved in cows that ovulate in response to GnRH-1. Bello et al. (2006) reported an increase of 25% in the proportion of cows ovulating after GnRH-2 if they ovulated to GnRH-1. We observed a 30% increase in the proportion of females ovulating after GnRH-2 if they ovulated or regressed a follicle in response to the GnRH-1 treatment. If only ovulation is considered as a positive response to GnRH-1 treatment, the increase in proportion in females ovulating after GnRH-2 is 15%, which is similar to a previous study (13%; Saldarriaga et al., 2006). In the current study, ovulation rate after GnRH-2 was influenced by follicular size at time of treatment. Bovine follicles achieve ovulatory capacity after deviation, around 10 mm in diameter (Sartori et al., 2001). Acquisition of ovulatory capacity is consistent with an increased expression of LH receptor in granulosa cells of the dominant follicle near the expected time of deviation. Xu et al. (1995) showed and increase in LH receptor mRNA on granulosa cells from non-detectable levels 2 days (6.7 mm) after the emergence of a new follicular wave to high levels 4 days (10.8 mm) after the emergence of the follicular wave. Similarly, Bao et al. (1997) found an increased LH receptor mRNA comparing small (7.8 mm), medium (10.8 mm) or large (15 mm) follicles. Furthermore, studies in vivo demonstrated that a 4-mg dose of LH induced ovulation in all cows when the

largest follicle was ≥ 12 mm, in 17% when it was 11 mm, and no ovulation when it was ≤ 10 mm (Sartori et al., 2001). Development of ovulatory capacity is related to follicular size in a LH dose-dependent manner. Sartori et al. (2001) reported that follicles that had undergone deviation and had reached a diameter of 10 mm ovulated in response to a high dose (24 or 40 mg) of LH, but not a low dose (4 mg).

Follicular size before ovulation has been associated with fertility (Vasconcelos et al., 2001; Peters and Pursley, 2003; Perry et al., 2005). Early induction of ovulation with GnRH can cause reduced luteal function in cattle and ultimately, reduced fertility (Taponen et al., 1999). Perry et al. (2005) reported that GnRH-induced ovulation of follicles ≤11 mm resulted in decreased pregnancy rates and increased late embryonic/fetal mortality. This decrease in fertility was associated with lower concentrations of estradiol on the day of AI, and decreased circulating concentrations of P4 after AI. Similarly, Vasconcelos et al. (2001) concluded that ovulation of small follicles (11.5 mm) have reduced fertility compared to large follicles (14.5 mm), possibly by the development of a smaller CL and decreased concentrations of P4. Lack of data makes it difficult to draw conclusions regarding the absolute follicular size at which fertility is

reduced, and could be influenced by differences in species (*Bos taurus* vs *Bos indicus*), breed (beef vs dairy), physiological status and age.

In the current study we observed that females that became pregnant had larger follicles (13 mm) at GnRH-2 than those that did not become pregnant (11.5 mm). This finding is in agreement to a recent report where Ovsynch-synchronized dairy cows that became pregnant had a significantly larger follicle diameter (15.8 mm) at TAI compared to those that did not become pregnant (14.5 mm; Lopes et al., 2006). Furthermore, a higher proportion of cows that had a ≥10 mm follicle at GnRH-2 become pregnant compared to those with follicles < 10 mm. These results are in agreement with a report by Borsato et al. (2004) where *Bos indicus x Bos taurus* heifers where synchronized using a combination of EB, CIDR insert and TAI. Heifers that become pregnant had a larger follicular diameter at TAI compared to those that did not become pregnant. Furthermore, a greater proportion of females with follicles larger than 10 mm become pregnant after TAI compared with those with follicles smaller than 10 mm (Borsato et al., 2004).

In conclusion, results from the current experiments indicate that presynchronization with GnRH 7 days before the initiation of the CO-Synch + CIDR protocol in *Bos indicus*-influenced cattle does not improve TAI pregnancy

rate. Presynchronization did increase the proportion of females ovulating after GnRH-1 but this was not reflected in an increased proportion of females with a synchronized follicular wave or ovulation after GnRH-2. Comparison of current results with previous reports indicates that pregnancy rates are probably greater when females are TAI 66 h after the PGF injection and removal of the progesterone source. Additional trials to confirm this speculation under optimal conditions are warranted and underway. Alternative methodologies to enhance the recruitment of a new follicular wave and improve synchronization of ovulation should be investigated, while trying to balance the cost/benefit of increases in the number of times animals have to be handled. In many cases, the latter consideration precludes the extensive utilization and adoption of this reproductive management tool by extensive beef cattle operations.

CHAPTER IV

ASSESSMENT OF VAGINAL ELECTRICAL RESISTANCE AS AN INDICATOR OF FOLLICULAR MATURITY AND SUITABILITY FOR TIMED ARTIFICIAL INSEMINATION IN COWS SUBJECTED TO A SYNCHRONIZATION OF OVULATION PROTOCOL

Introduction

Currently, only 5 to 7% of the beef cows in United States are inseminated artificially (NAHMS, 1997). Nonetheless, it is predicted that the practice of AI in pure-bred and commercial beef cattle operations will increase with the introduction of sexed semen (Hohenboken, 1999; Seidel, 2003). The main reasons for the low rate of adoption of AI among beef producers are the problem of accurate detection of estrus and the poor and inconsistent results of the protocols used to synchronize the onset of estrus and/or ovulation.

Several electronic devices have been developed to alleviate the need for visual estrus detection in cattle and to provide a precise determination of the onset of estrus. Commercially-available electronic technologies for estrus

detection are based on changes in physical activity (pedometers), changes in electrical and chemical properties of the reproductive tract (intravaginal resistance probes), or mounting activity (mount detectors) (Reviewed in Rorie et al., 2002).

The electrical resistance of reproductive tissues and their secretions has been studied not only in cattle but in buffaloes (Gupta and Purohit, 2001), sheep (Bartlewiski et al., 1999), goats (Rezac et al., 2001), horses (Ley et al., 1981), pigs (Rezac et al., 2003), foxes (Boue et al., 2000), rhinoceros (Bowers et al., 2005), rats (Singletary et al., 2005), guinea pigs (Bartos and Sedlacek, 1977), macaques (Fischer et al., 1990) and women (Brown et al., 2000). Vaginal electrical resistance (VER) fluctuates with stage of the estrous cycle, is highest during the luteal phase and declines during the follicular phase. The lowest VER values are correlated with the preovulatory LH peak (Schams et al., 1977; Aboul-Ela et al., 1983; Canfield and Butler, 1989). Changes in estradiol and progesterone regulate the degree of hydration of vaginal tissue and electrolyte content of reproductive tract secretions (Lewis et al., 1989). These changes increase the conductivity and decrease the electrical resistance of the vaginal wall during estrus (Feldmann et al., 1978).

Vaginal electrical resistance has been measured and used as an aid in estrus detection and for timing of insemination in cattle (Schams et al., 1977; Foote et al., 1979; Canfield and Butler, 1989). Leidl and Stolla (1976) found a correlation between low VER readings and higher pregnancy rates. Likewise, a recent report using a commercially-available probe indicates that readings obtained with the device accurately reflect estrous activity, with readings obtained every 12 h more reliable than 12-h visual observations (Wehner et al., 1997). Scipione and Foote (1999) found that both low milk progesterone and low VER values 21 to 23 days after insemination provided an early and accurate indication of a need for re-insemination.

Vaginal electrical resistance provides useful information about the course of luteolysis and development of preovulatory antral follicles in sheep (Bartlewski et al., 1999). Likewise, VER successfully predicted the stage of the estrous cycle, ovarian status, and ovulation in buffaloes (Gupta and Purohit, 2001). Follicular size has been associated with fertility in cattle, thus strategies might be developed to indirectly assess synchronization success (follicular size) before TAI. If this were possible, then females that were not synchronized would not be inseminated, thus saving the semen investment costs for cattle unlikely to conceive.

The current study was designed to retrospectively determine the efficacy of VER for identifying cows without a mature preovulatory follicle at TAI following a synchronization of ovulation protocol and as a prospective decision aid for determining cows that should not be bred.

Materials and Methods

Study Locations and Animal Protocols

This experiment was conducted at the Texas Agricultural Experiment Station, Beeville, Texas. The Institutional Agricultural Animal Care and Use Committee of the Texas A&M University approved in advance all procedures used in these studies.

Cattle

Two hundred and thirty three Brahman x Hereford (F1) females (nulli-, primi- and multi-parous) were utilized. Cattle were maintained in Coastal Bermuda and Kleingrass pastures and were required to have a minimum BCS of 5 (1-9 scale, 1 = emaciated, and 9 = obese) and if suckled be at least 50 days postpartum (DPP) at TAI.

Synchronization Procedure

All females received the CO-Synch + CIDR treatment for synchronization of ovulation (Fig. 6). The regimen included a CIDR insert (1.38 g of P4; Pfizer Animal Health, New York, NY) plus a GnRH (100 μg; Cystorelin®) injection on day 0, removal of the CIDR 7 day later (day 7) coincident with an injection of PGF (25 mg; Lutalyse®; Pharmacia & Upjohn Co., Kalamazoo, MI). Sixty-six hours following CIDR removal and PGF injection (day 10), all females were artificially inseminated and received a second injection of GnRH (100 μg; Cystorelin®). All females were turned with bulls 5 days after TAI for a 90-day breeding season. Pregnancy rates to TAI were determined by transrectal ultrasonography (Dynamic Imaging, Concept/MCV, equipped with a 5-7.5 MHz linear array probe; Livingston, UK) 30 day after TAI.

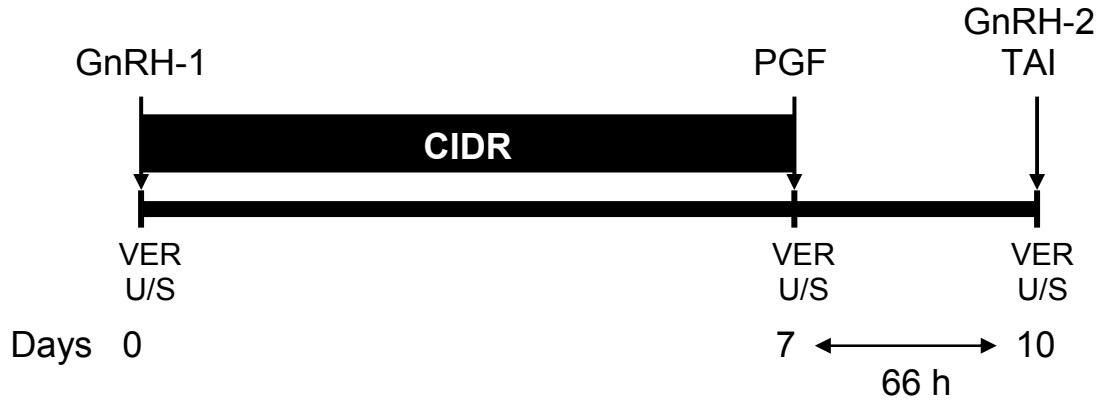

Fig. 6. Experimental protocol for synchronization of ovulation and timeline of events. All cattle received the CO-Synch + CIDR treatment for synchronization of ovulation. The regimen included a CIDR insert plus a GnRH injection on day 0 (GnRH-1), removal of the CIDR 7 days later coincident with an injection of Prostaglandin F2α (PGF). On day 10 (66 h after CIDR removal and PGF injection), all cattle were timed AI (TAI) and received a second injection of GnRH (GnRH-2). Vaginal electrical resistance (VER) measurements and ultrasound (U/S) examinations were performed on days 0, 7 and 10

Vaginal Electrical Resistance Readings

A commercially-available device (Ovascan; Animark Inc., Aurora, CO) was used to determine VER on days 0, 7, and 10 (Fig. 6).

The portable device consists on a main unit with a digital screen to display readings and a stainless steel detachable probe; it runs on rechargeable batteries. The probe was disinfected daily before each use and tested in a sodium chloride solution for calibration as recommended by the manufacturer's manual. The vulvar area of each female was cleaned with a paper towel and the probe was introduced in the vagina by spreading the vulva to avoid

contamination. The probe was rotated and moved back and forth 2 to 3 times and then held in place during 10 to 15 seconds or until the readings on the display stabilized. After each use VER determination, the probe was wiped with a clean paper towel to remove contamination and placed into Chlorhexidine solution (0.03%) until the next cow or heifer was in place for examination. Before each subsequent measurement, the probe was thoroughly rinsed with water and shaken to remove any excess water. Vaginal electrical resistant readings were taken by the same operator.

Ultrasonography

Transrectal ultrasonography of ovaries to evaluate follicular and luteal structures was performed at TAI on day 10 in all cattle and in a subset (n=98) on day 0, 7, and 10 (Fig. 6). Ultrasound examinations were performed by the same operator. Follicular structures were measured and an image of the dorsal and lateral view of each ovary was then obtained.

Statistical Analysis

Effects of animal, time and pregnancy outcome and its interaction on VER and follicular sizes and effects of pregnancy outcome on follicular size difference (follicle size at day 10 minus follicle size at day 7) and VER difference (VER on day 10 minus VER on day 7) were evaluated using the GLM procedure (SAS

Inst. Inc., Cary, NC). When a significant F-value was identified the LSD test was used to contrast means. After accounting for significant sources of variation, appropriate comparisons of pregnancy rates were made using chi-square analysis (PROC FREQ of SAS). Pearson correlation coefficient was applied to determine any linear correlation between variables of physiological interest.

Results

Mean (± SEM) age, BCS, BW, and DPP of all the females used in the study were 7.2 ± 0.3 yr, 5.2 ± 0.1, 538 ± 5.3 Kg and 77 ± 1.1 days, respectively (Table 8). Mean VER for the 233 females was greatest (101.4 ± 0.8) on day 0 and declined ($P < 0.01$) to 95.2 ± 0.8 and 82 ± 0.8 ohms, respectively, on days 7 and 10 (Fig. 7). Mean VER for the subset of 98 females examined with ultrasound on days 0, 7 and 10 decreased ($P < 0.01$) from 103.8 ± 1.2 to 95.5 ± 1.2 and 85.1 ± 1.2 ohms, respectively. Follicular sizes in these females on days 0, 7 and 10 were 10.1 ± 0.23, 10.2 ± 0.23, and 12.3 ± 0.25 mm, respectively. We observed a low negative but highly significant relationship ($r = -0.38$; $P < 0.001$) between VER and follicular size on days 0, 7, and 10. Follicular diameter and VER for the subset of 98 females are illustrated in Fig. 8.

Table 8

Mean (±SEM) BCS, DPP, BW, and Age in *Bos indicus*-influenced females used in the experiment

BCS	DPP (d)	BW (kg)	Age (yr)
5.2 ± 0.1	77 ± 1.1	538 ± 5.3	7.2 ± 0.3

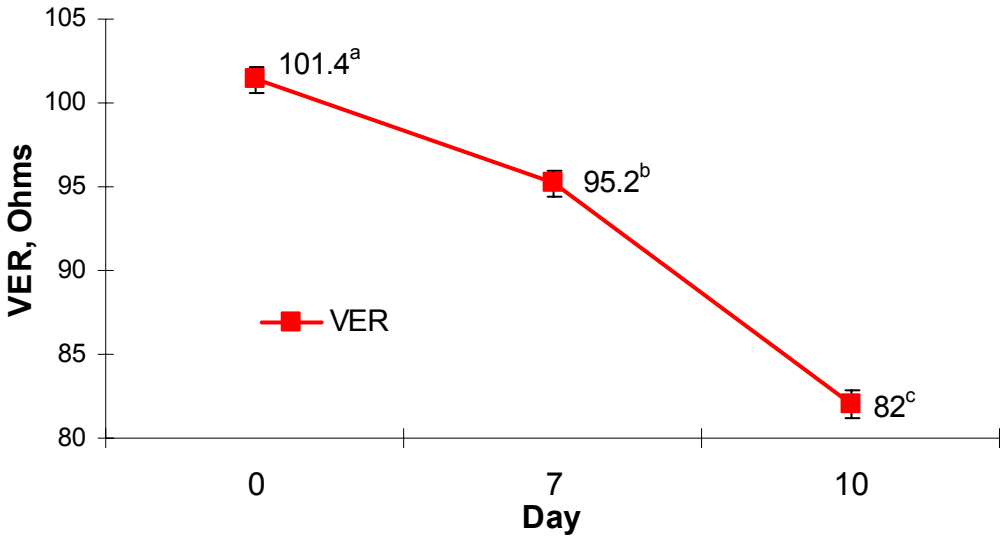

Fig. 7. Vaginal electrical resistance (VER) at different times during the synchronization protocol. (^{a,b,c} differ $P < 0.01$; n = 233)

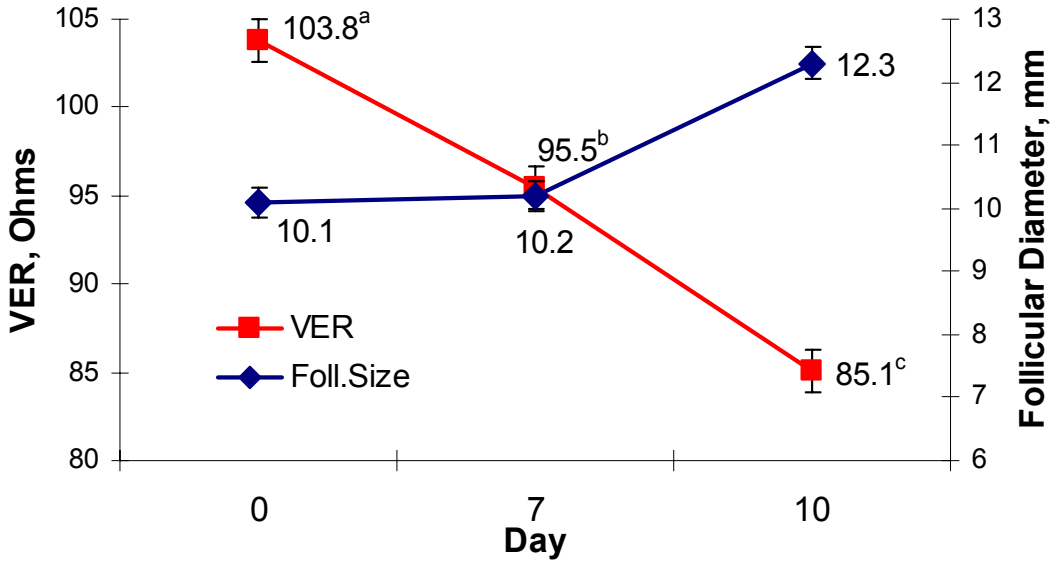

Fig. 8. Follicular diameter and vaginal electrical resistance (VER) at various times during the synchronization protocol. ([a,b,c] differ $P < 0.01$; $n = 98$)

The interaction of time and follicular size had an effect ($P = 0.05$) on pregnancy outcome (pregnant or not pregnant after TAI; Fig. 9) and VER ($P = 0.05$; Fig. 10). Mean follicular sizes on days 0, 7, and 10 for females that conceived were 10 ± 0.38, 10.4 ± 0.38, and 13 ± 0.4 mm, respectively. For females that did not conceive after TAI, follicular sizes were 10.2 ± 0.26 mm, 10 ± 0.26 mm, and 11.5 ± 0.27 mm respectively. Mean VER values on days 0, 7 and 10 were 100 ± 1.2, 96 ± 1.2, and 80.4 ± 1.2 ohms, respectively, in females conceiving after TAI and 102.9 ± 1, 94.4 ± 1, and 83.6 ± 1 ohms in those that did not conceive to TAI. Furthermore, the follicular size difference (follicle size at day 10 minus

follicle size at day 7) and VER difference (VER on day 10 minus VER on day 7) differed ($P < 0.05$ and $P < 0.01$) between females that conceived (2.6 ± 1.6 mm and -15.6 ± 1.3 ohms, respectively) and those that did not conceive (1.5 ± 2.3 mm and -10.8 ± 1 ohms, respectively). Timed-AI pregnancy rate was positively correlated with follicular size on day 10 ($r = 0.16$; $P < 0.05$) and follicular size difference ($r = 0.24$; $P < 0.05$), and negatively correlated with VER on day 10 (-0.15; $P < 0.05$) and VER difference (-0.18; $P < 0.01$). Relationships are summarized in Table 9.

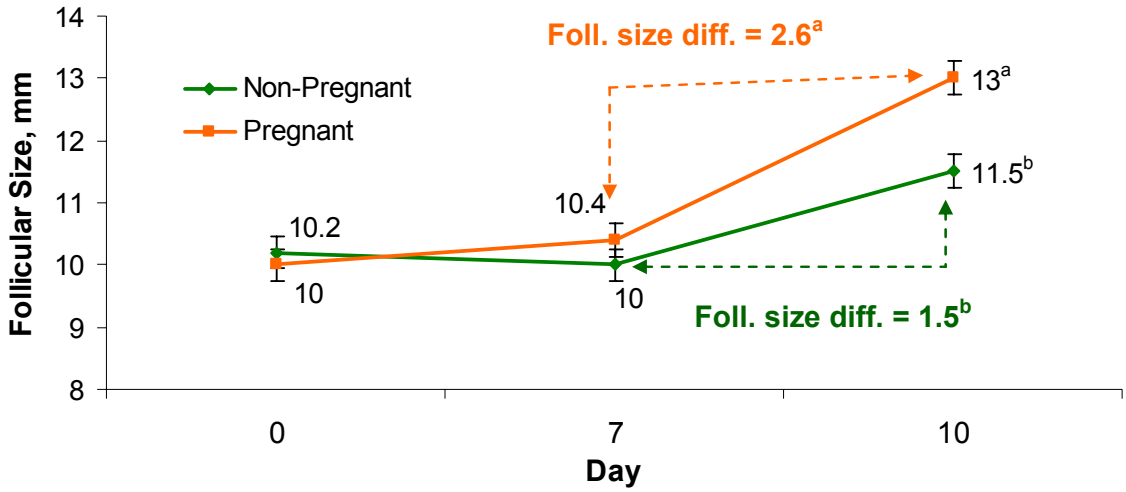

Fig. 9. Follicular diameter at various times during synchronization in females that either conceived or did not conceive to timed AI (TAI). Follicular size difference (Foll. Size Diff.) refers to: follicle size at day 10 minus follicle size at day 7. [a,b] differ $P < 0.05$

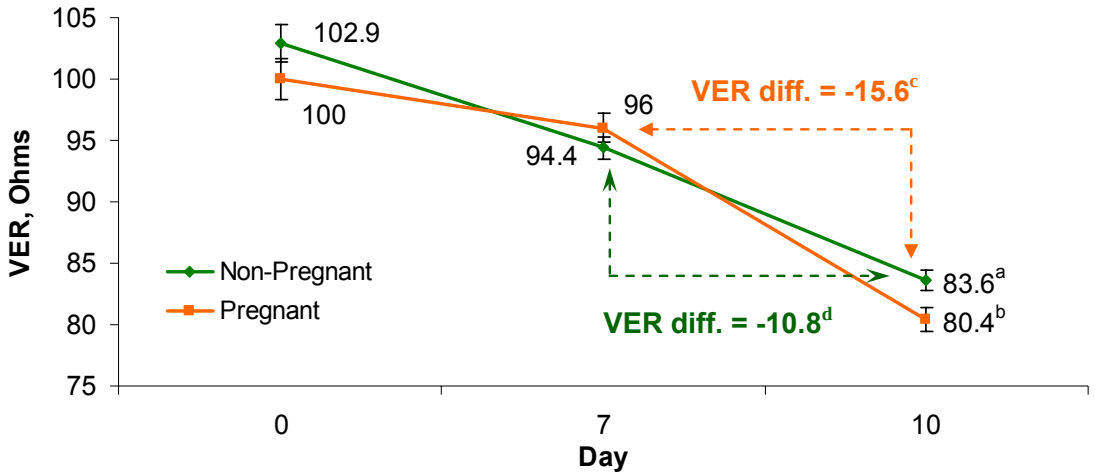

Fig. 10. Vaginal electrical resistance (VER) at various times during synchronization in females that either conceived or did not conceive to timed AI (TAI). VER difference (VER diff.) refers to: VER on day 10 minus VER on day 7 [a,b] differ $P < 0.05$ and [c,d] differ $P < 0.01$

Table 9

Relationships (Pearson correlation coefficient) of follicular size, change in follicular size, vaginal electrical resistance (VER) and change in VER to time AI (TAI) pregnancy rates

Variable	r	P - Value
Follicular Size at TAI	0.16	< 0.05
VER on TAI	-0.15	< 0.05
Follicular Size Difference[a]	0.24	< 0.05
VER Difference[b]	-0.18	< 0.01

Post hoc stratification of females into those with a small (< 10 mm; 44%) or large (≥ 10 mm; 84%) follicles at TAI revealed a greater ($P < 0.01$) ovulation rate for those with large compared to small follicles. Mean VER on day 7 and 10 were not different in animals with small (95.6 ± 1.8 and 83.7 ± 1.6) or large (95.6 ±

1.8 and 82 ± 1.6) follicles at TAI. Similarly, VER difference (VER diff.; ohms) did not differ between females with small (-11.9 ± 2) and large (-13.5 ± 2) follicles at TAI (Fig. 11). Timed-AI pregnancy rate was greater ($P < 0.01$) for females with large follicles than those with small follicles (Fig. 12). To contrast this information with the VER difference, we transformed the VER difference readings into two categories: Negative (< 0 ohms) or Neutral/positive (≥ 0 ohms). Pregnancy rates did not differ (39.4 ± 3.5 vs 29.4 ± 7.9%) between females represented in the negative and Neutral/positive categories (Fig. 12). A total of 11.4% (10/88) of females that became pregnant after TAI had a neutral/positive VER difference.

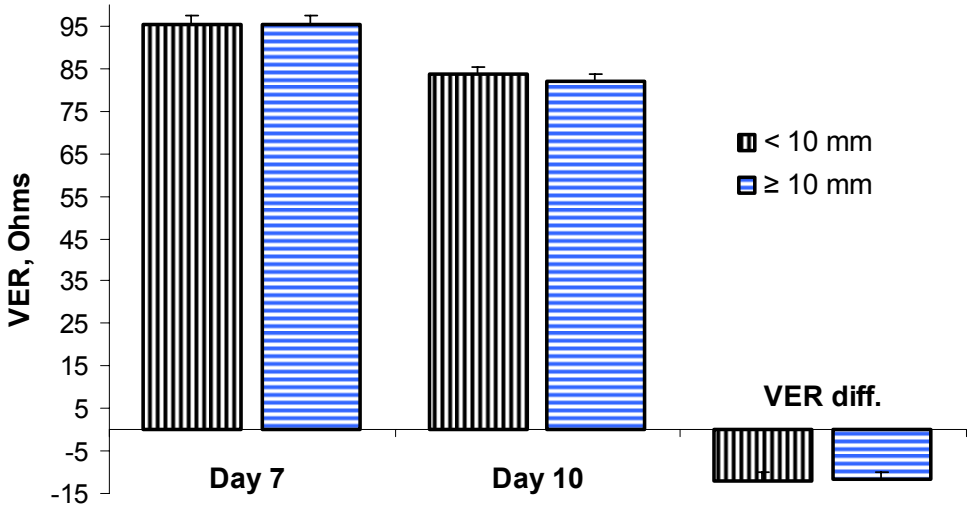

Fig. 11. Vaginal electrical resistance (VER) and VER difference (VER diff.; VER on day 10 minus VER on day 7) in relation to follicular diameter at timed AI (TAI; day 10)

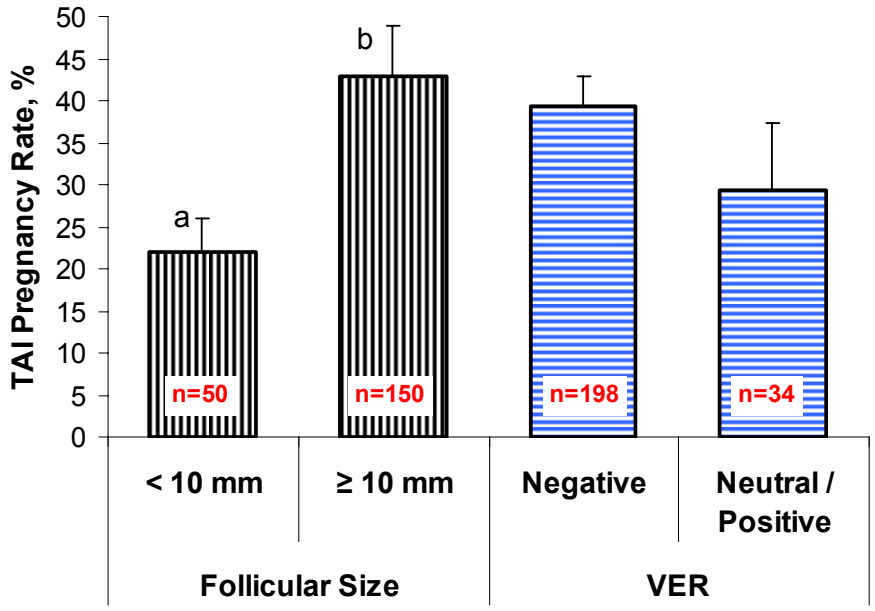

Fig. 12. Timed AI (TAI) pregnancy rate in relation to follicular diameter on day 10 and VER difference categories (Negative or Neutral / Positive). [a,b] differ $P < 0.01$

Discussion

Using a commercially-available device, the current study examined the efficacy of VER readings to identify cows without a mature preovulatory follicle at TAI. Overall, VER values observed in the current study were relatively greater than those presented in previous reports (Wehner et al., 1997; Scipioni and Foote, 1999). These differences may be the result of changes in intravaginal probe designs. Other factors such as depth of probe insertion in the vagina (McCaughey and Patterson, 1981; Aboul-Ela et al., 1983; Kitwood et al., 1993), position of the probe within the vagina (dorsal or ventral; Foote el at., 1979; Heckman et al., 1979), pressure against the mucous membrane (Leidl and Stolla, 1976), pathological conditions of the reproductive tract (Leidl and Stolla, 1976) and technician (Foote el at., 1979) can influence the results. Changes in VER at different times during the synchronization protocol were comparable to changes reported during the estrous cycle (Aboul-Ela et al., 1983).

Similar to previous studies in buffaloes (Markandeya et al., 1993; Gupta and Purohit, 2001), VER and developmental stage of the largest follicle were correlated in cattle in the current study. This is the first experiment reporting a correlation between follicular size assessed via ultrasonography and VER in cattle. However, electrical resistance of vaginal tissue has been correlated with

circulating levels of P4 and estradiol in cattle (Lewis et al., 1989) and sheep (Bartlewski et al., 1999), and the lowest values have been reported to be near estrus and coincident with the time of the LH peak (Schams et al., 1977; Aboul-Ela et al., 1983; Canfield and Butler, 1989). Changes in estradiol and P4 regulate the degree of hydration of vaginal tissue and electrolyte content of reproductive tract secretions (Lewis et al., 1989). Although VER seems to be controlled primarily by circulating concentrations of P4, it also changes in response to shifts in the estradiol:P4 ratio when P4 concentrations are decreasing (Bartlewski et al., 1999). In the current study, mean follicular size was similar on day 0 and day 7, but P4 was greater on day 7 compared to day 0 due to the presence of the CIDR. Thus, VER values should have been the same or slightly greater on day 7 compared to values on day 0. We hypothesize that presence of a CIDR during a 7-day period might generate inflammation of the vaginal mucosa; increasing the amount of extracellular fluid (edema) that eventually will reduce the tissue electrical resistance (Lewis et al., 1989).

We found that the interaction of pregnancy outcome and time had an effect on both follicular size and VER. Follicular size is an indicator of ovulatory capacity (Sartori et al., 2001) and it has been associated with fertility. Thus, in the current study, females that conceived to TAI had larger follicles compared to

those that did not conceive. This is in agreement with previous reports in which follicular size at the time of ovulation was associated with an increase in fertility (Vasconcelos et al., 2001; Perry et al., 2005; Lopes et al., 2006). Furthermore, we observed that TAI pregnancy rate was positively correlated with follicular size and negatively correlated with VER. The difference in VER at TAI between females that conceived or did not conceive might be explained by a greater rate of follicular growth and larger follicles in females conceiving to TAI. Follicle size is associated with estradiol production (Ireland and Roche, 1982; Bello et al., 2006) and a defining characteristic of the dominant follicle is a greater capacity for estradiol production (Fortune et al., 2001). Bartlewski et al. (1999) suggested that VER is controlled by the P4:estradiol ratio when P4 is low, such as during the follicular phase of the estrous cycle, or in the case of this study, after the induced luteolysis and removal of the external source of P4.

Marked variations among and within animals make single VER observations unreliable (Feldman et al., 1978, Rorie et al., 2002). Single measurements of the electrical resistance every second (Gartland et al., 1976) or third (Foote el at., 1979) day was demonstrated to be too infrequent and unreliable in distinguishing physiological states in cattle. Accurate identification of estrus requires measurements at least every 12 h (Canfield and Butler, 1989)

or more often (Aboul-Ela et al., 1983) to identify the changes in resistance over a period of time. Given that a single measurement allows only a limited evaluation, repetitive measurements are required in order to establish individual female baselines and associated declines in VER. We used VER on day 7 in the present study as a baseline to determine its decline over the 66- to 72- h pattern of decline to day 10. We detected a small (4.8 ohms) but statistically significant difference in VER between days 7 and 10 in females that conceived (-15.6 ohms) vs those that did not (-10.8 ohms).

Stratification of follicular structures into a small (<10 mm) or large (≥10 mm) category was made based on the ability of follicles to reach ovulatory competence around 10 mm in diameter (Sartori et al., 2001). As expected, a greater ovulation rate was observed in females with large follicles compared to small follicles and is in agreement with previous reports (Sartori et al., 2001). Additionally, an association between follicular size and fertility was observe in this study. Based on the follicular categories defined above, a greater proportion of females with large follicles at TAI become pregnant compared to those with small follicles. To the contrary, the VER difference was not significant (1.6 ohms) between females with large or small follicles, making this an unreliable measure to estimate follicular size. Furthermore, arrangement of VER difference

values into categories demonstrated that pregnancy rates did not differ between females with negative and neutral/positive readings. Surprisingly, some females that became pregnant had a neutral/positive difference. These results are interpreted to mean that VER, as applied in this study, does not permit consistent estimation of the grade of follicular maturity in synchronized females. The relatively small differences detected between the variables evaluated make VER methodology unreliable for this purpose. However, assessment of follicular size via ultrasonography is a very good methodology for detecting cows and heifers that have failed to respond optimally to synchronization and thus are less likely to conceive to the fixed TAI. Alternatively, more frequent VER measurements could possibly result in the more accurate identification of cows suitable for insemination. However, the financial costs associated with extra handling during synchronization would likely not be cost-effective and the added stress of the extra handling would also negatively impact reproductive performance.

CHAPTER V

SERUM PROGESTERONE CONCENTRATIONS IN OVARIECTOMIZED COWS BEARING NEW OR PREVIOUSLY USED CIDR DEVICES WITH OR WITHOUT AUTOCLAVING

Introduction

Intravaginal devices containing progestogens/progesterone have been used for more than 40 years with the aim of controlling the estrous cycle for use in AI programs. In the 1960s, sponges impregnated with progesterone were used in cattle (Carrick and Shelton, 1967) to synchronize the onset of estrus and in the early 1970s the progesterone releasing intravaginal device (PRID®) was developed (Mauer et al., 1975). The latter consisted of a stainless steel spiral coated with silicone rubber and impregnated with progesterone.

In the late 1980s, a new intravaginal device was developed and named CIDR-B® and it was first marketed in New Zealand in 1987 and was formulated to deliver progesterone (P4) for a 12-day period (Rathbone et al., 1997, 2001). The CIDR insert has been used in cattle during the last 20 years primarily in

protocols to synchronize estrus and/or ovulation (Macmillan and Peterson, 1993), as well as to resynchronize the return to estrus of previously inseminated cattle (Chenault et al., 2003; Colazo et al., 2006), as a presynchronization treatment (Schafer et al., 2006), as a supplemental source of P4 after AI (Stevenson et al., 2006), to induce estrous cycles (Perry et al., 2004) and to treat ovarian follicular cysts (Gumen and Wiltbank, 2005). The CIDR is a T-shaped vaginal insert containing 1.9 g of P4 (Canada) or 1.38 g of P4 (United States) in silicone molded over a nylon spine (Mapletoft et al., 2003). The original CIDR insert (1.9 g) was re-engineered to reduce the initial drug load to 1.38 g, decreasing the residual P4 remaining in the silicon after use, while maintaining biological performance (Rathbone et al., 2002).

Serum P4 concentrations have been shown to be similar in cows receiving a CIDR device with 1.38 g or 1.9 g of P4 (Rathbone et al., 2002), but peak concentrations occurring within 1 h after insertion are greater in cows receiving the 1.38 g device (Santos et al., 2004). The residual P4 load after a 7-day insertion period of the 1.38-g CIDR is 0.72 g (Rathbone et al., 2002), thus having the potential for reutilization. A single use is recommended by the manufacturer to prevent the potential transmission of venereal and blood borne diseases, but re-

utilization of CIDR inserts has been widely reported (Martinez et al., 2003; Stevenson et al., 2003; Colazo et al., 2004a, 2006).

Previously used CIDR inserts (containing 1.38 or 1.9 g of P4 when new) were able to suppress estrus during the 7-day insertion period in dairy and beef females (Richardson et al., 2002; Beal et al., 2003). No differences were observed in pregnancy rate to fixed-time AI between cattle synchronized with a new or once-used CIDR insert (Colazo et al., 2004a). Cerri et al., (2005) found no differences in concentrations of plasma P4 in lactating dairy cows receiving a new (1.38 g of P4) or an autoclaved insert used previously for 7 days. Different approaches have been used to clean, disinfect or sterilize inserts in studies reporting CIDR reuse. There are apparently no reports comparing serum concentrations of P4 produced by washed and autoclaved CIDRs. Therefore, the objective of this study was to compare serum concentrations of P4 in ovariectomized cows receiving new, re-used disinfected (DIS), and re-used autoclaved (AC) CIDR inserts (Pfizer Animal Health, New York, NY).

Materials and Methods

The experiment was conducted at the Texas Agricultural Experiment Station, Beeville, Texas. The Institutional Agricultural Animal Care and Use

Committee of the Texas A&M University approved in advance all procedures used in these studies.

Animals and Study Locations

Five non-lactating, bilaterally ovariectomized (OVX) Brahman x Hereford (F-1) cows and one OVX Hereford cow were used in a replicated 3 x 3 Latin square study. Each experimental period was determined to 7 days, with at least 48 h between different periods to minimize any carry-over effects from the preceding treatment.

Cows were housed in pens (dry-lot) measuring 25.6 x 9.6 m; and were fed a forage-based (Coastal Bermuda hay) diet.

Procedures

Three different insert treatments were used: 1) New, 2) Re-used disinfected (DIS), and 3) Re-used autoclaved (AC). All re-used CIDR inserts were obtained from cows involved in a synchronization of ovulation experiment, had been inserted for 7 days and were handled as follows: Immediately after removal, they were placed in an empty bucket, washed thoroughly with soap and water, with emphasis on trying to remove mucus and debris that had accumulated in empty spaces between the silicon layer and the T-shaped body.

Inserts for the DIS treatment were soaked in a Chlorhexidine Gluconate solution for 2 h (0.03%), rinsed thoroughly with water, allowed to air-dry and placed in zip-lock bags for storage. For the AC treatment, CIDRs were not soaked in disinfectant but were autoclaved at 121°C and 724 mm Hg for 20 min, allowed to cool and placed in zip-lock bags for storage before use.

Sampling

Blood samples were collected via coccygeal tail vessel using evacuated 10-ml tubes and 20 g bleeding needles at 0, 10, 30, 60, 180, 480 min relative to time of insertion of CIDRs, daily until day 7 and at 30, 60, 180 min relative to time of removal. Blood samples were placed on ice immediately after collection and remained on ice until transported to the laboratory. Samples were allowed to clot at room temperature for approximately 1 h before centrifugation at 3000 x g for 30 min. Serum was collected and stored at -20°C until hormone analysis using a commercial RIA kit for progesterone (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA). Samples were assayed in duplicate. Intra- and interassay CV were 8.01 and 10.4% respectively and sensitivity was 0.05 ng/mL.

Statistical Analysis

All data was subjected to ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC) as for a Latin square design.

Results

Mean (± SEM) BW of all animals was 604 ± 9 kg, and did not differ between experimental periods (P = 0.81). Mean (± SEM) serum concentrations (ng/mL) of P4 during the 7-day period of insertion (Fig. 13 and 14) were greater (P < 0.03) for New (3.7 ± 0.2) and AC (3.4 ± 0.3) than for DIS CIDRs (2.8 ± 0.2). These effects were created primarily by differences occurring during the first 8 h after CIDR insertion (Fig. 14 and 15). Within this interval, mean concentrations differed (P < 0.05) among all treatments (New, 4.6 ± 0.5; DIS, 2.7 ± 0.3; AC, 6.0 ± 0.7).

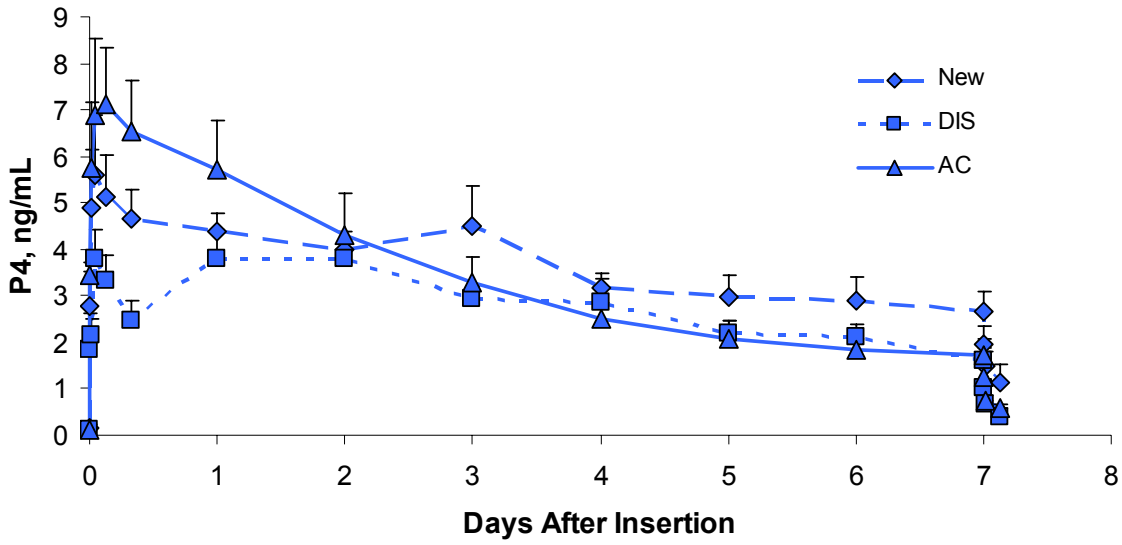

Fig. 13. Mean (± SEM) serum concentrations of progesterone (P4, ng/mL) in ovariectomized cows bearing New, re-used disinfected (DIS) or re-used autoclaved (AC) CIDR inserts during a 7-day insertion period

The majority (61 %) of maximum peak values occurred between 10 and 180 min after insertion. Mean concentrations during this period (New, 4.6 ± 0.5; DIS, 2.8 ± 0.3; AC, 5.8 ± 0.7) differed ($P < 0.01$) among all treatments (Fig. 14). Analysis demonstrated that cows bearing DIS CIDRs had a smaller ($P < 0.05$) area under the curve (AUC) during the first 4 days after insertion compared to New or AC CIDRs (Table 10). Autoclaved CIDRs had a greater AUC compared to New and DIS during interval from CIDR insertion (0 h) to 8, 24 and 48 h (Table 10; $P < 0.05$).

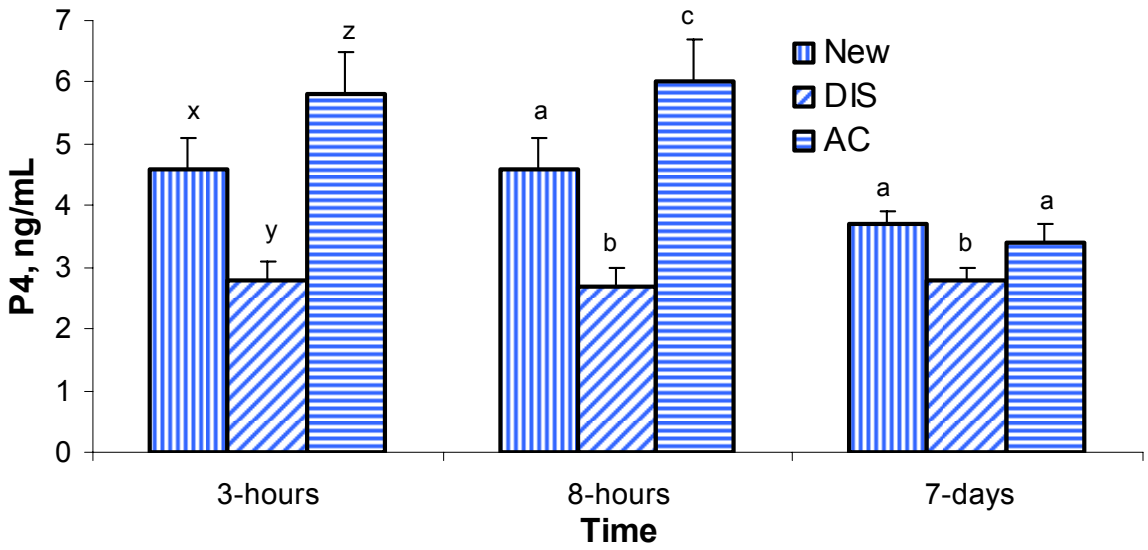

Fig. 14. Mean (± SEM) serum concentrations of progesterone (P4, ng/mL) in ovariectomized cows bearing New, re-used disinfected (DIS) or re-used autoclaved (AC) CIDR inserts during the first 3 or 8 h relative to insertion and the 7-day insertion period. Different letters within time differ. (^{x,y,z} $P < 0.01$; ^{a,b,c} $P < 0.05$)

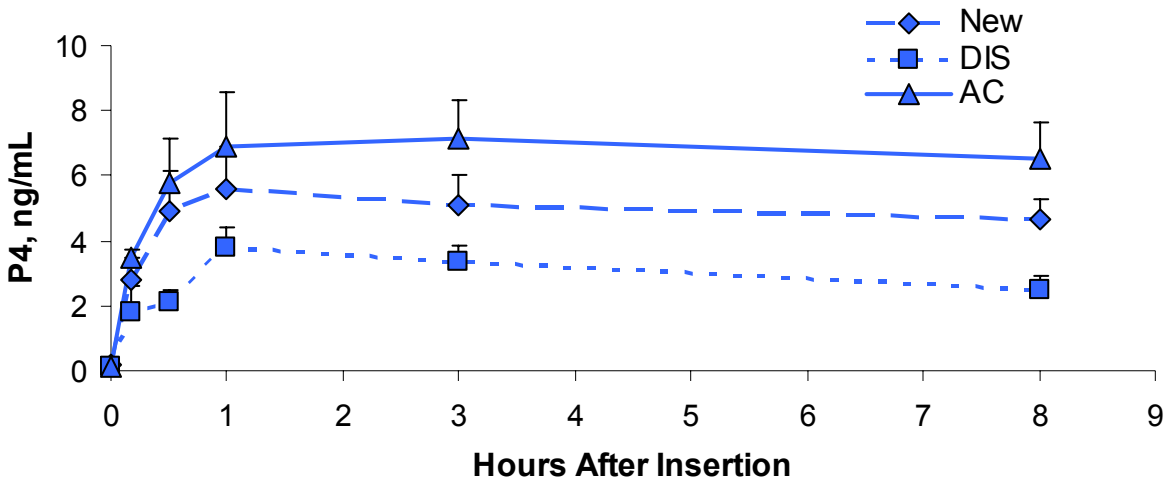

Fig. 15. Mean (± SEM) serum concentrations of progesterone (P4, ng/mL) in ovariectomized cows bearing New, re-used disinfected (DIS) or re-used autoclaved (AC) CIDR inserts during the first 8 h after insertion

Table 10
Area under the serum progesterone concentration–time curve of ovariectomized cows bearing New, re-used disinfected (DIS) or re-used autoclaved (AC) CIDR inserts for different intervals relative to insertion. Values are LS means.

Interval	Treatment			SEM ±
	NEW	DIS	AC	
0 - 10 min	0.25	0.17	0.3	0.05
0 - 30 min	1.5[a]	0.8[b]	1.8[a]	0.19
0 – 60 min	4.2[a]	2.3[b]	5[a]	0.4
0 – 3 h	14.9[a]	9.4[b]	19[a]	1.5
0 – 8 h	39.3[a]	24[b]	53[c]	4
0 – 24 h	111[a]	74[b]	151[c]	8
0 – 48 h	212[a]	165[b]	271[c]	13
0 – 72 h	314[a]	246[b]	362[a]	19
0 – 96 h	406[a]	315[b]	432[a]	26
0 – 120 h	479	376	486	32
0 – 144 h	550	428	533	39
0 – 168 h	616	472	575	44

[a,b,c] Means within row with uncommon superscripts letters differ ($P < 0.05$)

Discussion

Serum concentrations of P4 obtained during the 7-day insertion period of a new CIDR in the current study are comparable to those in a previous report using the 1.38 g-CIDR (Rathbone et al., 2002). Concentrations of P4 obtained with the DIS CIDR were also similar to those reported by Martinez et al. (2006) using a re-used insert originally containing 1.9 g P4 in ovariectomized cows. Progesterone concentrations in the 3 treatments used in the current experiments peaked within the first 3 h after insertion and reached plateaus that were sustained for about 8 h. This was followed by a constant decrease until removal on day 7. Macmillan et al. (1991) reported that following CIDR (1.9 g) insertion in ovariectomized heifers, plasma progesterone concentrations increased to approximately 8.7 ng/ml by 6 h and then decreased to 6.8 ng/ml and 2.5 ng/ml on d 1 and 12 after insertion, respectively. Furthermore, Cerri et al. (2005) compared plasma P4 concentrations after insertion of a new or a 7-day used, autoclaved CIDR. In both treatments, concentrations of P4 increased immediately after insertion, reached a plateau at 90 min and followed the same pattern during the remainder of the insertion period.

While it is possible that lower P4 concentrations observed with the DIS CIDRs compared to the AC inserts were caused by an extended exposure to the

disinfectant solution, it is more likely that steam sterilization of the CIDR in the AC group increased the rate of elution during the first few hours after insertion compared to DIS. Such an effect indicates that the autoclaving process modifies in some way the structure of the implant or the disposition of the P4 within the insert. Plasma profiles of progesterone resulting from re-used, gas-sterilized PRID devices were lower compared to the autoclaved PRID devices where steady-state plasma concentrations following re-insertion were observed to be very similar to an unused insert (McPhee et al., 1983). Results were attributed to formation of a large quantity of crystalline progesterone on the surface of the autoclaved PRID (McPhee et al., 1983). Structural and functional similarities exist between the PRID and CIDR inserts. Both inserts are made using micronized progesterone in a silicon rubber skin which is molded into a nylon (CIDR) or stainless steel structure (PRID; Rathbone et al., 1998). Due to this similarity, it is possible that the heat-sterilization process used in the current study may have resulted in the same effect observed previously in autoclaved PRID inserts.

Different approaches have been used to clean, disinfect or sterilize inserts in studies reporting CIDR reuse. Colazo et al. (2004) soaked used CIDRs in a povidone iodine-based detergent solution for approximately 2 h, followed by

scrubbing, rinsing with water, air-drying, and steam sterilization in an autoclave. Others have used schemes restricted only to chemical disinfection (Van Cleeff et al., 1992; Martinez et al., 2006), gas sterilization (Schmitt et al., 1996b) or autoclaving (Cerri et al., 2005). The efficacy of any of these methods in terms of preventing disease transmission has not been proven.

Padula and Macmillan (2006) demonstrated that changes in uterine and vaginal microflora in early postpartum beef cows occur after the insertion of a CIDR for 14 days. Microbial culture of swabs of CIDR inserts after removal yielded intense growth of bacteria. The predominant species isolated were *Pseudomonas aeruginosa* and *Actinomyces pyogenes* (Padula and Macmillan, 2006). Furthermore, P4 has an immunosuppressive effect on the uterus (Hansen, 1998). Therefore, reutilization of CIDR inserts has the potential to transmit pathogens and spread disease. Nonetheless, there are apparently no reports of disease transmission related to the re-use of CIDR inserts. More studies on CIDR re-use may be in order because of the popularity of their reuse.

Conclusions

Autoclaving may be the best option when re-using CIDR inserts because it creates greater concentrations of P4 immediately after insertion and reduces maximally the risk of disease transmission.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A presynchronization treatment with GnRH 7 days before the onset of the CO-Synch + CIDR protocol to synchronize ovulation **did not** yield better pregnancy rates compared to non-presynchronization in *Bos indicus*-influenced females in south Texas. Nonetheless, presynchronization optimized the ovulatory response after GnRH-1, but failed to improve the incidence of a new follicular wave after GnRH-1. Timed AI pregnancy rate observed in cattle synchronized with the CO-Synch + CIDR indicates the possibility that TAI at 66 h is preferred over 48 h. Synchronization of ovulation in well-nourished *Bos indicus*-influenced cattle with the CO-Synch + CIDR and TAI at 66 h could yield a relatively high (> 50%) pregnancy rate which is comparable to that obtained in *Bos taurus* females. However, more trials with greater numbers of females will be necessary to confirm this speculation. In addition, alternative methodologies to enhance the recruitment of a new follicular wave and improve synchronization of ovulation should be investigated. The implementation of a VER methodology to indirectly assess follicular maturity as applied in this study

does not permit consistent estimation of the grade of follicular maturity in synchronized females. However, assessment of follicular size via ultrasonography is a very good methodology for detecting cows and heifers that have failed to respond optimally to synchronization and thus are less likely to conceive to the fixed TAI. Feasibility of reusing CIDR is an attractive methodology to help reduce the initial investments in products. Steam sterilization of used CIDR inserts is a better means to guarantee greater circulating P4 concentration and reduce risks of disease transmission.

Improved pregnancy rates, detection of females with lower odds to conceive, and reduction of treatment costs could enhance the cost:benefit of a synchronization of ovulation protocol, making it more affordable and encouraging beef cattle producers to endeavor to utilize genetic improvement through fixed TAI.

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APPENDIX A

LIST OF ABREVIATIONS

μg	microgram
μl	microliter
AC	autoclaved
AUC	area under the curve
AI	artificial insemination
BCS	body condition score
BMP	bone morphogenetic protein
CIDR	controlled internal drug release
CL	corpus luteum
CV	coefficient of variation
DIS	disinfected
DPP	days postpartum
E-17β	estradiol-17β
EB	estradiol benzoate
EGF	epidermal growth factor
EV	estradiol valerate

F1	first filial generation
FDA	food and drug administration
FSH	follicle stimulating hormone
g	grams
GDF-9	growth and differentiation factor-9
GnRH	gonadotropin releasing hormone
h	hours
hCG	human chorionic gonadotropin
Kg	kilogram
LH	luteinizing hormone
m	meter
min	minute
mg	milligram
MGA	melengestrol acetate
MHz	megaHertz
mL	milliliter
mm	millimeter
NAHMS	national animal health monitoring system
ng	nanogram

NRC	national research council
OVX	ovariectomized
P4	progesterone
PGF	prostaglandin F2α
PRID	progesterone releasing intravaginal device
RIA	radioimmunoassay
SAS	statistical analysis system
SEM	standard error of the mean
SMB	Syncro-Mate-B
TAI	timed-AI
VER	vaginal electrical resistance

APPENDIX B

LABORATORY PROCEDURE

Progesterone RIA

Single Antibody RIA Kit, Diagnostic Products Corporation, Los Angeles, CA

References:
Diagnostic Products Corporation Coat-A-Count Progesterone Kit, L.A.,CA.
Jones, E.J., Armstrong, J.D., Harvey, R.W., 1991. J. Anim. Sci. 69, 1607-1615.
Simpson, R.B., Armstrong, J.D., Harvey, R.W., 1992. J. Anim. Sci. 70, 1478-1487.

1. Iodinated Product: Iodination grade hP4.

2. Antibody: Anti-human P4 coated tubes.

3. Standards: Human serum with added P4. Range 0.1 – 20.0 ng/ml.

4. Reference: Human standard preparation added to bovine serum.

5. RIA Procedure:
    A. Begin and complete assay
        I. Pipette in non-coated polypropylene tubes
            NSB – 100 μl of 0 Std
        II. Pipette in antibody coated tubes
            0 Std – 100 μl
            Std – 100 μl
            Ref – 100 μl
            Unknowns – 100 μl

III. Pipette 1 ml of ^{125}I –P4 provided in the kit to all tubes including two Total Count non-coated polypropylene tubes.
IV. Vortex tubes briefly and incubate at room temperature for 3 h.
V. Pour off supernatant.

6. Count radioactivity of each tube using a gamma counter.

VITA

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Publications

Zuluaga, J. F., J. P. Saldarriaga, D. A. Cooper, J. A. Cartmill, R. L. Stanko, and G. L. Williams. 2006. Effects of presynchronization with GnRH on conception rates and ovarian events in *Bos indicu*s-influenced females synchronized with CO-Synch + CIDR. J. Anim. Sci. 84 (Suppl. 1): 151

Zuluaga, J. F., J. P. Saldarriaga, D. A. Cooper, J. A. Cartmill, and G. L. Williams. 2006. Assessment of vaginal electrical resistance (VER) as an indicator of follicular maturity and suitability for timed AI in cows subjected to a synchronization of ovulation protocol**.** J. Anim. Sci. 84 (Suppl. 1): 149

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Saldarriaga J. P., D. A. Cooper, J. A. Cartmill, **J. F. Zuluaga**, R. L. Stanko, and G.L. Williams. 2006. Ovarian, hormonal and reproductive events associated with synchronization of ovulation and timed appointment breeding of *Bos indicus*-influenced cattle using intravaginal progesterone, GnRH and PGF2α. J. Anim. Sci. (in press).