SYNCHRONIZATION OF FOLLICULAR WAVE EMERGENCE AND OVULATION FOR REPRODUCTIVE BIOTECHNOLOGIES

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INTRODUCTION

The expanding knowledge of the roles of the corpus luteum (CL) and follicular waves in the bovine estrous cycle has resulted in renewed interest in the prediction of finely controlled ovulation synchronization. The intention of the following discussion is to provide an overview of the most used treatments to synchronize the emergence of follicular waves, follicular development and induction of ovulation. These protocols were designed for utilization in reproductive biotechnologies, such as artificial insemination (AI) and embryo transfer. A brief introduction about the normal events occurring in the estrous cycle will be given, followed by the discussion to determine how these events impact on the design and effectiveness of a synchronization program. This review is based on the general idea that ovarian response to exogenous stimulation is subjected to the physiologic status of the ovaries at the time of treatments for synchronization of both follicular wave emergence and ovulation.

The endogenous control of the bovine estrous cycle involves the secretion of a number of hormones from the brain, ovaries and uterus. Some of the main hormones are gonadotrophin releasing hormone (GnRH) from the hypothalamus, follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland, estrogen, progesterone and inhibin and other factors from the ovary and prostaglandin F2α (PGF) from the uterus. Although there has been a tremendous progress in the last 10 years, details of these endocrine interrelationships remain under investigation. The length of the bovine estrous cycle depends on the CL lifespan. The demise of the CL occurs about Day 17-18 in the normal cycling, nonpregnant bovine female. It is also known that regression of the CL is caused by PGF secreted by the nonpregnant uterus. This process results in a rapid fall in concentrations of circulating progesterone, while blood concentrations of LH and follicular growth is further stimulated. The dominant follicle of the last follicular wave becomes the preovulatory follicle and secretes increasing amounts of estradiol, which causes changes in the oviduct and uterus, behavioural estrus, and a preovulatory release of LH. The preovulatory LH peak results in maturation of the oocyte, ovulation 24-32 h after the onset of estrus and luteinization of the ovulated follicle to form a secreting corpus hemorrhagicum (CH). Growth and development of the CH into a fully functional CL causes progestational changes in the oviduct and uterus. Those changes facilitate embryonic development and pregnancy. If pregnancy is not established, the cycle will begin again.

The identification of individual follicles by ultrasonography has demonstrated that ovarian follicles in cattle grow in waves. A wave of follicular development is represented by the growth of several antral follicles followed by selection of a dominant follicle and regression of all other subordinate follicles. In the absence of luteal regression, the dominant follicle eventually regresses and a new follicle wave begins. Most cows exhibit 2 or 3 waves of follicular development during the estrous cycle. In general, the first wave of follicles begins to grow around time of ovulation. The selection of a dominant follicle becomes evident at the time of follicular deviation, which occurs approximately 3 days after a wave has emerged (Ginther et al., 1996). If a cow has two follicular waves,

the second wave of follicular development begins approximately by Day 10 post-ovulation. The follicle destined to ovulate apparently arose from this cohort in cows with two follicular waves, whereas the ovulatory follicle arose from a third follicular wave that started on Day 16 in cows with three follicular waves.
Historically, control of the estrous cycle has been achieved by lengthening or shortening the luteal phase. However, follicular development had remained uncontrolled. In order to more efficiently manipulate the estrous cycle, the control of both luteal and follicular dynamics is essential. During the last 10 years, there has been a copious amount of studies conducted to evaluate treatments to control follicular waves. The resulting treatments have been incorporated into estrus synchronization programs, which have also been improved with treatments intended to induce a synchronous ovulation facilitating a single fixed-time AI (FTAI).

METHODS OF CONTROL OF THE ESTROUS CYCLE

The bovine estrous cycle can be shortened (long acting estradiol or prostaglandin) or lengthened (progestins). Advances had been slow since the first attempts to control the bovine estrous cycle by progesterone (Christian and Casida, 1948) or estrogens (Wiltbank et al., 1961) or the combination of both (Wiltbank et al., 1965). New approaches to the manipulation of the bovine estrous cycle have been applied without impairment of fertility.

CONTROL OF LUTEAL FUNCTION

PROSTAGLANDINS

After approximately 14 days of progesterone secretion, the endometrium secretes 6-h pulses of PGF for approximately 36 h and PGF concentrations increased in uterine secretions and uterine vein plasma (Hansel et al., 1975). Responsiveness to PGF has been shown to increase progressively from 6 to 8 days post-estrus and the CL remains responsive for the remainder of diestrus (Momont and Seguin, 1984). After PGF treatment of beef heifers on Days 7 to 20 after estrus, the length of the estrous cycle was significantly reduced and pregnancy rate following estrus detection and AI (72.5%) did not differ from that in controls (73.0%; Roche, 1974).
It has been demonstrated that the length of the interval from PGF treatment to estrus depends upon the stage of development of the dominant follicle present at the time of treatment. The administration of PGF on Days 3 to 10 and 7 to 16 of the estrous cycle resulted in different intervals from treatment to estrus and it was attributed that these varying intervals depended on growth or atresia of large antral follicles, but no conclusions were stated (Macmillan and Henderson, 1984). In a more detailed study involving ultrasonography, the effects of day of PGF treatment were further examined based on the selection and development of the ovulatory follicle (Kastelic et al., 1990a). The interval from PGF treatment to ovulation was shorter in heifers treated on Day 5 after ovulation (growing/early static; 3.0 days) than in those treated on Day 12 (regressing phase of Wave 1 or early growing phase Wave 2, 4.5 days). Treatment on Days 5 or 8 was followed by ovulation of the dominant follicle of Wave 1 (near its maximum diameter at the time of treatment), whereas treatment on Day 12 was followed by ovulation of the dominant follicle of Wave 2. This follicle was small at treatment and required more time to attain ovulatory size. It was concluded that the interval from PGF treatment to estrus depended on the time of growth up to the

ovulatory size. The rationale for synchronization of follicular waves prior to inducing luteal regression in estrus synchronization programs was established in this study.

PROGESTERONE AND PROGESTINS

Progesterone was initially used in 1948 to alter the length of the cycle by serial daily injections starting on the Day 14 of the estrous cycle (Christian and Casida, 1948). Although estrus and ovulation were prevented in heifers treated with 50 mg of progesterone, heifers came into estrus 5 or 6 days later after suspension of treatment. The dose of 25 mg of progesterone was effective in suppressing estrus in all treated heifers, but ovulation was prevented only in 50% of heifers (Christian and Casida, 1948). Thus, when progesterone is given at an appropriate dose, it suppresses estrus, LH surge and ovulation.

Later studies demonstrated that progesterone or progestins given for a term longer than the normal life span of the CL (i.e., >14 days) resulted in synchronous estrus after treatment was discontinued, but fertility was poor (Trimberger and Hansel, 1955). When a progestin treatment was administered, ovulation was prevented; but increased LH pulsatility occurred after the CL regressed (Cooperative Regional Research Project, 1996). The administration of progesterone suppresses LH pulse frequency if given to cattle bearing a CL, which in turn, causes suppression of dominant follicle growth in a dose-dependent fashion (Adams et al., 1992b). However, the dose of progestins used to control the estrous cycle in cattle (melengestrol acetate [MGA] in the feed, or progestin-impregnated devices) has relatively less suppressive effects on LH secretion than progesterone from the CL and following luteal regression, the increased LH pulse frequency leads to development 'persistent' follicles (Custer et al., 1994; Kojima et al., 1995; Savio et al., 1993). These follicles contain aged, infertile oocytes (Custer et al., 1994; Mihm et al., 1999, Revah and Butler, 1996) and insemination following progestin withdrawal results in poor fertility (reviewed in Larson and Ball, 1992 and Odde, 1990).

One of the first developed progesterone-releasing devices was the PRID (progesterone release intravaginal device). Initially, PRIDs were placed by 18 days with plasma progesterone greater than 1 ng/mL with a high retention (90%). The progestin "norgestomet" has been used in regimens of estrus synchronization. Commercially, two implant types have been in the market "hydron" (SMB, Syncro-Mate B 6 mg of norgestomet contains; Merial, Iselin, U.S.A.) and Crestar (contains 3 mg of norgestomet; Intervet International, Boxmeer, Holland). The norgestomet implant SMB has been removed from the market since a long time ago. Therefore, the only commercial norgestomet implant is Crestar. The most used protocol for both consists of implant placement for 9 days plus an intramuscular injection of 5 mg norgestomet and 3 mg EV at the time of insertion. This type of protocols has been extensively used with varied results (Odde, 1990). However, when the standard Crestar protocol was compared with a double dose of prostaglandin, Crestar resulted in greater conception rates to detected estrus (Kastelic et al., 1999).

Other progestin or progesterone-releasing devices have recently been used in synchronization protocols, such as CIDR (controlled internal drug release; Pfizer Animal Health) DIV-B (Syntex, Argentina) that contains 1 g of progesterone (Cutaia et al., 2001; Moreno et al., 2001; Tríbulo et al., 2002; Bó et al., 2003) or similar to this one, the TRIU-B (Elastécnica, distributed by Biogénesis, Argentina) that also contains 1 g of progesterone (Carcedo et al., 2003). Another more recent intravaginal device is the Cue-Mate® (Bioniche Animal Inc. Health, Ontario, Canada) that contains 1.56 g of progesterone (Cavalieri et al., 2004, McDougall et al., 2004).

Melengestrol acetate (MGA) is orally administered progestin in the Canadian market. The most common MGA-based estrus synchronization protocol consisted of 14 days of

MGA feeding followed by an injection of PGF 17-19 days later, with insemination at detected estrus (Lamb et al., 2000). There is a recent trend for the utilization of MGA in short-term protocols for estrus synchronization. However, the risk of persistent follicles and low fertility still remains when there is no attempt to control follicular growth. Short-term MGA programs that included treatment with estradiol or GnRH to synchronize follicular wave emergence have been designed (Kastelic et al., 1996, 1997; Thundathil et al., 1999). Estradiol-17β treatment at the beginning of an MGA protocol in beef cattle resulted in more consistent estrus and pregnancy rates (to AI after detected estrus) than GnRH or Controls (Thundathil et al., 1999). These synchronization systems may be promising if a treatment to synchronize ovulation for FTAI are included.

MANIPULATION OF FOLLICULAR DEVELOPMENT

ESTRADIOL

Originally, treatments with estrogens were given to cause regression of the CL (Wiltbank et al., 1961). Shortly after, estradiol was used to induce luteolysis in short treatments with progestins (Ulberg and Lindley, 1960; Wiltbank et al., 1965; Wiltbank and Kasson, 1968). A dose of 5 mg of estradiol valerate (EV) was administered in the second day of an eight-day treatment with 400 mg/animal/day dihydroxi-progesterone (DHPA) in the ration (Wiltbank and Kasson, 1968). The treatment resulted in 95% of the heifers detected in estrus in a 96-h period, with an acceptable conception rate (54%; Wiltbank and Kasson, 1968). Thereafter, progestin-based protocols were shortened to 9 days with an im injection of EV administered at the beginning of the treatment to induce the luteolysis.

Recently, it was observed that the administration of 5 mg EV along with a norgestomet implant (Syncro-Mate-B, SMB) resulted in regression of antral follicles (Bó et al., 1991), and emergence of a new follicular wave at a relatively predictable time thereafter (Bó et al., 1993). When EV was administered in the late static or regressing phase, emergence of the next follicular wave was attributed to the extended suppression of plasma FSH concentrations (Bó et al., 1993). Therefore, the high estrous response and acceptable pregnancy rate obtained in earlier studies by Wiltbank and Kasson (1968) were not only due to the luteolytic effects of EV, but also to the EV-induced follicle turnover.

Estradiol-17β has been shown to suppress follicular growth when given to progestin-treated cattle regardless of the stage of follicle development at the time of treatment (reviewed in Bó et al., 1995a). Overall, emergence of a new follicular wave occurred 4.3 ± 0.2 days after treatment (range: 3 to 5 days) in 44/47 females (94%; Bó et al., 1995a, 1995b). The intramuscular injection of 5 mg E-17β resulted in increased plasma estradiol concentrations reaching peak concentrations 18 h later and decreased circulating FSH concentrations (Bó et al., 1994). Once estradiol concentrations declined, synchronous FSH release and emergence of a new follicular wave occurred (Bó et al., 1994). These studies were later confirmed by experiments in ovariectomized cows and intact heifers (Martínez et al., 2003a; 2004). In ovariectomized cows that received an intravaginal progesterone-releasing (IVP) device one day before or at the time of treatment, E-17β (5 mg) with or without progesterone (100 mg) caused a significant increase of the plasma LH concentration between 18 and 24 h, with suppression of plasma concentrations of FSH from 6 to 48 h. Once circulating concentrations of estradiol decreased by 60 h, FSH returned to concentrations previous to treatment (Martínez et al., 2003a; 2004). In intact cattle, a follicular wave emerges approximately one day after an FSH surge (Adams et al., 1992a). Therefore, the time of FSH resurgence (after E-17β treatment) in ovariectomized cows corresponds to the

FSH surge previous to wave emergence in intact females. It is also known that progesterone inhibits the growth of the dominant follicle (Adams et al 1992b). Progesterone has also been incorporated with the initial treatment of estradiol for follicular wave synchronization to prevent an estrogen-induced LH surge (Bó et al., 1994). Even though a treatment with E-17β resulted in an initial LH surge in ovariectomized cows, a significant reduction in plasma LH concentrations until 84 h post-treatment was also observed (Martínez et al., 2003a). In intact cattle, these events would cause regression of FSH-dependent follicles (by estradiol) and LH-dependent follicles (by the synergistic action of estradiol and progesterone), followed by emergence of a new follicular wave after FSH resurgence.

The doses of progesterone used in synchronization protocols can be considered marginal because treatment with estradiol-17β in ovariectomized cows, treated with a progesterone-releasing device, induced an LH increase by 16 to 24 h later (Martínez et al., 2003a). In intact cattle, the absence of a CL may be the cause for the lack of response to estradiol treatment in a small number of females (6%; Bó et al., 1995). The lack of response in cattle with low levels of endogenous progesterone was further examined. An experiment was designed to characterize follicular dynamics in beef cows that received a progesterone-releasing device and an im injection of E-17β with or without progesterone between days 16 and 18 of the estrous cycle (Kastelic et al., 2004). Thirteen of 36 cows ovulated during the three following days to treatment (Control, 8.3%, and both treated groups, 50%). Follicular wave emergence was more synchronous in the E-17β group than in the Control and E-17β/progesterone groups. When the progesterone-releasing devices were removed, the diameter of the dominant follicle was larger in the Control group than in the treated groups, three cows that did not ovulate (two in the Control group and one in the E-17β/progesterone group) and 16.6% of cows developed persistent follicles. It was concluded that length of follicular dominance and ovulation time were affected by treatments. Therefore, if an ovulation synchronization protocol is intended for FTAI, fertility may be impaired.

Although E-17β seemed to be highly efficient in synchronizing follicular wave emergence, other estradiol esters have also been investigated. Estradiol benzoate (EB; Caccia and Bó, 1998, Burke et al., 1999), EV (D'Occhio et al., 1996; Colazo et al., 2004) or estradiol cypionate (ECP; Colazo et al., 2003) have been used in combination with different progestin sources in synchronization programs.

In a comparative study, administration of progesterone and different types of estradiol in CIDR-treated, ovariectomized cattle suppressed plasma concentrations of gonadotrophins (Martínez et al., 2004). It was noteworthy that 5 mg of E-17β induced an initial increase in plasma LH concentrations, whereas the same dose of EB or EV did not. Suppression of FSH concentrations was followed by resurgence at an interval that corresponded to the persistence of elevated plasma estradiol concentrations. In that regard, the prolonged suppression of plasma FSH in cows given EB or EV was consistent with reports of delayed and more variable intervals to emergence of a new follicular wave when 5 mg of either of these esters were used (Bó et al., 1993).

The type of estradiol utilized in synchronization protocols has different effect on ovarian dynamics depending on the dose and route of administration. The effects of different doses of E-17β were examined by Bó et al. (1994). When E-17β was given to norgestomet-implanted beef heifers at different doses (2.5, 5.0 or 10.0 mg), the 5.0 mg E-17β dose resulted in the least variable interval from treatment to follicular wave emergence (Bó et al., 1994). Similarly, experiments were carried out using EB given by different route and dose. The im administration of 5 mg EB, 24 hours after the insertion of an IVP device, resulted in a fairly synchronous emergence of a new follicular wave (median = 5 days; range 5 to 7) after treatment while when the same dose was

administered by an intravaginal capsule; there was a greater variability in the response (median = 5.5; range 1 to 7; Bó et al., 1996). When 2.5 mg EB was used by im route in Hereford cows, the interval from treatment to follicular wave emergence (median = 4 days) was shorter and less variable than when a greater dose was used (5 mg; Caccia and Bó, 1998). In another study in Hereford cows, the direct comparison between E-17β and EB favored the use of 5 mg of E-17β or 1 mg of EB (3.7 ± 0.5 and 3.4 ± 0.6 days for 5 mg of E-17β or 1 mg of EB, respectively; Martínez et al., 2004). In IVP-treated beef heifers, a smaller dose of 1 mg EB resulted in similar interval to wave emergence (Martínez et al., 2004). Moreno et al. (2001) demonstrated that the use of 2 mg of EB combined with 50 mg of progesterone in beef cows induced a synchronization of the follicular wave less variable without the need for additional progesterone. This was later confirmed in dairy cattle (McDougall et al., 2004) and field studies in beef cattle (Colazo et al., 2004). In conclusion, appropriate doses of E-17β or EB effectively synchronized follicular wave emergence and ovulation (Martínez et al., 2004); the optimum doses for synchronization of follicular wave emergence in these experiments were 5 mg E-17β (cows and heifers) or 1 mg EB (heifers).

Once optimum doses of estradiol were determined in progestin-based synchronization protocols, the next step was to evaluate the effect of estradiol in PGF-based synchronization programs. Therefore, an experiment was designed to determine the effects of dose of EB and/or progesterone on follicular and luteal dynamics in a two dose PGF-based protocol in beef heifers (Martínez et al., 2005). Beef heifers (n=28; weight=350 to 450 kg) received 500 µg cloprostenol on Day -7. On Day 0, heifers were randomly allocated to 9 treatment groups to receive 0, 1 or 2 mg of EB and 0, 50, or 100 mg of P im in canola oil in a 3x3 factorial design. A second PGF treatment was administered on Day 14. The day of follicular wave at the time of treatment tended to vary among groups (P=0.08), and the diameter of the dominant follicle also differed (P<0.05). That was expected because after a single dose of PGF, there is variation in the interval to ovulation (Kastelic et al., 1990). The interval from treatment to wave emergence was shorter (P<0.05) in heifers that received 2 mg EB (4.6 ± 0.3 d) than in those that did not receive EB (5.9 ± 0.6 d), while the 1 mg EB group (5.1 ± 0.6 d) was intermediate. The interval to wave emergence in the 2 mg EB group was the least variable (P<0.05). There was no effect of EB (P=0.72) on the diameter of the CL at the time of the second PGF, but there was an effect of progesterone treatment (P=0.01). Treatments also influenced the variability of the interval from the second PGF to ovulation. There was an effect of time (P<0.01) and a progesterone-by-time interaction (P=0.06) on plasma progesterone concentrations, while estradiol concentrations were affected by EB dose, time and EB-by-time interaction (all P<0.01). Plasma concentrations of FSH were modified by time (P<0.01) and tended to be affected by EB-by-progesterone interaction (P=0.11). To summarize the effects of treatments, EB administered 7 days after a single injection of PGF affected follicular development, while progesterone treatment at that time appeared to influence CL function. In this study, a second dose of estradiol (i.e., 1 mg EB) given 24 h after the second PGF treatment could have resulted in a higher synchrony of ovulation.

The use of EV to synchronize follicular wave emergence was originally compared with the same dose of E-17β (Mapletoft et al., 1999; Colazo et al., 2004b). An experiment compared the effectiveness of two norgestomet implants (Crestar and Syncro-Mate B; SMB) placed for 9 days (with PGF at the time of removing it implants), and the combination of 5 mg of E-17β and 100 mg of progesterone (EP) or 5 mg of EV and 3 mg of norgestomet (N) im which were administered at the time of implant insertion. There was no effect of implant. Treatment with EV negatively affected the CL diameter. Although follicular wave emergence occurred earlier and was less variable in the EP

group (3.6 ± 0.1 days) that in the N group (5.7 ± 0.2 days), intervals from the removal of implant to estrus and ovulation were shorter in N heifers (45.7 ± 11.7 and 74.3 ± 12.6 h) that in EP heifers (56.4 ± 14.1 and 83.3 ± 17.0 h). Short interval from implant removal to estrus (38/56 cows were detected in estrus by 36 h; the interval in heifers was 48.0±4.4 h) was also observed by Kastelic et al. (1999) in an study in which a protocol consisting of Crestar plus EV was compared to a protocol using two doses of PGF. In addition, luteolytic effect of the EV injection was beneficial because there was no functional CL present in the ovaries at the time of norgestomet implant removal. Later, a second experiment evaluated the effect of different doses of EV on follicles and synchrony of estrus and ovulation in beef cows (n=43). On Day 0, all cows received a progesterone-releasing device plus an injection of 1, 2, 5 mg of EV im and another group did not receive additional treatment (Control). On Day 7, progesterone-releasing devices were removed and PGF was injected. Follicular wave emergence occurred within 7 days in 7/10 (70%) Control cows and 31/32 (97%; P<0.04) cows given EV. The interval from treatment to follicular wave emergence was longer (P<0.03) in cows given 5 mg EV (4.8 ± 1.2 days) that in those that received 1 mg (3.2 ± 0.9 days) or 2 mg (3.4 ± 0.8 days) of EV, whereas in Control cows, the interval was intermediate (3.8 ± 2.0 d). These results suggested that at an appropriate dose, estradiol esters as EB or EV could be used for the synchronization of follicular wave emergence in cattle.

As with EB and EV, attempts to control ovarian follicular dynamics have been carried out. Originally, experiments in heifers treated with 0.5 mg ECP had follicular waves emerging with the same variability as in the non-treated control heifers (Thundathil et al., 1997). The use of ECP for synchronization of follicular waves was further investigated in our laboratory. Several experiments were carried out to investigate the use of ECP for the synchronization of the follicular wave and the ovulation in beef heifers treated with a IVP device (Colazo et al., 2004a). The interval from treatment to follicular wave emergence was longer and more variable in heifers treated with ECP than in those given E-17β (4.1 ± 0.4 days versus 3.3 ± 0.1 days). These results confirm that ECP is not suitable to be used in synchronization of follicular wave emergence.

In summary, although the treatment with progestins and estradiol was used over several years to synchronize estrus (Wiltbank et al., 1965), their effect on follicular dynamics was not known until recently with the advent of the ultrasonography. It was also concluded that treatment with estradiol suppressed the growth of antral follicles, and that suppression was more profound when estradiol was administered with a progestin (Bó et al., 1994). The mechanism involved in the suppression of follicular growth seemed to involve inhibition of FSH through a systemic route (Bó et al., 2000a). Thereafter, FSH surges will occur in a predictable time following the emergence of a new follicular wave (Bó et al., 1994; Martínez et al., 2004). Except ECP, esters of estradiol used in lower doses accurately synchronized follicular wave emergence.

GONADOTROPHIN RELEASING HORMONE OR LUTEINIZING HORMONE

Although GnRH directs the estrous cycle in cattle, there is an endogenous control of its secretion. The use of exogenous GnRH offers the possibility of controlling follicular events during the estrous cycle. GnRH has been commercially available since the 1970's and is used widely (Drost and Thatcher, 1992), predominantly for the treatment of follicular cysts in cattle (Kittok et al., 1973). More recently, the effect of GnRH on follicular dynamics has been investigated. Treatment with GnRH resulted in ovulation of the largest follicle present at the time of treatment, but not in all cattle (Macmillan and Thatcher, 1991). It was concluded that these changes could have altered the normal wave patterns of follicular development. In another study, heifers treated with GnRH 32 h before ovulation had a shortened interval from the LH surge to the emergence of the

first follicular wave (Bodensteiner et al., 1996). After GnRH treatment, the dominant follicle would ovulate and a new follicular wave would emerge within 2 days after treatment (Twagiramungu et al., 1995). However, administration of GnRH at random stages of the estrous cycle has not always resulted in ovulation (Kastelic and Mapletoft, 1998). It was suggested that ovulation depended on the stage of development of the dominant follicle (Twagiramungu et al., 1995). The use of GnRH at different and variable stages of the first follicular wave was investigated in two preliminary studies (Prescott et al., 1992; Silcox et al., 1993), but results were inconclusive. In a more recent study (Martínez et al., 1999), the administration of GnRH or LH to heifers at precise stages of the first follicular wave (Days 3, 6, or 9 after spontaneous ovulation) resulted in ovulation in 56% and 78% of heifers, respectively. Specifically, ovulation occurred within 36 h in 67%, 100% and 67% of heifers treated with pLH and in 89%, 56% and 22% of heifers treated with GnRH on Days 3, 6 or 9, respectively. Follicular wave emergence was only induced within 2 days when ovulation of the dominant follicle occurred (Martínez et al., 1999). The remaining non-ovulated heifers had a new follicular wave emerging spontaneously. These results were similar to a study in which 3 different commercial presentations of GnRH (gonadorelin; Cystorelin®, Merial Canada, Victoriaville, Quebec, Canada; Fertagyl®, Intervet Canada, Whitby, Ontario, Canada; Factrel®, Wyeth-Ayerst Canada, Montreal, Quebec, Canada) were compared in beef heifers (Martínez et al., 2003b). Although gonadorelin diacetate tetrahydrate preparations induced an increased number of ovulations (not statistically significant) compared to gonadorelin hydrochloride, all commercial preparations resulted in similar mean interval to wave emergence (2.0±0.4, 2.2±0.4, and 2.1±0.3 d for Cystorelin, Fertagyl and Factrel, respectively).

FOLLICULAR ABLATION

Physical methods of follicular ablation, such as cautery, have been used to remove antral follicles (Ko et al., 1991; Adams et al., 1992a, 1993a, 1993b). Studies of follicular ablation by electrocautery demonstrated that removal of the dominant follicle hastened the emergence of the next follicular wave (Adams et al., 1992a, 1993a). Based on this, ultrasound-guided transvaginal follicle aspiration at random stages of the estrous cycle was used to synchronize follicular wave emergence and to improve the synchrony of ovulation after PGF treatment (Bergfelt et al., 1994). An FSH surge occurred after ablation of all follicles $\geq$5 mm in the ovaries, resulting in emergence of a new follicular wave within 2 days after aspiration. Treatment with PGF 4 days after ablation resulted in synchronous ovulation. A greater synchrony of ovulation was obtained when two doses of PGF were given 12 h apart (at 4.0 and 4.5 days after follicular ablation) and GnRH or pLH was administered 24 h later to induce ovulation (Brogliatti et al., 1998). When ultrasound-guided follicular aspiration was used to synchronize follicular wave emergence in IVP-based estrus synchronization programs, synchronous follicular wave emergence occurred within 1.0 ± 0.1 days after ablation, and ovulation occurred 3.0 to 4.5 days after IVP device removal, with pregnancy rates to AI after estrus detection of 65% (Martínez et al., 2000a). Although follicular ablation offers a great alternative for synchronization of follicular wave emergence and ovulation, this technique may be more appropriate for synchronization of follicular wave emergence in superstimulation protocols; follicle ablation induces a highly synchronized follicular wave emergence but it requires appropriate instrumentation and facilities which makes it less feasible under field conditions. More recently, we have shown that ablation of the two largest follicles in the ovaries were adequate to induce synchronous follicle wave emergence in a superstimulatory program (Baracaldo et al., 2000).

COMPARISON OF WAVE EMERGENCE SYNCHRONIZATION TREATMENTS IN AN ESTRUS SYNCHRONIZATION PROGRAM

Except the original and specific studies on synchronization of follicular wave emergence by Bó et al. (1991; 1993; 1994; 1995b; 2000), most of the protocols that followed have been as part of estrus or ovulation synchronization programs. In an estrus synchronization protocol, Bó et al. (1995a) showed that the combination of estradiol-17β (E-17β) and progesterone synchronized follicular wave emergence in IVP-treated beef cattle, resulting in 75% of heifers ovulating between 72 and 84 h following device removal and PGF administration. This treatment protocol was considered to have great potential in ovulation synchronization programs. Estradiol and progesterone in combination have been used in other progestin-based protocols (Macmillan et al., 2003). An injection of progesterone (100 mg) or an IVP device for 24 h on Day 10 of a 17-day norgestomet protocol in *Bos indicus* heifers synchronized follicular wave emergence and ovulation (Cavalieri et al., 1998). Treatment with 100 mg progesterone provided a shorter interval from implant removal to estrus (38.4 ± 2.6 h) than 200 mg progesterone (61.5 ± 3.9 h; Cavalieri et al., 1998). The use of the combination of 2 mg EB and 200 mg progesterone at the beginning of a progesterone-releasing device treatment induced a better synchrony of follicular wave emergence than progesterone alone (Cavalieri et al., 2003). This demonstrates that progesterone may not be necessary when estradiol is given when a progesterone-releasing device is used. In this case, estradiol caused a higher suppression of plasma LH concentrations, which may have facilitated the suppression of dominant follicles.

In our laboratory, 5 mg of E-17β and 100 mg progesterone have been given on Day 0 of a 7-day MGA program with PGF treatment on the last day of MGA feeding. The synchronized pregnancy rate in heifers without a CL (apparently not cyclic) was higher in the group treated with estradiol and progesterone than that in the non-treated Control group (52% vs 20%, respectively; Kastelic et al., 1997). The low pregnancy rates in the Control group receiving MGA without follicular wave synchronization can be a direct result of the development of persistent follicles that ovulated aged oocytes after MGA withdrawal.

More recently, an experiment was conducted to compare treatments for the synchronization of follicular wave emergence in IVP-treated cattle for synchronization of estrus and ovulation and to determine pregnancy rate following AI at observed estrus (Martínez et al., 2000a, Figure 1). A IVP device was inserted at random stages of the estrous cycle in 67 crossbred beef heifers (Day 0 = beginning of the experiment) that were randomly allocated to receive 1) no further treatment (Control); 2) 5 mg of E-17β plus 100 mg of progesterone (E/P group); 3) 100 µg of GnRH (GnRH group); or 4) transvaginal ultrasound-guided follicular ablation of all follicles ≥5 mm (FA group). The devices were removed on Day 9, 8, 6, or 5 after insertion, in Control, E/P, GnRH, or FA groups, respectively, so the dominant follicle of the induced wave would be exposed to exogenous progesterone for similar intervals in each group. Treatment with PGF was done twice, at device removal and 12 h later. Heifers were inseminated approximately 12 h prior to ovulation. Results are shown in Table 1. The interval from treatment to follicular wave emergence was longest in the E/P and Control groups, while it was the least variable in the E/P and FA groups. The proportion of heifers displaying estrus was higher in the Control than in the FA group (94 vs. 65%, respectively; $P<0.05$) and intermediate in E/P and GnRH groups (87 and 75%, respectively). Pregnancy rates were not significantly different among groups. Results supported the hypothesis that synchronous follicular wave emergence results in synchronous follicle development and, following device removal, synchronous estrus and ovulation with high pregnancy

rates to AI. The synchrony of estrus and ovulation in the E/P, GnRH, and FA groups suggested that these treatments, in combination with a IVP device, could be adapted to FTAI programs.
Later, all designed protocols consisted of a treatment to synchronize follicular wave emergence, with or without a source of progestin followed by PGF at progestin removal, and a treatment to synchronize ovulation for FTAI (Figure 2).

SYNCHRONIZATION OF OVULATION USING ESTRADIOL

High accuracy in the time of ovulation becomes important for fixed-time AI. If a follicular wave is synchronous, it will be possible to predict the day to induce ovulation with estradiol (Lammoglia et al., 1998), LH (Brogliatti et al., 1998) or GnRH (Pursley et al., 1995). Estradiol has been used to synchronize estrus in PGF-based estrus synchronization protocols (Dailey et al., 1983, 1986; Peters et al., 1977; Welch et al., 1975) and induced estrus, LH release and ovulation in CIDR-treated cattle (Lammoglia et al., 1998). The use of estradiol and progesterone in estrus synchronization protocols was based on the hypothesis that treatment will synchronize emergence of a new dominant follicle capable of ovulating after PGF-induced induced luteolysis, with a highly acceptable pregnancy rates following AI.
Estradiol benzoate has been used to induce estrus in PGF-treated cattle (Welch et al., 1975; Peters et al., 1977; Dailey et al., 1983). In IVP-treated cattle, the administration of 0.38 (heifers) or 1.0 mg (cows) of EB 24 to 30 h after device removal resulted in estrus in 86 and 100% of the cattle, respectively. The treatment also resulted in an LH surge between 16 and 20 h later, with higher pregnancy rates than in untreated cattle (Lammoglia et al., 1998). Different intervals from estradiol treatment to ovulation have been investigated. In an experiment designed to explore the timing of EB treatment (0 or 24 h) after IVP device removal, Cutaia et al. (2001) observed and PGF-ovulation interval from 66 to 84 h after treatment with 1 mg EB at 24 h. The administration of 1 mg of either E-17β or EB 24 h after IVP device removal consistently induced synchronous ovulation approximately 52 h later (Martínez et al., 2004). An experiment on the comparison of EB treatment (1 mg im) at 12, 24 or 36 h after IVP removal in beef heifers indicated that EB resulted in a better synchrony of ovulation than in the non-treated Control group. There was not difference in the mean interval to ovulation after treatment at 24 or 26 h; however, EB given at 12 h after IVP removal resulted in a shorter interval (Martínez et al., 2002).
Based on our studies of dosages (please, see previous section of control of follicular wave emergence), several experiments were later conducted. An experiment was designed to compare the effects of E-17β and EB on the interval to emergence of a new follicular wave in heifers treated with a IVP device and on the induction of ovulation following device removal (Martínez et al., 2004). Forty pubertal beef heifers received a IVP device on random days of the estrous cycle (Day 0), and were assigned to four groups in a 2x2 factorial design; half of the heifers received 5 mg of E- 17β plus 100 mg of progesterone and the other half received 1 mg of EB plus 100 mg of progesterone by intramuscular injection. After device removal and PGF treatment on Day 7, each group was randomly subdivided to receive an injection 24 h later (Day 8) of either 1 mg of E-17β or 1 mg of EB to induce LH release and ovulation. There was no effect of estradiol treatment on the mean intervals to wave emergence or ovulation (Table 2). In a second experiment with the same design, the efficacy of the two different estradiol preparations was tested in a IVP-based FTAI program in 84 lactating beef cows at random stages of the estrous cycle (Martínez et al., 2000b). All cattle were inseminated 30 h after the

second injection of estradiol (i.e., 54 h after device removal). Among the four treatment groups, there were no differences in the proportion of animals that displayed behavioral estrus (16/21, 19/21, 17/21, 17/21) or that became pregnant to fixed-time AI (14/21, 67%; 13/21, 62%; 11/21, 52%; and 15/21, 71%), for the E-17β/E-17β, E-17β/EB, EB/E-17β, and EB/EB groups, respectively). Results suggest that E-17β and EB. Each given at an appropriate dose can be used interchangeably in the synchronization of follicular wave emergence and ovulation for fixed-time AI in cattle treated with IVP. The same EB/EB protocol was used in IVP-treated beef heifers resulting in a high pregnancy rate to FTAI (76%) compared to Controls treated with only and inseminated to detected estrus (conception rate, 48%; pregnancy rate 38%; Martínez et al., 2000b). Therefore, this protocol was used thereafter in field situations with a highly acceptable pregnancy rates.

These estradiol-based synchronization programs have the advantage of high fertility after FTAI. However, there is a need for increased animal handling which involves four trips to the chute. These could be reduced if the treatment to induce ovulation were given at the time of IVP device removal by using a long lasting estrogen that allows for the cows to come into estrus and ovulate at an appropriate time for FTAI. In an attempt to compare fertility after FTAI (48 h after progestin removal) after estradiol in a protocol using sponges of medroxiprogesterone in suckled beef cows, it was concluded that EB given 0 or 24 h after the end of a progestin treatment had the same effect on ovulation rate and timing, incidence of short-lived CL, plasma progesterone concentrations and pregnancy rate after FTAI (Ross et al., 2004). This study suggested that EB could be used at the time of progestin removal, but the appointed insemination should be performed earlier than the traditional AI at 52-56 h.

Another alternative, ECP, a slow release and long circulating estradiol ester, the only licensed estradiol in the North American market, and. As mentioned previously, ECP was used in attempts to synchronize follicular wave emergence. More recently, four experiments were designed to study and compare the effects of ECP (with or without injectable progesterone), E-17β, and EB on follicular dynamics, time of ovulation, and pregnancy rate to FTAI in protocols based on the use of a progesterone-releasing device (Colazo et al., 2003). In addition, the duration of progesterone treatment (8 days versus 9 days with a progesterone-releasing device) on the interval from device removal to ovulation was determined. In Experiment 1, heifers (n = 24) received 1 mg ECP or 1 mg ECP plus 50 mg of a commercial progesterone (CP) preparation i.m. on Day 0 (device insertion). Eight or 9 days later, devices were removed, PGF was administered and heifers were allocated to receive 0.5 mg ECP i.m. concurrently (ECP0) or 24 h later (ECP24). There was no effect of treatment on mean day of follicular wave emergence (3.9 ± 0.4 days). Interval from device removal to ovulation was affected only by duration of device treatment (88.3 ± 3.8 h versus 76.4 ± 4.1 h; 8 days versus 9 days, respectively). In Experiment 2, 58 heifers received 100 mg progesterone and either 5 mg E-17β or 1 mg ECP i.m. on Day 0. Seven (E-17β group) or 9 days (ECP group) later, progesterone device were removed, PGF was administered and heifers received ECP (as in the Experiment 1) or 1 mg EB 24 h after device removal, with FTAI 58–60 h after device removal. Follicular wave emergence was later and more variable in heifers given ECP than in those given E-17β (4.1 ± 0.4 days versus 3.3 ± 0.1 days), but pregnancy rate was unaffected (overall, 69%; P = 0.2). In Experiment 3, 30 heifers received a progesterone-releasing device and 5 mg E-17β, with or without 100 mg progesterone (P) i.m. on Day 0. On Day 7, devices were removed and heifers received ECP as described in Experiment 1 or no estradiol (Control). Intervals from device removal to ovulation were shorter in ECP0 (81.6 ± 5.0 h) and ECP24 (86.4 ± 3.5 h) groups than in the Control group (98.4 ± 5.6 h). In Experiment 4, heifers (n = 300)

received a progesterone-releasing device, E-17β, P, and PGF (as in Experiment 3) and after device removal were allocated to three groups (as in Experiment 2), with FTAI 54–56 h (ECP0) or 56–58 h (ECP24 and EB24) after device removal. Pregnancy rate did not differ among groups (overall, 63.6%, P = 0.96). In summary, although 1 mg ECP (with or without progesterone) was less efficacious than 5 mg E-17β plus 100 mg progesterone for synchronizing follicular wave emergence, 0.5 mg ECP (at device removal or 24 h later) induced a synchronous ovulation with an acceptable pregnancy rate to fixed-time AI. These results suggested that although ECP is not effective in synchronizing follicular wave emergence, it could be utilized to synchronize ovulation with success.

DEVELOPMENT OF GnRH-BASED REGIMENS FOR SYNCHRONIZATION OF OVULATION

The first attempts of using GnRH in an estrus synchronization program involved GnRH treatment to synchronize follicular wave emergence, followed by PGF given 6 (Twagiramungu et al., 1992) or 7 days later (Wolfenson et al., 1994) to cause luteolysis of the new-formed and/or old CL. Further, a 10-day estrus synchronization program consisting of a 6-day interval between GnRH and PGF treatments, followed by a second GnRH treatment 48 h after PGF, was proposed for improving the precision of estrus without reducing fertility (Twagiramungu et al., 1995). In another study, an ovulation synchronization program consisting of a first injection of GnRH followed by PGF 7 days later, a second GnRH injection 48 hours after PGF treatment, and AI from 0 to 24 hours later (Pursley et al., 1995). This method improved the precision of estrus, and permitted fixed-time insemination without adversely affecting conception rate. Cows receiving the second GnRH treatment had a higher rate of ovulation than Control cows receiving only saline (97% vs. 77%, respectively). The first injection of GnRH apparently induced LH release and ovulation of the dominant follicle present at that time, resulting in the emergence of a new follicular wave within 2 days after treatment. The administration of PGF 7 days after treatment apparently induced the regression of the original and/or the induced CL. The second GnRH injection was given to induce LH release and synchronous ovulation of the new, GnRH-induced, dominant follicle. This program has been called “Ovsynch” (Seguin, 1997) and has been widely used on dairy farms in North America over the last few years (Wiltbank, 1997). It seems to be especially efficacious in dairy farms in which cows in estrus are not effectively detected (Wiltbank, 1997). GnRH has not resulted in acceptable synchronization estrus and ovulation when used in synchronization protocols in heifers. It has also been suggested and confirmed that the Ovsynch protocol is more efficacious in lactating dairy cows than in heifers (Seguin, 1997). Although, it has not been studied the cause of this inconsistency, it has been attributed to poor synchronization of wave emergence after the first treatment, and by the time PGF treatment was given 6 or 7 days later, the interval to estrus was highly great variable. In dairy cattle, ovulation rate after the first injection of GnRH was 85% in cows and 54% in heifers (Pursley et al., 1995). Furthermore, if spontaneous luteolysis occurred during the period between GnRH and PGF treatment, heifers would express estrus (Roy and Twagiramungu, 1999), since GnRH did not result in ovulation of the dominant follicle. When GnRH treatment was followed by PGF 7 days later, a high number of cattle was observed in estrus over the following 5 days (Thatcher et al., 1993). The use of a second GnRH treatment given at the time of AI (48 h after PGF) in cows seemed to facilitate fixed-time AI (Cosynch; Geary et al., 2001).

As mentioned in the preceding paragraph, although the specific causes of low fertility of an Ovsynch program in heifers has not been elucidated, it has been reported that many heifers were detected in estrus between the first injection of GnRH and PGF. This may be one of many factors responsible for the low pregnancy rates after FTAI. It has been attributed to the lack of response to the first GnRH injection. As described previously, when a GnRH treatment was given to beef heifers on Day 3, 6, or 9 of the first follicular wave, 56% ovulated and therefore, started a new follicular wave within 2 days (Martínez et al., 1999). Therefore, if approximately half of the heifers ovulated and had an induced follicular wave emergence, there will be a high number of unresponsive heifers with a regressing CL that will come into estrus before PGF treatment. One of the hypotheses to overcome this problem was the reduction of the interval from the first GnRH to PGF treatment would reduce the number of heifers detected in estrus during that period of time (i.e., 6 days a instead of 7 days). However, it was found that 9% of heifers showed estrus during a 6-day interval while 12% of heifers were detected in estrus during 7-day interval (Roy and Twagiramungu, 1999). In an experiment performed in lactating beef cows, there was no difference in pregnancy rates between a 6-day (54%) vs. 7-day (53%) Ovsynch program (Martínez et al., 2002).

Another hypothesis to prevent cattle from coming into estrus before or at PGF treatment was based on the utilization of a progesterone source during the interval from the first GnRH and PGF treatment. Furthermore, it was proposed that the increase in circulating progesterone concentrations may cause suppression of the dominant follicle that may have not responded to GnRH. Therefore, the use of a progestin-releasing device or MGA feeding may be a alternative to suppress estrus display during an Ovsynch program for FTAI. An estrus synchronization protocol consisting of a 6-day CIDR insertion with GnRH given at insertion and PGF treatment given and CIDR removal resulted in an acceptable pregnancy rate (65%) to AI after estrus detection (75%; Martínez et al., 2000a). However, the asynchrony of follicular growth at the time of the second GnRH treatment has not been reduced sufficiently to obtain acceptable pregnancy rates to fixed-time AI in heifers.

A series of experiments were conducted to determine the benefits of using a progesterone-releasing device in a GnRH- or pLH-based, Ovsynch-type FTAI program in beef cattle (Martínez et al., 2002b). In the first experiment, Simmental cows (n = 148) and heifers (n = 48) were treated in a 7-day Cosynch program and randomly assigned to receive no further treatment (Group 1) or a progesterone-releasing device concurrent with the first GnRH treatment (Day 0; Group 2). Pregnancy rates were not different (P = 0.79) in cows (Group 1, 45%; n = 71 vs. Group 2, 43%; n = 77). However, pregnancy rates were higher (P<0.05) in heifers treated with a progesterone-releasing device (68%; n = 25) than in Cosynch controls (39%; n = 23). Data suggest that although there was no apparent benefit in lactating beef cows, the use of a progesterone-releasing device increased fertility after FTAI in an Ovsynch-type program in heifers.

Porcine LH has been also used to synchronize follicular wave emergence (Martínez et al., 1999). Although pLH treatment in beef heifers resulted in a higher number of ovulations of the dominant follicle (78%) than GnRH treatment (56%; Martínez et al., 1999), fertility seemed to be similar in pLH-based Ovsynch-type protocols. Therefore, an experiment was designed to determine whether a progesterone-releasing device would improve pregnancy rates to a single fixed-time insemination in an Ovsynch-type, estrus synchronization program in 49 beef heifers in which porcine luteinizing hormone (pLH) was used in lieu of GnRH (Martínez et al., 2002b). Heifers were randomly assigned to three treatment groups; the first group received 12.5 mg of pLH on Day 0, PGF on Day 7, and 12.5 mg of pLH on Day 9 with AI 12 h later (pLH/ Ovsynch), while the second group (pLH/IVP) was similarly treated, with the addition of a progesterone-

releasing device from Days 0 to 7. Heifers in the third group (EB/IVP) received an injection of 1 mg of EB and 100 mg of progesterone on Day 0 and a IVP device from Day 0 to 7. Heifers were given PGF on Day 7 (at the time of device removal) and 1mg i.m. of EB on Day 8, with AI on Day 9 (52 h after PGF). The proportion of heifers in estrus was significantly greater in the EB/IVP (94%) and pLH/IVP (71%) groups than in the pLH/Ovsynch group (41%), whereas pregnancy rates were significantly higher in the EB/IVP group (75%) than in the pLH/Ovsynch group (38%), with the pLH/IVP group (65%) intermediate. Overall, in a Cosynch fixed-time breeding program in lactating beef cows, the use of a progesterone-releasing device did not influence pregnancy rates. However, the use of a IVP device in a 7-day Cosynch program utilizing GnRH or a 7-day Ovsynch program utilizing pLH significantly improved pregnancy rates in heifers.
It has also been shown that a progesterone-releasing device used in Cosynch protocols applied at different herd locations increased overall pregnancy rates in beef cows in good body condition (58%), compared to Control cows treated only with Cosynch (48%; Lamb et al., 2001). It is noteworthy that progesterone-releasing devices increased pregnancy rates in anestrous cows in that study (Lamb et al., 2001). In another study replicated over multiple sites, Lucy et al. (2001) showed that CIDR devices increased the synchrony of estrus and pregnancy rates in noncycling cattle. However, noncycling cattle had a lower pregnancy rate than their cycling herd-mates. Furthermore, the use of a norgestomet implant increased estrous rates in cows assigned to estrus synchronization programs consisting of an injection of GnRH followed 7 days later by PGF treatment (Stevenson et al., 2000). Treatment with GnRH and a norgestomet implant in lactating beef cows initially induced ovulation of the dominant follicle present at the time of treatment, synchronizing follicular wave emergence, which was later followed by higher concentration of estradiol and progesterone (Thompson et al., 1999). In this study, greater GnRH-induced LH release and pregnancy rates were observed after FTAI when a norgestomet implant was included in an Ovsynch program.

COMBINED TREATMENTS

As we have observed, there are alternative hormones to be used in synchronization programs. In our laboratory, an experiment was designed to compare progestins and methods of synchronizing wave emergence and ovulation in a FTAI program (Martínez et al., 2002c). Angus-cross heifers (n =503) were allocated into two synchronization groups and three treatment groups (2x3 factorial design) at random stages of the estrous cycle (Day 0=beginning of the experiment; Figure 3). At that time, heifers either received IVP devices (n =257) or were started on 0.5 mg animal^{-1} d^{-1} of MGA (; n=246) and given injections of 2 mg of EB plus 50 mg of progesterone, 100 µg of GnRH or 12.5 mg of pLH. The last feeding of MGA was given the morning of Day 6, and on Day 7, devices were removed and all heifers received PGF. Consistent with their treatment on Day 0, heifers were given either 1 mg EB 24 h after PGF and inseminated 28 h later or 100 µg GnRH or 12.5 mg pLH 48 h after PGF and concurrently inseminated. Heifers were exposed to bulls for 17 days, starting approximately 20 Day after fixed-time AI. Although estrus rates differed (P≤0.01), there was no difference in pregnancy rates among groups (P<0.3; Table 4). Overall, results suggest that the oral progestin (MGA) and the progesterone-releasing intravaginal device (CIDR) are equally efficacious, and that in combination with GnRH, pLH or EB, either can be used effectively to synchronize estrus and ovulation for fixed-time AI. The present study is apparently the first published report of a concurrent comparison of these six treatment protocols for fixed-time AI. As pregnancy rates to FTAI were not significantly different among treatments (overall rate,

58.0%), factors other than pregnancy rate (e.g., costs and management conditions) may influence the protocol selected. For example, CIDR devices are more expensive than MGA, but they can be used in both confined cattle and those at pasture. In regard to the latter, it is often difficult to ensure uniform intake of MGA in pastured cattle. In any case, the results of this experiment provide several options for fixed-time AI.

More recently, a large experiment was conducted to determine pregnancy rates following FTAI in heifers: (1) given GnRH or ECP to synchronize follicular wave emergence and ovulation in a IVP-based protocol; and (2) fed diets supplemented with flax or sunflower seeds (Colazo et al., 2003). At two locations, Angus and crossbred Angus heifers (n=983) were examined ultrasonically to confirm reproductive maturity and randomly allocated to six synchronization groups in a 2x3 factorial design (Figure 4). On Day 0 (beginning of treatments), heifers received a progesterone-releasing device and either 100 mg GnRH i.m. (n = 492) or 1 mg ECP plus 50 mg progesterone i.m. (n = 491); in these groups, device removal and PGF treatment were done concurrently on Days 7 and 8.5, respectively. Heifers were rerandomized to receive 0.5 mg ECP i.m. at device removal or 24 h later (with FTAI 58–60 h after device removal in both groups), or 100 mg GnRH i.m. concurrent with FTAI (52–54 h after device removal). The heifers were fed a barley silage-based diet for 50 days (from Day -25 to 25) supplemented with 1 kg/heifer per day of flax seed (n = 321), sunflower seed (n = 324), or no oilseed (n = 338). Following FTAI, heifers were also observed for estrus and reinseminated until 33 ± 1 d after FTAI, when heifers were sent to pasture and exposed to bulls for 57 days. Pregnancy rates to FTAI were not influenced by location (53.4 and 57.9% for Locations A and B, respectively, P=0.20) or by diet (52.1, 57.0, and 57.7% for Control, flax seed and sunflower seed, respectively, combined for both locations; P=0.46). Pregnancy rate was not affected by treatments given at device insertion (Table 5). However, pregnancy rate in heifers given ECP 24 h after device removal (ECP24) was higher (P<0.005) than in those given ECP at device removal (ECP0) or GnRH at FTAI (GnRH52). Overall pregnancy rate for AI (FTAI and reinsemination) was 79.8% (785/983) and fall pregnancy rate was 95.9% (943/983; Table 4). To summarize results, pregnancy rate to FTAI was not significantly affected by treatment, given at insertion of a progesterone-releasing device, to synchronize follicular wave emergence, and 0.5 mg ECP given 24 h after device removal (to synchronize ovulation) resulted in the highest pregnancy rate.

Following the idea of control of follicular wave emergence in a synchronization protocol based on the use of two doses of PGF, an experiment on breeding management was conducted. The objective was to determine reproductive performance following AI in beef heifers given estradiol to synchronize ovarian follicular wave emergence and estradiol or GnRH to synchronize ovulation in a two-dose PGF-based protocol. In Experiment 1, 561 cycling (confirmed by ultrasonography), Angus heifers received 500 mg cloprostenol, i.m. twice, 14 days apart (days 0 and 14) and were equally allocated to four groups in a 2x2 factorial design. On Day 7, heifers received either 2 mg estradiol benzoate (EB) and 50 mg progesterone (P), i.m. in oil (EBP group) or no treatment (NT group). Half the heifers in each group received 1 mg EB, i.m. in oil on Day 15 (24 h after the second PGF treatment) with TAI 28 h later (52 h after PGF), and the other half received 100 mg GnRH, i.m. on Day 17 (72 h after PGF) concurrent with FTAI. All heifers were observed for estrus twice daily from days 13 to 17; those detected in estrus more than 16 h before scheduled appointed AI were inseminated 4–16 h later and considered nonpregnant to FTAI. Overall pregnancy rate (approximately 35 days after AI) was higher in heifers that received EBP than those that did not (61.6% versus 48.2%, respectively; P<0:002); but was lower in heifers that received EB after PGF than those that received GnRH (50.0% versus 59.8%; P<0:02). Although estrus was

detected prior to TAI in 77 of 279 heifers (27.6%) treated with EBP (presumably due to induced luteolysis), they were inseminated and 53.2% became pregnant. Overall pregnancy rates were 51.4, 68.3, 45.0, and 55.0% in the NT/ GnRH, EBP/GnRH, NT/EB, and EBP/EB groups, respectively (P<0:05). A second experiment was designed with the same objectives as the previous study. However, since there were heifers detected in estrus before the appointed AI suggesting that EB/progesterone could have caused it, E-17β was given instead for wave emergence synchronization. Consequently, 401 cycling, Angus heifers were used with an experimental design was identical to the previous one, except that 1.5 mg estradiol-17β (E-17β) plus 50 mg progesterone (E-17βP) and 1 mg E-17β were used in lieu of EBP and EB, respectively. All heifers receiving E-17β 24 h after the second injection of PGF (NT/E-17β and E-17βP/E-17β) were TAI 28 h later without estrus detection, i.e. 52 h after PGF. Heifers in the other two groups received 100 mg GnRH, i.m. 72 h after PGF and were concurrently inseminated; heifers in these two groups that were detected in estrus prior to this time were inseminated 4–12 h later and considered nonpregnant to FTAI. Estrus rate during the first 72 h after the second PGF treatment was higher (P<0.05) in the E-17βP/GnRH group (45.0%; n ¼ 100) than in the NT/GnRH group (16.0%; n = 100), but conception rate following estrus detection and AI was not different (mean, 57.4%; P=0.50). Overall pregnancy rate was not significantly different among groups (mean, 46.9%; P=0.32). In summary for these two studies, the use of EB or E-17β to synchronize follicular wave emergence and estradiol or GnRH to synchronize ovulation in a two-dose, PGF-based protocol resulted in acceptable fertility to FTAI. However, when 2 mg EB was used to synchronize follicular wave emergence, early estrus occurred in approximately 28% of heifers, necessitating additional estrus detection. Therefore, a combination of estrus detection and FTAI in a two-dose PGF protocol resulted in highly acceptable pregnancy rates.

It is clear that in order for artificial insemination (or FTAI) to make an impact on the cattle industry, the necessity of estrus detection must be minimized. Estrus synchronization will reduce the amount of time that is required for estrus detection but it will not eliminate it. In addition, some methods of estrus synchronization have resulted in unacceptable levels of fertility. As with any breeding program, high levels of management are necessary for acceptable pregnancy rates after artificial insemination. All animals must be cycling before the initiation of the program or results can be disappointing.

New knowledge, generated by endocrine analysis combined with the ultrasonographic examination of the reproductive tract of the cow performed on daily basis, has provided new strategies for the control of the estrous cycle in cattle. One of the main findings was the identification of the reason for asynchrony of estrus and ovulation after an injection of PGF, which is the stage of follicle development at the time of treatment. It has also been determined that follicular wave emergence can be manipulated with steroid or peptide hormones. In addition, the same hormones can be used to accurately induce a synchronous ovulation. Obviously, additional studies with large numbers of animals are necessary to improve and confirm the efficacy of the approaches described in this paper.

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Table 1. Mean (± SEM) intervals from treatment to follicular wave emergence (WE), to estrus, and to ovulation in heifers given treatments to synchronize WE.

Intervals (Days)	Control	Estradiol-17β + progesterone	GnRH*	Follicular ablation
Treatment to WE	3.5±0.6^{a}	3.4±0.1^{a}	1.5±0.3^{b}	1.0±0.1^{b}
Range	(-2 to 8)^{x}	(3 to 4)^{z}	(-1 to 4)^{y}	(0 to 2)^{z}
n	18	16	16	17
PGF to estrus	2.3±0.2	2.2±0.2	2.2±0.2	2.5±0.1
Range	(1.5 to 4.5)	(1.5 to 3.0)	(1.5 to 3.5)	(2.0 to 3.5)
n	17	14	13	11
PGF to ovulation	3.6±0.2	3.4±0.1	3.5±0.1	3.8±0.1
Range	(2.5 to 5.5)	(3.0 to 4.5)	(2.5 to 4.5)	(3.0 to 4.5)
n	18	16	16	16**

* Although 8 of 16 heifers ovulated in response to GnRH treatment, data points are based on all 16 heifers in the group

** One outlier excluded for this data point.

^{ab} Means, and ^{xyz} variances within rows with different superscripts are significant ($P<0.05$).

Table 2. Mean (± SEM) intervals (and their range) from estradiol treatment to follicular wave emergence and from CIDR removal to ovulation in CIDR-treated beef heifers after administration of estradiol-17β or estradiol benzoate to synchronize follicular wave emergence and ovulation (Experiment 2).

	Estradiol-17β	Estradiol benzoate
Induction of follicular wave emergence		
No. of heifers	16	16
Interval (d) from estradiol treatment to		
Wave emergence	3.4±0.5	3.7±0.6
Range	-3 to 5	-5 to 6
Excluding non-responding heifers		
No. of heifers	15	15
Wave emergence (d)	3.9±0.2	4.3±0.2
Range (d)	3 to 5	3 to 6
Induction of ovulation		
Interval (h) from CIDR removal to		
Ovulation	75.8±3.0	77.3±1.9
Range	72 to 120	72 to 96

Table 3. Mean (±S.D.) intervals from treatment with 0, 1, 2 or 5 mg estradiol valerate at progesterone-releasing device insertion to follicular wave emergence (FWE), and from CIDR removal to estrus and ovulation (Experiment 3)

	Dose of estradiol valerate (mg)			
	0	**1**	**2**	**5**
Interval from treatment to FWE (d)				
No. of cows	10	11	10	10
Mean	2.2 a	2.5 a	3.4 a	4.8 b
S.D.	3.3 x	2.3 x	0.8 y	1.2 y
Range	−4 to 6	−4 to 4	2 to 5	3 to 7
Interval from CIDR removal to Estrus (h)				
No. of cows	9	11	10	8
Mean	58.0	53.5	61.2	59.3
S.D.	19.4	11.2	14.1	17.1
Range	36 to 96	36 to 72	48 to 84	48 to 96
Ovulation (h)				
No. of cows	10	11	10	10
Mean	86.4	85.1	91.2	90.0
S.D.	18.6	12.5	14.1	22.1
Range	60 to 120	72 to 108	72 to 120	72 to 132

Within the row with different letters (a, b) differed significantly ($P<0.05$). Standard deviations within the row with different letters (x, y) differed significantly ($P<0.001$).

Table 4. Estrus rates, interval from PG to estrus, and pregnancy rates in beef heifers receiving a CIDR device or fed MGA and given GnRH, pLH or EB for the synchronization of follicular wave emergence and ovulation for fixed-time AI[1]

	CIDR			MGA		
	GnRH	pLH	EB	GnRH	pLH	EB
No. heifers	103	102	52	101	97	48
Estrus %	65.0[a]	60.8[a]	92.3[b]	35.6[c]	33.0[c]	91.7[b]
PG to estrus h						
Mean	47.1	47.8	47.3	48.0	48.8	48.3
Standard deviation[2]	8.2[x]	10.3[x]	3.8[y]	2.9[y]	3.0[y]	1.8[z]
Pregnancy rate to AI %						
Overall	65.0	55.9	61.5	52.5	55.7	60.4
Detected in estrus	67.2	61.3	62.5	61.1	62.5	59.1
Not detected in estrus	61.1	47.5	50.0	47.7	52.3	75.0
Fall pregnancy rate %	75.7	77.4	78.8	73.3	72.2	77.1

[abc] Mean and percentages without a common superscript differ ($P<0.05$)
[xyz] Variances without a common superscript differ ($P<0.05$)
[1] Please refer to Figure 3 for treatment schedule; PG was administered to all heifers on d 7 at CIDR removal (1 d after the end of MGA feeding)
[2] Standard deviation was calculated for simple effects to show differences in variation of the interval from PG to estrus but statistical comparisons were done with variances.

Table 5. Pregnancy rates to fixed-time AI, after rebreeding, and fall pregnancy rates in beef heifers receiving a CIDR and given 100 µg GnRH or 1 mg estradiol cypionate (ECP) and 50 mg Progesterone 5% (to synchronize follicular wave emergence), and subsequently given 0.5 mg ECP at CIDR removal (ECP0) or 24 h later (ECP24), or 100 µg GnRH concurrent with AI (GnRH52) to synchronize ovulation.

	GnRH			ECP		
	ECP0	ECP24	GnRH52	ECP0	ECP24	GnRH52
No. heifers	162	165	165	160	165	166
Fixed-time AI [1]						
No. pregnant	78	109	89	90	107	80
Pregnancy rate (%)	48.1[a]	66.1[b]	53.9[a]	56.2[ab]	64.8[b]	48.2[a]
Rebreeding [1]						
No. pregnant	47	31	40	41	31	42
Pregnancy rate (%)[2]	77.2	84.8	78.2	81.9	83.6	73.5
Fall pregnancy examination [3]						
No. pregnant	152	159	159	158	157	158
Pregnancy rate (%)	93.8	96.4	96.4	98.8	95.2	95.2

[ab] Percentages without a common superscript differ (P<0.001); ECP/ECP0 tended to differ from GnRH/ECP24 (P<0.07) and ECP/ECP24 (P<0.1)
[1] Pregnancy rate based on ultrasonography
[2] Accumulated pregnancy rate after two inseminations; percentages tended to differ (P<0.09).
[3] Pregnancy rate based on rectal palpation

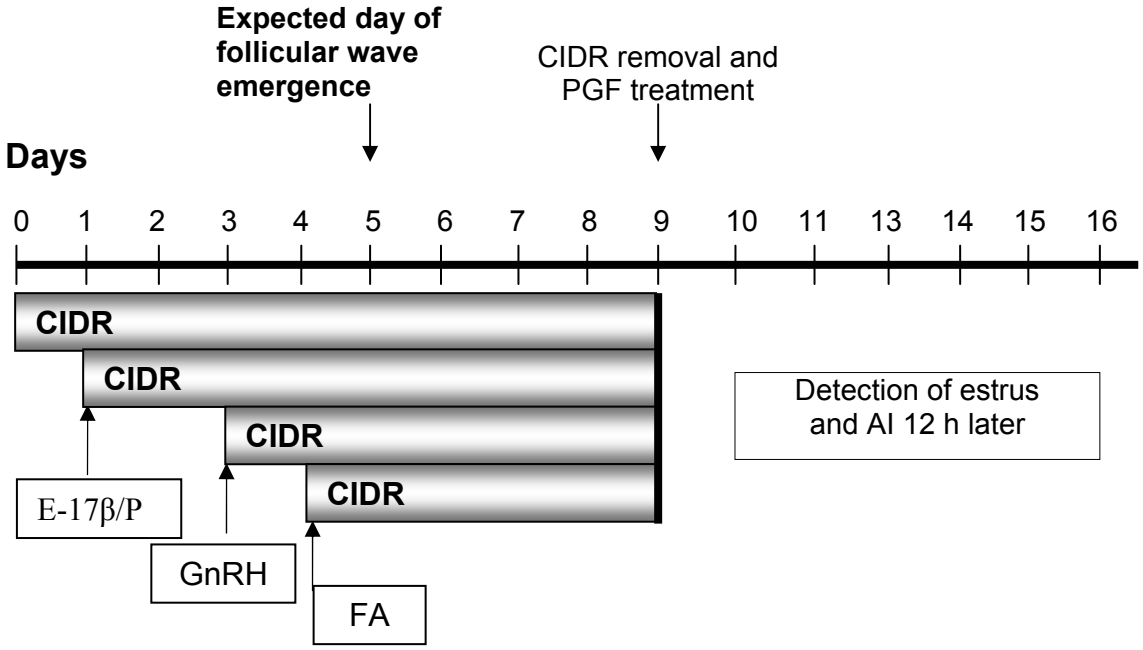

Figure 1. Schedule of synchronization treatments and AI in CIDR-treated beef heifers. Heifers received no further treatment (Control), estradiol-17β/ progesterone (E-17β/P), gonadotrophin releasing hormone (GnRH), or ultrasound-guided follicular ablation (FA) at the time of CIDR insertion in the respective groups.

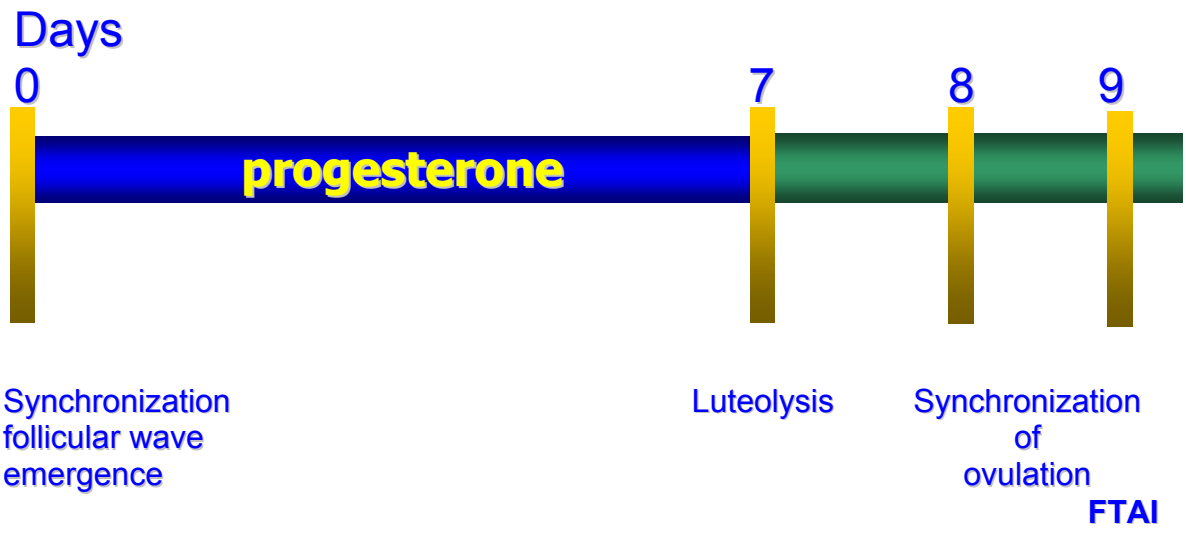

Figure 2. Schedule of an ideal ovulation synchronization protocol for FTAI in cattle. It is based on a short progestin treatment with a treatment to synchronize follicular wave emergence. This allows for the growth of a newly recruited dominant follicle, which will ovulate when a second hormonal treatment is induced after progestin removal and PGF. If estradiol is given for induction of ovulation, the treatment is administered 24 h after PGF, but if GnRH or pLH is given, the treatment can be administered at 48 h and AI could be performed concurrently.

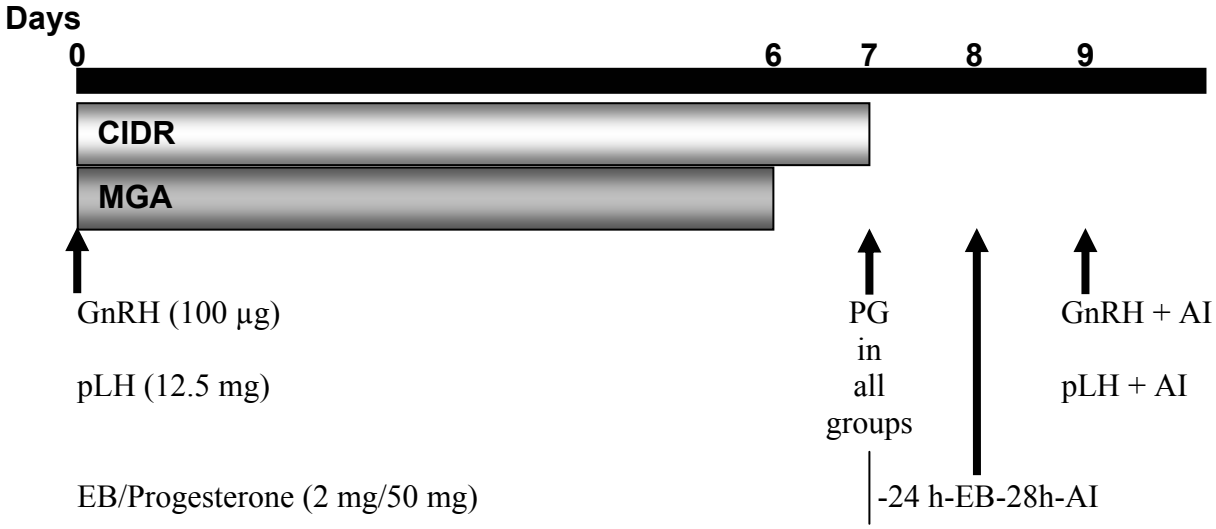

Figure 3. Schematic outline of the experiment (arrows indicate times of administration of treatments). The CIDR devices were removed after 7 Day and MGA was fed once daily from Days 0 to 6. Heifers were treated with GnRH, pLH or estradiol benzoate (EB) and progesterone on Day 0 and all received PG on Day 7. Heifers that received GnRH or pLH received a second injection of the same hormone 48 h after PG (Day 9) and were inseminated concurrently, while those in the EB group received EB 24 h after PG (Day 8) and were inseminated 28 h later (52 h after PG; Day 9).

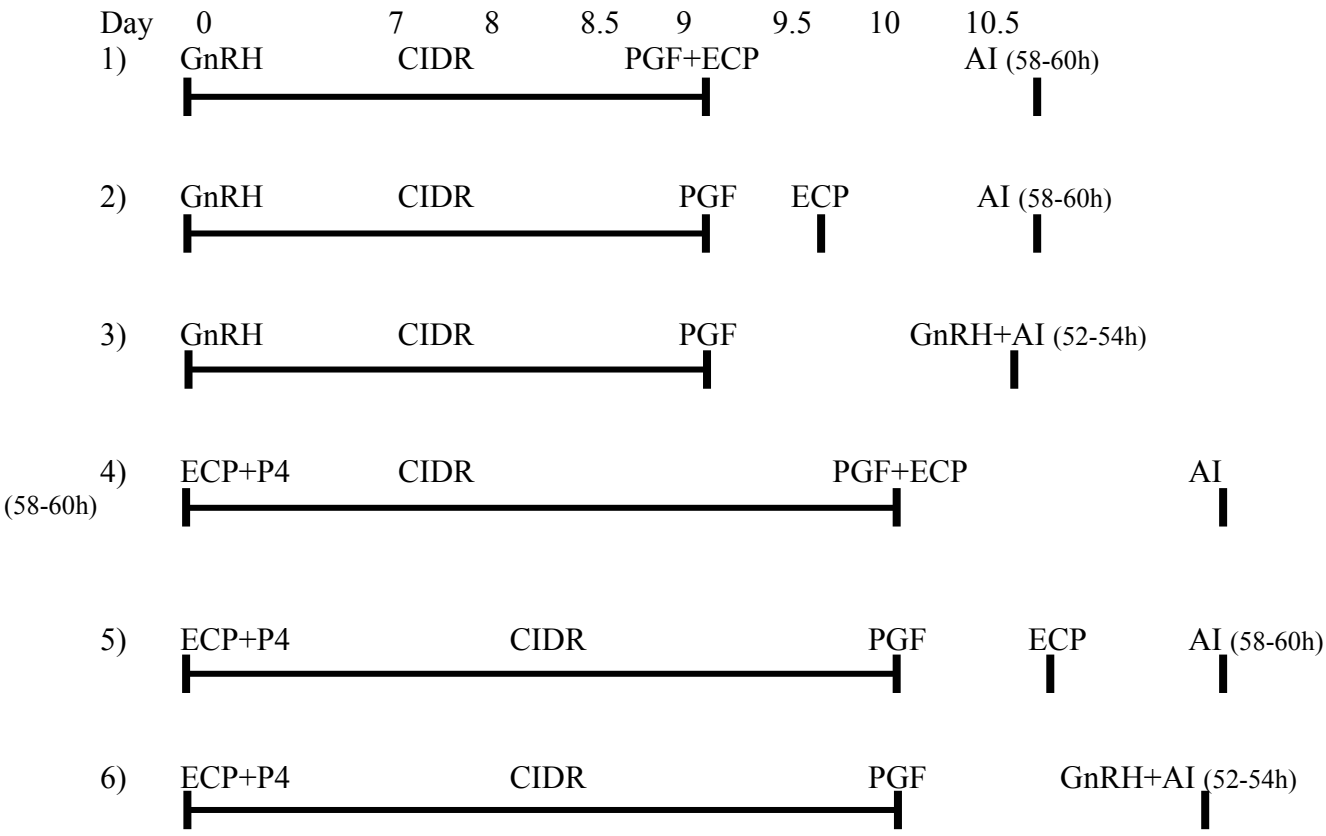

Figure 4. Schematic representation of the timeline of treatments in the six different estrus synchronization protocols. Treatments with ECP (1 mg) and progesterone 5% (P4; 50 mg) or GnRH (100 mg) were given at CIDR insertion to induce a new follicular wave emergence. In all protocols, PGF (25 mg dinoprost) was administered at CIDR removal. For synchronizing ovulation, ECP (0.5 mg) was given at CIDR removal or 24 h later and GnRH (100 mg) was given concurrently with AI 52 h after CIDR removal.