The use of controlled internal drug release devices for the regulation of bovine reproduction[1]

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ABSTRACT: Our knowledge of bovine estrous cycle physiology has expanded greatly in recent years, primarily with the advent of ultrasonography to monitor ovarian follicles. With increased knowledge, new methods of manipulating ovarian function have become available. The use of controlled internal drug release (CIDR) devices for the synchronization of estrus in cattle is now well accepted throughout the world. In fact, Canada and the United States are among the last countries in the world to have CIDR devices available for use in bovine practice. The use of CIDR devices, along with other hormones that are already on the market (e.g., gonadotropin releasing hormone) has permitted fixed-time artificial insemination with high pregnancy rates in beef cattle. New approaches, such as the use of estradiol in CIDR-based protocols, offer novel and exciting ways to manipulate the bovine estrous cycle. Recent studies suggest that steroid hormones readily available on the veterinary pharmaceutical market, such as estradiol cypionate and injectable progesterone, can be successfully used to synchronize follicular wave emergence and ovulation in CIDR-based, fixed-time artificial insemination programs. Experiments described in this report include several protocols that do not require detection of estrus, thereby permitting fixed-time artificial insemination in beef cattle. Over a 5-yr period, pregnancy rates to a single fixed-time artificial insemination have ranged from 55 to 77% in heifers and slightly less in lactating beef cows.

Key Words: Artificial Insemination, Cattle, Controlled Release, Estradiol, Gonadotropin-Releasing Hormone, Luteinizing Horomone

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Introduction

The keys to successful estrus synchronization are closely synchronized, rapid declines in circulating progestin concentrations and synchronous growth and ovulation of a viable follicle. However, $PGF_{2\alpha}$ is effective only when a fully developed corpus luteum (**CL**) is present (approximately d 7 to 18 of the cycle; Momont and Seguin, 1984) and withdrawal of exogenous progestin is effective only if either natural or induced regression of the CL has occurred. Although current techniques for estrus synchronization with $PGF_{2\alpha}$ are successful (Odde, 1990; Larson and Ball, 1992), variation in ovarian follicular wave dynamics results in poor synchrony of estrus and ovulation (i.e., the induction of luteolysis when a dominant follicle is mature will result in estrus and ovulation in 2 to 3 d, whereas the interval will be much longer if another follicle must be recruited from a new follicular wave) (Kastelic and Ginther, 1991).

Various progestins have been utilized for estrus synchronization. Progestin treatment for more than 14 d will synchronize estrus, but fertility at the induced estrus will be reduced (Wiltbank et al., 1965; Roche, 1974). Fortunately, these effects are transitory and are not apparent at the next estrus. Alternatively, shorter progestin treatment protocols (e.g., 7 to 10 d), with $PGF_{2\alpha}$ given before or at the termination of progestin treatment, have been devised to improve fertility (Odde, 1990; Macmillan and Peterson, 1993). However, these

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protocols do not result in sufficient synchrony of estrus and ovulation for fixed-time AI. In addition, pregnancy rates were low when short-term treatments were initiated during the late luteal phase (i.e., after d 14), due to the development of a persistent follicle (Savio et al., 1993; Stock and Fortune, 1993; Custer et al., 1994; Kinder et al., 1996). Poor fertility after long-term progestin treatments or short-term treatments initiated late in the estrous cycle has been attributed to prolonged maintenance of the dominant follicle and ovulation of an aged oocyte (Ahmad et al., 1995; Revah and Butler, 1996). These results emphasize the need to synchronize follicular development to ensure the presence of a viable, growing dominant follicle at the time of progestin withdrawal and/or $PGF_{2\alpha}$ treatment.

Synchronization of Follicular Wave Emergence

Follicle Ablation. Elimination of the dominant follicle results in the emergence of a new follicular wave by removing the suppressive effect of follicular products (e.g., estradiol and inhibin) on circulating concentrations of FSH (Ko et al., 1991; Adams et al., 1993). Transvaginal ultrasound-guided follicle aspiration induced synchronous wave emergence within 2 d in heifers, and $PGF_{2\alpha}$ given 4 d later resulted in synchronous ovulation (Bergfelt et al., 1994).

Gonadotropin-Releasing Hormone. It has been shown that GnRH will induce ovulation or luteinization of a growing dominant follicle present at the time of treatment (Macmillan and Thatcher, 1991). Protocols that utilize GnRH and $PGF_{2\alpha}$ have been developed for fixed-time AI in beef and dairy cattle. The Ovsynch treatment protocol (Pursley et al., 1997) consists of an injection of GnRH followed by $PGF_{2\alpha}$ 7 d later, a second injection of GnRH 48 h after $PGF_{2\alpha}$ treatment, and fixed-time AI approximately 15 h later. Others have used a similar protocol in beef cattle with an interval of 6 d between the first GnRH treatment and $PGF_{2\alpha}$ (Roy and Twagiramungu, 1999).

Estradiol. Although treatment with progestin and estradiol has been used for several years to synchronize estrus (Wiltbank et al., 1965), it was not until recent discoveries of the effects of estradiol on follicular development that the physiological basis of these treatments was fully appreciated. In a series of experiments, estradiol treatment suppressed antral follicle growth, and suppression was more profound when estradiol was given with a progestin (Bó et al., 1994). The mechanism responsible for estrogen-induced suppression of follicular growth appeared to involve suppression of FSH through a systemic pathway (Bó et al., 2000). Thereafter, FSH surges occurred at defined times, and a new follicular wave emerged (Bó et al., 1994).

The administration of 5 mg of estradiol-17β (**E-17β**) to progestin-treated heifers (Bó et al., 1994) resulted in regression of antral follicles, followed by the emergence of a new follicular wave (on average) 4.3 d later (Bó et al., 1995), whereas the same dose of estradiol benzoate (**EB**) resulted in emergence of a new follicular wave 5.4 d later (Bó et al., 1996). More recently, Caccia and Bó (1998) showed that treatment with 1, 2.5, or 5 mg of EB (plus 50 mg of progesterone) resulted in a median interval from treatment to follicular wave emergence of 4.0 d in CIDR-treated beef cows; furthermore, this interval was significantly more synchronous in cows given 2.5 mg vs. those given 5 mg of EB. Estradiol valerate (Mapletoft et al., 1999) and estradiol cypionate (**ECP**; Thundathil et al., 1997; Colazo et al., 2002) at doses of 5 and 1 mg, respectively, resulted in longer and more variable intervals to follicular wave emergence than E-17β. The effects of lesser doses of estradiol valerate apparently have not been studied, whereas a dosage of 0.5 mg ECP appeared to be marginally efficacious (Thundathil et al., 1997).

The Controlled Internal Drug Release Device

The controlled internal drug release (**CIDR**) device has recently been approved in Canada (Bioniche Animal Health, Belleville, ON, Canada) and the United States (Pharmacia Animal Health, Kalamazoo, MI) for synchronization of estrus in beef cattle and dairy heifers. The CIDR is a T-shaped vaginal insert containing 1.9 g of progesterone (Canada) or 1.38 g of progesterone (United States) in silicon molded over a nylon spine. Although plasma concentrations of progesterone are identical between the two devices, the model marketed in the United States apparently exhausts its supply of progesterone earlier than the Canadian model (H. D. Hafs, personal communication). The CIDR is inserted into the vagina by a specialized applicator (Macmillan et al., 1991) that collapses the wings for insertion; expulsion of the CIDR causes the wings to straighten, which confers retention by pressure on the vaginal wall. A thin nylon tail attached to the end of the CIDR is exteriorized through the vagina and is used to remove the device. Label directions (for AI) indicate that the device should be in the vagina for 7 d; $PGF_{2\alpha}$ is given 24 h before device removal and estrus detection begins 48 h after device removal. Because of the short treatment period (7 d), the incidence of persistent follicles is reduced. The CIDR device is well suited to various approaches used to synchronize ovarian follicular development and ovulation.

Following CIDR insertion in ovariectomized cows, plasma progesterone concentrations increased to near luteal levels (5 to 7 ng/mL) by 24 h and then decreased to concentrations of 2 to 3 ng/mL after 2 to 3 d, where they remained until CIDR removal on d 7 (Martínez, 2002). Plasma progesterone concentrations declined to baseline by 12 h after CIDR removal. Administration of 100 mg of progesterone at CIDR insertion increased plasma progesterone concentrations by 2 ng/mL over that of a CIDR alone in ovariectomized cows (Martínez, 2002), with similar increases expected in ovary-intact cattle.

Table 1. Mean (± SEM) intervals to follicular wave emergence (WE), estrus, and ovulation, and pregnancy rates in controlled internal drug release-treated heifers given treatments to synchronize wave emergence

	Control	E-17β + progesterone	GnRH[a]	Follicular ablation
Intervals, d				
Treatment to WE	3.5 ± 0.6[b]	3.4 ± 0.1[b]	1.5 ± 0.3[c]	1.0 ± 0.1[c]
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[d]
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
Pregnancy rates				
Number	14/18	13/16	11/16	10/16
%	78	80	69	65

[a]Although 8 of 16 heifers ovulated in response to GnRH treatment, data points are based on all 16 heifers in the group.
[b,c]Means within rows with different superscripts differ ($P < 0.05$).
[d]One outlier excluded for this data point.
[x,y,z]Variances within rows with different superscripts differ ($P < 0.05$).

Estrus Synchronization and Fixed-time Artificial Insemination

The following is a very brief summary of studies done in the authors' laboratories, showing how the CIDR device can be use in estrus synchronization programs in beef cattle; many have been published in abstract form and are referenced accordingly. The first experiment was designed to investigate synchronization of ovarian follicular wave emergence in CIDR-treated cattle for synchronization of estrus and ovulation and to determine pregnancy rate following AI at observed estrus (Martínez et al., 2000a). A CIDR was inserted at random stages of the estrous cycle in 67 crossbred beef heifers (d 0 = the first day 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 CIDR devices were removed on d 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 $PGF_{2\alpha}$ was done twice, at CIDR removal and 12 h later. Heifers were inseminated approximately 12 h prior to ovulation. Results are shown in Table 1. Although the interval from treatment to follicular wave emergence was longest in the E/P and Control groups, it was the least variable in the E/P and FA groups. The proportion of heifers displaying estrus was higher in the Control vs. 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 CIDR 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 CIDR, could be adapted to fixed-time AI programs.

The Use of Estradiol. Estradiol has been used to synchronize follicular wave emergence (Bó et al., 1991; 1993) and several studies have investigated the use of different estradiol preparations in progestin-based synchronization programs (Lammoglia et al., 1998; Burke et al., 1999; Martínez et al. 2000b). In addition, EB has been used to induce estrus in $PGF_{2\alpha}$-treated cattle (Welch et al., 1975; Peters et al., 1977; Dailey et al., 1983). In CIDR-treated cattle, the administration of 0.38 (heifers) or 1.0 mg (cows) of EB 24 to 30 h after CIDR removal resulted in estrus in 86 and 100% of the cattle, respectively (Lammoglia et al., 1998). Furthermore, in endocrine studies, EB treatment resulted in an LH surge between 16 and 20 h later, with significantly higher pregnancy rates than in untreated cattle (Lammoglia et al., 1998).

An experiment was designed to compare the effects of E-17β and EB on the interval to emergence of a new follicular wave in CIDR-treated heifers and on the induction of ovulation following CIDR removal (Martínez et al., 2002a). Thirty-two pubertal beef heifers received a CIDR device on random days of the estrous cycle (d 0), and were assigned to four groups in a 2 × 2 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 CIDR removal and $PGF_{2\alpha}$ treatment on d 7, each group was randomly subdivided to receive an injection 24 h later (d 8) of either

Table 2. Mean (± SEM) intervals from treatment with estradiol-17β (E-17β) or estradiol benzoate (EB) to follicular wave emergence and from PGF treatment (and controlled internal drug release [CIDR] removal) to ovulation in CIDR-treated beef heifers

	Treatments			
	E-17β/E-17β	E-17β/EB	EB/E-17β	EB/EB
Number of heifers	8	8	8	8
Interval from estradiol treatment to				
Wave emergence, d	3.9 ± 0.2	3.9 ± 0.3	4.0 ± 0.3	4.5 ± 0.3
Range, d	3 to 5	3 to 5	3 to 5	3 to 6
Interval from CIDR removal to				
Ovulation, h	79.5 ± 6.0	73.5 ± 1.5	72.0 ± 0.0	81.0 ± 3.0
Range, h	72 to 120	72 to 84	72 to 72	72 to 96

1 mg of E-17β or 1 mg of EB to induce LH release and ovulation. Heifers were examined ultrasonographically to monitor follicular dynamics and to detect 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 CIDR-based fixed-time AI 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 CIDR 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 can be used interchangeably in the synchronization of follicular wave emergence and ovulation for fixed-time AI in CIDR-treated cattle.

Gonadotropin-Releasing Hormone-Based Protocols. The Ovsynch protocol is much more efficacious in lactating dairy cows than in heifers (Seguin, 1997). Although the cause of this discrepancy is not known, ovulation following the first injection of GnRH occurred in 85% of cows and only 54% of heifers (Pursley et al., 1995). In addition, 19% of heifers showed behavioral estrus before the injection of $PGF_{2\alpha}$, dramatically reducing fertility to fixed-time AI (Wiltbank, 1997). In an experiment designed to confirm these results, GnRH treatment during the growing, early static, or regressing phases of development of the dominant follicle of the first follicular wave induced ovulation in 56% of beef heifers, and wave emergence occurred only when ovulation was induced; therefore, GnRH does not consistently induce emergence of a new follicular wave in beef heifers (Martínez et al., 1999).

Several experiments were conducted to determine the benefits of using a CIDR device in a GnRH-based, Ovsynch-type, fixed-time-AI 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-d Cosynch program and randomly assigned to receive no further treatment (Group 1) or a CIDR device concurrent with the first GnRH treatment (d 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 CIDR-treated heifers (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 CIDR device may make Ovsynch-type programs feasible in heifers.

A second experiment was designed to determine whether a CIDR 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 place of GnRH (Martínez et al., 2002b). Heifers were randomly assigned to three treatment groups; the first group received 12.5 mg of pLH on d 0, $PGF_{2\alpha}$ on d 7, and 12.5 mg of pLH on d 9 with AI 12 h later (pLH/Ovsynch), while the second group (pLH/CIDR) was similarly treated, with the addition of a CIDR device from d 0 to 7. Heifers in the third group (EB/CIDR) received an injection of 1 mg of EB and 100 mg of progesterone on d 0 and a CIDR device from d 0 to 7. Heifers were given $PGF_{2\alpha}$ on d 7 (at the time of CIDR removal) and 1 mg i.m. of EB on d 8, with AI on d 9 (52 h after $PGF_{2\alpha}$). The proportion of heifers in estrus was significantly greater in the EB/CIDR (94%) and pLH/CIDR (71%) groups than in the pLH/Ovsynch group (41%), whereas pregnancy rates were significantly higher in the EB/CIDR group (75%) than in the pLH/Ovsynch group (38%), with the pLH/CIDR group (65%) intermediate ($P < 0.05$). Overall, in a Cosynch fixed-time breeding program in lactating beef cows, the use of a CIDR device did not influence pregnancy rates. However, the use of a CIDR device in a 7-d Cosynch program utilizing GnRH or a 7-d Ovsynch program utilizing pLH significantly improved pregnancy rates in heifers.

It has also been shown that the use of a CIDR device 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 CIDR 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. Therefore, reproductive status can affect pregnancy rates in cattle given CIDR devices.

Combined Treatment Protocols. It was hypothesized that combinations of these treatments would be more efficacious than traditional approaches for synchronizing estrus and ovulation for fixed-time AI. Three experiments were conducted to evaluate methods of synchronization of estrus and ovulation in cattle for fixed-time AI (Martínez et al., 2000b). In the first experiment, a 7-d EB/CIDR treatment protocol was compared to a 7-d GnRH/CIDR treatment protocol or a simple 7-d CIDR protocol with the administration of $PGF_{2\alpha}$ at the time of CIDR removal. Pregnancy rate in the EB/CIDR group (76%) was higher than in the GnRH/CIDR (48%) or CIDR-treated, Control (38%) groups ($P < 0.01$). In addition, the percentage of heifers that displayed behavioral estrus in the EB/CIDR (100%) and CIDR-treated, Control (83%) groups was higher than in the GnRH/CIDR group (55%; $P < 0.01$).

A larger experiment was designed to compare progestins and methods of synchronizing wave emergence and ovulation in a fixed-time AI program (Martínez et al., 2002c). Angus-cross heifers (n = 503) were allocated into two synchronization groups and three treatment groups (2 × 3 factorial design) at random stages of the estrous cycle (d 0). At that time, heifers either received CIDR devices (n = 257) or were started on 0.5 mg·animal^{-1}·d^{-1} of melengestrol acetate (**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 d 6, and on d 7, CIDR devices were removed and all heifers received $PGF_{2\alpha}$. Consistent with their treatment on d 0, heifers were given either 1 mg EB 24 h after $PGF_{2\alpha}$ and inseminated 28 h later or 100 μg GnRH or 12.5 mg pLH 48 h after $PGF_{2\alpha}$ and concurrently inseminated. Heifers were exposed to bulls for 17 d, starting approximately 20 d after fixed-time AI. Although estrus rates differed ($P < 0.01$), there was no difference in pregnancy rates among groups ($P > 0.3$; Table 3). 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 fixed-time AI 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.

Table 3. Pregnancy rates following a single, fixed-time insemination in controlled internal drug release (CIDR)- or melengestrol acetate (MGA)-treated beef heifers in which follicular wave emergence and ovulation were synchronized with GnRH procine LH (pLH) or estradiol benzoate (EB)

	CIDR			MGA		
	GnRH	pLH	EB	GnRH	pLH	EB
Number of heifers	103	102	52	101	97	48
Estrus rate, %	66[a]	61[a]	92[b]	36[c]	33[c]	92[b]
Conception to AI, %	65	56	62	52	56	60
Conception to bull, %[d]	67	62	70	70	63	74
Total pregnancy rate, %	88	83	88	85	84	90

[a,b,c]Percentages with different superscripts differ ($P < 0.01$).
[d]Heifers not conceiving to AI.

A final series of experiments were conducted to determine the benefit of progesterone along with EB in the synchronization of follicular wave emergence in cattle treated with a CIDR and to determine the effect of interval from the second EB treatment to AI on pregnancy rates to fixed-time AI (Whittaker et al., 2002). Previous studies (Bo et al., 1994) suggested that including progesterone with estradiol might improve efficacy in synchronizing follicular wave emergence; it was hypothesized that the greatest benefit would be in cattle without a functional CL at the time of treatment. In the first experiment, lactating beef cows (n = 175) received a CIDR device on d 0 and were concurrently injected with either 2 mg of EB or 2 mg of EB plus 100 mg of progesterone. On d 7, CIDR were removed and all cows received an injection of $PGF_{2\alpha}$. On d 8, (approximately 24 h after CIDR removal), cows received an injection of 1 mg of EB and were inseminated on d 9, starting approximately 28 h after EB treatment. Overall pregnancy rate to fixed-time AI was 67%; pregnancy rate in those treated with EB alone was 64%, whereas those treated with EB plus progesterone was 70% ($P > 0.4$). In a replicate experiment in lactating beef cows and heifers (n = 137), results were similar, but the pregnancy rates differed by only 4%. Moreover, the inclusion of progesterone did not improve pregnancy rates in cattle in proestrus (13/28, 46%) or metestrus (12/19, 63%) at the beginning of treatment ($P = 0.3$). In a third experiment (unpublished), 391 lactating beef cows were treated similarly, except that inseminations were done 23 to 33 h after the second estradiol treatment. Calving rates did not differ among groups, but numerically more cows inseminated late (from 29.5 to 33.5 h after EB treatment) calved to the fixed-time AI. This trend was confirmed in a subsequent unpublished experiment involving 226 lactating beef cows. Although there would appear to be considerable flexibility in insemination time following CIDR removal and EB treatment, later insemination times (e.g., 34 to 38 h) should be investigated further. In addition, results do not provide convincing support for the use of progesterone along with estradiol benzoate at the time of CIDR insertion.

Effects of Cyclicity

Studies were conducted to determine the effects of reproductive status (noncycling vs. cycling) in a 7-d pLH/CIDR-based Cosynch program for fixed-time AI (Kastelic et al., 2001). Seventy-seven Hereford-cross heifers were confirmed to be cycling and 22 were confirmed to be noncycling by plasma progesterone analysis. Following CIDR removal, heifers were monitored electronically (HeatWatch) for estrus, but all were fixed-time inseminated concurrent with the second pLH treatment (d 9). There was no significant difference between cycling and noncycling heifers for rate of synchronous estrus, and pregnancy rate to fixed-time AI (58%) was not significantly affected by reproductive status (cycling vs. noncycling). Although only 78% of the heifers were puberal at the time of treatment, 97% had a functional CL 7 d after fixed-time AI. However, numerically more heifers in the cycling group became pregnant, which is consistent with the results of the study reported by Lucy et al. (2001).

Resynchronization

A great deal of the genetic potential of AI bulls is not utilized because few producers take the time to rebreed cattle that do not conceive in an estrus synchronization program; time saved in a timed-AI program would be lost by watching for return to estrus in nonpregnant cattle. It was hypothesized that the knowledge and technology developed in these experiments could make it feasible to synchronize return to estrus (and ovulation) as part of a total breeding program. Macmillan and Peterson (1993) had previously reported that the reinsertion of a used CIDR device at midcycle and subsequent removal on d 21 resulted in all repeats occurring over a 3-d period. Therefore, several experiments were conducted to determine the efficacy of progestins for resynchronization of return to estrus in heifers not pregnant to fixed-time AI.

In a preliminary experiment (unpublished), a used CIDR was placed in 79 heifers from d 13 to 20 after fixed-time AI, and the remaining 80 heifers were untreated controls. Mounting was monitored electronically (HeatWatch) for 6 d after CIDR removal, and AI was done 6 to 12 h after the onset of estrus. The mean interval from fixed-time AI to the return to estrus was 22 d (range, 4 d) in the CIDR-treated group vs. 19 d (range, 7 d) in the Control group ($P < 0.001$; variance, $P < 0.07$), but estrus rates and conception rates did not differ.

A subsequent experiment was designed to compare the use of a used CIDR and MGA and to investigate whether the addition of estradiol to a resynchronization program would increase the synchrony of estrus and pregnancy rates to a single reinsemination (Martínez et al., 2001). Fixed-time inseminated heifers (n = 651) were randomly assigned to seven groups for resynchronization (n = 93 per group). Heifers received no treatment (Control), MGA (0.5 mg·animal^{-1}·d^{-1}; three groups), or a used CIDR (three groups) for 7 d, starting 13 ± 1 d after fixed-time AI. The three treatment groups were 1) no further treatment; 2) 0.5 mg of E-17β plus 50 mg of progesterone on d 13; or 0.5 mg of E-17β plus 50 mg of progesterone on d 13 and 0.5 mg of E-17β on d 21 (48 h after the last feed of MGA or 24 h after CIDR removal). Heifers were inseminated 6 to 12 h after first detection of estrus. Variability in return to estrus was greater ($P < 0.001$) in the Control group than in progestin-treated groups. Conception and pregnancy rates in heifers given a CIDR (65 and 61%, respectively) were higher ($P < 0.01$) than those given MGA (50 and 40%), but were not different from Controls (62 and 55%). In summary, following fixed-time AI, progestins (used CIDR or MGA) and estradiol-17β can be used to resynchronize follicle waves, estrus, and ovulation, facilitating a synchronous reinsemination of nonpregnant heifers. However, used CIDR devices seemed more efficacious than MGA in this study. In a follow-up study (our unpublished results), 979 beef heifers that had been fixed-time inseminated received a used CIDR device from d 13 to 20. The overall pregnancy rate to fixed-time AI was 56%. After CIDR removal on d 20, 336 heifers were detected in estrus between d 21.5 and 25.5, with a mean and mode of 22.5 d. Ninety heifers (21% of those found to be nonpregnant by ultrasound examination on d 28) were found to be not pregnant, even though they were not detected in estrus. Of the 336 heifers that were reinseminated, 238 (71%) became pregnant, for an overall pregnancy rate of 81% to two inseminations, with 4 d of estrus detection; in the previous study, untreated (control) heifers were detected in estrus over a 10-d period.

Commercial Preparations of Steroid Hormones

Although EB and E-17β were both shown to be very efficacious for the synchronization of follicular wave emergence and ovulation for fixed-time AI in CIDR-treated cattle, neither estrogen preparation is commercially available in Canada or the United States. However, a much longer-acting ester, **ECP** (Pharmacia Animal Health, Orangeville, ON, Canada) is available to practicing veterinarians. Three experiments were conducted to investigate the use of ECP for synchronizing follicular wave emergence and ovulation in beef heifers treated with a CIDR device (Colazo et al., 2002). In the first experiment, ECP was shown to be very efficacious in inducing ovulation of the dominant follicle of an E-17β-synchronized wave; 19 of 20 ECP-treated heifers ovulated between 72 and 96 h after CIDR removal, confirming earlier studies in GnRH-treated cattle (reviewed in Thatcher et al., 2001). In a second experiment, follicular wave emergence was more variable ($P < 0.01$) in CIDR-treated heifers given ECP (n = 30) than in those given E-17β (n = 28; 4.0 ± 0.26 d vs. 3.3 ± 0.15 d), but there was no difference in pregnancy rates to fixed-time AI when ECP was given 24 h after CIDR removal to synchronize ovulation (overall mean, 71%; $P > 0.2$).

A larger experiment was conducted to compare ECP plus a commercial source of progesterone with GnRH in a CIDR-based, fixed-time AI program (Colazo et al., 2002). On d 0, all heifers (n = 979) received a CIDR and were randomly allocated to receive either 100 μg of GnRH (n = 491) or 1 mg of ECP plus 50 mg of progesterone (Progesterone 5%, Vétoquinol N-A Inc., Lavaltrie, QC, Canada; n = 488). The CIDR devices were removed and $PGF_{2\alpha}$ was given on d 7 or 8.5 in the GnRH and ECP groups, respectively. Heifers were further subdivided to receive 0.5 mg of ECP at CIDR removal or 24 h later (with AI 58 to 60 h after CIDR removal) or a second injection of GnRH at the time of AI (52 to 54 h after CIDR removal). There was no difference in pregnancy rates between groups treated with GnRH (276/491, 56%) or ECP (277/488, 57%) on d 0. However, pregnancy rate was higher ($P < 0.01$) in heifers receiving ECP 24 h after CIDR removal (216/331, 65%) than at CIDR removal (168/320, 52%) or GnRH at AI (169/328, 51%). Data demonstrate that commercially available steroids can be used successfully to synchronize follicular wave emergence and ovulation in a CIDR-based, fixed-time AI program in beef heifers.

Use of Controlled Internal Drug Release Devices in Superstimulation Protocols

Precise control of ovarian function is essential for successful superovulation. Although gonadotropin treatments are usually initiated on d 8 to 12 of the estrous cycle to coincide with emergence of the second follicular wave, superstimulatory response can be adversely affected if these treatments are not initiated precisely at wave emergence (Nasser et al., 1993). Superstimulatory treatments can be initiated at an optimal time by synchronization of follicular wave emergence in CIDR-treated donor cattle, eliminating the need for estrus detection and the obligatory delay of 8 to 12 d. One approach involves transvaginal ultrasound-guided follicle ablation at random stages of the estrous cycle to synchronize wave emergence, followed by FSH 1 d after ablation, and $PGF_{2\alpha}$ 48 h later (Bergfelt et al., 1997). It was found that the timing of estrus could be controlled most accurately when a progestin implant was inserted for the period of superstimulation and two injections of $PGF_{2\alpha}$ were administered on the day of implant removal. In a more recent study, ablation of the two largest follicles was shown to be as efficacious in synchronizing follicular wave emergence for superstimulation as ablating all follicles ≥5 mm (Baracaldo et al., 2000), thereby eliminating the need to identify the dominant follicle. Therefore, ultrasound-guided follicular ablation can be used (along with a CIDR) to eliminate the effects of a dominant follicle prior to initiating gonadotropin treatments.

The reported asynchrony in follicular wave emergence (from 3 d before to 5 d after treatment; Martínez et al., 1999) suggests that GnRH or pLH may not be feasible for superstimulation. Indeed, when GnRH or pLH were compared to E-17β for the synchronization of follicular wave emergence prior to superstimulation (Deyo et al., 2001), the number of ova/embryos collected was reduced in the GnRH- or pLH-treated cattle. Therefore, the use of GnRH or pLH to synchronize follicular wave emergence prior to initiating superstimulatory treatments is not recommended.

The preferred approach is to use estradiol to synchronize follicular wave emergence in CIDR-treated donor cows. On d 0 (random and unknown stages of the estrous cycle), a CIDR is inserted and an injection of 5 mg of E-17β plus 100 mg of progesterone is given to synchronize follicular wave emergence. Four days later, gonadotropin treatments are initiated and CIDR are removed 48 to 72 h later, 12 h after a first injection of $PGF_{2\alpha}$. Inseminations are done 12 and 24 h after the onset of estrus (or 60 and 72 h after the first $PGF_{2\alpha}$ injection). Data from several experiments and commercial embryo transfer records show that this approach is very practical, and superovulatory responses were at least as high as when treatments were initiated around the time of emergence of the second follicular wave (reviewed in Bó et al., 2002).

The use of estradiol esters (e.g., EB or estradiol valerate) has also been investigated. Treatment with 2.5 mg of EB and 50 mg of progesterone given at CIDR insertion resulted in synchronous emergence of a new follicular wave 3 to 4 d later (Caccia and Bó, 1998). Superstimulatory treatments initiated 4 d after the administration of 5 mg of E-17β plus 100 mg of progesterone, 2.5 mg of E-17β plus 50 mg of progesterone, or 2.5 mg of EB plus 50 mg of progesterone resulted in superovulatory responses comparable to those initiated 8 to 12 d after estrus (reviewed in Bó et al., 2002). Treatment with 5 mg of estradiol valerate plus 3 mg of norgestomet resulted in less synchronous follicular wave emergence and a lower superovulatory response than 5 mg of E-17β plus 100 mg of progesterone (Mapletoft et al., 1999). Unfortunately, lower doses of estradiol valerate have not been investigated. Collectively, these studies demonstrate that exogenous control of follicle wave emergence offers the advantage of initiating superstimulatory treatments at an optimal time for follicle recruitment, regardless of the stage of the estrous cycle. The treatment is practical, easy to follow by farm personnel, and more importantly, the need for estrus detection and waiting 8 to 12 d prior to initiating gonadotropin treatments is eliminated.

Implications

Variable responses have been one of the most frustrating limitations of estrus synchronization and superovulation in cattle. However, protocols that control both ovarian follicles and luteal function have provided opportunities for fixed-time artificial insemination (without estrus detection). Inserting a controlled internal drug release device and synchronizing ovarian follicular development consistently resulted in high pregnancy rates to fixed-time artificial insemination, regardless of stage of the estrous cycle. Similarly, used controlled internal drug release devices were beneficial for resynchronization of heifers not pregnant to fixed-time artificial insemination. Although variability in response to superstimulation has not been completely eliminated, protocols involving synchronization of follicular wave emergence in controlled internal drug release-treated cattle offer the convenience of initiating treatments immediately or at a self-appointed time, without estrus detection and without adversely affecting the superovulatory response or number of transferable embryos.

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