Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle

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The effects of concentration of progesterone in plasma on development and fertility of the first wave dominant follicle were studied in cattle. To identify a source of exogenous progesterone that would permit extension of the first wave dominant follicle, nonlactating Holstein cows ($n = 6$) received on day 8 of two successive oestrous cycles an injection of PGF_{2α} (25 mg) and a new (1.9 g of progesterone (Period 1)) or used (≈ 1.2 g of progesterone (Period 2)) CIDR-B device that was removed on day 17. Control cows ($n = 6$) received a new CIDR-B device on day 8 that was removed on day 17 and a PGF_{2α} injection (25 mg) on day 17. Ultrasonography and collection of blood samples were performed on alternate days throughout the experiment. Plasma concentrations of progesterone and oestradiol were different between treatments ($P < 0.0001$ and $P < 0.05$, respectively). The dominant follicle was maintained until day 17 and ovulated upon removal of the intravaginal device in 1 of 6, 6 of 6 and 0 of 6 in new CIDR-B, used CIDR-B and control groups, respectively ($P < 0.01$). The preovulatory dominant follicles were 14.2 ± 1.6 mm, 20 ± 1.3 mm and 10 ± 1.3 mm, respectively ($P < 0.001$) on day 17. There were fewer 5–9 mm follicles in cows having a persistent dominant follicle ($P < 0.01$). The interval to onset of oestrus was negatively correlated with size of the dominant follicle on day 17 ($P < 0.001$). In Expt 2, the fertility of oocytes ovulated from new (PGF_{2α} on day 7; T1; $n = 91$) and persistent dominant follicles (PGF_{2α} on day 7 and a used CIDR-B device inserted on day 7 and withdrawn on day 16; T2; $n = 91$) was tested using Holstein heifers. Size of the dominant follicle and plasma concentrations of progesterone and oestradiol on days 7 (T1) and 16 (T2) were different between treatments: 11.3 ± 0.2 versus 16.2 ± 0.3 mm ($P < 0.001$); 4.2 ± 0.2 versus 2.9 ± 0.3 ng ml⁻¹ ($P < 0.01$) and 3.5 ± 0.3 versus 11.7 ± 1.7 pg ml⁻¹ ($P < 0.01$), respectively. Pregnancy rates at first artificial insemination were 64.8% (46 of 71) and 37.1% (26 of 70) for new and persistent dominant follicles, respectively ($P < 0.01$). Pregnancy rates at second service were 50% and 52.8%, respectively. Low plasma concentrations of progesterone, therefore, resulted in persistency of the dominant follicle and temporarily impaired fertility.

Introduction

Follicular development in cattle occurs in a wave-like manner (Rajakoski, 1960). In primates, each follicular wave proceeds through the stages of recruitment, selection and dominance (Goodman and Hodgen, 1983). During the luteal phase of the oestrous cycle in heifers and cows, two or three waves of follicular development normally occur (Savio *et al.*, 1988, 1990a; Sirois

and Fortune, 1988; Knopf *et al.*, 1989; Driancourt *et al.*, 1991). Each wave concludes with the development of a single dominant follicle, which suppresses the growth of other follicles larger than 4 mm in diameter (Savio *et al.*, 1993).

Regardless of the final number of follicular waves characteristic of each oestrous cycle, there is a consistent follicular turnover at midcycle (days 10 to 14 (Savio *et al.*, 1988, 1990a; Sirois and Fortune, 1988; Knopf *et al.*, 1989; Driancourt *et al.*, 1991)). The first dominant follicle appears unable to suppress growth of other follicles continuously, and emergence of a new follicular wave can be detected by ultrasonography. Demise of the first dominant follicle at midcycle is due to the negative feedback effect of progesterone from the corpus luteum on LH secretion (Savio *et al.*, 1990c, 1993). In the absence of a normal corpus

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Received 7 April 1992.

luteum and in an environment with low progesterin, resulting in an increased LH pulse frequency, the first dominant follicle continues to grow and suppress growth of other follicles well beyond normal limits (> 20 days; Savio *et al.*, 1990c, 1992).

There is evidence of impaired fertility in cattle following oestrus synchronization treatments that involve endocrine manipulations for extended periods (e.g. 15 days or more; for review see Beal *et al.*, 1988; Odde, 1990). When such procedures were used during periods in which the corpus luteum regressed, progesterone concentration at the end of treatment was probably lower than during a normal luteal phase (Van Cleeff *et al.*, 1992). It is suggested that under these conditions of low plasma progesterone concentrations, the dominant follicle continued growing and led to the development of a persistent follicle which induced a subfertile oestrus.

Objectives of the present study were to: (1) identify a source of exogenous progesterone that could prevent ovulation and permit the extension of the first follicular wave beyond mid-cycle; and (2) test whether heifers that ovulate persistent first wave dominant follicles have reduced fertility compared with non-persistent first wave dominant follicles.

Materials and Methods

Experiment 1

Twelve nonlactating Holstein cows were assigned randomly to either a treated group ($n = 6$), which was monitored for two consecutive experimental oestrous cycles, or a control group ($n = 6$). Cows were maintained on natural pasture (*Cynodon dactylon*) and had free access to supplementary maize silage and peanut hay. Oestrous cycles were synchronized using a norgestomet implant (6 mg; Syncro-mate-B; CEVA Laboratories, Inc., Overland Park, KS) inserted subcutaneously on the outer surface of the ear and left in place for 9 days. In addition, 25 mg PGF_{2α} (PGF_{2α} tam salt, Lutalyse, Upjohn Co., Kalamazoo, MI) were injected (i.m.) 2 days before withdrawal of the norgestomet implant. The day of oestrus was designated as day 0.

On day 8 of the initial oestrous cycle, cows ($n = 6$) received an injection (25 mg, i.m.) of PGF_{2α}. Simultaneously, a CIDR-B device (Controlled Internal Drug Release – Bovine, Eazi-breed, AHI Plastic Co., Hamilton), containing 1.9 g of progesterone was inserted into the vagina. The device was left in place for 9 days (Period I). A group of cows ($n = 6$) that had a new CIDR-B device inserted on day 8 and removed on day 17, and received a PGF_{2α} injection (25 mg) on day 17 was used as controls.

During the second oestrous cycle (Period II), the cows treated in Period I received similar treatment, except that CIDR-B devices previously used during the 9 days of Period I were used instead of new CIDR-B units. Used CIDR-B devices have lower amounts of progesterone and the rationale for the treatment sequence was to identify a CIDR-B device with a release level of progesterone appropriate for maintaining persistence of the first wave dominant follicle. A previous study (Van Cleeff *et al.*, 1992) indicated that a CIDR-B device used for 9 days previously contained approximately 1.2 g progesterone and resulted in reduced concentrations of plasma progesterone than in cows that had new CIDR-B devices inserted. Different

concentrations of progesterone in peripheral blood would therefore be expected from CIDR-B devices that had different initial contents of progesterone (1.9 versus 1.2 g of progesterone for new and used CIDR-B devices in Periods I and II, respectively).

A sequence of new (Period I) and used (Period II) CIDR-B devices was followed on a within cow basis to permit each cow the opportunity to use progesterone from a new device, in the absence of a corpus luteum, as a characteristic of that cow and for the device to be used again in Period II in the same manner. Previous studies (Savio *et al.*, 1990c, 1993) indicated that induced persistence of a dominant follicle had no carry-over effect on turnover of dominant follicles in the subsequent oestrous cycle.

An assessment of the relationship between dominant follicle development at the time of luteolysis and interval to onset of oestrus was completed by giving control cows an injection of PGF_{2α} on day 17. It was anticipated that, at day 17, 50% of the control cows would have an active dominant follicle that originated from the second follicular wave, whereas the other 50% were expected to be in interphase period between second and third follicular waves (Savio *et al.*, 1990a, c).

Follicular development was assessed by examining cows every second day in both experimental periods by transrectal ultrasonography from day 8 until ovulation. An Equisonic LS 1000 linear-array ultrasound scanner equipped with a 7.5 MHz transducer (Tokyo Keiki Co. Ltd, Tokyo) was used. During each examination, the total number of follicles > 3 mm was determined and ovarian maps were drawn to record the size and relative positions of follicles ≥ 5 mm with respect to each other and to other ovarian structures (e.g. corpus luteum).

Blood samples were collected before each ultrasound session by jugular venepuncture using heparinized tubes (Vacutainer, Becton Dickinson Vacutainer Systems USA, Rutherford, NJ) and samples were stored in an ice bath. Plasma was separated by centrifugation (1800 g for 30 min) and stored at -20°C until assayed for progesterone (Knickerbocker *et al.*, 1986) and oestradiol (Tortonese *et al.*, 1990) as modified by Badinga *et al.* (1992). Intra- and interassay coefficients of variation were 6.3 and 10%, respectively, for progesterone and 7.1 and 11.3%, respectively, for oestradiol. The sensitivity of the assays was 0.1 ng ml^{-1} and 1.4 pg ml^{-1} for progesterone and oestradiol, respectively.

Experiment 2

Experiment 2 was designed to test the fertility of the first wave dominant follicle at two different stages of development (days 9–10 and 17–18 of the oestrous cycle, respectively) in heifers of a commercial dairy herd (Larson's Dairy, Okeechobee, FL). A group of 189 Holstein heifers, 14–17 months old and weighing between 300 and 370 kg, was used. Heifers were in very good body condition and were healthy. They were maintained on natural pasture (*Cynodon dactylon*) and received 3 kg supplement of concentrate per animal per day.

Heifers received two i.m. injections of 25 mg PGF_{2α} given 11 days apart to synchronize oestrous cycles. Behavioural oestrus was monitored three times a day (06:00 h, 12:00 h and 18:00 h) for 90 min each time, from days 1 to 5 after the second PGF_{2α} injection. Visual observation for signs of oestrus was aided by

Fig. 1. Mean ($\pm$ SEM) plasma concentrations of progesterone (ng ml^{-1}) in cows that received (arrow) an injection of $\text{PGF}_{2\alpha}$ (25 mg) and a new (●—●) or used (△—△) CIDR-B device on day 8 of the oestrous cycle ($n = 6$ per treatment). Cows in the control group (○—○; $n = 6$) had a new CIDR-B device inserted (arrow) on day 8 and received a luteolytic dose (25 mg) of $\text{PGF}_{2\alpha}$ on day 17 (arrow) of the oestrous cycle. CIDR-B devices were removed on day 17 of the oestrous cycle.

use of a modified tail-paint system (Macmillan *et al.*, 1988). A total of 164 heifers (86.8%) were detected to be unequivocally in oestrus during the observation period; the proportion of animals detected in oestrus during each day of the observation period were: 2 (1.2%), 19 (11.6%), 109 (66.5%), 20 (12.2%) and 14 heifers (8.5%) on days 1, 2, 3, 4 and 5, respectively. An additional group of 18 animals (9.5% of the total) was detected to have some degree of alteration in the tail-paint during the observation periods. Although visual signs of oestrus were not obvious in these animals, they were considered to be synchronized and were used in the experiment. The day on which the majority of heifers were detected to be in oestrus (day 3 after the second $\text{PGF}_{2\alpha}$ injection) was considered day 0.

After synchronization, animals were randomly assigned within day of detected oestrus (1 to 5 after second injection of $\text{PGF}_{2\alpha}$) and precision of the detection of oestrus (visual + tail-paint or only tail-paint alteration) to two treatments, 1 and 2. Treatment 1 (T1, new follicle; $n = 91$) was designed to cause ovulation of new dominant follicles and Treatment 2 (T2, persistent follicle; $n = 91$) was designed to cause ovulation of a dominant follicle made to persist from the first follicular wave. In T1, $\text{PGF}_{2\alpha}$ injection (25 mg i.m., Lutalyse) was given on day 7 of the synchronized oestrous cycle. Most animals in this group were expected to be in oestrus between 48 and 72 h after $\text{PGF}_{2\alpha}$ injection and to ovulate the first wave dominant follicle. For T2 (persistent follicle), $\text{PGF}_{2\alpha}$ injection (25 mg i.m., Lutalyse) was given on day 7 of the synchronized oestrous cycle. In addition, a previously used and gas sterilized (ethylene oxide at room temperature for 24 h) CIDR-B device (CIDR-B devices used previously for 9 days in different heifers from the same herd) was inserted on day 7 and withdrawn on day 16. On the basis of data from Expt 1, the first wave dominant follicle present at day 7 should continue growing and be the active dominant follicle at day 16 of the oestrous cycle. Consequently, the

ovulatory follicle in this treatment would have persisted for about 9 days longer than the ovulatory follicle in T1.

Different days of $\text{PGF}_{2\alpha}$ injection were used in Expts 1 and 2 (days 8 and 7 of the oestrous cycle, respectively) because in heifers the dominant follicle on day 7 of the oestrous cycle is already in a plateau period, whereas a comparable event in mature cows appears to be delayed by about 2 days (Savio *et al.*, 1990a).

The interval between the onset of oestrus and time of artificial insemination was standardized by adapting the time of $\text{PGF}_{2\alpha}$ injections and CIDR-B withdrawal to the average expected time for onset of oestrus (56 h in T1; Savio *et al.*, 1990b; and 40 h in T2; Expt 1). Timing for breeding of each treatment to the breeding routine of the farm was optimized by treating the animals so that in most of the cows oestrus occurred in late afternoon or the evening of the day before the day of expected insemination. Consequently, $\text{PGF}_{2\alpha}$ injections were given to all the animals (T1 and T2) between 14:00 and 16:00 h on day 7 of the oestrous cycle. The CIDR-B devices (T2) were withdrawn at 08:00 h on day 16 of the oestrous cycle.

A subsample ($n = 40$) of heifers from each treatment group was examined by ultrasonography on day 7 (T1) or day 16 (T2) to determine the size of the dominant follicle (the largest follicle detected). Blood samples were also collected at ultrasound examinations by jugular venepuncture. Plasma was obtained by centrifugation (1800 g for 30 min) and stored at -20°C until assayed for progesterone and oestradiol.

During the breeding period, oestrus detection was aided by the use of a special chalk (All-Weather Paintstick, La-Co Industries, Inc./Markal Company, Chicago, IL) as used routinely on the farm. The heifers were observed for behavioural signs of oestrus three times a day (06:00, 12:00 and 18:00 h for 90 min each time) during days 2–5 of the expected oestrous period (days 9–12 for T1 and days 17–19 for T2). During the remainder of the breeding period, heifers were observed for behavioural oestrus and chalk condition for 20 min each morning. Animals detected to be in oestrus were inseminated artificially with frozen semen of known good fertility. The semen used was from three different bulls, which were proportionally balanced between treatments. All inseminations were performed by the same operator once a day (morning), and all animals detected in oestrus during the previous 24 h were inseminated each morning. Pregnancy diagnosis was assessed by rectal palpation at 65 days after first service.

Statistical analysis

Data were analysed statistically by analysis of variance using the GLM procedure of the Statistical Analysis System (SAS, 1988). Size of the dominant follicle and plasma concentrations of progesterone and oestradiol, as well as the total number of follicles in each class (Class I (3 to 4 mm), Class II (5 to 9 mm) and Class III (≥ 10 mm)) were analysed as repeated measures. The split-plot model included treatment, cow(treatment), day and treatment $\times$ day. Differences between means were evaluated by preplanned orthogonal contrasts. Means in Expt 2 were compared using analysis of variance. Proportional responses were compared using χ^2 analysis.

Fig. 2. Different patterns of development of the first ($\square$ — $\square$) second ($\blacklozenge$ — $\blacklozenge$) and third (∇ — ∇) wave dominant follicles detected in Expt 1 (means $\pm$ SEM; $n = 6$ per treatment). (a) and (b) The patterns of growth of dominant follicles of cows that had (a) three ($n = 3$) or (b) two ($n = 3$) follicular waves in control group (new CIDR-B device inserted between days 8 and 17 and PGF_{2α} injection on day 17). (c) and (d) The patterns of dominant follicle development in cows that received an injection of PGF_{2α} on day 8 and had a new CIDR-B device inserted between days 8 and 17; (c) corresponds to those oestrous cycles with two follicular waves ($n = 5$) and (d) to the cow ($n = 1$) that had one follicular wave. (e) The sustained growth of the dominant follicle of the first follicular wave ($n = 6$) in cows that received a PGF_{2α} injection on day 8 and had a used CIDR-B device inserted between days 8 and 17.

Fig. 3. Mean ($\pm$ SEM) plasma concentrations of oestradiol (pg ml⁻¹) in cows that received (arrow) an injection of PGF_{2α} (25 mg) and a new ($\bullet$ — $\bullet$) or used ($\triangle$ — $\triangle$) CIDR-B device on day 8 of the oestrous cycle ($n = 6$ per treatment). Cows in the control group ($\circ$ — $\circ$; $n = 6$) had a new CIDR-B device inserted (arrow) on day 8 and received a luteolytic dose (25 mg) of PGF_{2α} on day 17 (arrow) of the oestrous cycle. CIDR-B devices were removed on day 17 of the oestrous cycle.

Results

Experiment 1

Plasma concentrations of progesterone were different ($P < 0.001$) among treatments (Fig. 1) and ranged from 9 to 12 ng ml⁻¹ for control cows, 4 to 5 ng ml⁻¹ for cows receiving a new CIDR-B device (Period I) and were about 2 ng ml⁻¹ for cows receiving a used CIDR-B device (Period II).

The frequencies of oestrous cycles with an extended lifespan of the first dominant follicle (one out of six in the new CIDR-B period (I) and six of six in the used CIDR-B period (II)) were also different ($P < 0.01$). Five cows in the new CIDR-B period and none in the used CIDR-B period therefore developed a new follicular wave during the experimental oestrous cycles. In other words, one cow of the new CIDR-B period and six cows of the used CIDR-B period maintained the first dominant follicle and ovulated spontaneously on removal of the CIDR-B device. All cows in the control group had follicular turnover at midcycle and developed a second follicular wave. The second follicular

Table 1. Mean number of Class I, II and III follicles in nonlactating Holstein cows that received an injection of PGF_{2α} and a new or used CIDR-B device on day 8 of the oestrous cycle. CIDR-B devices were removed on day 17 of the oestrous cycle. Cows in the control group had a new CIDR-B inserted between days 8 and 17 and received an injection of PGF_{2α} on day 17 of the oestrous cycle

Day	Class I (3–4 mm) ^a			Class II (5–9 mm) ^b			Class III (≥ 10 mm) ^c		
	New CIDR	Used CIDR	Control	New CIDR	Used CIDR	Control	New CIDR	Used CIDR	Control
8	14.7 ^d	15.3 ^d	6.3 ^d	1.3 ^d	2.3 ^d	1.8 ^d	1.3 ^d	1.1 ^d	1.0 ^d
10	8.7 ^d	11.3 ^d	12.3 ^d	2.5 ^d	2.0 ^d	1.7 ^d	1.1 ^d	1.1 ^d	1.2 ^d
12	12.2 ^d	19.8 ^e	12.0 ^d	3.5 ^d	1.1 ^e	3.5 ^d	1.1 ^d	1.0 ^d	1.0 ^d
14	4.5 ^d	17.8 ^e	7.3 ^d	1.5 ^d	0.3 ^e	2.7 ^d	1.0 ^d	1.0 ^d	1.3 ^d
16	8.0 ^d	22.2 ^e	11.0 ^d	2.8 ^d	0.6 ^e	4.1 ^d	1.1 ^d	1.0 ^d	1.8 ^d
18	15.2 ^d	22.3 ^d	14.8 ^d	2.3 ^d	1.0 ^e	5.5 ^d	1.1 ^d	1.0 ^d	1.5 ^d

^a*P* = 0.07 for treatment effect.

^b*P* = 0.009 for treatment effect.

^c*P* = 0.18 for treatment effect.

Within a day and follicle class, means followed by different superscripts differ (orthogonal contrast; *P* < 0.05).

Pooled SEM = 1.0, 0.3 and 0.1 for Class I, II and III, respectively.

Fig. 4. Best fit quadratic regression of the effect of the size of the preovulatory dominant follicle at the time of luteolysis or progesterone withdrawal on the time to onset of oestrus in nonlactating Holstein cows (*n* = 18).

Table 2. Mean ($\pm$ SEM) diameter of the dominant follicle, and plasma concentrations of progesterone and oestradiol on days 7 and 16 of the oestrous cycle in Holstein heifers that ovulated a new (day 7; T1) or persistent (day 16; T2) first wave dominant follicle

	T1 (Day 7)	T2 (Day 16)
Dominant follicle (mm)	11.3 $\pm$ 0.2	16.2 $\pm$ 0.3***
Progesterone (ng ml ⁻¹)	4.2 $\pm$ 0.2	2.9 $\pm$ 0.3**
Oestradiol (pg ml ⁻¹)	3.5 $\pm$ 0.3	11.7 $\pm$ 1.7**
Number of heifers	40	40

Significant difference between T1 and T2: ***P* < 0.01 and ****P* < 0.001.

waves in new CIDR-B and control groups were detected on days 12 $\pm$ 2.4 (*n* = 5) and 11.3 $\pm$ 1 (*n* = 6) of the oestrous cycle, respectively (Fig. 2). In three of six cows in the control group, a third follicular wave was detected on day 16.3 $\pm$ 0.3, and was developing at the time of PGF_{2α} injection and CIDR-B withdrawal on day 17 of the oestrous cycle. The single persistent dominant follicle of the used CIDR-B treatment (Fig. 2e) continued to grow after day 8 and reached a diameter of about 20 mm by day 16 of the experimental oestrous cycle.

Plasma concentrations of oestradiol were different (*P* < 0.05) among treatments (Fig. 3). They were low (< 5 pg ml⁻¹) on day 8 of the oestrous cycle in all treatments, and remained low until day 18 in the control group. In treated cows (new and used CIDR-B devices) plasma concentrations of oestradiol were consistently higher (*P* < 0.05) than in the control group (Fig. 3).

Overall follicular development was analysed by categorizing follicles according to size (Class I (3–4 mm), Class II (5–9 mm) and Class III (≥ 10 mm)). Fewer Class II follicles were detected by ultrasonography during the used CIDR-B period (in which cows had a persistent dominant follicle) than in the same animals during new CIDR-B period or for cows in the control group (*P* < 0.01; Table 1). By contrast, cows with a used CIDR-B device had a greater number of Class I follicles on days 12, 14 and 16 of the oestrous cycle than cows in new CIDR-B device or the control group (*P* < 0.05; Table 1). Frequency of Class III follicles did not differ among treatments or days.

Average sizes ($\pm$ SEM) of the dominant follicles on day 17 of the oestrous cycle were 14.2 $\pm$ 1.6 mm, 20 $\pm$ 1.3 mm and 10.0 $\pm$ 1.3 mm for new and used CIDR-B treatments and control group, respectively (*P* < 0.001). The size of the dominant follicle at the time of induced luteolysis or CIDR-B withdrawal on day 17 of the oestrous cycle affected the interval to onset of oestrus (*P* < 0.001; Fig. 4). Average interoestrous intervals ($\pm$ SEM) were 19.7 $\pm$ 0.30, 18.8 $\pm$ 0.15 and 20.6 $\pm$ 0.19 days, for new and used CIDR-B treatments and control group, respectively. All cows ovulated the preovulatory follicle on the day

Table 3. Number of heifers detected in oestrus for 5 days after induced luteolysis on day 7 (T1) or withdrawal of a used CIDR-B device on day 16 with luteolysis induced on day 7 (T2)

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Total
T1	0	17 (23.9%)	53 (74.6%)	1 (1.4%)	0	71 (100%)
T2	0	58 (82.8%)	6 (8.6%)	3 (4.3%)	3 (4.3%)	70 (100%)

Chi-square (4 dominant follicles) = 64.5; $P < 0.001$ for treatment by day distribution (2×5 factorial).

Table 4. Conception rates after first and second service by artificial insemination and interval between services (mean $\pm$ SEM) in Holstein heifers that received a PGF_{2a} injection (25 mg) on day 7 (T1) or heifers that also received PGF_{2a} on day 7 but had used CIDR-B devices inserted between days 7 to 16 (T2)

	T1	T2
Number of heifers	71	70
Number pregnant first service	46	26
Conception rate first service	64.8%**	37.1%
Interval between first and second service (days)	23.5 $\pm$ 3.1	25.1 $\pm$ 1.4
Conception rate second service	50% (12/24)	52.8% (19/36)

**Chi-square = 10.749; $P < 0.01$ for distribution of pregnant and nonpregnant heifers between T1 and T2.

after the day of standing oestrus. Thus, there is no evidence that a persistent dominant follicle had a delay in ovulation. However, small differences in time to ovulation could not be determined precisely since ultrasonography was done only once per day.

Experiment 2

The dominant follicle was 4 mm larger at day 16 for T2 than for T1 at day 7, and this was associated with a concentration of progesterone that was 1.3 ng ml⁻¹ lower and a concentration of oestradiol that was 8.2 pg ml⁻¹ higher in plasma (Table 2).

The number of animals detected in oestrus within 5 days after PGF_{2a} (T1) or CIDR-B withdrawal (T2) was similar between treatments (71 of 91 (78%) and 70 of 91 (77%) for T1 and T2, respectively). However, the distributions of days to onset of oestrus after induced luteolysis or CIDR-B withdrawal differed ($P < 0.001$) between groups of heifers (Table 3). Most of the heifers in T1 were detected in oestrus during day 3 after PGF_{2a} injection (74.6%), whereas in T2 the majority of heifers were detected in oestrus during day 2 (82.8%). The number of heifers that were not detected in oestrus within 5 days after PGF_{2a} injection (T1) or CIDR-B withdrawal (T2) was the same (19 of 91 in each group). In addition, one heifer in T1 was eliminated from the experiment because oestrous signs were observed on the day of PGF_{2a} injection. Two heifers were

eliminated in T2: one because a CIDR-B device was lost during the experimental period; the other because its cervix could not be penetrated at artificial insemination.

Heifers inseminated at oestrus induced by the presence of a persistent dominant follicle had a reduced conception rate (Table 4). Interoestrous intervals of heifers that did not conceive were the same between treatments. No carry-over effects of the persistent dominant follicle that ovulated in T2 were detected since conception rate to second service was the same between groups.

Discussion

The induction of a low progesterone (about 2 ng ml⁻¹) environment resulted in persistence and increased size of the dominant follicle and higher plasma concentrations of oestradiol, which is in agreement with the studies of Savio *et al.* (1990c, 1993). In addition, the oestrus induced by these persistent follicles had lower fertility. Factors related to final follicular or oocyte development or to early pregnancy events appear to be altered.

The importance of different peripheral concentrations of progestins, acting through the negative feedback effect on LH secretion, on follicular development in cattle has been reported by Savio *et al.* (1990c), Sirois and Fortune (1990), and Taylor and Rajamahendran (1991). The present experimental model provided a predictable endocrine environment that was associated with persistence of the dominant follicle.

In agreement with previous studies (Savio *et al.*, 1988, 1990a; Sirois and Fortune, 1988; Knopf *et al.*, 1989; Driancourt *et al.*, 1991), normal plasma concentrations of progesterone from the corpus luteum plus a new CIDR-B device (control group) resulted in consistent follicular turnover at midcycle. By contrast, when previously used CIDR-B devices were inserted after PGF_{2a}-induced luteolysis on day 8 of the oestrous cycle, concentrations of plasma progesterone were reduced and none of the cows developed a new follicular wave after development of the first follicular wave. In these animals, the first dominant follicle continued growing and was still the dominant follicle on day 17 of the oestrous cycle. This response was associated with low concentrations of progesterone (about 2 ng ml⁻¹) in plasma. In addition to sustained follicular growth, functional dominance was also observed as there was a suppression in the number of other follicles more than 4 mm in diameter.

Follicular persistence and sustained dominance were also reported using a similar model with norgestomet and PGF_{2a} (Savio *et al.*, 1990c, 1993). The presence of an active dominant

follicle was associated with a large number of small (≤ 4 mm) and very few medium size (5 to 9 mm) follicles. By contrast, the development of a new follicular wave occurred with an increase in the number of 5–9 mm follicles, which is consistent with the rise in the frequency of Class II follicles (5–9 mm) on days 12 to 18 detected in the cows that developed a second follicular wave (new CIDR-B and control groups) in the present study. Furthermore, at the same time as the increase in Class II follicles there was a decrease in the number of Class I follicles (new CIDR-B and control groups on days 12, 14 and 16). There appears to be a dynamic equilibrium in the frequency of ovarian follicular populations across follicular classes which is detectable by ultrasonography. This balance of follicles among classes is regulated by the presence of an active dominant follicle. Lack of follicular dominance results in an increase in FSH concentration (Adams *et al.*, 1992; Badinga *et al.*, 1992) which permits further growth of small follicles (recruitment of a new follicular wave) and selection of a new dominant follicle.

Intermediate plasma concentrations of progesterone (3 to 5 ng ml⁻¹) provided by the use of new CIDR-B devices in the absence of a corpus luteum (Expt 1, Period I) resulted in a mid-cycle turnover of the dominant follicle in five of six cows. Continued development of the first dominant follicle of the oestrous cycle throughout the luteal phase in cyclic cows is a rare event. There is only one report showing the development of a single dominant follicle during an oestrous cycle of normal duration in cattle (Savio *et al.*, 1988). It is therefore possible that the intermediate blood concentrations of progesterone (about 4 ng ml⁻¹) provided by new CIDR-B devices in Period I were at the threshold for the regulation of normal follicular turnover. This hypothesis is supported by data of Ireland and Roche (1982) and Roberson *et al.* (1989) showing variable patterns of LH secretion in cows with different plasma concentrations of progesterone. Since normal follicular turnover occurred in five of six cows in the new CIDR-B group, progesterone is the only secretory product from the corpus luteum required for an indirect regulatory effect on dominant follicle turnover. The results reported here indicate that follicular turnover in cattle is altered by progesterone. Previous studies (Savio *et al.*, 1990c, 1993) indicate that progestin-induced alterations in basal LH secretion regulate follicular turnover.

In agreement with previous reports (Sirois and Fortune, 1988; Roberson *et al.*, 1989), size of the dominant follicle at the time of progesterone withdrawal is negatively related to the interval to onset of oestrus. The intercept of the regression (135.68 h) indicates that, theoretically, about 5.6 days may be required for a small follicle (< 3 mm (sensitivity of ultrasonographic detection)) to grow and induce the onset of oestrus, which supports previous data of Fortin and Seguin (1984). The quadratic association indicates that follicles ≥ 15 mm require approximately the same amount of time before induction of oestrus.

The very good synchrony and high fertility in T1 agree with data from previous studies in which induced luteolysis occurred at this stage of the oestrous cycle in heifers (Macmillan and Henderson, 1983/84; Tanabe and Hann 1984; Watts and Fuquay, 1985; Thatcher *et al.*, 1989; Savio *et al.*, 1990b; Kastelic *et al.*, 1990). In T2, the precise synchrony of oestrus in heifers is also in agreement with previous studies (Beal *et al.*, 1988; Odde, 1990). In addition, the lower pregnancy rate (37.1%) of the

synchronized oestrus in T2 explains why extended progestin treatments (> 15 days) often result in poor fertility (Beal *et al.*, 1988; Odde, 1990). If progestin treatments were initiated at mid- or late luteal phases of the oestrous cycle, low progestin environments would induce extended periods of follicular growth of a single dominant follicle (Sirois and Fortune, 1990; Rajamahendran and Taylor, 1991) with subsequent low fertility. Similarly, this also explains why short-term oestrous synchronization treatments (7 days) with progestagens (melengestrol acetate initiated during late stages of the oestrous cycle ($> \text{day } 13$, (Beal *et al.*, 1988; Brink and Kiracofe, 1988; Patterson *et al.*, 1989)) had lower fertility. A low progesterone or progestin environment could result in persistence of the dominant follicle leading to a depression in fertility. The earlier induction of oestrus following removal of the used CIDR-B device in T2 is probably due to the presence of a more advanced dominant follicle and a quicker return of progesterone concentrations to basal values following withdrawal of the used CIDR-B device in the absence of a corpus luteum compared with a PGF_{2 α} -induced luteolysis in T1.

The reason for the lower fertility of the extended dominant follicles is not known. However, 5 to 6 days are considered enough for a small antral follicle to be selected and to reach preovulatory size (this study; Fortin and Seguin, 1984). Growth periods between 5 days and the normal lifespan of an active dominant follicle in cattle (about 9 days) may therefore be optimal. Follicular growth extended beyond this period probably increases the risk of inducing a persistent dominant follicle that exerts a potentially detrimental effect on fertility.

Comparable intervals between first and second services in T1 and T2 (Expt 2) heifers suggest that late embryo mortality was not a cause for the lower fertility of T2. Moreover, similar pregnancy rates to the second service in T1 and T2 also preclude the possibility of a long-term carry-over effect of the persistent dominant follicle on fertility. Adverse environmental conditions (e.g. heat stress) were not factors since conditions were identical during the critical breeding days in both groups. In addition, heifers in Florida are not considered susceptible to heat stress-induced low fertility (Badinga *et al.*, 1985). Consequently, fertilization failure or early embryo mortality remains the most likely cause of the poor fertility detected in T2. Possible reasons for the low fertility condition in T2 may be an alteration of the oocyte or a follicular factor related to sperm attraction (Ralt *et al.*, 1991). Alternatively, an abnormal oviductal or uterine environment derived from high and sustained concentrations of plasma oestradiol or both factors may reduce fertility. This indicates the importance of follicular synchronization to improve the present methods of oestrous synchronization (Roche and Ireland, 1981; Macmillan and Henderson, 1983/84; Thatcher *et al.*, 1989, 1993). A precise oestrous synchronization method should control both synchronized luteolysis (or progesterone withdrawal) and size and maturity of the dominant follicle. Most current methods of oestrous synchronization in cattle are based only on the management of blood concentrations of progesterone. The most commonly used procedures for manipulating blood concentrations of progesterone (or progestins) involve PGF_{2 α} -induced luteolysis or provision of exogenous progesterone (for example PRIDs, CIDRs) or progestins such as norgestomet (Syncro-mate-B) and melengestrol acetate.

In conclusion, dominant follicle development, preovulatory follicular growth and oestrous synchronization in cattle result from the interplay between concentration of plasma progesterone, stage of the follicular wave and time of luteolysis. More precise timing of onset of oestrus can be expected when progesterone withdrawal is coincident with the presence of a fully mature dominant follicle. However, fertility of the synchronized oestrus is influenced by the persistence or turnover of the dominant follicle. Cows ovulating persistent dominant follicles have lower pregnancy rates. Coordination of induced luteolysis with follicular development should be considered to ensure normal fertility following oestrous synchronization treatments.

The authors thank the staff at the Dairy Research Unit and Larson's Dairy, Okeechobee, FL for care of the animals; J. Larson for providing the heifers for Expt 2 and especially to K. E. Vinton and C. Gould (Larson's Dairy) for assistance in managing and breeding the animals. Research was supported partially by the Florida Dairy Check-off Program and Bard grant IS-1475-88. This is Journal Series No. R-02753 of the Florida Agricultural Experiment Station.

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