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Manipulating patterns of ovarian follicle development in cattle with progesterone and gonadotrophin releasing hormone to produce oestrous cycles with two or three follicle waves

B.A. CLARK, F.M. RHODES, C.R. BURKE, S.R. MORGAN AND K.L. MACMILLAN[1]

Dairying Research Corporation (Ltd), Private Bag 3123, Hamilton, New Zealand.

ABSTRACT

This study tested the hypothesis that oestrous cycles with two follicle waves created using progesterone, could be converted to oestrous cycles with three follicle waves using gonadotrophin releasing hormone (GnRH).

Oestrus (Day 0) was synchronised in 24 Friesian cows. On Day 2, a progesterone containing device (CIDR) was inserted into the vagina of 16 cows for 10 days. Eight of these animals received 8µg of buserelin (GnRH) at device removal. Eight untreated animals formed the Control group. Daily transrectal ultrasonography showed that the oestrous cycles of the cows treated only with a CIDR device had two ovarian follicle waves compared to 1/8 in CIDR+GnRH and 3/8 in Control groups ($p < 0.01$). The average length of the oestrous cycle was shorter in the CIDR-only group (19.3 ± 0.5 days), compared to the CIDR+GnRH (20.9 ± 0.2 days) and Control (22.3 ± 1.1 days) groups ($p < 0.05$).

These results demonstrate that strategic administration of GnRH can convert progesterone induced two-wave oestrous cycles into a three-wave pattern.

Keywords: cattle; progesterone; gonadotrophin releasing hormone; oestrous cycle; ovarian follicle.

INTRODUCTION

Our understanding of the bovine pattern of ovarian follicle growth and development has been extended through the use of transrectal ultrasonography. Growth of ovarian follicles >5 mm occurs in waves. There are usually two or three waves per cycle which emerge at intervals of about 7-10 days (Savio *et al.*, 1988; Sirois and Fortune, 1988). A dominant follicle emerges from each wave. The dominance of this follicle inhibits the growth of other follicles until it either ovulates or becomes atretic, depending on whether the phase of dominance is associated with luteolysis or not (Sirois and Fortune, 1988). Manipulation of the follicular wave pattern can be acheived by administering buserelin, an agonist of gonadotrophin-releasing hormone (GnRH), during the dioestrous phase of the oestrous cycle (Macmillan *et al.*, 1989). Administering GnRH alters the size distribution of ovarian follicles, increasing the number of medium sized follicles and reducing the number of large follicles (Macmillan and Thatcher, 1991). Progesterone can also be used to manipulate follicle wave patterns. Elevated progesterone during the metoestrous phase of the cycle reduced the size of the first dominant follicle (DF) and the corpus luteum (CL) and was also associated with premature luteolysis as a consequence of producing cycles with one or two follicle waves (Burke *et al.*, 1994). Progesterone given at metoestrus followed by GnRH given at dioestrus extended cycle length to 20 days when compared to progesterone alone where cycle length was 16 days (Lynch and Macmillan, 1996a). This extended cycle length is thought to be due to the conversion of two wave oestrous cycles to three wave ones.

The novel aim of this study was to demonstrate using ovarian ultrasonography that two wave oestrous cycles created using progesterone are converted to those comprising three waves by GnRH.

MATERIALS AND METHODS

From a larger number of nonlactating Friesian cows, 24 animals were observed in oestrus following a single injection of 25mg dinoprost tromethamine (Lutalyse™, Upjohn, Auckland), a synthetic analogue of prostaglandin $F_2\alpha$. Each of 16 animals had a progesterone device (CIDR™, InterAg, Hamilton) inserted into the vagina on Day 2 of the oestrous cycle (day of synchronised oestrus = Day 0) for a period of 10 days. Eight of these animals received no further treatment (CIDR-only) while the others were injected with 8µg of a GnRH analogue, buserelin, (Receptal(, Bomac Laboratories, Auckland) (CIDR+GnRH) at device removal on Day 12. A further 8 animals formed an untreated control group.

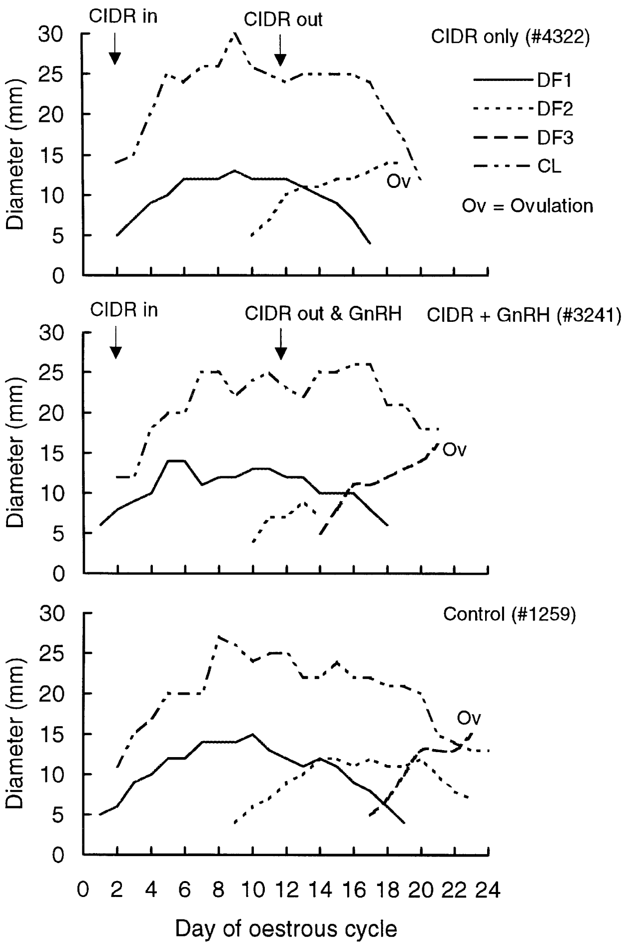
Ovaries were examined daily by transrectal ultrasonography beginning on Day 0. All follicular or luteal structures greater than 3 mm in diameter were sketched and graphed. Initiation of an ovarian follicle wave was defined as the day on which a large dominant follicle (>8 mm) was retrospectively traced to being 4-5 mm in diameter. Blood samples were collected via coccygeal venipuncture from Day 0 until Day 23 or ovulation. The blood was immediately placed on ice before being centrifuged at 1500 g for 20 minutes. The plasma was frozen at -20°C until hormone analysis. Concentrations of progesterone were determined in a single assay using a commercial RIA kit (Coat-A-Count™, DPC, California, USA). Intra-assay coefficients of variation were 26.3, 7.1 and 7.7% for standard concen-

[1]Present address: University of Melbourne, Veterinary Clinical Centre, 250 Princess Highway, Werribee, Victoria 3030, Australia.

tration of 0.4, 3.0 and 4.5 ng/ml, respectively. The minimum detectable concentration was 0.14 ng/ml. Animals were observed twice daily for any behavioural signs of oestrus with the aid of tailpaint.

All continuous variables were analysed by analysis of variance and Students t-test. Categorical values were analysed by Chi square.

FIGURE 1: Pattern of CL and follicle growth in a representative cow from each of the three treatment groups.



RESULTS

The average (± sem) length of the oestrous cycle in cows of the CIDR-only group was shorter than either of the other groups ($p<0.05$; Table 1). Typical patterns of CL and follicular growth for respective cows from each treatment group during this monitored oestrous cycle are represented in Figure 1. The number of cows that had cycles with two ovarian follicle waves also varied with treatment ($p<0.01$; Table 1). The average interval between the emergence of the first and second follicular waves was 9.1 ± 0.3 days and this did not differ between groups ($p>0.1$). Neither did the average day of emergence of the second DF (10.7 ± 0.2 days, $p>0.1$). The average interval between emergence of second and third waves was less in the CIDR+GnRH group than in the Control group (4.1 ± 0.6 vs 6.8 ± 0.7 days; $p<0.05$). An accessory CL was formed

TABLE 1: Number of follicle waves and length of oestrous cycle (± sem) of animals receiving a CIDR device between Days 2-12, with GnRH on Day 12 (CIDR+GnRH) or without (CIDR-only) as compared to untreated controls.

Treatment group	Number of Follicle Waves		Length of Oestrous Cycle
n = 8	Two	Three	(days)
Control	3	5	22.3± 1.1^{a}
CIDR only	8	0	19.3± 0.5^{b}
CIDR+GnRH	1	7	20.9± 0.2^{a}

^{ab} different superscripts indicate significant differences ($p<0.05$)

in 5/8 CIDR+GnRH animals following ovulation of the second DF. Treatment had no effect on the size of the ovulatory follicle ($p>0.1$). Luteolysis occurred sooner ($p<0.05$) among cows in the CIDR-only group where plasma progesterone concentrations declined to <1ng/ml by Day 16.6 ± 0.4 compared to Day 18.0 ± 0.3 and Day 19.9 ± 1.2 for the CIDR+GnRH and Control groups, respectively.

DISCUSSION

This trial has described how ovarian follicle wave patterns during the oestrous cycle in cattle can be profoundly manipulated by progesterone and GnRH. Insertion of a CIDR device on Day 2 of the cycle for 10 days with no other treatment resulted in shorter oestrous cycles, premature luteolysis and ovulation of the second DF. The additional administration of GnRH on Day 12 resulted in the turnover of the second wave DF and initiation of a third follicle wave. Induction of accessory CL by GnRH was observed in 5/8 animals of the CIDR+GnRH group, which is consistent with previous reports (Macmillan and Thatcher, 1991; Webb *et al*., 1992).

Oestrous cycle length was shortest in the CIDR-only group (Table 1) following premature luteolysis and ovulation of the second wave DF. In the present study oestrous cycle length was not reduced as much (19 days) after metoestrous progesterone treatment as previous studies have shown, with ranges from 16-18 days (Macmillan *et al*., 1991; Burke *et al*., 1994; and Lynch and Macmillan, 1996a). Early progesterone treatment did not alter the number of days between emergence of the first and second follicle waves for CIDR-treated groups compared to the Control group. No differences were observed in the size of the first DF due to progesterone treatment. This differs from the findings of Burke *et al*. (1994), who reported that progesterone administration for 4-5 days during metoestrus suppressed the size of the first DF. These differences may have been due to a different animal type in the current study. Lynch and Macmillan, (1996a) reported that administration of progesterone beginning at metoestrus reduced cycle length to 16 days, but this effect was negated by an injection of GnRH at device removal on Day 12, and cycle length was extended to 20 days. This is in agreement with the current findings, where GnRH converted progesterone-induced short cycles back to those of normal length.

There are conflicting reports on the effect of a CIDR device inserted post-insemination on pregnancy rates (PR). Macmillan and Peterson (1993) showed an increased PR if a device was inserted 6-8 days post insemination, whereas Van Cleeff *et al*. (1996) found that a device inserted 2 days after insemination for 7 days resulted in suppressed fertility. The combination treatment of a post-insemination CIDR device inserted for 10 days followed by a GnRH injection at CIDR device removal resulted in increased PR and fewer return intervals of less than 21 days compared with untreated controls (Lynch and Macmillan, 1996b). However in a later trial, this same treatment (CIDR+GnRH) did not increase PR compared to cows with no post-insemination intervention (Taufa *et al*., 1998). Nevertheless, this combined treatment does overcome the depressive effects of metoestrous progesterone treatment on PR (P. Lynch, unpublished). Future research will investigate the effect of modifying hormonal profiles and follicular wave patterns post-insemination on embryo development and the uterine environment.

In conclusion, we have demonstrated that two wave oestrous cycles created by progesterone administration from metoestrus (Day 2), can be converted to three wave cycles when GnRH is given 10 days later (Day 12).

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