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Effect of luteolysis on control of ovarian follicles using oestradiol benzoate and progesterone in cattle

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ABSTRACT

This study aimed to determine whether progesterone from intravaginal insertion of a CIDR device could substitute for luteal progesterone and facilitate oestradiol benzoate (ODB)-induced control of ovarian follicle waves in the event of luteolysis. Luteolysis was induced by an injection of prostaglandin- $F_{2\alpha}$ on Day 13 of the oestrous cycle in 24 lactating dairy heifers. Concurrent treatments on Day 13 were: i) 1 mg ODB i.m. (ODB1); ii) CIDR for 6 days (CIDR); iii) 1 mg ODB i.m. plus CIDR for 6 days (ODB1/CIDR); or, iv) 2 mg ODB i.m. plus a CIDR for 6 days (ODB2/CIDR). Examination of the ovaries using ultrasonography showed that oestrous cycles comprising 2 follicle waves predominated among ODB1 and CIDR groups; whereas combination treatments produced mostly 3-wave cycles ($p < 0.05$). Day of ovulation was 15.4 ± 0.3 , 21.2 ± 0.2 and 22.5 ± 0.2 for ODB only, CIDR only and 1 or 2 mg ODB plus CIDR cows, respectively ($p < 0.01$). The interval from day of emergence to ovulation of the ovulatory follicle was less ($p < 0.01$) in cows treated with ODB only (6.0 ± 0.3 days), or ODB plus a CIDR (7.2 ± 0.6 days), compared to a longer interval in those receiving a CIDR only (11.0 ± 0.8 days).

These results showed that progesterone from a CIDR device was able to facilitate ODB-induced follicle wave turnover at the time of luteolysis in lactating dairy heifers.

Keywords: Ovarian follicles; progesterone; oestradiol benzoate; cattle.

INTRODUCTION

The potential benefits of advancing and concentrating the breeding pattern in seasonal dairy herds through the use of oestrous synchronisation and artificial insemination (Macmillan *et al.*, 1990) are traditionally compromised by reduced fertility when progestin-based treatments are used (Xu *et al.*, 1996). Subnormal fertility is associated with a prolonged period of low circulating concentrations of progesterone which leads to the development of aged ovulatory follicles (Savio *et al.*, 1993) with incompetent oocytes (Mihm *et al.*, 1994). Oestradiol benzoate (ODB) may be incorporated with initiation of progesterone treatment to improve fertility (Day *et al.*, 1997) by promoting follicle wave turnover and preventing the development of persistent follicles (Burke *et al.*, 1997a). Follicle turnover occurs as a consequence of the negative feedback action of progesterone and oestradiol on gonadotrophic support for follicular growth (Kinder *et al.*, 1996). Turnover is not assured when a precipitous decline in progesterone to basal concentrations (i.e. < 1 ng/ml) occurs within 48 h of ODB treatment (Burke *et al.*, 1997b). In a herd of cattle having random oestrous cycles, 10-20% will be at a stage where the event of luteolysis may prevent the ability of ODB to promote follicle turnover.

The objective of the present study was to determine whether or not the negative effect of a precipitous decline in progesterone during luteolysis on follicle wave control using oestradiol benzoate could be prevented by concurrent insertion of an intravaginal progesterone releasing device (CIDR).

MATERIALS AND METHODS

First calving Friesian (F; $n=12$) and Jersey (J; $n=12$) heifers of high genetic potential were used in this study. Mean live-weights and body condition scores (CS) were 377 ± 8.3 kg and 4.3 ± 0.1 CS in F, and 296.8 ± 9.0 kg and 4.4 ± 0.1 CS in J heifers, respectively. These animals had resumed oestrous cycles by 56 ± 2.2 days postpartum. At this time a progesterone releasing device (CIDRTM, InterAg, Hamilton) was inserted into the vagina for 10 days with an i.m. injection of 500 μ g cloprostenol sodium (PGF; EstrumateTM, Pitman-Moore NZ Ltd, Upper Hutt) 4 days before device removal. Subsequent detection of oestrus (designated as Day 0) was aided by the use of tail paint.

The ovaries of every cow were examined by transrectal ultrasonography from Day 7 to subsequent ovulation. Location and diameter of all follicular and luteal structures ≥ 3 mm in diameter were recorded. Emergence of an ovarian follicle wave was defined as the day on which a large dominant follicle (DF; > 8 mm) was retrospectively traced to being 4-5 mm in diameter.

Allocation to treatments was balanced for breed and timing of emergence of the second dominant follicle (DF2). On Day 13, every heifer received 500 μ g cloprostenol sodium i.m. to induce luteal regression. Additional administrations at this time comprised: i) 1 mg ODB i.m. (ODB1; $n=7$); ii) intravaginal insertion of a CIDR for 6 days (CIDR; $n=6$); iii) 1 mg ODB i.m. plus a CIDR for 6 days (ODB1/CIDR; $n=5$); or, iv) 2 mg ODB i.m. plus a CIDR for 6 days (ODB2/CIDR; $n=6$).

Blood samples were collected from a coccygeal vessel of every animal each day from Day 13 until subsequent

oestrus. Samples were immediately placed on ice before being centrifuged at 1500 g for 20 min. Plasma was stored at -20°C until hormone analysis. Concentrations of progesterone were determined in a single assay using a commercial RIA kit (Coat-A-Count, DPC, USA). Intra-assay coefficients of variation were 12.1, 6.72 and 6.36 % for standard concentrations of 0.4, 3.0 and 4.2 ng/ml, respectively. The minimum detectable concentration was 0.15 ng/ml.

Data were analysed by using analysis of variance, Student's t-tests and chi squared analysis. Results are presented as means (\pm sem).

RESULTS

A precipitous decline in concentrations of plasma progesterone between Day 13 and 14 occurred in all animals and confirmed ultrasonic assessment that luteal regression was successfully achieved by the PGF injection on Day 13. The magnitude of the decline was greatest in animals of the ODB1 group (6.5 ± 0.9 ng/ml; $p < 0.05$) since other treatment sequences involved insertion of a CIDR device and concentrations of progesterone remained elevated between 1.8 ± 0.1 to 4.1 ± 0.2 ng/ml during device insertion (Fig. 1).

FIGURE 1: Mean (\pm SEM) concentrations of progesterone in plasma of animals which received a luteolytic dose of PGF on Day 13 of the oestrous cycle plus: i) 1 mg ODB i.m. (ODB1; n=7); ii) intravaginal insertion of a CIDR for 6 days (CIDR; n=6); iii) 1 mg ODB i.m. plus a CIDR for 6 days (ODB1/CIDR; n=5); or, iv) 2 mg ODB i.m. plus a CIDR for 6 days (ODB2/CIDR; n=6).

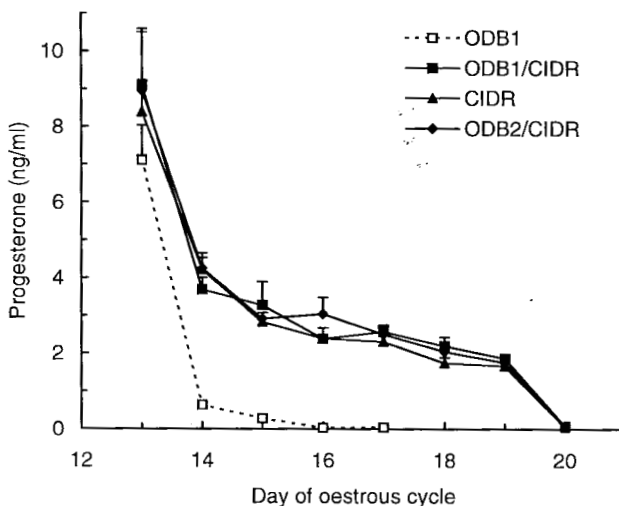
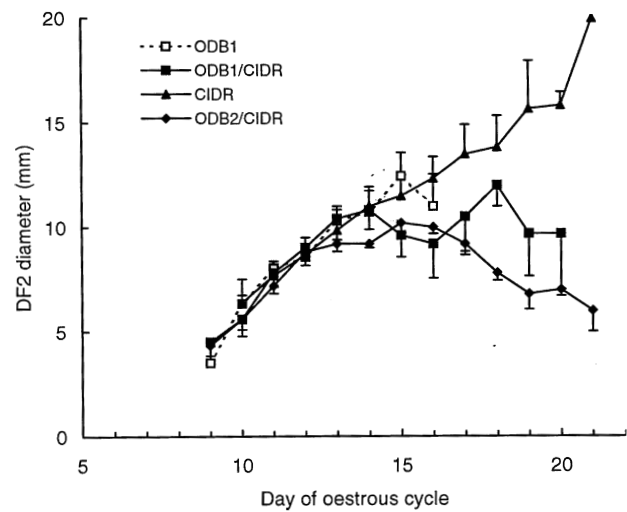


TABLE 1: Follicle wave patterns and characteristics of the ovulatory follicle in animals which received a luteolytic dose of PGF on Day 13 of the oestrous cycle plus: i) 1 mg ODB i.m. (ODB1; n=7); ii) intravaginal insertion of a CIDR for 6 days (CIDR; n=6); iii) 1 mg ODB i.m. plus a CIDR for 6 days (ODB1/CIDR; n=5); or, iv) 2 mg ODB i.m. plus a CIDR for 6 days (ODB2/CIDR; n=6). The ovulatory follicle is the third dominant follicle (DF3) except in the case of ODB1 treatment where it is the second dominant follicle. Values are means (\pm sem).

	ODB1	CIDR	ODB1/CIDR	ODB2/CIDR
(2 vs. 3-waves)	7 vs. 0	5 vs. 1	1 vs. 4	0 vs. 5
Day DF3 emerged	-	13 (-)	15.8 (0.8)a	16.2 (0.4)a
Growth rate of DF3 (mm/day)	-	1.4 (-)	1.6 (0.1) a	1.6 (0.1) a
Day of ovulation	15.6 (0.3)a	21.2 (0.2)b	22.2 (0.4)c	22.8 (0.2)c
Age of ovul. follicle (days)	6.0 (0.3)a	11.0 (0.8)b	7.8 (1.1)a	6.6 (0.5)a
Diam. of ovul. follicle (mm)	13.0 (0.7)a	18.7 (1.2)b	16.6 (1.2) b,c	14.4 (1.0)a,c
Expressed oestrus	6 / 7	6 / 6	5 / 5	3 / 5

abc different superscripts indicate differences between columns ($p < 0.05$)

FIGURE 2: Mean (\pm SEM) diameter of the second dominant follicle (DF2) of animals which received a luteolytic dose of PGF on Day 13 of the oestrous cycle plus: i) 1 mg ODB i.m. (ODB1; n=7); ii) intravaginal insertion of a CIDR for 6 days (CIDR; n=6); iii) 1 mg ODB i.m. plus a CIDR for 6 days (ODB1/CIDR; n=5); or, iv) 2 mg ODB i.m. plus a CIDR for 6 days (ODB2/CIDR; n=6).



Mean timing of emergence of the DF2 among all heifers was Day 9.5 ± 0.3 and the diameter of this follicle on Day 13 was 10.0 ± 0.3 mm. In a heifer assigned to the ODB2/CIDR group, the DF2 had not emerged by Day 13 and all follicular data pertaining to this animal were omitted from general analyses. Subsequent development of the DF2 beyond Day 13 varied between treatment groups (Fig. 2; $p < 0.05$). In 7/7 and 5/6 heifers in ODB1 and CIDR groups, respectively, the DF2 persisted to become the ovulatory DF thus producing almost exclusively 2-wave cycles in these groups (Table 1). However, the timing of ovulation was different between these groups ($p < 0.05$; Table 1). The ovulatory follicle of animals in the CIDR group was generally older and larger at ovulation compared to other groups (Table 1).

In contrast to the follicle wave patterns in either of the ODB1 and CIDR groups, the oestrous cycle of most animals (9/10) which received ODB and a CIDR device comprised 3 waves of follicles. The developmental characteristics of DF3 among these 2 groups were similar ($p > 0.1$; Table 1). The interval from CIDR device withdrawal to ovulation was greater with the combination treatments (3.5 ± 0.2 days) as compared to CIDR treatment

alone (2.2 ± 0.2 days; $p < 0.05$).

Three of the 24 animals were not detected in oestrus; two belonged to the ODB2/CIDR group and the other to the ODB1 group.

DISCUSSION

A successful model for investigating methods to control follicle wave patterns in cattle is described. Treatments initiated on Day 13 will typically ensure the presence of a newly emerged healthy DF and a corpus luteum that is receptive to a luteolytic dose of PGF. The precipitous decline in progesterone during luteolysis (similar to that seen in treated animals, Fig. 1) begins the proestrus phase and has a stimulatory effect on the DF. Follicle wave turnover may be most difficult to achieve by hormonal means during these conditions.

In agreement with previous results, ODB alone was unable to induce follicle turnover when administered at the time of luteolysis (Burke *et al.*, 1997b). The use of progesterone alone resulted in the development of large persistent DF. This follicle ovulated 2 days after removal of the CIDR device compared to 3 days in animals treated with ODB and a CIDR device. We would expect lower fertility in the former group of animals with persistent DF (Savio *et al.*, 1993). It was only when ODB and progesterone were administered in combination, that effective control of follicle waves was established. The implication for oestrus synchrony is that insertion of a CIDR device with at least 1 mg ODB i.m. should promote follicle turnover and a fertile oestrus, even when treatments are initiated around the time of luteolysis.

No differences were observed between animals injected with 1 or 2 mg ODB at insertion of a CIDR device. This is in contrast to findings of a recent field trial in which a greater proportion of cows were detected in oestrus 48 h after removal of a CIDR device, following 1 mg ODB compared to 2 mg ODB i.m. at device insertion (Day *et al.*, 1997). The small numbers of animals used in the present study may have resulted in an inability to detect any significant differences. Alternatively, heifers may be equally sensitive to 1 or 2 mg ODB in contrast to mature cows used in the field study.

We conclude that progesterone provided by a CIDR device was able to substitute for luteal progesterone in the

event of luteolysis, and facilitate ODB-induced control of ovarian follicle waves. Neither ODB nor a CIDR device alone was able to promote turnover in this animal model. Future studies will determine whether turnover can be achieved by bolus administration of progesterone with ODB, or with a CIDR device.

ACKNOWLEDGEMENTS

The authors thank Gwyn Verkerk and Pat Laboyrie for their assistance. Thanks also to Trish O'Donnell for undertaking the progesterone assay. This study was supported by FRST contract 97DRC601/1 and InterAg, Hamilton.

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