Effect of betamethasone treatment on luteal lifespan and the LH response to GnRH in dairy cows

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Summary. Betamethasone (a synthetic glucocorticoid, 15 mg) was administered i.m. twice daily for 10 days to 4 regularly cycling dairy cows, beginning on Day 10 of the oestrous cycle. Luteal function, monitored by plasma progesterone, was extended by 7, 9, 19 and 20 days, respectively. Luteal function in the next cycle was normal. Endogenous cortisol values were suppressed for 14, 13, 34 and 27 days, respectively. Pituitary responsiveness to 20 µg GnRH was assessed by LH measurement on Days –1, +3 and +7 relative to the start of betamethasone treatment. There was a progressive decrease in peak LH concentrations after each GnRH challenge compared to control cows. Hourly measurements of PGF-2α metabolite during the expected period of luteolysis failed to reveal normal increases.

It is suggested that betamethasone caused prolonged luteal function, either by directly inhibiting PGF-2α release, or by suppressing pituitary stimulation of follicular growth and hence lowering oestradiol concentrations, since it is known that PGF-2α and oestradiol act synergistically to cause luteolysis.

Introduction

Altered adrenal function has an influence on reproductive function in dairy cattle (Wagner & Li, 1982; Moberg, 1984). Repeated injections of synthetic adrenocorticotrophin (ACTH_{1–24}) or infusions of cortisol have been shown to reduce the luteinizing hormone (LH) response to gonadotrophin-releasing hormone (GnRH) (Matteri & Moberg, 1982; Li & Wagner, 1983). Treatment with betamethasone (a synthetic glucocorticoid) for 9 days extended luteal function by an average of 10 days when administered starting on Day 10 of the oestrous cycle (Kanchev *et al.*, 1976). The aim of the present experiment was to establish whether such a prolonged luteal lifespan was due to a diminished LH response to GnRH, which might delay follicular development, or a direct action on the uterus to prevent luteolytic release of prostaglandin F-2α.

Materials and Methods

Animals. Fifteen non-pregnant non-milked mature Friesian cows were bled by venepuncture three times a week for 5 weeks to establish the regularity of pretreatment luteal function by progesterone measurements. After treatment, similar luteal monitoring was continued for 10 weeks. Cows were housed inside in chained standings under natural lighting and fed hay and water *ad libitum*. An indwelling catheter was inserted into one jugular vein the day before periods of frequent sampling. All blood samples were centrifuged immediately at 1000 g and the plasma stored at –15°C.

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During late June, beginning on Day 10 of unsynchronized oestrous cycles, adrenal function was suppressed in 4 cows by i.m. administration of 30 mg betamethasone (Betsolan: Glaxovet, Uxbridge, U.K.) in two half doses per day (09:00 and 23:00 h) for 8 days, and 20 mg and 10 mg on the 9th and 10th days of treatment, respectively. Blood samples (10 ml) were taken from 2 of the 4 cows every 30 min for 1 h before and 12 h after the first and seventh betamethasone injection for cortisol analysis. All cows were treated i.m. with 750 mg oxytetracycline (Terramycin Q: Pfizer, Sandwich, U.K.) on alternate days during betamethasone treatment. The samples taken for luteal monitoring (see above) after treatment were also analysed for cortisol.

On Days -1, +3 and +7 relative to the start of betamethasone treatment, each cow was given i.v. 20 µg GnRH (gonadorelin: Hoechst U.K. Ltd, Milton Keynes, U.K.) in 2 ml saline (0.9% (w/v) NaCl) through the jugular vein sampling catheter. Blood samples (10 ml) were taken for LH analysis every 15 min for 0.5 h before and 3 h after each GnRH injection. Four control cows were given 20 µg GnRH i.v. on Days 12 and 16 of untreated oestrous cycles, and bled as above.

The betamethasone-treated cows were also bled hourly for 12 h for prostaglandin metabolite analysis on Days 19, 20 and 21 (expected) of the treatment cycle, betamethasone treatment having begun on Day 10 of the cycle. Seven control cows were bled similarly on Days 19, 20 and 21 of untreated oestrous cycles; 2 cows (Nos 27 & 65) provided data for 2 consecutive days.

Hormone analysis. Radioimmunoassay was by methods characterized and verified in this laboratory and reported elsewhere. Current intra- and interassay coefficients of variation were 8.3% and 12.8% for the progesterone assay (Kanchev *et al.*, 1976), 9.2% and 14.7% for the cortisol assay (Alam *et al.*, 1986), 5.8% and 6.2% for the LH assay (Alam & Dobson, 1986), and 9.4% and 10.3% for assay for 13,14-dihydro-15-keto prostaglandin F-2α (PGFM: Kaker *et al.*, 1984). Assay sensitivities, defined as $2 \times$ standard deviation from buffer control, were 15, 100, 500 and 50 pg/ml, respectively, for each assay. There was <1.0% cross-reaction of cortisol in the progesterone assay, and *vice versa*. Dexamethasone did not cross-react with any of the assays.

Results

Luteal function, as assessed by plasma progesterone measurements, was extended by 7, 9, 19 and 20 days, respectively in the 4 betamethasone-treated cows: progesterone remained at luteal phase concentrations (1.0–6.0 ng/ml) for 4, 6, 15 and 17 days, respectively, after the end of the 10-day betamethasone treatment which began on Day 10 of the oestrous cycle (exemplified in Fig. 1). Luteal lifespan during the cycle before the treatment cycle was normal, as was the cycle occurring after the end of treatment. All the control cows had normal luteal function, as assessed by progesterone profiles.

Analysis of plasma samples collected more frequently (every 30 min) before and after the first and seventh betamethasone injections revealed a suppression of cortisol concentrations by 60 min after the first injection, reaching baseline by 90–120 min. Values remained low until the second injection. Samples taken before and after the seventh injection were all low.

Cortisol concentrations remained low in all cows during betamethasone treatment, returning to normal at various intervals (Table 1). None of the cows appeared clinically affected by adrenal suppression. From cortisol concentrations, Cow 133 appeared least influenced by betamethasone, whereas Cow 731 did not fully regain adrenal function for at least 10 weeks. The results in Table 1 show that longer periods of complete adrenal suppression resulted in longer extensions of luteal lifespan. However, subsequent luteal activity began before cortisol plasma concentrations returned to pre-treatment values (Table 1). Concentrations of cortisol in 4 control cows remained relatively constant (1–6 ng/ml) throughout the sampling period.

The LH responses to 20 µg GnRH injections are given in Table 2: results are shown for each cow due to the variation in response between individual cows. There appeared to be no effect of betamethasone treatment on LH values before GnRH injection (mean of only 3 samples; data not shown), whereas the peak concentration of LH on a within-cow basis was lower on Day 3 of betamethasone treatment, and lower still on Day +7, compared with the response before betamethasone. Control cows given 20 µg GnRH i.v. on Days 12 and 16 of the cycle had peak LH concentrations of 14–25 ng/ml. Betamethasone treatment also reduced the area under the LH response curve, in parallel with the LH peak values (data not shown). All cows produced maximum LH concentrations 15–30 min after GnRH and returned to baseline (1–2 ng/ml) 2–3 h afterwards.

Fig. 1. Concentration of plasma cortisol and progesterone in 2 cows treated with beta-methasone (30 mg/day) for a 10-day period (shown by the horizontal bar). The length of oestrous cycles is indicated.

Table 1. Data concerning adrenal suppression before and after betamethasone treatment (30 mg/day) and relationship with luteal function

	Cow number			
	402	133	13	731
Cortisol conc. (ng/ml)*				
Before treatment	3.63 ± 2.50 (3)*	1.99 ± 1.20 (3)	0.65 ± 0.30 (3)	6.2 ± 1.50 (3)
During treatment	0.16 ± 0.08 (6)	0.34 ± 0.19 (6)	0.40 ± 0.26 (6)	0.48 ± 0.37 (6)
End of treatment to recovery	0.13 ± 0.07 (5)	0.13 ± 0.07 (3)	0.12 ± 0.05 (11)	0.15 ± 0.07 (14)
'Recovered' period	2.45 ± 2.62 (17)	4.00 ± 2.70 (18)	0.51 ± 0.29 (12)	0.25 ± 0.13 (9)
Adrenal suppression after 10 days of betamethasone (days)	14	13	27	34
Extension of luteal lifespan (days)	7	9	19	20
Day of next cycle when plasma cortisol values increased	8	3	10	17

*Values are mean ± s.d. for the no. of samples in parentheses.

Concentrations of PGFM during betamethasone treatment did not reveal the characteristic pattern of release expected on Days 19, 20 and 21 of the oestrous cycle (Fig. 2). The sampling catheter came out of Cow 402 part-way through the sampling period; hence these results were discarded. There were far fewer samples with concentrations > 100 pg/ml in the treated cows, compared to control cows bled on similar days of the cycle.

Table 2. Peak values of LH (ng/ml) in response to 20 µg GnRH i.v. in control cows and in those treated with betamethasone i.m. (30 mg/day for 10 days)

	Control cows				Betamethasone-treated cows			
	51	391	6	888	731	13	133	402
Day - 1 (before start of betamethasone)	—	—	—	—	14.4	21.7	20.1	6.4
Day + 3 (after start of betamethasone, ≡ Day 12 of oestrous cycle)	14.1	14.1	25.3	15.4	8.8	9.5	5.5	3.5
Day + 7 (after start of betamethasone, ≡ Day 16 of oestrous cycle)	21.0	17.5	15.1	14.6	5.4	6.5	3.9	2.5

Fig. 2. Concentrations of 13,14-dihydro,15-keto prostaglandin F-2α (PGFM) in hourly samples taken (a) on expected Days 19, 20 and 21 of a cycle when betamethasone treatment (30 mg/day) was begun on Day 10, and (b) on Days 19, 20 and 21 of oestrous cycles in the control group of cows.

Discussion

Betamethasone-induced extension of luteal life-span by 7–20 days concurs with the 5–18-day extension reported previously (Kanchev *et al.*, 1976). Similar results have been obtained in many more animals (L. N. Kanchev, unpublished observations) although such detailed hormone analyses were not carried out. The progressive decline in LH responses to each GnRH challenge in the treated cows, but not control cows, suggests an action of betamethasone at the pituitary level (influence on the hypothalamus was not investigated in this study). Since destruction of ovarian follicles results in failure of luteolysis (Villa-Godoy *et al.*, 1985) and oestradiol exerts a synergistic effect with prostaglandin F-2 α in cows (Hixon *et al.*, 1983), it is suggested that the prolonged luteal function brought about by betamethasone treatment may be (a) a result of failure of follicular growth due to inadequate LH release, which is required during normal follicular growth (Rahe *et al.*, 1980); or (b) the result of direct inhibition of prostaglandin release. On the other hand, the absence of PGFM release over Days 19, 20 and 21 of the extended cycle might be expected in the absence of increasing oestradiol concentrations from the follicles, since oestradiol causes PGFM release (Thatcher *et al.*, 1984). Further considerations of the effect of oestradiol on oxytocin and its receptors and their role in luteolysis are beyond the scope of the present experiment.

Analysis of cortisol in samples taken frequently before and after the first and seventh betamethasone injections confirmed suppression of adrenal activity by 120 min after the first injection. Concentrations did not increase between subsequent injections. The longer the adrenal suppression after betamethasone treatment, the greater was the prolongation of luteal function. It was not evident from the LH responses to GnRH during betamethasone treatment which cows were more severely affected. Luteal function and, presumably, prior follicular growth, began in each cow before the return to normal cortisol concentrations. It is possible that normal pituitary responses to GnRH were regained before any changes in plasma cortisol could be detected by radioimmunoassay. Alternatively, pituitary LH cells may have regained functional integrity before pituitary ACTH cells. Whatever the reason, increased response of the pituitary to release LH presumably led to follicular growth.

There was no evidence of betamethasone suppression of progesterone concentrations, either directly or via a reduction in luteotrophic support. This relationship was not examined in great detail in the present experiment, but administration of antiserum to bovine LH from Days 12 to 16 of the oestrous cycle resulted in increased cycle length, due to a failure of follicles to develop to mature size, rather than failure of luteolysis (Snook *et al.*, 1969). Betamethasone treatment in the present experiment did not appear to affect spontaneous tonic LH secretion. Only 3 samples were assessed before GnRH stimulation; confirmation of an effect on tonic LH patterns would require monitoring for at least 12 h with 15 min sampling.

The results of this experiment provide an example of altered adrenal function, decreasing pituitary release of LH and thereby influencing reproductive function by extending luteal lifespan. It is, however, appreciated that pharmacological doses of a potent glucocorticoid have been used in the present study; hence, further work is required to examine the effects, on reproduction, of more moderate alterations in adrenal function.

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