Effect of endogenous and exogenous progesterone on the oestradiol-induced LH surge in dairy cows

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Summary. Four cows released an LH surge after 1·0 mg oestradiol benzoate administered i.m. during the post-partum anoestrous period with continuing low plasma progesterone. A similar response occurred in the early follicular phase when plasma progesterone concentration at the time of injection was <0·5 ng/ml. Cows treated with a progesterone-releasing intravaginal device (PRID) for 8 days were injected with cloprostenol on the 5th day to remove any endogenous source of progesterone. Oestradiol was injected on the 7th day when the plasma progesterone concentration from the PRID was between 0·7 and 1·5 ng/ml. No LH surge occurred. Similarly, oestradiol benzoate injected in the luteal phase of 3 cows (0·9–2·1 ng progesterone/ml plasma) did not provoke an LH surge. An oestradiol challenge given to 3 cows 6 days after ovariectomy induced a normal LH surge in each cow. However, when oestradiol treatment was repeated on the 7th day of PRID treatment, none released LH.

It is concluded that ovaries are not necessary for progesterone to inhibit the release of LH, and cows with plasma progesterone concentrations >0·5 ng/ml, whether endogenous or exogenous, did not release LH in response to oestradiol.

Keywords: oestradiol; progesterone; LH; cows

Introduction

Sustained high plasma progesterone concentration, either endogenous (e.g. from a persistent corpus luteum: Lamming & Bulman, 1976) or exogenous (e.g. progesterone treatment: Ulberg et al., 1951; Britt & Ulberg, 1971; Mauer et al., 1975), will block oestrus and ovulation in cows. Endogenous progesterone concentrations of >0·3 ng/ml in the luteal phase of cows also blocked the release of LH induced by oestradiol benzoate (Hobson & Hansel, 1972; Alam & Dobson, 1987). It is surprising that treatment with exogenous progesterone has no effect on the pattern or amount of LH released in response to oestradiol treatment in intact prepubertal heifers (Swanson & McCarthy, 1978) or in ovariectomized cows (Short et al., 1973; Hausler & Malven, 1976; Short et al., 1979).

The discrepancies between the effects of endogenous and exogenous progesterone are difficult to explain and the present experiments were designed to examine whether the surge release of LH induced by oestradiol benzoate in intact and ovariectomized cows is affected by the source of progesterone, the existence of the ovaries, or both these factors.

Materials and Methods

Five experiments were conducted each on 3 or 4 intact (10–70 days post partum) or ovariectomized multiparous lactating Holstein/Friesian cows weighing between 450 and 550 kg. The cows were housed together in tied stanchions and were fed concentrates, with hay and water ad libitum. In each experiment, 1·0 mg oestradiol benzoate (Intervet
Laboratories Ltd, Cambridge, U.K.) in 2 ml arachis oil was injected i.m. An indwelling catheter was inserted into one jugular vein the day before periods of frequent sampling.

Heparinized blood samples were collected at the time of injection and every 2 h from 16 to 32 h after oestradiol treatment, in order to monitor the LH response which normally occurs during this period (Alam & Dobson, 1987). Blood samples were also collected by caudal venepuncture twice weekly to monitor the ovarian cycle. All blood samples were centrifuged immediately at 1000 g and the plasma stored at −15°C. In addition, ovarian activity was assessed weekly by palpation per rectum.

**Experiment 1.** Four intact cows were treated 3 times with oestradiol benzoate. All cows were anoestrous at the start of the experiment but 2 cows spontaneously started ovarian activity. Injection of 500 μg cloprostenol (Estrumate: Coopers, Crewe, Cheshire, U.K.) was given 12 h before each of the last two treatments in each cow to remove any luteal tissue.

**Experiment 2.** Three cows were treated with oestradiol benzoate in the luteal phase of the oestrous cycle.

**Experiment 3.** Four intact cows (2 in mid-cycle and 2 anoestrous) were treated with a progesterone-releasing intravaginal device containing 1.44 g progesterone (PRID: Ceva, Watford, Herts, U.K.). The oestradiol benzoate capsule incorporated in the PRID for luteolytic purposes was removed before insertion to exclude any influence in the present experiments. After 5 days, cloprostenol was injected in all cows to prevent the release of endogenous progesterone. Injection of oestradiol benzoate was given 7 days after insertion of the PRID.

**Experiment 4.** Three ovariectomized cows were treated with oestradiol benzoate 1 week after bilateral ovariectomy, carried out through a left flank incision under general anaesthesia.

**Experiment 5.** At 2 weeks after ovariectomy, 3 cows were given oestradiol benzoate, 7 days after insertion of a PRID (without the oestradiol capsule).

**Hormone analyses.** Plasma LH and progesterone concentrations were measured by radioimmunoassay, as described by Alam & Dobson (1986) and Kanchev et al. (1976), respectively. Current intra- and interassay coefficients of variation over the range of concentrations measured in this study were, respectively, 5.8% and 6.2% for LH and 8.3% and 12.8% for progesterone. Assay sensitivities, defined as twice standard deviation from the buffer control, were 0.5 and 0.015 ng/ml, respectively.

A rise in LH value in two consecutive samples above 10.0 ng/ml after oestradiol benzoate was considered as a surge of LH; the lowest peak height was 33.0 ng/ml. Using each animal as its own control, the response to oestradiol benzoate in different experiments was compared in terms of time of onset, duration and height of the LH surge after treatment.

**Results**

The different responses in the various experiments are depicted in Fig. 1. Data from only one treatment per cow in Exp. 1 are presented as an example.

**Experiment 1**

All 4 cows released a normal surge of LH after 11 out of 12 oestradiol benzoate treatments in the absence of a corpus luteum. One cow (with pyometra) failed to respond to the first oestradiol benzoate challenge 7 days post partum. Plasma progesterone concentration in all cows was <0.14 ng/ml at the time of oestradiol benzoate treatment.

**Experiment 2**

The 3 cows treated with oestradiol benzoate in the luteal phase had plasma progesterone concentrations of 0.9, 2.1 and 2.1 ng/ml respectively, at the time of treatment. None of the cows had an LH surge after treatment.

**Experiment 3**

All cows had elevated progesterone concentrations 3–5 days after the insertion of a PRID. A substantial decrease in progesterone concentration was recorded 12 h after treatment with
cloprostenol in 2 (cyclic) cows 5 days after PRID insertion. The concentration of progesterone in all cows at the time of oestradiol benzoate treatment 7 days after PRID insertion was between 0-7 and 1·5 ng/ml. The LH concentration in cows remained basal (<2·5 ng/ml) throughout the period of the experiment and not one LH surge was recorded. Progesterone concentrations 12 h after the removal of the PRID were <0·14 ng/ml, indicating that the increased concentration of progesterone during this experiment was exogenous.

Experiment 4

All 3 ovariectomized cows released a normal LH surge in response to oestradiol benzoate, although the peak response was delayed (at 32 h) in one cow.
Experiment 5

Plasma progesterone concentrations were 0.9, 1.0 and 1.0 ng/ml for each cow at the time of oestradiol benzoate treatment, 7 days after the insertion of the PRID. None of these cows exhibited an LH surge.

Discussion

All normal cows, whether in the post-partum anoestrous period, in the follicular phase of the cycle, or ovariectomized, released a normal LH surge in response to oestradiol benzoate when the plasma progesterone at the time of treatment was <0.5 ng/ml (Exps 1 & 4). The hypothalamo–pituitary axis was capable of releasing a normal LH surge in response to oestradiol benzoate.

The cow which once failed to respond had pyometra, the bacterial endotoxins from which can prevent a normal LH surge in cows (Peter & Bosu, 1987). The condition, however, was cured and the cow responded normally to subsequent oestradiol benzoate treatments.

None of the luteal-phase cows with plasma progesterone concentrations between 0.9 and 2.1 ng/ml released an LH surge in response to oestradiol benzoate treatment (Exp. 2) in agreement with the suggestion that endogenous progesterone concentrations of >0.3 ng/ml prevent LH release in response to oestradiol (Hobson & Hansel, 1972; Short et al., 1979; Alam & Dobson, 1987).

Intact and ovariectomized cows with plasma progesterone concentrations >0.7 ng/ml during exogenous progesterone treatment (PRID; Exps 3 & 5), failed to release a normal LH surge in response to oestradiol benzoate treatment. This is in contrast to previous studies reporting failure of exogenous progesterone to block oestradiol-induced LH release in intact (Swanson & McCarthy, 1978) or ovariectomized (Short et al., 1973; Hausler & Malven, 1976) cows. In each of these studies 25–50 mg progesterone were administered by i.m. injections given 12–24 h apart, which would elevate plasma progesterone concentrations for a short time only (Short et al., 1973). This mode of administration may have been insufficient to maintain high enough progesterone concentrations within the brain–hypothalamo–pituitary complex to suppress LH release. This would not, however, explain the failure of progesterone implants to suppress oestradiol-induced LH release in ovariectomized cows (Short et al., 1979). These implants blocked oestrus in intact cows for up to 45 days. It was suggested that the luteal tissue may metabolize oestradiol or produce a substance other than progesterone, which exerted an inhibitory effect on LH release (Short et al., 1979). In the present study (Exps 3 & 5) the influence of the corpus luteum was removed by cloprostenol treatment or ovariectomy; the inhibition was therefore due solely to exogenous progesterone. The present study shows that ovaries are not necessary for progesterone to inhibit the surge release of LH and that progesterone in sufficient concentration, whether endogenous or exogenous, can block oestradiol-induced release of LH.

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References


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