The effect of pretreatment with progesterone on the oestrous response to oestradiol-17ß benzoate in the post-partum dairy cow

S. McDOUGALL, C.R. BURKE, K.L. MacMILLAN AND N.B. WILLIAMSON[1]

Dairying Research Corporation, Ruakura Agricultural Centre, Hamilton, New Zealand.

ABSTRACT

The objective of the experiments was to test the hypothesis that pretreatment with progesterone would increase the proportion of animals exhibiting behavioural oestrus following injection of oestradiol-17ß benzoate (OB).

An initial dose response trial was completed and 600 µg of OB induced behavioural oestrus in 50% of post-partum non-cycling dairy cows.

Post-partum cows were randomly allocated to be pretreated with either a CIDR-B (Carter Holt Harvey, Hamilton, NZ) device containing 1.9 g of progesterone or a blank CIDR-B device for 5 days. All cows were injected with 600 µg of OB 48 hours after CIDR-B device removal. The cows were observed for oestrous behaviour for 15 minutes, 4 times daily at 6 hourly intervals for 60 hours after OB injection. There were 81% (13/16) of progesterone pretreated cows detected in oestrus compared with 39% (7/18) of non-progesterone pretreated cows. Progesterone pretreatment apparently sensitises cows to the behavioural effects of exogenous OB.

Keywords Progesterone, oestrogen, post-partum anoestrus, oestrous behaviour, dairy cattle.

INTRODUCTION

European and North American studies have shown that only 10 to 30% of cows demonstrate a behavioural oestrus concurrent with their first post-partum ovulation (Lamming and Bulman, 1976; Boyd and Munro, 1979; Van der Weil *et al.*, 1979; Fagan and Roche, 1986). However, this percentage increases at the second and subsequent ovulations and the low initial percentage of expression of oestrus may be due to the lack of central nervous system exposure to progesterone before the first ovulation (Lamming and Bulman, 1976; Fagan and Roche, 1986).

Oestradiol-17ß benzoate (OB) can induce behavioural oestrus in ovariectomised cows (Asdell *et al.*, 1945; Melampy *et al.*, 1957; Carrick and Shelton, 1969; Katz *et al.*, 1980; Davidge *et al.*, 1987; Allrich *et al.*, 1989) with the proportion of cows responding being dependent on the dose (Asdell *et al.*, 1945; Carrick and Shelton, 1969; Cook *et al.*, 1986). However, the interval from injection to onset of oestrus and the duration of oestrus are not altered by increasing OB concentrations except at very high doses (Cook *et al.*, 1986). There is no relationship between the plasma concentration of oestrogen and the duration or strength of oestrous behaviour at a natural oestrus (Glencross *et al.*, 1981; Coe and Allrich, 1989) or at an oestrus as a result of luteolysis induced by prostaglandin F2α (Walton *et al.*, 1987) suggesting there is a threshold in oestrogen concentration above which oestrus is expressed.

Progesterone treatment before an OB injection reduces the dose of oestrogen required to induce oestrous behaviour in ovariectomised ewes (Robinson *et al.*, 1956), but not in ovariectomised does (Sutherland and Lindsay, 1991). In contrast, pretreatment with progesterone injections for 5 days increased the dose of OB required to induce oestrus in ovariectomised cows (Carrick and Shelton, 1969) and reduced the proportion displaying behavioural oestrus (Davidge *et al.*, 1987). A single injection of progesterone simultaneously, or 48 hours before OB injection does not change the proportion of cows exhibiting oestrus (Allrich *et al.*, 1989). However, simultaneous administration of OB and progesterone increased the intensity of oestrous behaviour (Walter and King, 1984).

The absolute time post-partum may influence the behavioural response to endogenous and exogenous oestrogen. Cows ovulating for the first time more than 30 days after parturition are significantly more likely to exhibit oestrus than herd mates ovulating for the first time less than 30 days after parturition (54% vs 20% respectively; Fagan and Roche, 1986). The intensity of behavioural oestrus increased with post-partum interval in ovariectomised, OB-treated milking cows (Britt *et al.*, 1986).

The aim of the present experiments was to determine whether progesterone pre-treatment of intact, non-cycling post-partum cows before oestrogen injection increased the proportion of cows exhibiting behavioural oestrous and whether the time from treatment to onset of oestrus and subsequent duration of oestrus was affected.

MATERIALS AND METHODS

Experiment 1

Dose response trial

Twenty cows which were between 11 and 26 days post-partum were stratified into 3 age groups (2, 3 and >3 years) and then randomly assigned to 1 of 5 groups. Cows in each group were then injected with either sterile 0.9% sodium chloride or 200, 400, 600 or 800 µg of OB i.m. (Oestradiol-17ß benzoate SA, Intervet, Sydney, Australia). A milk sample was collected for progesterone analysis on the day of injection. They were tail-painted three days before OB injection and spray raddled at the time of injection (Macmillan *et al.*, 1988) and were observed for oestrous behaviour 3 times daily (7.15 am, midday and 4.45 pm) for 3 days after injection. Any cow observed to stand to be ridden by another cow or which had more than 75% of its tail paint removed was regarded as being in oestrus. The minimum dose

[1] Department of Veterinary Clinical Sciences, Massey University, Palmerston North, New Zealand.

found to induce oestrus in approximately 50% of cows was selected for use in Experiment 2.

Experiment 2

Effect of progesterone pretreatment

Milk samples from each of 70 mixed-age Friesian and Friesian cross, spring-calving cows, which were between 1 and 4 weeks post-partum, were collected on days -10, -7 and -4 before subsequent CIDR-B treatment. Any cow which displayed behavioural oestrus or had a milk progesterone concentration of >2.5 ng/ml was removed from the experiment before treatment. The remaining cows were divided into pairs matched on calving date and age. Within the pairs, the animals were randomly assigned to one of two treatments:

1. A CIDR-B device containing 1.9 g of progesterone was inserted intravaginally for 5 days. An injection of 600 μg of OB was administered i.m. 2 days after device removal.
2. A blank device was inserted for 5 days, and an injection of 600 μg of OB was administered i.m., 2 days after device removal.

The reproductive tract of each cow was examined per rectum immediately before device insertion. Any animal found with palpable uterine or ovarian pathology was removed from the trial. Tail paint was also applied at this time, and at device removal the paint strip was covered with red aerosol raddle.

The cows were observed for oestrous behaviour at pasture for 15 minute periods 4 times daily, at noon, 6 pm, midnight and 6 am for 60 hours following OB injection. A cow was assumed to be in oestrus if she stood still while being mounted by a herdmate.

Milk samples for progesterone concentration determination were also collected on the day of CIDR-B device insertion and on the day of OB treatment. Any cow that ovulated (milk progesterone concentration >2.5ng/ml) or was detected in oestrus during the treatment period was retrospectively removed from the experiment.

The milk progesterone concentration was estimated in the unpreserved, whole milk samples using a commercially available heterologous, solid phase, competitive binding Enzyme Linked Immunosorbent Assay (Ovucheck, Cambridge Veterinary Sciences, Ely, Cambridgeshire, CB6 1SE, UK). The absorbance was read at 405 nm on a Biorad model 3550 spectrophotometer (Biorad Laboratories, Richmond, California, 94804, USA). The standard curve was plotted as log dose by absorbance and a linear regression performed. The unknown concentrations were calculated from the equation. Data collection and calculation were done by commercially available software (Biorad microplate manager - data analysis software, Biorad Laboratories, Richmond, California, 94804, USA). The sensitivity of the assay (upper 95% confidence interval of milk samples from 10 ovariectomised cows) was 0.4 ng/ml and the inter and intrassay variation were 15.6% and 9.2%, respectively.

Statistical Analyses

The proportion of cows expressing oestrous behaviour and the number of observation periods at which each cow was observed in standing oestrus within three days of OB injection was compared by χ^2 analysis among treatments. The time from OB treatment to first standing oestrus among treatments was compared by one way analysis of variance. Logistic regression analysis was performed to compare days post calving and response to treatment.

Results are expressed as mean ± standard deviation unless otherwise noted.

RESULTS

Experiment 1

Dose response trial

Milk progesterone concentration was >2.5 ng/ml in 3 cows on the day of the OB injection; 1 in each in the 0, 200 and 400 μg OB groups None of these animals was detected in oestrus and each was removed from further analysis. None of the cows treated with less than 600 μg OB displayed behavioural oestrus, but 2/4 of the 600 ng OB group and 4/4 of the 800 ng OB group did so. The 600 μg dose was selected for the second experiment.

Experiment 2

Effect of progesterone pretreatment

Twenty-six cows were removed from analysis due to ovarian or uterine pathology, detection of behavioural oestrus or elevated milk progesterone concentration (>2.5 ng/ml) by the day of CIDR-B treatment. Thus 44 cows were initially treated with a CIDR-B or blank CIDR-B. A further 10 cows were removed before final analysis because they had shown behavioural oestrus or a rise of progesterone concentration on or before the day of OB treatment. Of the 34 remaining cows, 16 were pretreated with progesterone and 18 were pretreated with a blank device. There was no difference between groups in age, date of calving, weight, or milk production. Significantly more progesterone pretreated cows displayed behavioural oestrus than non progesterone pretreated cows (13/16 vs 7/18, respectively; $\chi^2 = 4.6$, df = 1, $P<0.05$). There was a strong trend for the progesterone pretreated cows to be detected in oestrus earlier (26.3 ± 7.0 vs 32.6 ± 5.9 hours, p=0.053). No cow was observed to be in standing oestrus at more than two consecutive observation periods. Of the cows observed in standing oestrus, there was no difference between treatments in the number of periods individual cows were seen in standing oestrus ($\chi^2 = 0.02$, df = 1, $P>0.1$). There was no effect of days post-partum on the proportion of cows exhibiting standing oestrus, nor was there an interaction between days post-partum and treatment.

DISCUSSION

In the present experiment, pretreatment for 5 days with progesterone was sufficient to "prime" the central nervous system so that 600 μg of OB was able to induce oestrus in 82.4% of animals, compared with 38.9% of non progesterone pretreated animals. The interval from OB treatment to onset of oestrus was similar to those previously published. They ranged between 22.7 and 25.4 hours in the ovariectomised cow (Melampy *et al.*, 1957; Carrick and Shelton, 1969; Katz *et al.*, 1980).

Previously reported experiments have failed to alter the oestrous response following progesterone "priming" (Carrick and Shelton, 1969; Davidge *et al.*, 1987; Allrich *et al.*, 1989). There are several factors that may explain the difference between the present and previous results. Firstly, all the cows used in the previous experiments were ovariectomised. Secondly, the progesterone was injected in an oily vehicle which may have prolonged its release and led to the progesterone concentration still being elevated at the time of the OB injection. Elevated

progesterone concentrations has been reported to block OB-induced behavioural oestrus in some studies (Ulberg *et al.*, 1951; Melampy *et al.*, 1957; Davidge *et al.*, 1987), but not in others (Walton and King, 1984). Thirdly, the duration of progesterone treatment may not have been appropriate. Allrich *et al.*, (1989) injected only a single dose of progesterone 48 hours before the OB injection.

Progesterone treatment alone is sufficient to initiate the endocrine events leading to oestrus in some intact post-partum cows (Bulman *et al.*, 1978; Ball and Lamming, 1983: Macmillan and Peterson, 1992). Thus, the "priming" effect of progesterone may have been mediated via the ovary as well as the central nervous system. In the intact cow, exogenous progesterone has been shown to affect the normal follicle turnover pattern (Sirois and Fortune, 1990), the oestrogen concentration and LH receptor concentration within the dominant follicle (Johnson *et al.*, 1991) and lengthen the interval from luteolysis to the preovulatory surge of luteinising hormone (Roberson *et al.*, 1989). In the present experiment, any effects of progesterone acting via the ovary cannot be distinguished from those acting directly on the central nervous system. Repeating the experiment in ovariectomised animals would allow determination of the role of progesterone "priming" on the central nervous system without possible ovarian interactions.

The optimum duration of progesterone pre-treatment, the interval from progesterone withdrawal to OB injection, and the optimal dose of OB have not been systematically studied in cattle. From one to five days of progesterone pretreatment by intramuscular injection (Carrick and Shelton, 1969; Davidge *et al.*, 1987; Allrich *et al.*, 1989) or five days by intravaginal sponge (Britt *et al.*, 1986) have been used. The interval from the last injection of progesterone or progesterone sponge removal to OB treatment has ranged from 36 to 72 hours (Carrick and Shelton, 1969; Britt *et al.*, 1986; Davidge *et al.*, 1987; Allrich *et al.*, 1989). In sheep, 48 hours was found to be the optimal interval from final progesterone injection to OB injection (Moore and Robinson, 1957). However, pre-treatment up to five days before ram introduction was also found to be effective (Pearce *et al.*, 1987). Thus, further experiments evaluating varying durations of progesterone pretreatment, the interval from progesterone withdrawal to OB injection and the doses of OB required are necessary to fully define the requirements for progesterone priming on OB-induced behavioural oestrus.

The hypothesis that pre-treatment with progesterone will increase the proportion of cows exhibiting oestrus in response to injected OB is accepted. However, the hypothesis that within the post-partum range of 23 to 51 days, the time post-partum affects the oestrous response to injected OB is rejected.

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