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# Hormonal induction of oestrus during the early postpartum period and the subsequent effects of nutrition on ovarian activity in dairy heifers

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## ABSTRACT

Our working hypothesis was that a significant period of severe underfeeding of dairy heifers during early lactation would not prevent the continuation of normal length oestrous cycles following hormonal induction of oestrus and ovulation in the early postpartum period (pp; 26.0 ( 0.7 d pp). Each of 16 Friesians (F) and 15 Jerseys (J) was treated with a CIDR device for 5 days, with one injection of prostaglandin-F2α the day before device removal and another injection 1.0 mg oestradiol benzoate 24 h after removal. The feed allowances of 8 F and 7 J heifers were reduced over the following 3 oestrous cycles, to a level where live-weights and milk yields were depressed finally by up to 15% and 36%, respectively, compared to fully fed contemporaries. Ovarian ultrasonography and monitoring of concentrations of milk progesterone (mP4) during the 1st (Cyc 1) and 3rd (Cyc 3) oestrous cycles showed that 3 Fs (1 FH and 2 FL) did not respond to initial treatment, and remained anovulatory throughout. Another 3 heifers (2 FL and 1 JL) became anovulatory after an initial positive response. The size of the first dominant follicle (DF1) and concentrations of mP4 were reduced by restricted feeding, but only during Cyc 3. This study has shown that a hormonal treatment can induce oestrus and ovulation in the majority of heifers which have been calved between 20 and 30 days. Subsequent restrictions in feeding level may convert some cycling heifers into an anoestrous state, although most will continue to cycle.

**Keywords:** Postpartum dairy heifer; nutrition; breed; ovary; reproduction.

## INTRODUCTION

Prolonged periods of postpartum anovulatory-anoestrus (PPA) are the main cause of infertility in New Zealand dairy cows. The most successful hormonal treatment developed to date involves 5 days of progesterone priming using a CIDR device, followed by an injection of a low dose of oestradiol benzoate (ODB; 1 mg) 24 h after device removal (McDougall *et al.*, 1992; Macmillan *et al.*, 1995). It is currently recommended that this treatment be initiated in the week preceding the planned start of mating (PSM) for animals which have been calved for at least 30 days.

Induction of oestrus an earlier time postpartum (pp; i.e., <30 d after calving) has the potential to improve reproductive efficiency. Firstly, ensuring that first ovulation occurs before the PSM should improve the fertility during the mating period (Whitmore *et al.,* 1974). Secondly, a treatment which can shorten the PPA period should provide an alternative to the practice of calving induction in some cases. Thirdly, it may be prudent to initiate oestrous cycles in susceptible stock (e.g. heifers, thin cows, etc.) before peak milk yields and feed shortages produce a chronic energy deficiency. However, the treatment would not be effective if a significant proportion did not respond; or, having responded, reverted back to an anoestrous state when challenged nutritionally at a later date.

This study tested the efficacy of hormonal induction of oestrus and ovulation in dairy heifers which had been calved 20 to 30 days; and aimed to determine whether these animals would continue to exhibit oestrous cycles, even when their feed intakes were severely restricted.

## MATERIALS AND METHODS

Sixteen Friesian (F) and 15 Jersey (J) heifers of high genetic merit calved normally during a 14 day period in July 1994. Each animal had a CIDR™ (InterAg, Hamilton) device inserted into the vagina from 20 to 30 days pp (26.0 ± 0.7 d pp) for 5 days. All animals were injected with prostaglandin-F2α (5 ml Lutalyse™, Upjohn, Auckland) on the 4th day of this treatment to induce luteolysis since 4 animals had previously ovulated as detected by ultrasonography and milk progesterone concentrations (mP4). Oestradiol benzoate (1 mg in peanut oil) was injected 24 h after device removal. The occurrence of behavioural oestrus was recorded at intervals of 2-4 h during the period of 16 to 28 h after this injection.

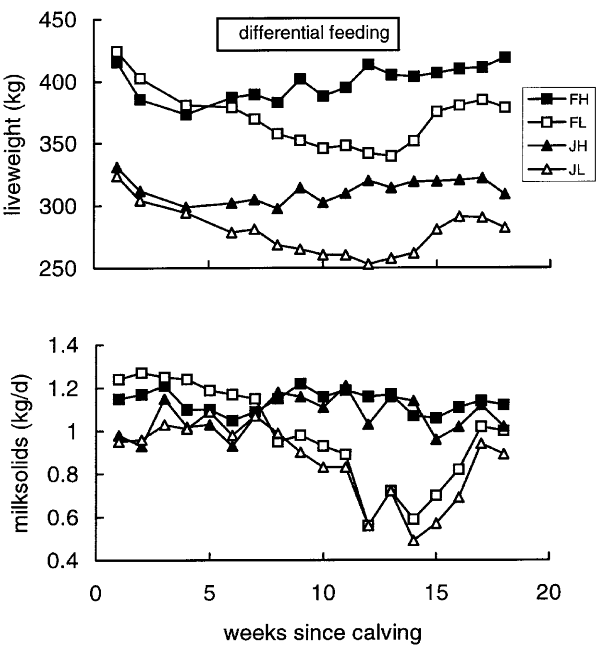
The ovaries of each animal were monitored by daily ultrasonography (Aloka SSD-210 DXII, 7.5 Mhz transducer) during the 1st (Cyc 1) and 3rd (Cyc 3) oestrous cycles after induction of oestrus. All follicular and luteal structures greater than 3 mm in diameter were sketched and the video image recorded on tape.

Pasture allowances for half the heifers of each breed were restricted (L) from 4 to 5 days after induction of Cyc 1 until the end of Cyc 3 (Weeks 5 to 13 pp), compared to fully fed contemporaries (H). Live-weights (LWT) and milk yields were measured twice weekly. These data were used to adjust feeding allowances, and to quantify the different nutritional states between the treatment groups (Mackle *et al.,* 1996).

Concentrations of progesterone were measured by RIA (Coat-a-Count, DPC, California) in composite milk samples collected 3 times weekly to confirm the occurrence of ovulation and the development of a functional corpus luteum (CL). Intra- and inter-assay coefficients of variation in standards of 0.4, 1.8 and 3.5 ng/ml were 17.8, 10 and 7%, and 21.8, 12 and 8.8%, respectively. The sensitivity of the assay was 0.08 ng/ml.

All data are expressed as mean ± standard error of mean. The effects of feeding level and breed were tested using general linear models for analyses of variance. The plateau diameter of the first dominant follicle (DF1) and average mid-luteal mP4 during the oestrous cycle were the parameters selected to represent the follicular and luteal activities of the ovaries. This project was approved by the Ruakura Animal Ethics Committee (DR003/13).

**FIGURE 1:** Mean live-weight (top) and milk solids (bottom) harvested in Friesian (F) and Jersey (J) heifers (n=31) which had either a restricted feed allowance during weeks 5 to 13 after calving (L), or were fully fed (H) throughout.




## RESULTS

The mean onset of oestrus occurred 19.7 ± 0.7 h after the injection of ODB in all but a single J heifer. Three F heifers (1 H and 2 L) were seen in oestrus but did not ovulate before the feeding treatments began. They remained anovulatory throughout the trial (Table 1).

Differential feeding allowances between Ls and Hs achieved contrasting live-weights and milk yields (Fig. 1). During the final 4 weeks of differential feeding, heifers in L groups were an average 55 kg (15%) lighter and produced 36% less milk solids (MS: 0.7 *vs* 1.1 kg MS/d) compared to fully fed heifers (P<0.001). Compensatory increases in live-weight and milk yields occurred in L heifers after feeding restrictions ended (Fig. 1). In association with restricted feeding, 3 (2 F's and 1 J) of the 13 L heifers which had ovulated initially ceased ovulating. None of the fully fed animals became anoestrus again, although one FH heifer failed to ovulate at the end of Cyc 1 following development of a cystic follicle.

**TABLE 1:** Some characteristics of the 1st (Cyc 1) and 3rd (Cyc 3) oestrous cycle following hormonal treatment to induce oestrus and ovulation in early postpartum Friesian (F) and Jersey (J) heifers which were either, fully (H) or restrictive (L) fed after the treatment.

| **Group (n=)** | **FH (8)** | | **FL (8)** | | **JH (8†)** | | **JL (7)** | |
|---|---|---|---|---|---|---|---|---|
| **Cycle** | **1** | **3** | **1** | **3** | **1** | **3** | **1** | **3** |
| Normal cycle* | 6 | 7 | 3 | 4 | 6 | 8 | 6 | 6 |
| Short cycle (<18 d) | - | - | 1 | - | - | - | - | - |
| Long cycle (>28 d) | - | - | - | - | 2 | - | - | - |
| Anovulatory | 1 | 1 | 2 | 4 | - | - | - | 1 |
| Became anovulatory | 1§ | - | 2 | - | - | - | 1 | -° |

† includes a cycling heifer which ovulated 6 days after ODB injection.
* A normal cycle is one which has an inter-ovulatory interval of 18 to 28 d, 2 or 3 follicle waves and mid-luteal mP4 >5 ng/ml.
§ developed a cystic follicle

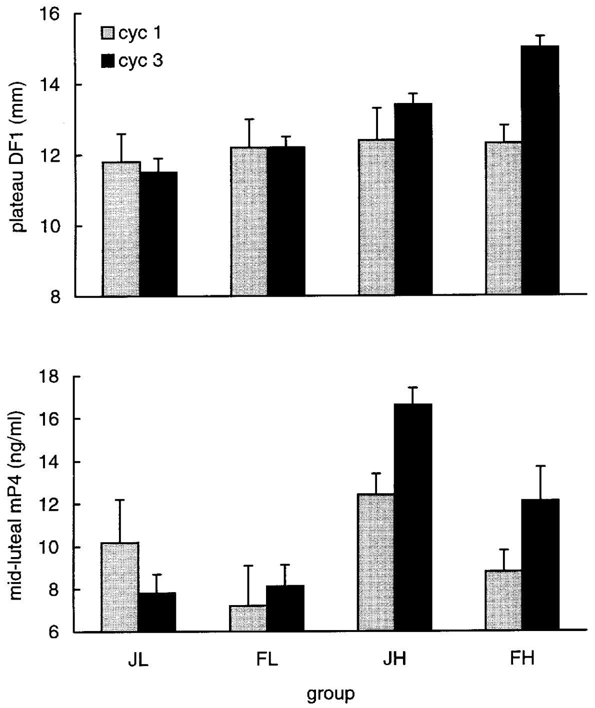
Mean plateau diameter of DF1 of Cyc 3 was greater in H's (14.1 ± 0.3 mm) compared to L's (11.8 ± 0.3 mm; P<0.01), and in F's compared to J's (14.0 ± 0.5 *vs* 12.6 ± 0.3 mm; P<0.01; Fig. 2). Similar differences were not evident during Cyc 1, although mid-luteal mP4 concentrations were higher in J's (11.4 ± 1.1 ng/ml) than F's (8.0 ± 1.0 ng/ml; P<0.05). The breed effect on mP4 was not observed at Cyc 3, but concentrations in H's were almost twice as great as those in L's (14.5 ± 1.0 verses 7.9 ± 0.6 ng/ml; P<0.001).

## DISCUSSION

This study demonstrated that 5 days of progesterone priming followed by an injection of ODB 24 h later, was an effective treatment for inducing oestrus and ovulation in F and J heifers between 20 and 30 days pp and for reducing the interval to normal cyclicity (Burke *et al.,* 1995). A subsequent restriction in feeding levels influenced ovarian activity, but most heifers continued cycling normally despite large losses in live-weight and milk yield.

Our previous studies in postpartum heifers have shown that the first spontaneous ovulation is seldom accompanied by oestrus and the ensuing oestrous cycle is usually short (i.e. 8-12 days), containing a single follicle wave (Burke *et al.,* 1995). The first and second oestrous cycles among animals in the present study were ‘normal’ with inter-ovulatory intervals between 18 and 28 days, 2 or 3 follicular waves in each cycle, and concentrations of progesterone in milk >5 ng/ml. Progesterone/ODB therapy effectively substituted for the short cycle that normally follows the first spontaneous ovulation after calving in heifers.

**FIGURE 2:** Mean plateau diameter of the first dominant follicle (DF1; top) and mid-luteal concentrations of progesterone in milk (mP4; bottom) during the 1st (Cyc 1) and 3rd oestrous cycles (Cyc 3) postpartum in Friesian (F) and Jersey (J) heifers (n=31). Animals received either a restricted feed allowance during weeks 5 to 13 after calving (L), or were fully fed (H) throughout.




Most of the heifers in the L group tolerated large losses in live-weight and milk yield (Fig. 1) without reverting to an anoestrous state. Cessation of ovulations occurred in 3 L group heifers (Table 1). Although this represented 20% of the L group which had ovulated following the treatment, numbers were insufficient for this effect to be statistically significant. It is now well known that underfeeding during the prepartum or postpartum periods will extend the calving to first ovulation/oestrus interval in Holstein-Friesians that are anoestrus (Randal, 1990). These effects could be due to suppression of pulsatile release of GnRH from the hypothalamus which leads to reduced secretion of LH by the pituitary gland by a mechanism associated with the effect of negative energy balance on metabolism (Randal, 1990). The same mechanism may be responsible for the conversion of ovulatory heifers into an anovulatory state.

A sustained period of restricted feeding reduced size and function of the structures on the ovary of cycling heifers (Fig. 2). A relationship between restricted dietary intake and reduced diameter of the DF1 was also observed in cycling beef heifers (Murphy *et al.,* 1991), and prolonged periods of negative energy balance depressed luteal activity in lactating Holstein cows (Villa-Godoy *et al.,* 1988). Restricted feeding allowances in non-lactating, cycling F cows altered some aspects of ovarian activity, but were rarely able to suppress oestrus cycling (Burke *et al.,* 1996). As was found in our previous work (Burke *et al.,* 1995), breed was also a significant factor on size and function of ovarian structures. Again, F heifers seemed less tolerant to underfeeding than Js. We have yet to find an reasonable explanation for these differences.

We conclude that 5 days of progesterone treatment followed 24 h later with 1.0 mg ODB is sufficient to stimulate oestrus and ovulation in Friesian or Jersey heifers between 20 and 30 days after calving. A subsequent restriction in feeding allowance reduced ovarian activity and resulted in some animals reverting to anoestrus, although the majority continued to cycle.

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