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# Patterns of luteinising hormone release and embryo recovery following superovulation of cycling and anoestrous lactating dairy cows and in non-lactating cows

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## ABSTRACT

Lactating dairy cows, either during postpartum anoestrus (LA; n=10) or returned to oestrous cyclicity (LC; n=10), and cycling non-lactating dairy cows (DC; n=10) were superovulated with follicle-stimulating hormone during a progesterone supplementation treatment, inseminated and embryos recovered non-surgically 7 days later. Blood samples were obtained during the period of oestrus to determine plasma concentrations of luteinising hormone (LH). Number of corpora lutea varied (14.6±1.6, 8.1±1.5, and 8.7±1.9 for LC, LA and DC cows, respectively; $P<0.02$), but not the total number of all ova and embryos, or the total number of transferable embryos per cow. There were no significant differences between groups in either mean time from start of sampling to peak LH (37.0±3.9, 34.5±3.6 and 36.2±4.5 hours for LC, LA and DC cows, respectively) or mean LH peak (12.5±3.6, 16.6±3.3 and 13.4±4.2 ng/ml for LC, LA and DC cows, respectively).

**Keywords:** dairy cow; superovulation; luteinising hormone

## INTRODUCTION

Postpartum cows can be successfully superovulated during the postpartum period, whether they are anoestrous or have resumed cycling (Kamimura *et al*., 1996; Rhodes *et al*., 1997). In the experiment described by Rhodes *et al.* (1997), numbers of *corpora lutea* (CL) were similar for cows whether they were still anovulatory anoestrous or had recommenced cycling during the postpartum period (9.9 and 9.5 CL/cow, respectively), but the numbers of embryos recovered from both groups were low (1.3 and 2.1 viable embryos per cow, respectively). The poor recovery rate of viable embryos in these cows may have been due to failure of insemination, or their postpartum or lactational status.

Postpartum anoestrus is associated with low levels of luteinising hormone (LH) release, probably associated with reduced pulsatile release of LH-releasing hormone from the hypothalamus (McDougall *et al*., 1995). Treatment of anoestrous cows with a progesterone-releasing intravaginal insert (CIDR™, InterAg, Hamilton, NZ) resulted in suppression of mean plasma LH concentrations from 5.0±1.5 ng/ml to 0.25±0.03 ng/ml. Superovulation treatment with a low-LH formulation of follicle-stimulating hormone (FSH: Ovagen™, ImmunoChemical Products Ltd, Auckland, NZ) also resulted in lower mean plasma LH concentrations and lower LH pulse frequency after CIDR‘ insert removal compared to cows which had received only a CIDR (Kamimura *et al*., 1996).

This study was initiated to compare circulating levels of plasma LH during the period of the preovulatory surge, superovulation rates and embryo recovery, in lactating anoestrous and cycling cows within 60 days postpartum, and in cows that were non-lactating and that had not been pregnant during the previous year.

## MATERIALS AND METHODS

A group of lactating dairy cows (Jersey and Friesian), which were 45-60 days postpartum, were examined by transrectal ultrasonography on two occasions five days apart for evidence of a CL structure in their ovaries. On the basis of this examination, treatment groups (LA: lactating anoestrous; LC: lactating cycling) consisting of 10 cows each, balanced for age and breed, were enrolled. The third treatment group, also balanced for age, consisted of 10 parous cows, known to be experiencing regular oestrous cycles but which were not lactating (DC: non-lactating cycling).

Superovulation treatment was initiated by insertion of an intravaginal progesterone-releasing insert (CIDR™) applied for 7 days (d0=day of CIDR™ insertion). From days 4 to 7, FSH (Ovagen™) was administered in a series of equal, twice daily, intramuscular (i.m.) injections, to a total dose of 14.4 and 10.8 mg for Friesian and Jersey cows, respectively. Prostaglandin (250mg Lutalyse™, Pharmacia & Upjohn, Auckland, NZ) was injected i.m. at the time of the 6$^{th}$ and 7$^{th}$ injections of FSH, and CIDR‘ inserts were removed at the second prostaglandin injection. Cows were inseminated at 12 and 24 h after detection of standing oestrus with frozen semen processed as a single batch from a bull of known high fertility.

Blood samples were obtained from each animal by coccygeal venipuncture into heparinised vacutainers. This was done at six-hourly intervals from the time of the 7$^{th}$ injection of FSH, until that animal had been inseminated twice. Blood samples were placed immediately into iced water and centrifuged at 1200g within 2 h of collection. An aliquot of plasma from each sample was stored at -20˚C until determination of plasma concentrations of LH. Seven days after oestrus, the number of CL was determined by transrectal ultrasonography of the ovaries. The cows were flushed using conventional non-surgical flushing, and unfertilised/degenerate oocytes and embryos were collected and evaluated.

Plasma concentrations of LH were determined in a single assay using a double-antibody RIA as described by McDougall *et al.* (1995). Intra-assay coefficients of variance were 13.6%, 12.7%, 7.7% and 9.8% for standard concentrations of 0.3, 1.0, 2.7 and 9.2 ng/ml.

Results are reported as mean ± sem. Variables examined for each cow were total number of CL, total number of unfertilised/degenerate oocytes and embryos recovered, total number of fertilised embryos, peak plasma LH concentration and time to peak plasma LH concentration.

A curve fitting procedure was used to determine peak parameters of the plasma LH concentration data. Data were analysed in a general linear model ANOVA procedure (SAS, Statistical Analysis Systems Institute Inc.) for effects of group status (LC, LA and DC) and breed.

## RESULTS

All cows were observed in oestrus within 24 hours after the last injection of FSH, and unfertilised oocytes and/or embryos were recovered from all except one cow in the DC group which had only one CL on ultrasound and was not flushed.

The LC cows had more corpora lutea (14.6±1.6, 8.1±1.5, and 8.7±1.9 for LC, LA and DC cows, respectively; $P<0.02$), but the total number of unfertilised/degenerate oocytes and embryos recovered (9.6±2.2, 8.8±2.0 and 4.2±2.6 for LC, LA and DC cows, respectively) and the total number of transferable embryos per cow (6.0±1.5, 3.7±1.3 and 1.5±1.7 for LC, LA and DC cows, respectively) did not differ significantly between treatment groups, and there were no significant effects of breed. Overall recovery rates (total number of all oocytes and embryos divided by number of CL) were greater in the LA cows than either the LC or the DC groups (108%, 66% and 48%, respectively). The proportion of transferable embryos recovered was similar for both LC and LA groups, which were higher than for the DC group (41%, 46% and 17% for LC, LA and DC cows, respectively).

There were no significant differences between groups or breed in either mean time from start of sampling (seventh injection of FSH) to peak plasma LH concentration (37.0±3.9, 34.5±3.6 and 36.2±4.5 hours for LC, LA and DC cows, respectively) or mean plasma LH peak concentration (12.5±3.6, 16.6±3.3 and 13.4±4.2 ng/ml for LC, LA and DC cows, respectively).

## DISCUSSION

All groups of cows were able to be successfully superovulated with the treatment protocol described, with the greatest overall response, as evidenced by the number of CL detected during transrectal ultrasonography at the time of flushing, in those cows that were lactating but had resumed oestrous cycles before the treatment began. Despite this, the total number of oocytes and embryos recovered did not vary between the treatment groups.

Overall recovery rates and the proportions of transferable embryos recovered from the lactating cows in the early postpartum period are superior to those reported by Rhodes *et al*. (1997). Furthermore, the better performance of the lactating cows over the DC group indicates that postpartum status was not detrimental to the outcome of superovulation. The low recovery rates of viable embryos described by Rhodes *et al.* (1997) may, therefore, have been associated with poor insemination performance.

The finding that plasma LH concentrations were similar for all groups of cows suggests that pituitary production of LH is not limiting in the cow with postpartum anovulatory anoestrus, thus supporting the contention of McDougall *et al.* (1995) that the limitation is in hypothalamic signals to the pituitary gland. Once the preovulatory surge was initiated, the LH concentrations achieved were adequate to induce successful ovulation.

These results indicate that lactating cows (cycling and anoestrous) can be successfully superovulated within 45-60 days of parturition and good numbers of viable embryos recovered. Further, superovulated lactating anoestrous cows can achieve plasma concentrations of LH during the preovulatory surge similar to those seen in superovulated cycling cows that are either lactating or non-lactating.

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