

INDUCTION OF CYCLIC ACTIVITY IN THE EARLY POSTPARTUM DAIRY COW

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Twenty-five lactating dairy cows were treated with either saline (control), 500 μg gonadotropin-releasing hormone (GnRH), GnRH + 10 mg estradiol 17β (E_2), E_2 , or GnRH + E_2 + 25 mg prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) at approximately 11 days postpartum. Blood samples were taken at the time of injection and then twice each week for about 30 days. Treatment with GnRH alone gave the largest LH peak; LH production appeared to be inhibited when E_2 was given with GnRH. Plasma estrogen concentrations were above 1 ng/ml for 12 h in all animals receiving estrogen alone or in combination. None of the treatments hastened initiation of cyclic activity which began at an average of 19 days postpartum. It seems unlikely that postpartum anestrus is an important constraint to early rebreeding in dairy cows.

On a administré à 25 vaches laitières en lactation, en injection intramusculaire, un soluté salé (témoin), 500 μg de gonadostimuline (GnRH), GnRH + 10 mg d'estradiol 17β (E_2), E_2 ou GnRH + E_2 + 25 mg de prostaglandine $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$), environ 11 jours après le vêlage. Des échantillons de sang ont été prélevés au moment de l'injection et ensuite deux fois par semaine pendant environ 30 jours. C'est le traitement au GnRH seul qui a donné le pic le plus prononcé de LH mais la production de LH semble avoir été inhibée quand GnRH était combiné à E_2 . La teneur en estrogène du plasma a dépassé 1 ng/ml pendant 12 h chez tous les sujets recevant l'estrogène seul ou en combinaison. Aucun des traitements n'a accéléré la reprise de l'activité cyclique laquelle a été observée environ 19 jours après le vêlage. Il ne semble pas que la période d'anoestrus soit une limite sérieuse à la remise en reproduction précoce chez les vaches laitières.

Several recent reports (see for example Britt 1974; Lauderdale 1975) emphasize the importance of reproductive efficiency in dairy cows on overall production. In general, as the period between calvings extends beyond a 365-day interval, the production of the cow during her lifetime declines (Britt 1974). Fertility is acceptable by as early as about 55 days postpartum (Britt 1974) and, since a 60-day rest period between lactations seems adequate (Louca and Legates 1968; Olds and Cooper 1970), provided animals are cycling, it should be possible to maintain a 365-day calving interval in even high producing dairy herds. Several previous reports have indicated

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substantial differences in the onset of cyclic activity in cows postpartum depending on nutrition and frequency of milking (Short et al. 1972; Oxenreider 1968; Bellows et al. 1974; Wiltbank and Cook 1958). These studies have consistently shown that dairy cows have a shorter postpartum anestrus interval than suckled beef cows and that dairy cows appear to be cycling as early as 20-30 days postpartum, with the first expression of estrus at an average of about 30 days postpartum. Huertes Vega et al. (1972) and Britt et al. (1974b) fed cows melengesterol acetate for 14 days beginning about 2 wk postpartum; those cows had fewer days open and required fewer services per conception during the subsequent

breeding period. These authors suggested that the reason for the better fertility was that the animals began cyclic activity earlier than they normally would.

In a subsequent report (Britt et al. 1974a), gonadotropin-releasing hormone (GnRH) was utilized on day 14 postpartum to promote ovulation and initiate cyclic activity. In the present experiment, four different treatments were designed to initiate cyclic activity in dairy cows. Cows were sampled twice weekly for blood progesterone analysis to determine when the first functional corpus luteum was established so that the day of ovulation could be estimated.

MATERIALS AND METHODS

Nine heifers and 16 Holstein cows were used from the University of Saskatchewan dairy herd. As they calved, they were assigned to each of five treatments (control, GnRH, GnRH + estradiol-17 β (E₂), E₂ and GnRH + E₂ + prostaglandin F_{2 α} (PGF_{2 α}). Treatment E₂ had one heifer and four cows, while the other treatments had two heifers and three cows. Animals were milked twice daily and were fed cereal grain silage and concentrate according to production based on National Research Council (USA) standards.

Treatments

All treatments were administered as intramuscular injections. When GnRH (500 μ g) and PGF_{2 α} (25 mg) were given, the two compounds were dissolved together in 7 ml of saline. (Doses of PGF_{2 α} refer to the free acid equivalent.) Estradiol-17 β (10 mg) was prepared as a 5 mg/ml solution in 30% ethanol-water and was injected at an intramuscular site separate from GnRH.

GnRH was given as a single intramuscular injection. This would be easier to administer than the gelatin capsule containing GnRH used by Britt et al. 1974a. Previous studies in cattle (Kaltenbach et al. 1974; Zolman et al. 1974) and sheep (Reeves et al. 1971) showed that animals near estrus gave a greater response to GnRH than did animals at other stages of the cycle. Hence, in the GnRH + E₂ treatment, E₂ was injected with the GnRH to determine whether the response to GnRH would be enhanced. The dose

of E₂ selected was based on the study of Foote and Hunter (1964) who demonstrated a reduced postpartum interval in cows treated with estrogen as compared to control animals. The GnRH + E₂ + PGF_{2 α} treatment was based on rationale already described plus three additional possible mechanisms. These are (a) to induce luteolysis if a corpus luteum were present and consequently increase the number of cycles and perhaps fertility (b) to determine whether the GnRH release and the subsequent peak of LH in the blood might be prolonged due to a slowed absorption of GnRH from the injection site. Manns (1975) reported that intramuscular injections of PGF_{2 α} gave relatively long PG peaks in the circulation, and speculated that since the half life of PG in the circulation is very short, the vasoconstrictor properties of PG might be reducing the absorption from the injection site (c) that there might be some interaction between estrogen, PG and GnRH which would alter the LH response and subsequent ovulatory activity. To ensure that as few cows as possible were cycling at the time of administration of the compounds, the animals were injected on an average of 11 days postpartum (Table 1). The day before injection, a jugular vein catheter was installed and a blood sample collected. The cows were injected at time 0 the following day and additional blood samples were taken at -1/2, 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h before and after treatment. The reproductive tract was palpated before and at 12-h intervals for 48 h after treatment. Beginning 3-4 days after treatment, cows were palpated and blood samples were collected for progesterone, estrogen, luteinizing hormone (LH) and prolactin analysis. Palpation and sampling continued at 3- to 4-day intervals until approximately day 40 postpartum. All samples were collected into heparinized tubes and the plasma was frozen until analysis.

Hormone Analyses

All hormones were measured by radioimmunoassay. Progesterone was assayed on hexane extracts of plasma using an antibody, the specificity of which has been described (Abraham et al. 1971). Estrogen was assayed on diethyl ether extracts of plasma using an antibody obtained from Dr. B. Caldwell. This antiserum shows a cross reactivity with estrone of approximately 80%; consequently, values from this assay are given as plasma estrogen. Steroid assays utilized tritiated steroids and

Table 1. Day of treatment, time of ovulation and interval to conception in lactating dairy cows

	Treatment					Overall mean \pm SEM
	Control	GnRH	GnRH + E ₂	E ₂	GnRH + E ₂ + PGF _{2a}	
Day of treatment	11.6	11.6	11.2	11.4	11.2	11.4
Day postpartum to ovulation (range and $\bar{x} \pm$ SEM)	12 - 23	11 - 23	9 - 32	10 - 35	14 - 36	19 \pm 2
Days postpartum to conception ($\bar{X} \pm$ SEM)	16 \pm 2	17 \pm 2	20 \pm 5	20 \pm 5	21 \pm 4	19 \pm 2
	103 \pm 20	100 \pm 17	105 \pm 15	100 \pm 16†	104 \pm 15‡	102 \pm 7§

†Includes only three cows; one cow developed a severe uterine infection; another cow was culled after a displaced abomasum.

‡Includes only four cows; only cow was culled for low productivity.

§Includes only 22 cows (see† and ‡).

charcoal separation procedures. Tritiated steroids were used to quantitate extraction from plasma; all values given are corrected for losses.

LH and prolactin were assayed with antisera developed in guinea pigs to NIH-LH-B₇ and NIH-P-B₃. Immunizations were done according to the method of Vaitukaitis et al. (1971). The prolactin antibody showed no significant cross reaction with growth hormone, LH, FSH or TSH; recovery and parallelism studies were conducted to validate the assay. The LH antibody showed no cross-reactivity with FSH or growth hormone but did cross-react strongly with TSH. To determine if this was solely due to LH contamination in the NIH-TSH, an animal was injected with 1 mg of thyrotropin-releasing hormone (TRH) and blood samples were taken before and after injection to measure plasma prolactin and LH concentrations. Such an injection would release TSH and prolactin (Convey et al. 1973; Kelly et al. 1973). Although there was a large increase in plasma prolactin subsequent to the TRH injection, there was no release of immunoassayable plasma LH. It is recognized that NIH-TSH preparations contain a substantial quantity of LH which has been rendered biologically inactive but which does retain its immunological reactivity. Consequently we believe the antibody to be specific for LH. Prolactin and LH were iodinated by minor modifications of the chloramine-T procedure (Greenwood et al. 1963). Precipitations were by double antibody. The buffer used for all hormone assays was 0.05 molar pH 7.5 phosphate containing 9.0 NaCl, 1.0 g disodium EDTA, 1.0 g gelatin, and 100 mg of methiolate per liter.

RESULTS AND DISCUSSION

In each instance when GnRH was administered, there was a peak in LH production between 2 and 4 h subsequent to GnRH administration (Fig. 1). However, when E₂ was given with GnRH there was a substantial depression in LH production compared to GnRH alone. Estrogen by itself did not stimulate LH production in the samples collected in this study. Blood estrogen concentrations were over 1 ng per ml in samples collected from 1 to 12 h after injection of E₂. By 24 h, the estrogen concentrations had declined to 100–500 pg per ml and by 3–4 days later when the next

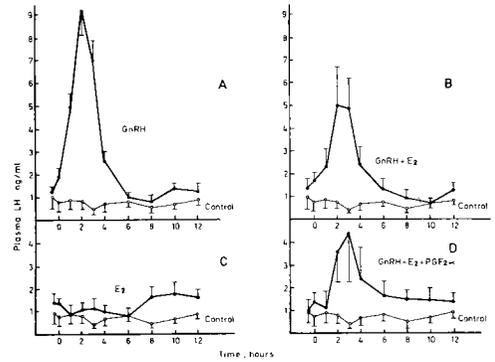


Fig. 1. Plasma LH (ng NIH-LH-B₇/ml) in control and treated cows (mean \pm SEM).

sample was taken they were at the usual basal levels of 2–20 pg per ml. Thus it would seem that the estrogen treatment resulted in extremely high estrogen concentrations and had a depressant effect on LH release from the anterior pituitary. It is known from much previous work that estrogens in high quantities can inhibit LH production from the anterior pituitary (Barraclough 1973), whereas at rather low concentrations, estrogen will result in increased production of LH by the anterior pituitary (Hobson and Hansel 1972).

Although there was some variation in progesterone concentration in treatment groups compared to control, the progesterone concentrations started to rise at approximately the same time in each of the treatment and control groups. Based on plasma progesterone concentrations, the time of ovulation was estimated on the assumption that ovulation would have occurred 4 days before the first perceptible rise in progesterone concentration (Table 1). There was very little difference in the estimated time of ovulation in any of the five groups and in fact the control group had the shortest time to ovulation. The three estrogen-treated groups did appear to have a slightly prolonged period of postpartum anestrus. This was not statistically significant because of the substantial range that was present in these experiments. Since the

control animals began to cycle so soon after calving, it is difficult to be certain if any of the treatments induced ovulation or if the cows were merely beginning to cycle normally. Rectal palpations frequently were quite unreliable at time of treatment because the uterus was still large and the ovaries were often difficult to reach. By about day 20, however, tracts were easily examined and the progesterone-secreting structures appeared to be normal corpora lutea.

All animals except three were rebred and kept in the herd. One animal in treatment E₂ was culled because of a displaced abomasum and another because of a prolonged uterine infection which did not respond to treatment. One animal in treatment GnRH + E₂ + PGF_{2α} was culled because of low productivity. The overall mean time of conception was 102 days for 22 of the 25 cows (Table 1).

When records of production on these animals in the lactation preceding the experiment were compared to time of initiation of cyclic activity and postpartum conception, no meaningful relationship was observed. The average production for all cows in the lactation preceding the experiment was 7,429 kg/305 days, while the comparable figure for cows during the lactation when the animals were studied was 7,513 kg. Also, the level of productivity in the lactation during which the animals

were bred did not appear to correlate with initiation of cyclic activity or with the date of conception. However, it should be emphasized that the number of animals in this study was much too small to draw any meaningful comparisons in that respect. It is rather interesting to note, however, that one animal, which produced over 10,000 kg of milk, ovulated 12 days postpartum and conceived to the second service 98 days postpartum.

No obvious relationship was observed in this study between plasma prolactin concentrations and initiation of cyclic activity (Table 2). Perhaps this is understandable, since the blood samples (except those taken in the first 3 days) were taken by vena puncture and one might argue that the resulting stress on the animals would initiate prolactin release which would preclude any correlations with other more meaningful physiological events.

The finding in this study that ovulation occurred on average in these 25 cows at 19 days postpartum indicated that length of postpartum anestrus in dairy cows was not an important constraint to early rebreeding. Previous results (Whitmore et al. 1974) showed that the level of fertility postpartum increased until about day 60–70 at which time there was a levelling off; subsequent to that, fertility did not improve markedly. Many producers wait 70–80 days before

Table 2. Plasma prolactin (ng NIH-P-B₃/ml) in relation to postpartum interval (values are $\bar{x} \pm$ SEM)

Days postpartum	Treatment					Overall
	Control	GnRH	GnRH + E ₂	E ₂	GnRH + E ₂ + PGF _{2α}	
10.4	17 ± 6	37 ± 11	73 ± 36	46 ± 36	20 ± 5	38.6 ± 10.1
11.4	28 ± 10	25 ± 7	28 ± 5	49 ± 25	29 ± 14	31.8 ± 4.4
12.4	21 ± 10	26 ± 5	39 ± 6	32 ± 10	19 ± 7	27.4 ± 3.7
16.4	10 ± 4	32 ± 20	22 ± 8	8 ± 4	25 ± 7	19.4 ± 4.6
20.0	14 ± 4	23 ± 8	21 ± 5	17 ± 5	14 ± 3	17.8 ± 1.9
23.4	11 ± 4	15 ± 5	22 ± 4	16 ± 6	14 ± 5	15.6 ± 1.8
26.9	9 ± 4	36 ± 13	41 ± 16	11 ± 4	10 ± 5	21.4 ± 7.0
30.4	12 ± 5	54 ± 37	88 ± 37	13 ± 5	23 ± 19	38.0 ± 14.6
34.4	12 ± 3	66 ± 45	64 ± 46	11 ± 5	13 ± 5	33.2 ± 13.0
37.6	60 ± 47	60 ± 36	60 ± 35	52 ± 49	45 ± 39	55.4 ± 3.0
41.0	65 ± 52	73 ± 43	65 ± 30	75 ± 63	70 ± 65	69.4 ± 1.9

rebreeding cows because they feel that earlier breeding may be difficult due to poor fertility and may cause reduced productivity per lactation. The present results and those of others show that earlier rebreeding should be possible and generally will increase lifetime milk productivity of a cow. For the producer starting to rebreed by 50–60 days postpartum, the major problems are likely to be common ones such as estrus detection, cystic ovaries and uterine infections.

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