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The Importance of Prolactin and the Milking Stimulus in the Artificial Induction of Lactation in Cows

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Abstract

Lactations were successfully induced in 15 out of 18 non-pregnant cows treated with oestradiol-17β (0·1 mg/kg body weight) and progesterone (0·25 mg/kg body weight) on days 1–7 (where day 1 is the first day of treatment). A further 23 out of 26 cows were successfully treated with oestradiol-17β and progesterone standardized at 40 and 100 mg/day respectively. No significant differences in milk yields were obtained by the additional treatments of twice daily milking from day 10, sustained elevation or suppression of plasma prolactin during the induction phase by reserpine and bromocryptine respectively, or by continuing oestradiol-17β injections alone on days 8–11. Levels of plasma prolactin less than 20 ng/ml were adequate for the preparation of the mammary gland for lactation to occur. The induced lactations of 24 monozygotic twin cows ranged from 20 to 87% of their respective siblings which had normal parturient lactations.

All 11 cows treated with the standard oestrogen–progesterone treatment plus reserpine had successfully induced lactations. In the absence of oestrogen and progesterone treatment, a short period of reserpine treatment followed by dexamethasone trimethylacetate failed to induce lactation in five cows.

Ninety per cent of induced cows ($n = 39$) and 91% of parturient cows ($n = 22$) conceived and subsequently calved following natural mating.

Introduction

Although lactations have been artificially induced in non-pregnant cows by 7 days of oestradiol-17β and progesterone injections, milk yields have been variable and generally lower than expected from normal parturient cows. In addition, approximately one-fifth of treated cows have failed to lactate (Smith and Schanbacher 1973, 1974; Narendran *et al.* 1974). The mammary glands of those cows unsuccessfully induced have not been fully developed after hormonal treatment and required regular milking for continued development (Narendran *et al.* 1974; Howe *et al.* 1975). In this study the effect of twice daily milking early in the induction phase on subsequent milk production was therefore studied.

Erb *et al.* (1976*b*) have found that the hormonal profile during induced lactation does not accurately duplicate that observed at parturition in that there was no rise in oestrogen and prolactin during progesterone decline and the peak of prolactin was lower than that normally observed at parturition. High plasma prolactin levels may be essential for optimal lactation as suppression of prolactin secretion by bromocryptine in the periparturient period in the cow severely inhibits lactation (Karg and Schams 1974). Recently, Collier *et al.* (1977) reported that induced milk yields in non-pregnant cows could be improved by the administration of the prolactin-stimulating tranquilizer reserpine following oestradiol-17β and progesterone treatment.

This paper reports our investigations into the effects of the pharmacological elevating and lowering of plasma prolactin during the induction phase, and of continuing oestrogen injections after the standard treatment, on both the timing of lactogenesis and induced milk yields. Attempts were also made to induce lactation by replacing the ovarian steroids with a combination of reserpine and dexamethasone trimethylacetate. Dexamethasone has been shown to be a potent lactogenic trigger in the cow (Fulkerson and McDowell 1975). Milk yields, milk composition and prolactin levels of artificially induced and normal parturient cows were compared.

Materials and Methods

Animals and Management

Two experiments were conducted in the period from June 1975 to May 1977. In experiment 1 (1975–1976) there were 18 sets of monozygotic twins. Within each twin set, one cow was pregnant and the other non-pregnant, having failed to conceive in the previous mating season. In experiment 2 (1976–77) there were 25 non-pregnant cows and six sets of monozygotic twins in which one cow was pregnant and the other non-pregnant. Two cows which failed to lactate on the standard and bromocryptine treatments respectively were retreated with oestrogen–progesterone and reserpine. The animals were of mixed breed but the Jersey and Friesian breeds predominated. In each year the animals were grazed together on perennial ryegrass–white clover pastures.

Prior to treatment the ovaries of all non-pregnant cows were examined by rectal palpation to confirm cyclic activity before treatments were started. Indwelling jugular cannulae were inserted 3 days prior to treatments commencing and during blood sampling cows were held in a race under quiet conditions. In experiment 1, 17 induced and 17 parturient cows, and in experiment 2, 22 induced cows were joined with a bull 2 months after treatment. Milking was discontinued when milk yields declined to 3 kg/day.

Hormones and Drugs

Stock solutions of progesterone (25 mg/ml) and oestradiol-17β (10 mg/ml) (Roussel Pharmaceuticals Pty Ltd, Melbourne) were prepared in absolute ethanol. The rauwolfia alkaloid, reserpine (Aldrich Chem. Co., U.S.A.) was dissolved in acetone (1 mg/ml), and the synthetic glucocorticoid, dexamethasone trimethylacetate (Opticortenol 0·5%, Ciba Geigy Aust. Ltd) was administered as commercially supplied. Bromocryptine (CB 154-Sandoz, Basle) was mixed with an equal weight of tartaric acid and then dissolved in 70% ethanol (50 mg/ml).

Experimental Procedure

Experiment 1

The 18 sets of monozygotic twin cows were treated as follows.

Group 1*a*: One member of each twin set calved normally and commenced milking immediately after parturition.

Group 1*b*: Nine non-pregnant siblings, twins to cows in group 1*a*, were injected subcutaneously twice daily for 7 days with 0·10 or 0·25 mg oestradiol-17β plus progesterone per kilogram body weight respectively. The first day of injection was taken as day 1 and the cows in this group commenced to be milked on day 10.

Group 1*c*: The remaining nine non-pregnant sibling twins were treated as in group 1*b* except that milking was delayed until day 24 or when their udders filled with milk, whichever came first.

Experiment 2

Twenty-five non-pregnant cows were allocated to five treatments of five animals on the basis of the previous year's milk production, so that the mean production of each group was similar. Cows in treatments 2*a*, 2*b*, 2*c* and 2*d* received a standard treatment of oestradiol-17β (40 mg/day) and progesterone (100 mg/day) for days 1–7 irrespective of live weight (range 250–400 kg).
Additional treatments were as follows.

Group 2*a*: None.
Group 2*b*: Subcutaneous injections of oestradiol-17β (10 mg) at 0800 and 1700 h on days 8–11.
Group 2*c*: Subcutaneous injections of bromocryptine (150 mg) at 0800 h on days −1, 0, 3, 6, 9, 12, 15 and 18.
Group 2*d*: Subcutaneous injections of reserpine (5 mg) at 0800 h on days 1, 6, 11, 16 and 21.
Group 2*e*: Subcutaneous injections of reserpine (5 mg) at 0800 h on days 1, 4 and 7 followed by single intramuscular injections of dexamethasone trimethylacetate (20 mg/day) at 0800 h on days 7, 8 and 9. There was no oestradiol-17β-progesterone treatment.

Six sets of monozygotic twins were treated as follows.

Group 2*f*: One non-pregnant member of each set of twins was treated as in group 2*d*.
Group 2*g*: The remaining sibling twins lactated after a normal pregnancy.

Milking of non-pregnant cows in groups 2*a*, 2*b*, 2*c*, 2*d*, and 2*f* commenced on day 21 or when their udders filled with milk, whichever came first. Cows in group 2*e* entered the milking shed on day 10 following the cessation of the dexamethasone trimethylacetate treatment. In experiments 1 and 2, treatment in all cows commenced within the 7 days immediately following standing oestrous and continued for 7 days.

Collection of Samples

Experiment 1

Blood samples were taken from three cows selected at random from group 1*c* and the respective sibling of each pair in group 1*a* using an indwelling jugular cannula each morning before treatment at 0700, 0730 and 0800 h. A mammary secretion was expressed from the teat sinus both morning and evening for each of these cows for lactose determinations on bulked 2-day samples. Sampling of blood and mammary secretions was continued until after lactation was established as indicated by the lactose contents of the mammary secretion in both induced and parturient cows.

Experiment 2

Blood samples were taken from the 25 cows in groups 2*a*, 2*b*, 2*c*, 2*d* and 2*e* at 0800 h on alternate days. Collection was via an indwelling jugular cannula inserted prior to treatment in all cows. In group 2*c*, blood sampling continued until lactation had been established. Once cows had entered the milking shed, sampling was delayed for at least 2 h after milking to ensure base-line plasma prolactin levels were being measured. Blood sampling of cows in all other treatments ceased when they entered the milking shed on day 21. Mammary secretions were also expressed at the time of blood sampling from all cows and the lactose content of these secretions was determined.

Chemical Analyses

Individual milk yields were recorded at each milking and once a week an evening–morning composite milk sample was taken and analysed for milk fat (Milkotester Mk III, Foss Electric, Hillerod, Denmark), and milk protein (Pro-milk, Foss Electric, Hillerod, Denmark).

To determine the timing of the onset of lactogenesis, the lactose content of the mammary secretion was estimated from glucose release following acid hydrolysis (Cowie *et al.* 1969).

Prolactin was assayed by solid-phase radioimmunoassay according to the method described by Fell *et al.* (1972). Levels of prolactin were expressed in terms of the NIH-P-S8 standard.

Statistical Analysis

As some cows failed to be successfully induced into lactation, the treatment groups were unequal in number and values within groups varied markedly. Data were therefore subjected to logarithmic transformation and as the variances within treatments were similar for all treatments, a pooled variance was used to compare means using Duncan's multiple range test.

Results

Number of Successfully Induced Cows

Fifteen out of 18 cows (groups 1*b* and 1*c*) and 12 out of 15 cows (groups 2*a*, 2*b* and 2*c*) which received oestradiol-17β and progesterone but no reserpine were successfully induced into lactation (see Table 1). All 11 cows treated with oestradiol-

Table 1. Proportions of cows successfully induced to lactate, the timing of lactogenesis, the levels of production and the plasma prolactin levels of cows treated as indicated

Dissimilar letters within columns and within groups 1*a*–1*c*, 2*a*–2*e*, and 2*f*–2*g* indicate significant difference ($P < 0.05$) between geometric means according to Duncan's multiple range test

Group and treatment[A]	No. of cows successfully induced	Timing of lactogenesis[B] (days)	Plasma prolactin levels (ng/ml) Days 1–7	Days 8–21	Total milk prodn (kg)	Total milk fat prodn (kg)	Total milk protein prodn (kg)
1*a* Parturient twin cows	18 calved				1863[a]	88·9[a]	68·7[a]
1*b* Milked from day 10	8/9				939[b]	60·0[b]	37·5[b]
1*c* Milked from day 24	7/9				823[b]	34·4[b]	29·7[b]
2*a* Standard treatment only	3/5	13·1[a]	16·3[a]	45·7[a]	1766[a]	72·1[a]	59·8[a]
2*b* Oestradiol-17β (20 mg/day) on days 8–11	5/5	20·0[b]	18·5[a]	41·7[a]	895[a]	38·5[a]	32·0[a]
2*c* Bromocryptine (150 mg/day) on days −1, 0, 3, 6, 9, 12, 15 and 18	4/5	29·1[b]	10·9[a]	12·6[b]	1463[a]	67·1[a]	55·4[a]
2*d* Reserpine (5 mg/day) on days 1, 6, 11, 16 and 21	5/5	11·4[a]	104·1[b]	158·5[c]	1392[a]	60·4[a]	49·6[a]
2*e* Reserpine (5 mg/day) on days 1, 4, 7. Dexamethasone (20 mg/day) on days 7, 8 and 9. No standard treatment	0/5		43·7[c]	64·6[a]			
2*f* Reserpine (5 mg/day) on days 1, 6, 11, 16 and 21	6/6				1412[b]	63·7[b]	49·6[b]
2*g* Parturient twin cows	6 calved				2458[a]	108·1[a]	83·1[a]

[A] In experiment 1 all cows received oestradiol-17β and progesterone (0·1 and 0·25 mg/kg body weight respectively) and in experiment 2 a standardized amount of oestradiol-17β (40 mg/day) and progesterone (100 mg/day) for days 1–7.

[B] The period in which the lactose content of the mammary secretion increased at a rate greater than 0·5% per day represents lactogenesis.

17β, progesterone and reserpine (groups 2*d* and 2*f*) had successful lactations. Two of these 11 cows were being retreated after having previously failed to lactate on treatments 2*a* and 2*c* respectively.

All five cows treated with reserpine and dexamethasone alone (group 2*e*) failed to be induced into lactation.

Time of Lactogenesis

Experiment 1

Lactogenesis was arbitrarily defined as the period when the lactose content of the mammary secretion increased at a rate greater than 0·5% per day. In the parturient twins, lactogenesis was in the immediate periparturient period. The timing of lactogenesis was more variable in their induced twin mates as indicated by the larger standard errors of the mean lactose contents (Fig. 1).

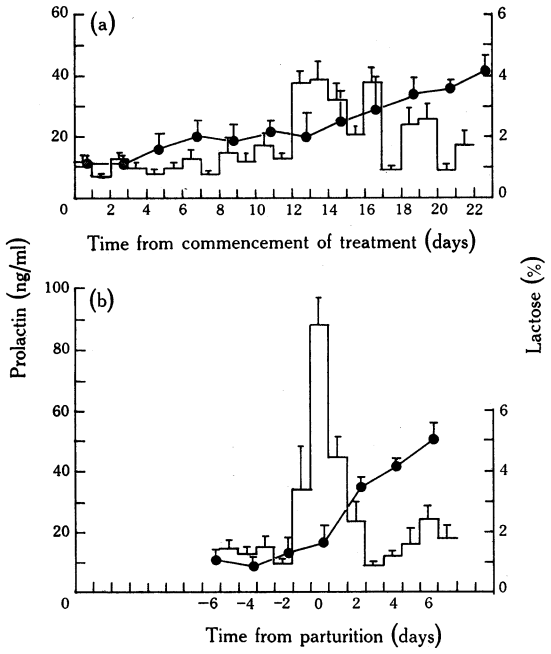

Fig. 1. Changes in plasma prolactin (—) and the concentration of lactose (●) in the mammary secretion of (*a*) three treated cows and (*b*) their three parturient twins. Day 1 is the first day of treatment in the treated twins (*a*) and day 0 is the day of parturition in the parturient twins (*b*). Vertical bars show standard error of the mean.

Experiment 2

Lactogenesis occurred later in the bromocryptine treated cows and cows which had extended oestradiol-17β injections (groups 2*b* and 2*c*) than in cows receiving the standard oestrogen–progesterone and reserpine treatments ($P < 0{\cdot}05$) (Table 1). This implies an association between minimal plasma prolactin and steroid levels for lactogenesis. However, in the bromocryptine treated cows there was no relationship between the onset of lactogenesis and the recovery of plasma prolactin levels (Fig. 2).

Level of Milk Production

Mean milk, milk fat and milk protein yields of cows successfully induced into lactation are given in Table 1. If induced lactations peaked at less than 3 kg milk/day they were considered to have failed and were excluded from the table.

Milking of cows in the period from day 10 to day 24 (group 1*b*) did not improve yields of induced lactation compared to cows milked from day 24 (group 1*c*). These yields were not improved by the pharmacological elevating or lowering of plasma prolactin during the induction period with reserpine (groups 2*d* and 2*f*) or bromocryptine (group 2*c*) respectively, or by additional injections of oestrogen on days 8–11 (group 2*b*).

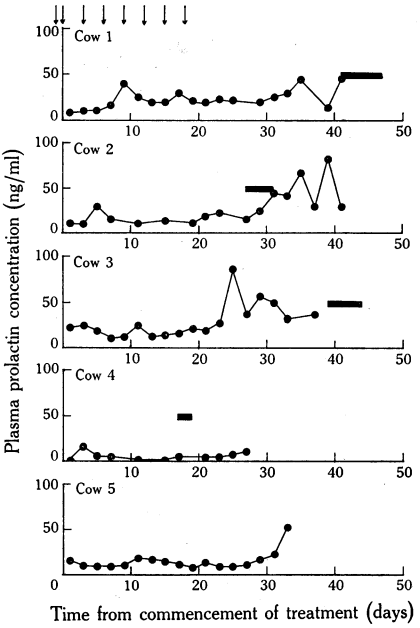

Fig. 2. Plasma prolactin levels (●) and lactogenesis (horizontal bars) for five individual cows treated with bromocryptine (↓). Bars indicate days in which the lactose content of the mammary secretion increased at a rate greater than 0·5% per day. Arrows indicate days on which 150 mg of bromocryptine were injected.

Plasma Prolactin

Experiment 1

The mean plasma prolactin levels of the three induced twins (group 1*a*) were unaltered for the first 12 days (11 ± 1 ng/ml, $n = 96$) but were significantly elevated from days 13 to 21 (25 ± 2 ng/ml, $n = 87$). However, the elevated plasma prolactin levels were still substantially lower than the prolactin peak which occurred at calving in their parturient siblings (87 ± 9 ng/ml, $n = 15$) (see Fig. 1).

Experiment 2

The results presented in Table 1 show that administration of the ovarian steroids (groups 2*a* and 2*b*) resulted in elevated prolactin levels in the post-treatment period (days 8–21) similar to those observed for the induced cows in experiment 1. Treatment of cows with bromocryptine (group 2*c*) significantly lowered plasma prolactin, and treatment with reserpine elevated plasma prolactin compared to all other cows. Cows treated with reserpine and a dexamethasone trigger only (group 2*e*) had plasma prolactin levels intermediate between cows treated with ovarian steroids alone (groups 2*a* and 2*b*) and a combination of ovarian steroids and reserpine (group 2*d*).

Reproductive Performance

In experiments 1 and 2, 35 out of 39 induced cows (90%) and 20 out of 22 parturient cows (91%) conceived and subsequently calved following natural mating.

Animal Health

In the two experiments, a total of 7 out of 48 induced cows had displaced hips which were probably caused by oestrous activity following treatment with oestrogen and progesterone. Two of these cows had to be subsequently destroyed.

Discussion

As with earlier reports (Smith and Schanbacher 1973; Collier *et al.* 1975) milk yields induced by the standard oestrogen–progesterone treatment were variable and substantially lower than those for normal parturient cows. Our attempts to improve induced milk yields by early milking (days 10–24), continuing the administration of oestradiol-17β alone on days 8–11, or by reserpine treatment were unsuccessful. It is difficult to reconcile our failure to confirm a recent report that administration of reserpine improves induced milk yields (Collier *et al.* 1977). There were differences in both the route and timing of the administration of reserpine injections which may have contributed to differences in milk yields, although in the period following oestrogen and progesterone administration when secretory cell development and differentiation is rapidly occurring (Groom *et al.* 1976) plasma prolactin levels were elevated in both studies. All 11 cows treated with oestradiol-17β, progesterone and reserpine had successfully induced lactations compared with 82% (n = 23) of those that did not receive reserpine. Two of these cows had previously failed on treatments without reserpine but residual effects of these treatments may have contributed to their successful induction on retreatment with oestrogen, progesterone and reserpine. It is unlikely that reserpine is acting via hormones other than prolactin because the most probable candidates in the cow, glucocorticoids and growth hormone, are unchanged by reserpine administration (Bauman *et al.* 1977).

Although the lowering of plasma prolactin levels by treatment with bromocryptine during the induction phase delayed lactogenesis, it did not appear to affect subsequent milk yields. That the onset of lactogenesis was not related to the restoration of prolactin levels (Fig. 2) suggests that elevated levels of prolactin are not essential for lactogenesis. However, the delay in the onset of lactogenesis indicates that bromocryptine has an effect on the steps leading up to lactogenesis, presumably by its affect on plasma prolactin levels or by a direct action on the mammary gland. When bromocryptine completely suppressed plasma prolactin levels in two cows treated with oestradiol-17β and progesterone, lactogenesis did not occur (Schams 1976). Low levels of plasma prolactin (greater than 0 but less than 20 ng/ml) during the induction phase therefore appear adequate to prepare the mammary gland for lactogenesis, although it may be delayed.

Dexamethasone trimethylacetate consistently triggers lactation in cows which have mammary glands prepared by prolonged administration of oestrogen and progesterone (Fulkerson and McDowell 1975). That none of the cows treated with reserpine to develop the mammary glands followed by a dexamethasone trigger (group 2*e*) responded indicates that the ovarian steroids may be essential for the induction of lactation in the cow.

In previous reports only half of the cows mated have conceived following the standard oestradiol-17β and progesterone treatment (Collier *et al.* 1975; Erb *et al.* 1976*a*). Although all 39 of the induced cows which were mated had previous reproductive failures, 35 of these cows conceived and subsequently calved indicating

that cows may be artificially induced into lactation for one year and be mated successfully to calve and have a natural lactation the following year. Some problems were encountered with cows causing physical injury to each other during oestrus. These problems could be overcome by management of cows in smaller groups.

In conclusion, this paper confirms earlier work that lactation can be induced in non-pregnant cows with 7 days of oestradiol-17β plus progesterone injections. It also shows that levels of prolactin ranging from less than 10 to greater than 200 ng/ml during the induction phase (days 1–21) have no effect on induced milk yields. Lactogenesis occurred later in cows with low plasma prolactin during this period but high plasma prolactin is not a prerequisite for lactogenesis in the cow. Reserpine appears to have improved the success rate of induced cows although this requires confirmation with a larger number of cows. Contrary to previous reports (Collier *et al.* 1975; Erb *et al.* 1976*a*), the reproductive performance of cows with induced lactations was equal to that of cows with parturient lactations. The production of milk from non-pregnant cows by the artificial induction of lactation for one year, particularly when they can be successfully mated to calve the following season, has practical application if the use of ovarian steroids is approved for this purpose.

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