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The Use of Oestrogen, Progesterone and Reserpine in the Artificial Induction of Lactation in Cattle

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Abstract

Oestradiol-17β, progesterone and reserpine were utilized in three experiments in an attempt to improve the success rate and the magnitude of hormonally induced lactations. Onset of lactogenesis induced by steroid administration was measured by a surge in the lactose content of the mammary secretion and lactations were regarded as being successfully induced if milk yields exceeded 3 kg/day. In all experiments, the dose rate of oestradiol-17β was standardized at the reduced level of 20 mg/day (approximately 0·05 mg/kg body weight daily), and progesterone maintained at 100 mg/day (approximately 0·25 mg/kg body weight daily) for the initial 7 days (days 1–7). The halving of the dose rate of oestradiol-17β from the commonly used 0·1 mg/kg body weight daily eliminated the occurrence of noticeable physical injuries in the 60 animals so treated.

Induced milk yields were not improved by either repeating the standard ovarian steroid treatment on days 16–22, extending the standard treatment for days 8–21 or continuing the steroid treatment at one-quarter the standard rate for days 8–21.

Addition of reserpine to an oestradiol-17β–progesterone treatment does improve the success rate of the hormonal induction of lactation. In experiment 1, 86% (n = 14) of heifers receiving reserpine were successfully induced into lactation compared to 45% (n = 15) which were not. In experiments 2 and 3, 95% (n = 19) of cows receiving both ovarian steroids and reserpine were successfully induced into lactation. However, when we consider only the milk yields of those cows sucessfully induced into lactation, reserpine (3 mg/day) administration on days 1, 6, 11, 16, 21 and 22–28, on days 22–28 alone, or on days 8, 10, 12 and 14 did not enhance induced yields.

In one experiment all eleven cows failed to be induced into lactation and this was attributed to an inadequate dry period (25–43 days).

Introduction

Lactations may be induced in non-pregnant cows and heifers but milk yields are variable and generally less than when following a normal pregnancy (Smith and Schanbacher 1973, 1974). A practical disadvantage of the oestradiol-17β (0·1 mg/kg body weight daily) and progesterone (0·25 mg/kg body weight daily) treatment which is normally used is that these high levels of oestrogens can cause intense oestrous activity which, together with relaxed ligaments, results in physical injuries such as fractured pelves in some cows (Peel *et al.* 1978). When above-average induced milk yields were associated with plasma hormone levels (Erb *et al.* 1976) it was concluded that the oestradiol-17β dose rate could probably be reduced by one-half with no deleterious effect upon milk yields. Such a reduction may also alleviate the problem of physical injuries occurring in treated cows.

Low plasma levels of prolactin in some cows can limit the yield of milk which is induced by steroid treatment (Erb *et al.* 1976). However, reports on the effects of prolactin stimulators such as reserpine and thyrotrophin releasing hormone during the treatment period upon subsequent induced milk yields are conflicting (Mose *et al.* 1975; Collier *et al.* 1977; Peel *et al.* 1978).

Typically, cows which have poor induced lactations have a large number of immature or developing ducts and alveoli (Narendran *et al.* 1974). The standard 7-day steroid treatment generally induced lactations which were at least equal to those induced by traditional schemes which employed low levels of steroid administration (e.g. 0·002–0·010 mg oestrogen/kg body weight daily and 0·002–0·500 mg progesterone/kg body weight daily) for many weeks (Meites 1961; Fulkerson 1978). A longer duration of twice daily injections of high levels of oestradiol-17β (e.g. 0·05–0·10 mg/kg body weight daily) and progesterone (0·25 mg/kg body weight daily) may be required to stimulate the development of a greater mass of secretory tissue which has differentiated for milk production.

The primary objective of our study was to improve induced milk yields by either increasing the duration of oestrogen and progesterone injections or by strategic regimes of reserpine in addition to the ovarian steroids, so as to stimulate prolactin. Pilot trials were conducted to determine a daily dose rate of reserpine which would successfully elevate plasma prolactin without undesirable side effects such as sedation or loss of appetite. It was also anticipated that the potential risk of injury to experimental cows could be minimized by reducing the oestradiol-17β dosage to 20 mg per cow daily which is approximately half the accepted dose rate. A further objective was to confirm earlier indications that reserpine administration improves the reliability of steroid treatments for the induction of lactation.

Materials and Methods

Animals and Management

Fifty cows and 29 heifers were used in a series of experiments conducted during 1977. Cows were either Jerseys or Friesians, and with the exception of pilot trial (*b*), were grazed together on perennial ryegrass–white clover pasture within any experiment. In pilot trial (*b*) the three cows were housed and stall-fed on a mixture of chaffed hay and oats. The cows in pilot trial (*b*) and a further five heifers and seven cows in experiment 1 were fitted with indwelling jugular cannualae and blood was collected under quiet conditions. Machine milking of treated cows in all experiments continued for at least 15 weeks.

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).

Experimental Procedure

Pilot Trials (February–March 1977)

Pilot trial (*a*). To determine the effects of repeated injections of reserpine on the health of the cow, 10 cows in late lactation received a single subcutaneous injection of reserpine (3 mg/day) on 7 consecutive days.

Pilot trial (*b*). Samples of venous blood were taken hourly from three cows for a control period of 24 h, and then hourly for a further 24 h following a single subcutaneous injection of reserpine (3 mg).

Experiments 1 (April), 2 (October) and 3 (November)

To synchronize oestrous cycles, all animals were given a single intramuscular injection of 500 μg of cloprostenol ('Estrumate', I.C.I. Australia Ltd). Seven days later the first oestradiol-17β–progesterone injection was given (day 1). Treatment details for each experiment follow.

Experiment 1

Cows were grouped on the basis of the previous year's milk production and heifers on the basis of live weight so that the mean milk production or live weights of each group were similar. Mean live weights of cows and heifers were 410 and 380 kg respectively. All animals received a uniform steroid treatment of oestradiol-17β (20 mg/day) and progesterone (100 mg/day) irrespective of live weight, administered as a single subcutaneous injection at 0800 h on days 1–7. Additional treatments were as follows.

Group 1*a*: Three cows and seven heifers; no further treatment.

Group 1*b*: Two cows and eight heifers; subcutaneous injections of oestradiol-17β (20 mg/day) and progesterone (100 mg/day) at 0800 h on days 16–22.

Group 1*c*: Three cows and seven heifers; subcutaneous injections of reserpine (3 mg/day) at 0800 h on days 22–28.

Group 1*d*: Three cows and seven heifers; subcutaneous injections of reserpine (3 mg/day) at 0800 h on days 1, 6, 11, 16, 21 and 22–28.

Group 1*e*: Six parturient heifers; no hormone treatment.

The calving of six contemporary Friesian heifers (group 1*a*) coincided with the induction of lactation in the 29 heifers. The induced milk yields could therefore be compared with parturient yields, although the parturient heifers were not grouped on the basis of live weight with the induced heifers.

Experiment 2

Five sets of identical twin cows were used and were allocated to groups on the basis of monozygosity. The basal treatment was as for animals in experiment 1 except that the steroids were administered in a divided dose (10 mg oestradiol-17β plus 50 mg progesterone) at 0800 and 1700 h on days 1–7. Additional treatments were as follows.

Group 2*a*: Single intramuscular injections of reserpine (3 mg) at 0800 h on days 8, 10, 12 and 14.

Group 2*b*: Single intramuscular injections of reserpine (3 mg) at 0800 h on days 8, 10, 12 and 14. Subcutaneous injections of oestradiol-17β (2·5 mg) plus progesterone (12·5 mg) at 0800 and 1700 h on days 8–21 (i.e. one-quarter the basal steroid treatment).

Experiment 3

Five sets of identical twin cows were allocated to groups and received the same basal treatment as for cows in experiment 2 (i.e. 10 mg oestradiol-17β plus 50 mg progesterone at 0800 and 1700 h on days 1–7). Additional treatments were as follows.

Group 3*a*: Single intramuscular injections of reserpine (3 mg) at 0800 h on days 8, 10, 12 and 14 (as for experiment 2).

Group 3*b*: Subcutaneous injections of oestradiol-17β (10 mg) plus progesterone (50 mg) at 0800 and 1700 h on days 8–21 followed by intramuscular injections of reserpine (3 mg) at 0800 h on days 22, 24, 26 and 28.

Collection of Samples

Pilot trial (*b*). Blood samples were taken from three cows by means of indwelling jugular cannulae at 1-h intervals for a control period of 24 h and then for a further 24 h following the administration of reserpine (3 mg).

Experiment 1: A mammary secretion was expressed at 0800 h on days 1, 7, 11 and then alternate days until day 32. Blood samples were collected from 16 animals at 0900 h on days 18–32.

Experiment 2: A mammary secretion was expressed at 0800 h on days 1, 7 and 15.

Experiment 3: A mammary secretion was expressed at 0800 h on days 1, 7, 11 and then on alternate days until the lactose content of the secretion had risen to levels similar to normal milk.

Chemical Analysis

Individual milk yields were recorded at each milking and once per week morning and evening milk samples were collected and combined to be analysed for milk fat ('Milko Tester' Mk III, Foss Electric, Hillerod, Denmark) and milk protein ('Pro-Milk' Mk II, Foss Electric, Hillerod, Denmark).

We have previously defined lactogenesis as the period when the lactose content of the mammary secretion increases at a rate greater than 0·5% per day (Peel *et al.* 1978). The lactose content of these mammary secretions 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-B-P2 standard. The within-assay coefficient of variation was less than 15% and the between-assay coefficient of variation ranged from 12 to 25% for different samples. To offset this between-assay variation, all samples to be compared were measured in the one assay.

Statistical Analysis

In pilot trial (*b*) the mean plasma prolactin levels for the 24-h periods both immediately before and after reserpine administration were compared by paired *t*-tests.

In previous work (Peel *et al.* 1978) induced milk yields in the 11-week period (weeks 5–15) of cows and heifers were significantly correlated to total induced milk yields ($r = 0\cdot92$, $n = 33$). Based on this finding, differences between groups were determined using the 11 weeks from week 5 to week 15, which then allowed the cows to be utilized in other experiments after week 15.

Data from experiment 1 were analysed in a one-way analysis of variance. Variances within groups were homogenous and mean milk yields, milk fat and milk protein, day of lactogenesis and plasma prolactin were compared using Duncan's multiple range test. In experiment 2 (five sets of twins) and experiment 3 (four sets of twins) a two-way analysis of variance was used to determine the significance of treatments.

Results

Pilot Trials

Effect of reserpine treatment (pilot trial a)

In cows given reserpine (3 mg/day) on 7 consecutive days, body temperatures before and after 7 days of treatment were 38·5°C and 38·4°C respectively (s.e. ±0·1°C). There was no change in daily milk yields, no loss of appetite and overt signs of drowsiness were not observed.

Changes in plasma prolactin (pilot trial b)

The mean plasma prolactin level increased from 33·3±3·9 ng/ml ($n = 66$) in the 24-h control period to 54·4±3·6 ng/ml ($n = 71$) ($P<0\cdot05$) in the 24 h following a single reserpine injection (3 mg) in three cows.

Experiments 1, 2 and 3

Number of cows induced to lactate

Animals which produce more than 3 kg milk per day were regarded as having successful lactations. In experiment 1, none of the treated cows and 19 out of 29 treated heifers lactated (see Table 1). In experiments 2 and 3, all cows with the exception of one in group 3*b* lactated.

Time of lactogenesis

In experiment 1, lactogenesis was delayed approximately 10 days by steroid administration on days 16–22 (see Table 2). In experiment 2, injections were continued on days 8–21 at one-quarter the standard rate. Although there were too few samples collected to use our normal criteria for lactogensis, there appeared to be no difference

Table 1. The effect of varying regimes of oestrogen, progesterone and reserpine on the initiation of lactation and subsequent milk production in cows and heifers

Dissimilar superscript letters within columns and within groups 1, 2 and 3 indicate significant differences ($P<0\cdot05$) between means according to t-tests

Group	Treatment[A]	No. of animals successfully induced[B]	Range of dry periods (days)	Timing of lacto-genesis[C] (days)	Plasma prolactin levels (ng/ml) days 18–32	Yields for the period weeks 5–15 (kg per cow)		
						Milk	Milk fat	Milk protein
1*a*	Steroid treatment only	3/10 (3/7)	25–43	18·3	$17\cdot3^{b}$	550^{b}	$27\cdot3^{ab}$	$18\cdot8^{b}$
1*b*	Steroid treatment repeated days 16–22	4/10 (4/8)	43	28·7	$24\cdot4^{b}$	551^{b}	$28\cdot1^{ab}$	$19\cdot3^{b}$
1*c*	Reserpine (3 mg/day) days 22–28	6/10 (6/7)	25–43	19·0	$17\cdot4^{b}$	489^{b}	$24\cdot7^{ab}$	$16\cdot8^{b}$
1*d*	Reserpine (3 mg/day) days 1, 6, 11, 16, 21, 22–28	6/10 (6/7)	43	19·8	$30\cdot8^{a}$	525^{b}	$23\cdot0^{b}$	$17\cdot5^{b}$
1*e*	Parturient heifers	6 calved				1033^{a}	$33\cdot0^{a}$	$27\cdot9^{a}$
2*a*	Reserpine (3 mg/day) days 8, 10, 12 and 14	5/5	151–343			797^{a}	$36\cdot4^{a}$	$28\cdot1^{a}$
2*b*	Reserpine (3 mg/day) days 8, 10, 12 and 14; oestradiol-17β (5 mg/day) and progesterone (25 mg/day) days 8–21	5/5	151–322			748^{a}	$31\cdot9^{a}$	$25\cdot3^{a}$
3*a*	Reserpine (3 mg/day) days 8, 10, 12 and 14	5/5	106–239	$10\cdot8^{b}$		707^{a}	$31\cdot0^{a}$	$25\cdot7^{a}$
3*b*	Steroid treatment repeated days 8–21; reserpine (3 mg/day) days 22, 24, 26 and 28	4/5	162–239	$25\cdot3^{a}$		452^{b}	$21\cdot4^{b}$	$16\cdot2^{b}$

[A] All treated cows received a steroid treatment of oestradiol-17β (20 mg/day) and progesterone (100 mg/day) for days 1–7.

[B] Values in parentheses exclude cows with dry periods of less than 50 days.

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

between groups in its timing. The lactose contents of the secretions increased from 0·90 and 0·75% on day 7 to 2·6 and 2·9% on day 15 in groups 2*a* and 2*b* respectively. In experiment 3, lactogenesis was delayed for approximately 15 days by the continued administration of high levels of oestradiol-17β and progesterone (i.e. the standard rate) from day 8 to day 21 (Table 1). In three of the five cows in group 3*b* there were two surges in the lactose content of the mammary secretion. The first occurred between days 10 and 13, reaching approximately 3·5% and then gradually declining until the second lactose surge which was accompanied by copious milk production at the cessation of the steroid administration.

Plasma prolactin

In experiment 1 blood plasma levels of prolactin were low for all groups in the period days 18–22 (Table 1).

Level of milk production

There were no differences in milk yields or milk fat and milk protein yields between the four groups which received hormones in experiment 1. The milk yield and milk protein production for these four groups were lower ($P<0\cdot05$) than for six contemporary Friesian heifers which calved at about the same time. There were no significant differences in milk fat production between groups.

In experiment 2 extension of steroid treatment, but at a lower dose rate, did not increase milk production (Table 1). In experiment 3 extension of the steroid treatment at the normal rate to day 21, and delaying administration of reserpine until the completion of steroid treatment (group 3*b*) resulted in lower induced milk yield, milk fat and milk protein ($P<0\cdot05$) than in group 3.

Mean yields of induced milk, expressed as a percentage of the previous parturient milk yields, were $64\pm3\%$ ($n = 12$) for cows treated with reserpine on days 8, 10, 12 and 14 (experiments 2 and 3) and $57\pm5\%$ ($n = 18$) for cows given the high oestrogen–progesterone treatment alone ($P<0\cdot1$) (Peel *et al.* 1978).

Discussion

Previous unpublished observations had indicated that seven successive daily injections of reserpine (5 mg/day) resulted in drowsiness, loss of appetite and the lowering of body temperature in cows with an average body weight of approximately 400 kg (i.e. a daily dose of 12·5 μg/kg body weight). The administration of reserpine (3 mg/day) did not cause these adverse symptoms. Although plasma prolactin was elevated for at least 12 h at this reduced level of reserpine, it did not reach the sustained high levels reported by Bauman *et al.* (1977) and Peel *et al.* (1978) for cows receiving reserpine at 5 mg/day. Plasma prolactin levels in animals which were treated with daily injections of reserpine (3 mg/day, groups 1*c* and 1*d*) were not significantly different from those in cows which received no reserpine treatment (groups 1*a* and 1*b*). It is possible that prolactin may have peaked and returned to basal levels because blood samples were not collected until 24 h after the last reserpine injection.

The risk of physical injuries occurring in cows treated with high levels of oestrogens was eliminated in these experiments by reducing the dosage of oestradiol-17β from 40 to 20 mg/day. None of the 60 animals treated with this lower level sustained any physical injuries compared to 7 out of 48 on the higher level in experiments reported previously (Peel *et al.* 1978).

The length of the dry period appears critical to the successful induction of lactation. In experiment 1 all 11 cows failed to be induced into lactation when the dry period ranged from 23 to 43 days. In contrast, 90% ($n = 64$) of cows with dry periods ranging from 82 to 343 days have been successfully induced into lactation [experiments 2 and 3 and in the experiments previously reported (Peel *et al.* 1978)]. It is apparent that the minimum dry period required before cows can be successfully induced into lactation is between 43 and 82 days. Cows which were treated but had dry periods of 43 days or less (experiment 1) are excluded from the remainder of this discussion. Our finding that only 66% ($n = 29$) of the heifers were successfully induced into lactation (experiment 1) indicates that the administration of the ovarian steroids on a once daily basis is probably inferior to giving them in a divided morning and evening regime.

We have previously found that all 11 animals treated with ovarian steroids plus reserpine successfully lactated compared with 27 out of 33 when reserpine was not administered (Peel *et al.* 1978). Including those results with the ones reported in this paper allows us to show that 93% ($n = 45$) of animals are successfully induced into lactation when reserpine is included in the regime compared to only 71% when it is not included. This confirms the reports of Collier *et al.* (1977) and Peel *et al.* (1978) that addition of reserpine to the ovarian steroid treatment improves the success rate of the hormonal induction of lactation. The mode of action of reserpine in improving this success rate is uncertain. Although reserpine elevates the plasma prolactin in cows, we have previously demonstrated that lactations may be hormonally induced even when basal plasma prolactin levels are suppressed to less than 20 ng/ml by bromocryptine administration (Peel *et al.* 1978).

When we consider only those animals with successfully induced lactations, reserpine administered on days 1, 6, 11, 16 and 21 or on days 22–28 as well did not improve yields. Secretory cells should be responsive to circulating prolactin in the period days 22–28 as oestrogen and progesterone levels are then low (Erb *et al.* 1976). It is apparent that basal prolactin levels in this period are adequate for lactogenesis. Furthermore, by expressing the cows' induced yields as a percentage of their previous parturient yields, we can show that cows receiving reserpine (3 mg/day) on days 8, 10, 12 and 14 (groups 2*a*, 2*b* and 3*a*) produced $64 \pm 3\%$ ($n = 12$) which is not significantly different from that of cows receiving no reserpine ($57 \pm 5\%$, $n = 18$) which was reported previously (Peel *et al.* 1978). In this comparison the cows receiving reserpine on days 8, 10, 12 and 14 were receiving only half the level of oestradiol-17β (20 mg/day) as cows not receiving reserpine. We are also assuming that cows grazing pastures have repeatable lactations in successive years. In this instance we considered the cows to be fully fed in both years. Despite these confounding factors, we tentatively conclude that addition of reserpine does not improve milk yields of those cows successfully induced into lactation. However, the definitive experiment comparing animals treated with the equivalent amount of ovarian steroids, with and without reserpine administration on days 8, 10, 12 and 14, remains to be done.

The postponement in onset of lactogenesis by 10 days when additional oestradiol-17β and progesterone was administered from day 16 to day 22 (group 1*b*) and from day 8 to day 21 (group 3*b*) reflected the inhibitory role of high plasma progesterone on lactogenesis (Kuhn 1969; Hartmann *et al.* 1973). Conversely, the administration of reserpine (group 2*b*) triggered lactogenesis even when progesterone (25 mg/day) was administered concurrently. This agrees with the findings of Fulkerson *et al.* (1976) that in the ewe, positive lactogenic stimuli can trigger lactogenesis even in the presence of high circulating progesterone levels. The initial surge in the lactose content of three out of five cows in group 3*b* is surprising as it occurred during the administration of high levels of oestradiol-17β and progesterone and without a positive hormonal stimulus. However, copious milk secretion accompanied only the second lactose surge which did not occur until after the cessation of steroid treatment.

The hypothesis that prolonging the oestrogen–progesterone treatment (groups 1*b*, 2*b* and 3*b*) would result in a greater mass of mature secretory tissue and would be reflected in higher yields of hormonally induced milk, proved to be incorrect. In fact, twins which were given oestradiol-17β (20 mg/day) and progesterone (100 mg/day) for 21 consecutive days had lower milk yields than their siblings (group 3*a*) which were treated similarly for only 7 days although differences in the timing of reserpine administration may be confounding this result. In the parturient cow there is a 10-fold increase in plasma oestrogen levels over the last month of pregnancy. The largest incremental change is during the 2–5 days prior to parturition (Hoffman *et al.* 1973; Smith *et al.* 1973). Since only 7 days of steroid treatment were just as effective as much longer regimes in terms of inducing lactation, it is probably a relatively short period of high levels of steroids that is essential for secretory tissue development and lactation.

In conclusion, average milk yields which approximated to 60% of expected parturient yields were reliably and safely induced with a combination of oestradiol-17β, progesterone and reserpine, provided the cows had a dry period of 82 days or more and the level of oestradiol-17β was approximately 0·05 mg/kg live weight daily. As milk yields were not increased by extending the duration of the steroid treatment, the more economical 7-day treatment with injections given twice daily was found to be optimal.

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