ARTIFICIAL INDUCTION OF LACTATION IN CATTLE: INITIATION OF LACTATION AND ESTROGEN AND PROGESTERONE CONCENTRATIONS IN MILK

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ABSTRACT

Thirty heifers were given 11 subcutaneous injections of 5 mg estradiol benzoate and 200 mg progesterone every 3 d to develop their mammary glands. Three days later groups of animals were treated with 1) 20 mg dexamethasone twice, 2) 500 µg cloprostenol thrice, 3) dexamethasone and cloprostenol, 4) oxytocin 4 IU six times, or 5) no further injections. Two further groups of six heifers each (6 and 7) were treated in a manner similar to groups 1 and 3 except the dose of estrogen to develop their mammary glands was doubled to 10 mg/3 d. Six lactating first calf heifers were controls (8). The proportion of animals lactating, combined milk yield for each group (kg), and mean days lactated were: 1) 5/6, 3831, 142; 2) 1/6, 912, 195; 3) 6/6, 4898, 194; 4) 3/6, 1066, 128; 5) 1/6, 293, 154; 6) 6/6, 6109, 130; 7) 6/6, 6265, 130; and 8) 6/6, 19,190, 251. The lactogenic response to dexamethasone and oxytocin is similar to that in sheep, but the response to cloprostenol indicates a species difference. Intensive blood sampling before and after injection of hormones, intended to trigger lactogenesis, showed that plasma prolactin rose to peaks above 210 ng/ml in cows of groups 2, 3, and 4 and were unchanged from the base below 40 ng/ml in groups 1 and 5. Monitoring of steroids after induction treatment showed estradiol-17β ranged between 35 and 400 pg/ml and 20 to 80 pg/ml in mammary secretion and plasma and progesterone concentrations were less than at diestrus. Low estrogen in the induction regimen for groups 1 to 5 reduced abnormal estrous behavior, but resulting milk production was 20% of normal. Increased estrogen improved milk production but also increased adverse side effects. Further refinement of the low dose steroid treatment is required.

INTRODUCTION

Earlier attempts to induce lactation artificially in ruminants by estrogen alone or combined with progesterone (22, 31) often met with low or variable rates of success and required lengthy treatment, which limited commercial application of those hormones. Smith and Schanbacher (27, 28) and Fulkerson and coworkers (10, 13, 14) reported substantial milk production from ruminants following hormone treatment for much shorter periods (7 to 40 d).
The method of Smith and Schanbacher (27, 28) has the advantage of a short treatment (7 d), and the high doses of estrogen administered are enough to initiate lactogenesis. However, this method also has produced commercially unacceptable side effects, especially abnormal estrous behavior and reduced fertility, cystic follicles, cystic corpora lutea, and chronic vaginal prolapse (23, 25, 30).

Fulkerson and coworkers used a more gradual build-up of hormones similar to endocrine events during normal mammogenesis (pregnancy) and lactogenesis (parturition) for inducing lactation (11). Lower doses of estrogen in a combined estrogen-progesterone priming phase were used over longer treatment than by Smith and Schanbacher (27, 28). Fulkerson and coworkers also showed that a number of hormones may provide a “trigger” to lactogenesis in ewes including glucocorticoids, oxytocin, prostaglandins, and estrogen (12, 13, 15, 16). No studies have been attempted in cattle with similar hormones after low-dose steroid regimens to develop mammary glands, although several of these hormones have been used with the Smith and Schanbacher (27, 28) regimen.

Our study attempted to modify the hormonal regimen used to induce lactation to remove some of the adverse side effects. To this end, the amount of estrogen used to “prime” the mammary glands was reduced to alleviate problems with continuous mounting behavior, and the ability of different hormones to initiate a less variable lactational response was tested. In addition, endogenous hormone changes around the time of lactogenesis and steroid residues in milk were measured.

MATERIALS AND METHODS

Animals

Forty-two nulliparous Friesian heifers that had shown cyclical estrous activity but had not been mated were in the experiment in winter as this was the time of year when typically infertile animals induced to lactate artificially were detected normally in dry land dairy herds. They ranged in age from 24 to 31 mo with a mean liveweight 376.4 ± 10.0 kg, and body condition score 4.8 (5). Six first-calf heifers served as controls (weight 390.0 ± 12.7 kg). All animals were fed adequately at pasture.
milked twice daily. Milk yields and samples for analysis were taken from all heifers on d 4, 7, 14, 21, 28, and 42 after treatment or calving. Thereafter milk yields were measured, and samples were taken every 30 d until d 180 (groups 1 and 3), until d 280 (group 8), or until d 130 (groups 6 and 7), when these heifers were dried off.

Milk Sampling

Samples of mammary secretion were analyzed for protein, lactose, and fat by Milko-Scan milk analyzer. Somatic cell numbers were counted in milk samples at 14 and 28 d and thereafter at monthly intervals. Milk samples also were taken from the same two heifers every 3 d until d 28 (groups 1, 4, and 5) or until d 36 (groups 2 and 3). These were stored at -15°C and assayed subsequently for estradiol -17β (E$_2$) and P. No samples were taken from heifers of groups 6 and 7 for E$_2$ and P assay.

Blood Sampling

Blood samples were taken by means of indwelling jugular cannulae left in place for the first 6 d from four heifers in groups 1 to 4, and from five heifers from group 5 at the following times: d 1, 1600 h; d 2, 0800, 1600 h; d 3, 0700, 0800, 0830, 0900, 1000, 1200, 1600, 2000 h; d 4, 0800, 1600 h; d 5, 0800 h. Plasma from these samples was stored at -15°C and assayed subsequently for prolactin and cortisol. In addition, further blood samples were taken by venipuncture every 3 d until d 28 (groups 1, 4, 5) or d 36 (groups 2, 3) and plasma assayed for E$_2$. No blood samples were taken from heifers of groups 6, 7, and 8 for prolactin, cortisol, or E$_2$ assay.

Hormone Assays

Cortisol. Plasma cortisol was determined by the radioimmunoassay procedure of Chandler et al. (1) with minor modifications. The cortisol antibody was raised in a rabbit by injection of a cortisol-21-hemisuccinate (Schwarz-Mann) conjugated to bovine serum albumin (BSA). The main crossreactions were with corticosterone (8%), cortisone (7%), and 11-deoxycorticisol (7%) (E.D. Horgan, Royal Perth Hospital, personal communication). Limit of detection of the assay was 40 pg/tube, coefficient of variation interassay 9%, and coefficient of variation intraassay 10%.

Prolactin. Prolactin in blood plasma was measured by the solid phase radioimmunoassay method of Fell et al. (9). Sensitivity of the assay was 1 ng/ml. Three standard samples were assayed in a number of assays to determine interassay precision. These samples were (mean ± SE) 254 ± 8.8 (n = 9), 8.2 ± 1.1 (n = 14), and 3.9 ± 1.1 (n = 12) ng/ml prolactin with corresponding coefficients of variation of 10, 7, and 5%.

Progestrone. Milk progesterone was assayed by radioimmunoassay by the method of Kulski et al. (20) with minor modifications. The progesterone antibody was raised in a sheep by injection of a progesterone-11-BSA conjugate. The main crossreactions were with deoxycorticosterone (21.2%) and 11-keto-progesterone (17.1%), and all other crossreactions were less than 10% (R. J. Scaramuzzi, personal communication). The limit of detection of the assay was 8 pg/tube and the coefficient of variation intraassay 10.1%. Mean recovery (± SE) of [3H]progesterone from mammary secretion was 96.0 ± 1.4% (n = 12).

Estradiol-17β in Milk. Duplicate .5-ml samples were mixed with 50 μl of .1 M sodium hydroxide containing 1267 dpm of labeled estradiol-17β (1, 2, 6, 7, 16, 17)-[3H] E$_2$, (New England Nuclear, Searle Nucleonics, Sydney, Australia) and extracted with 2 ml of diethyl ether. The extracts were evaporated, redissolved in .5 ml of hexane/ethylacetate (90:10 vol/vol), and chromatographed on .8 by 8-cm columns of Lipidex-5000. Samples were eluted sequentially with hexane/ethylacetate (90:10 vol/vol), then 4.5 ml (60:40 vol/vol), and finally with 2 ml of ethylacetate. The final eluate was evaporated and redissolved in 270 μl of buffer (.01 M phosphate, .15 M sodium chloride, .1% (wt/vol) BSA, pH 7.5). Two samples of 100 μl were removed for assay, and 50 μl counted to determine recovery. The antiserum used was raised in a rabbit and exhibited significant crossreactions only with estriol (1.7%) and estrone (.6%). A double antibody technique was used to separate free from bound hormone. The limit of detection of the assay was 6.0 pg/tube. Hormone in samples was corrected for individual recoveries (mean ± SE, 42.6 ± .7%; range 16 to 61; n = 181) but
not for assay blanks. Intraassay coefficient of variation was 14% (n = 6).

**Estradiol 17β in Plasma.** Samples of plasma were assayed as described but without chromatography. A pooled sample containing 69.6 ± 4.98 pg/ml of estradiol was used to estimate the coefficient of variation within assay of 16% (n = 6, one assay). The mean (± SE) recovery of [³H]estradiol from plasma was 97.0 ± 1.0% (n = 10).

**Animal Behavior and Reproduction**

Heifers were observed closely for abnormal estrous behavior during hormone treatment and in early lactation. Those in groups 1 to 5 were examined by rectal palpation 2, 4, and 7 wk after commencement of hormone injections to determine the state of reproductive tract and ovarian activity. All heifers were inseminated artificially as they came into estrus after either 45 d of milking in groups 1 to 5 or after 60 d in groups 6 to 8. If they had not conceived after two inseminations, they were mated to a Friesian bull.

**Statistical Analysis**

Treatment differences among groups in milk yield, fat, lactose, protein content, and days lactated were ascertained by separate analysis of variance on overall figures. Time effects were tested by analysis of variance of separate day (sampling date) data. In general, treatment effects did not alter with time, and so overall figures are presented. Days to first estrus and days to conception after hormone treatment were analyzed by analysis of variance and Student's t test (29). Responding groups were compared by analysis of variance at specific sampling times for differences in hormone concentrations.

**RESULTS**

**Milk Production and Composition**

Total yield for each group, proportion of animals responding to treatment, and mean milk yields of heifers induced to lactate (groups 1 to 7) and after a normal pregnancy (group 8) are in Table 1.

Production of milk (kg/cow) of heifers in groups 6 and 7 (which was about 60% of normal up until d 130) was higher than that of heifers in the other induced groups (P<.05). Similarly, milk production from heifers in groups 1 and 3 was higher than that of heifers in group 4 (P<.05). Appreciable quantities of milk (> 1 kg/d) were produced only from one heifer in each of groups 2 and 5. Average milk production from heifers induced to lactate after a "low estrogen" priming phase (groups 1 to 5) was only 20 to 26% of that for normally calved heifers (group 8) (P<.001). Lactation length was also shorter (P<.05).

**TABLE 1. Milk production for each group of heifers.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total milk produced/group (kg)</th>
<th>Animals lactating at d 30 (n)</th>
<th>Milk production of lactating heifers (kg/cow)</th>
<th>Days lactated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3831.4</td>
<td>5/6</td>
<td>766.3 ± 499.98a</td>
<td>141.6 ± 46.4</td>
</tr>
<tr>
<td>2</td>
<td>912.0</td>
<td>1/6</td>
<td>912.0 ± 195c</td>
<td>195 ± 32.7c</td>
</tr>
<tr>
<td>3</td>
<td>4897.5</td>
<td>6/6</td>
<td>816.3 ± 62.1a</td>
<td>194.2 ± 8.4b</td>
</tr>
<tr>
<td>4</td>
<td>1065.8</td>
<td>3/6</td>
<td>355.1 ± 169.9b</td>
<td>128 ± 32.7b</td>
</tr>
<tr>
<td>5</td>
<td>293.0</td>
<td>1/6</td>
<td>293.0 ± 72.4c</td>
<td>154 ± 6.1c</td>
</tr>
<tr>
<td>6</td>
<td>6109.0</td>
<td>6/6</td>
<td>1018.2 ± 25.5c</td>
<td>250.8 ± 6.1c</td>
</tr>
<tr>
<td>7</td>
<td>6265.0</td>
<td>6/6</td>
<td>1044.2 ± 72.4c</td>
<td>250.8 ± 6.1c</td>
</tr>
<tr>
<td>8</td>
<td>19,190.9</td>
<td>6/6</td>
<td>3198.5 ± 242.6d</td>
<td>250.8 ± 6.1c</td>
</tr>
</tbody>
</table>

a,b,c,d Numbers in the same column followed by different superscript letters differ (P<.05).

1 All heifers in groups 6 and 7 were lactating at the termination of this part of the experiment.
A mammary secretion that was either watery or yellow and viscous and high in protein (11 to 13% wt/vol) was expressed from all heifers that did not produce milk. Neither fat nor lactose content of this secretion rose above 1.5% (wt/vol), and all animals were dried off by d 20.

Milk composition from heifers of all groups was similar except for the initial 14 d when mammary secretion of heifers successfully induced to lactate (groups 1, 3, 4, 5, and 7) was higher \( (P<.05) \) in protein and fat and lower \( (P<.05) \) in lactose than normally calved heifers.

Somatic cell numbers of milk were low \( (<10^8 \text{ counts/ml}) \) and did not vary significantly among groups during the experiment.

Liveweight and Condition

There was no significant difference, assessed monthly among groups in liveweight or condition of animals during lactation.

Estrous Behavior and Reproductive Performance

Rectal palpation of reproductive organs of all heifers receiving hormone injections, at 2 and 4 wk after injections began, showed ovaries had regressed to a stage similar to that in prepubertal heifers. In contrast, 3 wk after cessation of hormone treatment, in all heifers examined at least one ovary was judged active. Regular cyclical estrous activity was observed subsequently in most heifers, and there were no significant differences between groups in either days to first estrus or conception. Only 6 of 30 heifers in groups 1 to 5 (the “low estrogen” priming phase groups) exhibited abnormal estrous behavior either during the latter stages of steroid treatment or within 10 d of treatment. Incidence of abnormal estrous behavior was higher in groups 6 and 7 (about 50%), but despite this, normal cycling activity had resumed by an average of 27 d after treatment, and 11 of the 12 heifers had conceived by d 73. Overall, three heifers were not in calf 90 d after treatment had ceased.

Hormones

**Prolactin.** Prolactin in plasma from all heifers was low (10 to 50 ng/ml) before hormones administered to initiate lactation. Within an hour of injection of either cloprostenol (group 2), dexamethasone plus cloprostenol (group 3), or oxytocin (group 4), prolactin rose to peaks (mean ± SE) of 265.4 ± 72.8, 279.5 ± 63.4, and 216 ± 58.8 ng/ml (Figure 1), which were not significantly different \( (SE = 23.17, F \text{ ratio } = .43) \). By 24 h after commencement of injections to trigger lactogenesis, blood plasma prolactin fell to 25 to 50 ng/ml for all groups except animals administered oxytocin, which remained higher at 50 to 100 ng/ml. Prolactin in heifers from group 1 (dexamethasone only) did not rise significantly after injections to trigger lactogenesis and neither did plasma prolactin in heifers that had no further injections (group 5).

![Figure 1](https://example.com/figure1.png)  

*Figure 1.* Concentrations of prolactin in plasma of heifers in treatment groups 1 to 5. Arrows indicate times of injections. Plotted points represent means for four heifers (group 5: five heifers), and standard errors are vertical bars. a) Group 1 (○—○), dexamethasone injection; group 3 (●—●), dexamethasone and cloprostenol injection; b) group 2 (Δ—Δ), cloprostenol injection; group 4 (▲—▲), oxytocin injection; group 5 (○—○), no further injections.
Figure 2. Concentrations of cortisol in plasma of heifers in treatment groups 1 to 5. Arrows indicate times of injections. Plotted points are means for four heifers (group 5: five heifers), and standard errors are vertical bars. a) Group 1 (○—○), dexamethasone injection; group 3 (●—●), dexamethasone and cloprostenol injection; b) group 2 (Δ—Δ), cloprostenol injection; group 4 (▲—▲), oxytocin injection; group 5 (○—○), no further injections.

Cortisol. Apart from the marked fall of plasma cortisol immediately after injection of dexamethasone in groups 1 and 3 (Figure 2a), cortisol of heifers in all other groups was variable and did not change significantly over the “trigger” phase.

Progesterone in Mammary Secretion. Concentration of P in mammary secretion was generally low (between 1.0 and 3.0 ng/ml (Figure 3) and less than that normally in milk of cows during the estrous cycle [range .5 to 15 ng/ml (21)]. Cyclical estrous activity resumed between d 20 and 30 in 5 of the 10 heifers from which samples were taken, and this was confirmed by measurement of P.

Estradiol-17β in Mammary Secretion and Plasma. There was no significant effect of treatment used to initiate lactation in groups 1 to 5 on E₂ in milk or plasma, so the concentrations represent the mean (± SE) of 10 determinations. Concentrations of E₂ in milk ranged between 35 to 400 pg/ml and were more variable than plasma but had fallen to below 65 pg/ml by 36 d after the end of induction treatment (Figure 3). Mean E₂ in plasma ranged from 40 to 80 pg/ml between days 20 to 30, then fell to below 30 pg/ml (Figure 3).

DISCUSSION

This experiment highlights the importance of adequate estrogen in a low dose steroid treatment to develop mammary glands and shows that with this regimen lactational performance is affected markedly by the hormones used to initiate lactation. Results from heifers in groups 1 to 5 allow direct comparison of effectiveness of each of the lactogenic stimuli at the dose rates reported. Most milk was produced by animals injected with 10 mg E₂ and 200 mg P every 3 d over 28 d to prime their mammary glands and then treated with dexamethasone (either alone or in combination with cloprostenol). When a lower dose of estrogen was used to develop mammary glands, the same hormones were the most effective at initiating lactation. Oxytocin-treated heifers produced only half as much milk, and only one heifer treated with either cloprostenol or no further hormone lactated.

The inability of cloprostenol to initiate lactation in cattle confirms other work in cattle (17) and contrasts with results of experiments on sheep (16).

Figure 3. Concentrations of estradiol-17β (○—○) and progesterone (●—●) in milk and of estradiol-17β (▲—▲) in plasma of two heifers from each of treatment groups 1 to 5. Plotted points are means for 10 heifers, and standard errors are vertical bars. Measures for d 30 to 36 were determined on four heifers only.
Oxytocin appears to be a weak lactogenic stimulus in cattle as only three of six treated heifers produced small amounts of milk (peak yield ca. 2.5 kg/d). This agrees with one study in sheep (12) but is at variance with other work in sheep (4, 15). In cattle oxytocin simply may act by preventing the build-up of mammary secretion in alveolar cells.

The influence of milking with associated oxytocin release may explain why one heifer lactated in each of groups 2 and 5. A similar result was reported in ewes (13). Elevation of glucocorticoids could not be linked to initiation of milk secretion heifers from groups 2 and 5 or in the three of six heifers injected with oxytocin that lactated. Although variable, there were no significant changes of cortisol in groups 2, 4, or 5 around time of injection of hormones at the end of the priming phase.

Prolactin in plasma rose significantly for 120 to 180 min in response to cloprostenol injection, but five of these six heifers did not lactate. Of course, the relatively short duration of elevated prolactin may have mitigated against a substantial lactation response. However, this result and the fact that heifers may be induced to lactate while being treated with the prolactin inhibitor CB154 (26), lend some support to the theory that only low prolactin is required to induce lactation with a regimen similar to current work. Results from heifers in group 1 agree with this. They did not show a prolactin peak in response to injections of dexamethasone, although substantial amounts of milk were produced.

These results are at variance with several studies designed to modify the Smith and Schanbacher induction regimen to improve milk yields and reduce variability of response. Both thyroid-releasing hormone and reserpine have stimulated prolactin secretion, reduced variability of response, and on some occasions increased milk yield (2, 3). The explanation of these conflicting results probably lies in the different physiological concentrations of steroid hormones used to prime the mammary glands in the current work and the regimen outlined by Smith and Schanbacher. It appears with the latter regimen that E2 and P prime the mammary gland so that it subsequently responds to elevated prolactin (d 8 to 35), which develops the gland and permits lactogenesis without the need for a milking stimulus or injection of further hormones (8). With the low-dose steroid regimen used here and elsewhere (10, 14, 17) other hormones are required, but it seems the need for a prolonged and substantial prolactin surge to initiate an artificially induced lactation is remote. From this and (26), prolactin of the order of 20 to 50 ng/ml is probably sufficient to permit lactogenesis in cattle induced with a low-dose steroid regimen.

The priming phase was longer, and the dose of estrogen administered was less in these experiments than is used commonly (26, 27, 28, 30). The aim was to use more physiological concentrations of steroid hormones associated with normal mammary gland development during pregnancy and to induce lactation without the high incidence of abnormal estrous behavior often observed by others (23, 25, 30). Although abnormal estrous behavior was reduced and subsequent reproductive performance was satisfactory after induction of lactation, only small amounts of milk were produced by heifers in groups 1 to 5 (20 to 26% of controls). This in part may be due to low basal prolactin during mammary gland development, because our heifers were induced to lactate in winter, similar to the results of Kensinger et al. (19), where milk yields and basal prolactin were lower in cows induced to lactate in winter compared to spring. However, the amount of E2 used to develop the mammary gland also strongly influences the success of induction treatment, because in groups 6 and 7, administered twice the dose of E2, milk yields were improved to yields similar to those reported by Erb (6) and Fulkerson (10). Abnormal estrous behavior also increased in these groups. There is a need to refine further the technique of steroid treatment to strike a balance between inducing commercially acceptable milk yields while minimizing the incidence of abnormal estrous behavior and cost of treatment.

The E2 and P in milk and plasma samples from 10 heifers induced to lactate generally were lower than (7, 18, 24, 30) and are below concentrations in cattle after normal calving (7, 21). This result is not unexpected, because quantities of exogenous hormones used in our work were less than in previous studies. The E2
in milk was greater than in plasma from induced cows and is probably due to the lower milk production of treated animals.

CONCLUSIONS

Our work extends techniques to initiate lactogenesis developed in the ewe to a comparison of their effectiveness in cattle. It points to a possible compromise between the extent to which economic milk yield from artificial induction of lactation techniques is achieved and adverse side effects of abnormal estrous behavior and low reproductive performance. It shows that if a low-dose steroid treatment is used to develop mammary glands, further hormonal stimulus to initiate lactation is required. Probably only basal prolactin is needed to permit lactogenesis under these conditions. In addition, it provides evidence of a difference between cattle and sheep in their lactogenic response to cloprostenol and a similarity in their lactogenic response to glucocorticoids and oxytocin.

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