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Artificial Induction of Lactation in Ewes: The Involvement of Progesterone and Prolactin in Lactogenesis

W. J. Fulkerson,[A] *R. D. Hooley,*[B] *G. H. McDowell*[A] *and L. R. Fell*[C]

[A] Dairy Research Unit, Department of Animal Husbandry, University of Sydney, Camden, N.S.W. 2570.
[B] Reproduction Research Section, University of Melbourne, S. S. Cameron Laboratory, Werribee, Vic. 3030.
[C] Dairy Research Centre, P.O. Box 217, Richmond, N.S.W. 2753.

Abstract

An attempt has been made to evaluate the importance of prolactin and 'progesterone withdrawal' for lactogenesis. The experimental model system used was the ovariectomized, non-pregnant ewe induced to lactate artificially by treatment with trigger hormone (either oestrogen, glucocorticoid or oxytocin) alone or in combination with progesterone. It appears from the results that prolactin is important in the lactogenic responses elicited by oestrogen and oxytocin but not as important in the response elicited by glucocorticoid. Moreover, the results suggest that, in the ewe, an appropriate positive hormonal stimulus will overcome the inhibitory influence of progesterone on lactogenesis.

Introduction

The development of regimes for artificial induction of lactation in the non-pregnant ewe (Fulkerson and McDowell 1974, 1975) has provided useful experimental models for studying the mechanisms by which various hormones or hormone analogues initiate lactation (Fulkerson *et al.* 1975) and thus the likely mechanisms of initiation of lactation in the parturient animal. Although the pregnant animal has been used in the past for this purpose (Meites *et al.* 1963; Tucker and Meites 1965; Delouis and Denamur 1967), results obtained may have been confounded by the changing endocrine status of the ewe during late pregnancy.

A number of workers have suggested that the decline in the levels of progesterone in peripheral blood over the periparturient period ('progesterone withdrawal') initiates milk secretion in various species (Shinde *et al.* 1965; Kuhn 1969; Hartmann *et al.* 1973). Results of recent studies at this laboratory (Fulkerson and McDowell 1974) are not consistent with this suggestion. Ewes 'primed' with 240 μg oestradiol benzoate plus 60 mg progesterone every third day for 60 days to develop the mammary glands then merely milked, produced only trivial amounts of secretion despite cessation of progesterone administration. In contrast, injections of 5 mg oestradiol benzoate plus 12·5 mg progesterone each day for 5 days after priming initiated essentially normal lactation.

The present studies were carried out to evaluate the effect of relatively high doses of progesterone on the lactogenic response and plasma prolactin levels in ovariectomized ewes induced to lactate with either oestrogen, dexamethasone or Syntocinon after priming.

Materials and Methods

Sheep

Thirty-six nulliparous crossbred (Border-Leicester × Merino) ewes with no apparent abnormalities of the mammary glands were ovariectomized 3 weeks before the experiment commenced. All ewes were accustomed to handling and could be bled from the external jugular vein with a minimum of restraint. The ewes were housed and fed as described previously (Fulkerson and McDowell 1974, 1975).

Hormones

Oestradiol benzoate (Schering AG, Berlin) and progesterone (Calbiochem, La Jolla, California) were dissolved in ethanol and administered either in ethanol or suspended in peanut oil. Dexamethasone trimethylacetate (Opticortenol, 0·5%) was obtained from Ciba-Geigy Aust. Ltd, Lane Cove, Australia. Syntocinon (Sandoz Ltd, Basle) was diluted with 0·9% (w/v) saline to give a solution containing 0·5 i.u./ml.

Experimental Procedure

The mammary glands of all ewes were developed, over 30 days (priming phase), by injecting subcutaneously progesterone plus oestradiol benzoate in peanut oil as described by Fulkerson *et al.* (1975). Ewes were then randomly allocated to nine groups of four and injected each day for a further 5 days (trigger phase) as follows:

Groups 1 and 2: 40 μg oestradiol benzoate in ethanol, subcutaneously, at 0800 and 1800 h.
Groups 3 and 4: 2·5 mg oestradiol benzoate in ethanol, subcutaneously, at 0800 and 1800 h.
Groups 5 and 6: 10 mg dexamethasone trimethylacetate, subcutaneously, at 0800 h.
Groups 7 and 8: 1 i.u. Syntocinon, intravenously, at 1000, 1400, 1800 and 2200 h.
Group 9: No further hormone.

Ewes in groups 2, 4, 6 and 8 also were injected subcutaneously with 20 mg progesterone in peanut oil at 0800 h each day during the trigger phase.

Collection of Samples

All ewes were milked by hand once daily (at 0830 h) for 6 days from the first day of the trigger phase. During the remaining 15 days of the experiment (lactation phase) ewes were milked twice daily at 0830 and 1600 h. Milk yields were recorded daily and subsamples were stored at −16°C pending analysis of lactose by the method of Cowie *et al.* (1969).

Plasma was prepared from heparinized blood collected at intervals prior to, during, and after the trigger phase. Whenever appropriate, blood samples were collected immediately before injection of hormone preparations. Samples were stored at −16°C until assayed for prolactin by solid-phase radioimmunoassay (Fell *et al.* 1972). Concentrations of prolactin were expressed in terms of the standard NIH-P-S8.

Results

Milk Yields

Three weeks after milking commenced, yields of mammary secretion from ewes in groups 3–6 (injected with high doses of oestrogen±progesterone or dexamethasone± progesterone during the trigger phase) were not significantly different and had reached 0·223±0·065, 0·164±0·033, 0·225±0·024 and 0·276±0·060 kg/day (mean±s.e.) respectively. At this time, yields of secretion from ewes in groups 1, 2 and 7 (low oestrogen±progesterone and Syntocinon without progesterone respectively) were similar (*c.* 0·50 kg/day). These yields were significantly lower ($P < 0·05$) than yields from ewes in groups 3–6 but were significantly higher ($P < 0·05$) than the trivial yields from ewes in groups 8 (Syntocinon+progesterone) and 9 (controls). Ewes in group 2 secreted substantially less milk over the first 2 weeks after milking commenced than ewes from group 1 (see Fig. 1).

Lactose Concentrations

By 2–3 days after milking commenced, levels of lactose in secretion from ewes in groups 1 and 3–7 ($> 4{\cdot}0\%$) were significantly higher ($P < 0{\cdot}05$) than levels in secretion from ewes in groups 2, 8 and 9 (*c.* $2{\cdot}5\%$) (see Fig. 1). However, by 2 weeks after milking commenced, levels of lactose for ewes in groups 1–7 were similar. When the experiment was terminated 3 weeks after milking commenced, levels of lactose for ewes in groups 1–7 (*c.* $5{\cdot}2\%$) were significantly higher ($P < 0{\cdot}05$) than levels for ewes in groups 8 (*c.* $3{\cdot}9\%$) and 9 (*c.* $3{\cdot}5\%$).

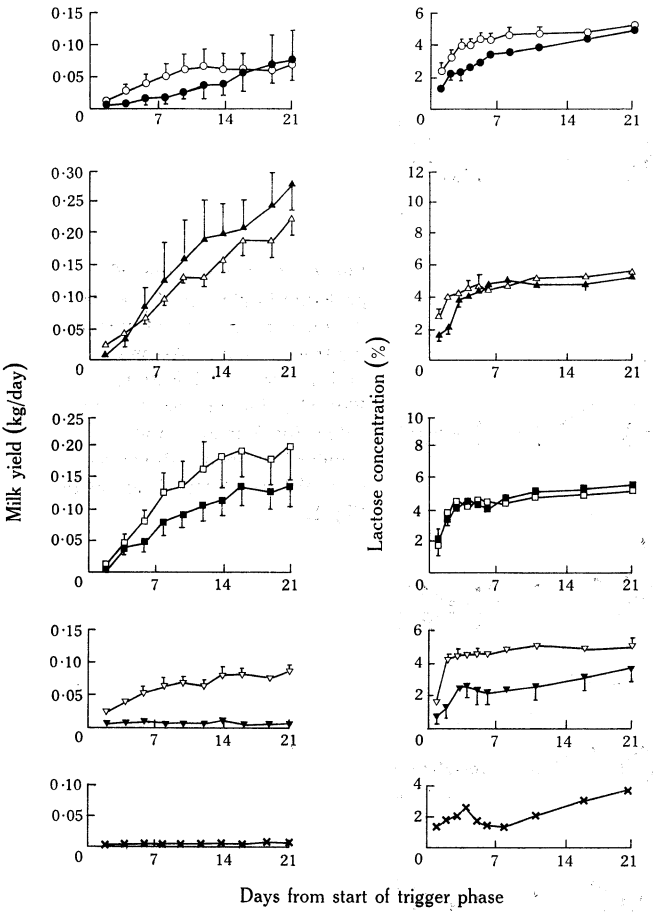

Fig. 1. Yields and lactose concentrations of mammary secretion from ewes in the nine groups treated during the trigger phase. ○ Group 1. ● Group 2. △ Group 3. ▲ Group 4. □ Group 5. ■ Group 6. ▽ Group 7. ▼ Group 8. × Group 9. Plotted points represent mean values for four ewes and standard errors are shown as vertical bars. Milking commenced on the first day of the trigger phase.

Prolactin Concentration

The levels of prolactin in plasma from all ewes were low and constant (20–40 ng/ml) prior to the commencement of the trigger phase (see Fig. 2). Four hours after the first injection of oestradiol benzoate (with or without progesterone) to ewes in groups 1–4, prolactin levels had reached peak values of $405{\cdot}1 \pm 204{\cdot}6$, $419{\cdot}0 \pm 59{\cdot}3$, $372{\cdot}5 \pm 110{\cdot}8$ and $350{\cdot}0 \pm 96{\cdot}2$ ng/ml (mean ± s.e.) respectively. There were no significant differences between these values. A similar pattern of prolactin release was observed for ewes in groups 7 and 8 (injected with Syntocinon) but peak levels

(202·7±67·3 and 96·4±3·4 ng/ml respectively) of prolactin were significantly less ($P < 0{\cdot}05$) than those for ewes in groups 1–4. Levels of prolactin in plasma from ewes in groups 5 and 6 (treated with dexamethasone) and 9 (controls) showed no marked rise at the commencement of the trigger phase.

By 24 h after commencement of the trigger phase levels of prolactin in plasma from ewes in groups 1–4, 7, and 8 had fallen to *c.* 40 ng/ml. There was an inexplicable rise in prolactin levels in plasma of all ewes during the third day of the trigger phase. Apart from this, levels of prolactin remained relatively low, but higher than during the priming phase.

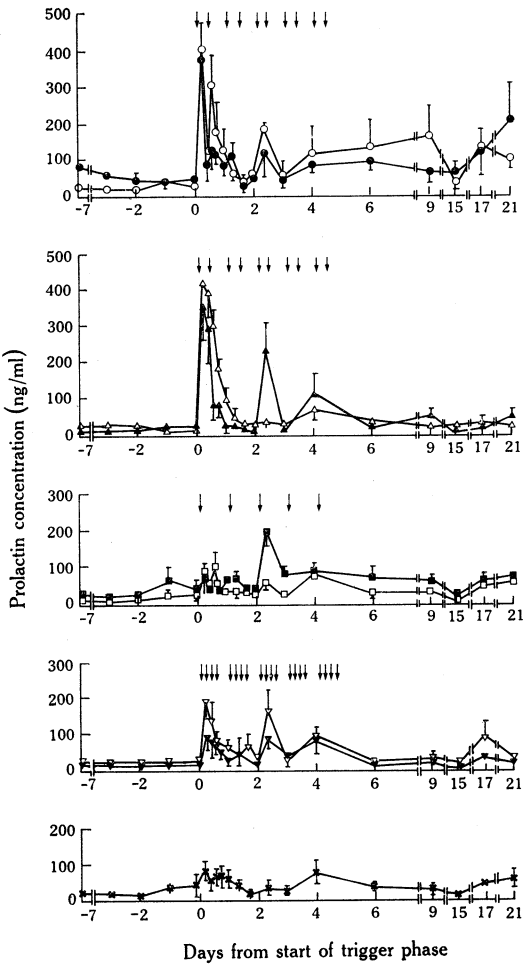

Fig. 2. Concentrations of prolactin in plasma of ewes in the nine groups treated during the trigger phase. Arrows indicate times of injections. Plotted points represent mean values for four ewes and standard errors are shown as vertical bars. Symbols are as in Fig. 1.

Discussion

The results of the present studies suggest that 'progesterone withdrawal' *per se* does not effect the initiation of lactation. Merely ceasing injections of progesterone to ovariectomized ewes following development of mammary glands clearly failed to initiate copious milk secretion. Similar results have been reported for intact ewes (Fulkerson and McDowell 1974). Moreover, injections of progesterone (20 mg/day)

had no effect on the lactogenic response elicited by dexamethasone (10 mg/day) or high doses of oestradiol benzoate (5 mg/day), suppressed the response to low doses of oestradiol benzoate (80 μg/day) and virtually eliminated the response to Syntocinon (4×1 i.u./day). Thus it seems reasonable to propose that the initiation of lactation will occur if an appropriate positive hormonal stimulus overcomes the inhibitory influence of progesterone on lactogenesis.

This proposal is apparently at variance with the conclusion of Hartmann *et al.* (1973) that progesterone withdrawal is the factor responsible for initiation of lactation in the ewe. In their experiments, ewes submitted to caesarean section on day 144 of gestation secreted substantial volumes of milk-like fluid when given either no further hormone treatment or injections of oestrogen. On the other hand, ewes injected with high doses of progesterone (125 mg/day) alone or in combination with oestrogen produced only trivial volumes of secretion. However, the possibility exists that the stress associated with caesarean section may have elicited release of ACTH (and hence glucocorticoids) and/or prolactin (cf. Nicol *et al.* 1960; Fell *et al.* 1973) in amounts sufficient to initiate lactation only in the absence of progesterone.

Results of previous experiments with rats (Kuhn 1969; Shinde *et al.* 1969) and sheep (Hartmann *et al.* 1973) are consistent with the finding of Turkington and Hill (1969) that progesterone affects lactose synthesis by suppressing synthesis of α-lactalbumin. In the present study, injections of progesterone failed to affect levels of lactose in secretion of ewes treated with dexamethasone or high doses of oestrogen, reduced lactose levels in secretion of ewes treated with low doses of oestrogen and suppressed, substantially, lactose levels in secretion of ewes treated with Syntocinon. These findings are consistent with our suggestion that progesterone modifies the response to a positive hormonal stimulus of insufficient magnitude.

Comparison of results for ewes treated with dexamethasone, high doses of oestrogen (5 mg/day) or Syntocinon in the absence of progesterone shows that Syntocinon was less effective than the other hormones in inducing lactation in ovariectomized ewes. Since the above triggers were equally effective in initiating lactation in intact ewes (Fulkerson *et al.* 1975), the results of the present study support the earlier suggestion that Syntocinon may interact with the ovary to exert its lactogenic effect (Fulkerson and McDowell 1975).

Results of previous studies (Fulkerson *et al.* 1975) suggested that prolactin is important for the lactogenic response elicited by oestrogen. In the present experiments both high and low doses of oestrogen elicited substantial increases in levels of prolactin in peripheral blood but yields of ewes injected with low doses of oestrogen were considerably less than those of ewes receiving high doses of oestrogen. Thus it is possible that oestrogen *per se* or a factor released by oestrogen, or both, may synergize with prolactin to give the lactogenic response. It is possible that growth hormone could synergize with prolactin and, indeed, it has been reported that exogenous oestrogen increases levels of growth hormone in the blood of sheep (Streumpler and Burroughs 1959).

The observed increase in prolactin levels following injection of Syntocinon is consistent with results of some workers (Bryant *et al.* 1970; Johke 1970; Fell 1973) but not others (Kühn *et al.* 1973; McNeilly and Hart 1973). In recent studies at this laboratory it was found that milk yields of ewes induced to lactate artificially with Syntocinon were reduced substantially if prolactin secretion was blocked by injection

of ergocryptine (Fulkerson *et al.* 1975). Thus, it seems reasonable to suggest that the lactogenic response to Syntocinon is dependent on prolactin.

In contrast to the findings with oestrogen and Syntocinon, no evidence for an increase in prolactin levels was obtained for ewes treated with dexamethasone. In spite of this, results of earlier studies showed that the lactogenic response to dexamethasone was reduced substantially if prolactin secretion was blocked with ergocryptine (Fulkerson *et al.* 1975). Thus, it appears that although an elevated level of prolactin is not essential for dexamethasone to initiate lactation, a full lactogenic response to dexamethasone does require an increased level of prolactin.

In conclusion, the ovariectomized, nulliparous ewe induced to lactate artificially has been used as a model to elucidate the involvement of various hormones in the initiation of milk secretion. It now remains for the action of these hormones to be confirmed and defined more precisely using *in vitro* techniques.

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