J. Reprod. Fert., Suppl. **30** (1981), 241–249 *Printed in Great Britain*

The effect of suckling upon the endocrine changes associated with anoestrus in identical twin dairy cows

J. F. Smith, E. Payne, H. R. Tervit, L. T. McGowan, R. Fairclough, R. Kilgour and P. G. Goold

Ministry of Agriculture and Fisheries, Research Division, Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand

Summary. Identical twin pairs of dairy cows (one twin being placed with 4 calves for multiple suckling while the other was machine milked) were used to study the hormonal changes associated with anoestrus. Experiments were conducted to examine the effect of multiple suckling on the interval to first oestrus *post partum*, concentrations of LH and progesterone *post partum*, and the LH response to LH-RH or oestradiol-17β. The effects of hormone therapy on anoestrus were also examined.

Multiple suckled cows had a 5–9-week longer interval to first oestrus *post partum* than did their non-suckling twins in all trials. There were no significant differences between the suckling and non-suckling cows in basal plasma levels of LH. The suckling cows showed a delay of 2 and 6 weeks *post partum* in their response to LH-RH and oestradiol-17β respectively when compared with their non-suckling twins. Treatment with a progesterone-releasing intravaginal device and bromocriptine to lower prolactin levels did not reduce the interval to the first post-partum oestrus while treatment with the progesterone device and PMSG reduced the interval to both first oestrus and conception. These findings suggest that suckling affects the response of the hypothalamic–pituitary axis to both LH-RH and oestradiol in the post-partum cow.

Introduction

Anoestrus is a major factor influencing the reproductive efficiency of New Zealand beef and dairy herds. Its incidence can be affected by the level of nutrition (Dunn, Ingalls, Zimmerman & Wiltbank, 1969) and by suckling (Graves, Lauderdale, Hauser & Casida, 1968; Moller, 1970). Despite many studies of ovarian and hormonal changes in the post-partum period in cattle (e.g. Henricks, Dickey, Hill & Johnston, 1972; Edgerton & Hafs, 1973; Britt, Kittok & Harrison, 1974; Arije, Wiltbank & Hopwood, 1974), our understanding of the hormonal mechanisms in anoestrus is incomplete.

This study was undertaken to investigate the effect of suckling upon the post-partum endocrine changes associated with anoestrus.

Materials and Methods

The experiments were conducted at the Ruakura Animal Research Station, Hamilton, New Zealand during the spring (August–November) of 1974, 1975, 1976 and 1977.

0449-3087/81/020241-09$02.00/0

Animals and management

Forty-five (45) pairs of identical twin dairy cows ranging from 2 to 10 years of age were used. They were a mixture of Jersey (15 pairs), Friesian (10 pairs) and Friesian × Jersey crossbreeds (20 pairs). All cows were run on ryegrass and white clover pasture for the whole year and received supplements of silage and hay when necessary during the winter periods. Hay was also available *ad libitum* to animals whilst restrained in stalls for bleeding. Cows and calves were weighed weekly.

The system of 'mothering on' for the suckled calves was that developed by Hudson (1977). The cow's own calf was removed at parturition before any contact had been made with it and 4 pre-selected hungry calves, which had been wiped with the dam's amniotic fluids, were introduced to the cow within 5 min of parturition. Two-year-old Jersey cows were placed with only 2 calves for suckling.

To facilitate the handling of animals all cows expected to calve in a 10-day period were treated so as to induce parturition over 2 days. Cows were injected (i.m.) with 20 mg dexamethasone (Ciba Geigy) at 10:30 h on a Wednesday. Those animals not calved by 18:30 h on Thursday were injected (i.m.) with 500 μg cloprostenol (ICI) and calving was usually completed by Friday. Cows were held in individual pens (10 × 10 m) and kept under constant observation.

Oestrous behaviour was checked daily by using vasectomized bulls fitted with chin-ball harnesses. This was supplemented by the use of tail-paint (Macmillan & Curnow, 1977).

All blood samples were collected by venepuncture into heparinized vacutainers and were immediately chilled in ice water until the plasma was removed and stored at −20°C.

Radioimmunoassays

Progesterone. The method used to measure plasma progesterone concentrations was that described by Fairclough, Hunter & Welch (1975). Plasma samples were extracted in duplicate with 4 volumes hexane and polyethylene glycol was used to separate free and bound steroid. The antiserum used was raised in sheep against a progesterone-11–BSA conjugate, and its cross-reactions were 4·2% with 20β-dihydroprogesterone, 0·6% with 20α-dihydroprogesterone, 1% with 17α-hydroxyprogesterone, and <0·1% with all other steroids tested. The smallest amount of progesterone which could be measured with precision was 0·15 ng progesterone/ml plasma.

The intra- and inter-assay coefficients of variation for a plasma pool assayed on 21 consecutive occasions and containing progesterone at a concentration of 3·6 ng/ml were 8·5% and 9·4% respectively.

Luteinizing hormone (LH) concentrations were measured as described by Knight, Peterson & Payne (1978). The sensitivity (twice the s.d. of the zero point) was 0·25–0·5 ng/ml plasma for baseline values. The intra- and inter-assay coefficients of variation were 16% and 18% respectively. All results are in terms of the standard (NIH-LH-B8).

Prolactin. This was analysed by double-antibody radioimmunoassay using antiserum raised in rabbits against bovine prolactin (NIH-P-B3). The method of assay was similar to that of Koprowski & Tucker (1971) adapted for an automatic diluter. The second antibody was raised in a sheep against rabbit globulin. Prolactin standards (NIH-P-B3) were diluted in plasma from hypophysectomized sheep.

Highly purified ovine prolactin (LER 860-2) was iodinated by the method of Langley (1974) which incorporates modifications of Fell *et al.* (1972) and Koprowski & Tucker (1971) into the method of Greenwood, Hunter & Glover (1963). Cross-reactivity of antisera to bovine LH, thyroid-stimulating hormone and growth hormone was negligible; 200 ng of each caused no change in binding of labelled prolactin.

Dilution of steer plasma showed parallelism to the standard curve. The apparent sensitivity of the method (twice the s.d. of the zero point) for the assays was 2–5 ng/ml plasma. The intra- and inter-assay coefficients of variation were 10·3% and 11·0% respectively for values > 10 ng/ml.

Experimental design

Experiment 1. Ten (10) pairs of twins were used in 1974 with one member of each twin pair allowed to suckle and the other being machine milked twice a day. The suckling cows and calves were run as a separate group with access to a similar allowance of pasture as the milked cows. All cows were bled twice each week and plasma samples were assayed for progesterone and LH. At 2-weekly intervals the suckling cows were placed under observation for 24 h and the incidence and extent of suckling activity recorded.

Experiment 2. Eight (8) pairs of identical twin cows were used in 1975 and managed as in Exp. 1. All cows were treated (i.m.) with 250 μg luteinizing hormone-releasing hormone (LH-RH; ICI) within 72 h of parturition and thereafter at intervals of 2 weeks for a period of 12 weeks. Blood samples were taken 15 min before injection of LH-RH, immediately after injection and then at 15-min intervals for 6 h. The plasma was stored and assayed for LH. During blood sampling the calves were separated from the cows that they sucked. Additional blood samples were taken twice weekly for progesterone assay. Ovarian changes were assessed weekly by palpation *per rectum*.

Experiment 3. In 1977, 5 pairs of identical twin cows were used to examine the basal levels of LH. One member of each twin pair was allowed to suckle several calves while her twin (non-lactating) was 'dried-off' immediately *post partum*. At 1 week after calving and every 2 weeks thereafter for 11 weeks all cows were bled once every 20 min for 6 h. Plasma samples were assayed for both LH and prolactin. Additional samples were taken twice weekly for progesterone assay.

Fifteen (15) pairs of identical twins were used to examine the response to oestrogen. One member of each pair was suckling and the other non-lactating. The presence of a positive feedback of oestradiol on LH release was examined in 5 pairs of twins at 1 and 7 weeks after parturition, in another 5 pairs at 3 and 9 weeks, and in a third group of 5 twin pairs at 5 and 11 weeks. The procedure adopted was similar to that of Radford, Nancarrow & Mattner (1978). Cows were injected (i.m.) with 500 μg cloprostenol at 18:00 h on a Tuesday; 27·5 h later (Wednesday at 21:30 h) they were injected (i.m.) with 500 μg oestradiol-17β in arachis oil and 10 h later (07:30 h Thursday) were bled every 30 min for 10 h. During blood sampling the suckling cows were separated from their calves. Additional samples were taken twice weekly for progesterone assay. Cows were managed in two herds (suckling and non-suckling) and given continuous access to similar pasture for the duration of the trial.

Experiment 4. In 1976, 24 pairs of identical twin cows were used and both members of each pair were given 4 calves to suckle. The cows and calves were grazed on pasture throughout the trial. At approximately 47 days *post partum* cows were allocated to one of the following three treatment groups: (A) controls, no treatment; (B) treated for 7 days with a progesterone-impregnated intravaginal device, PRID (Abbott Laboratories). At the time the device was inserted each animal was injected (i.m.) with 6 mg oestradiol valerate (Steraloids Inc.) + 250 mg progesterone in arachis oil. At PRID removal each animal was injected (i.m.) with 750 i.u. PMSG; (C) treated as in (B) except that the PMSG was omitted and the animals were injected (i.m.) with 80 mg bromocriptine (Sandoz) at time of PRID insertion and daily for the next 3 days. The allocation of twin pairs to treatments was on basis of breed and bodyweight. One member of each of 8 pairs of twins was placed in treatment group A or B, those in another 8 pairs in treatment group A or C and those in the final 8 pairs treatment groups B or C. Oestrous behaviour was monitored from 14 days *post partum*. Animals exhibiting oestrus after treatment

or after Day 47 in the controls were naturally mated. Twice weekly blood samples were taken from all cows between Days 30 and 80 *post partum* for progesterone assay. Cows in Group C were bled four times at 15-min intervals immediately before the first bromocriptine injection, on the day of the last injection and immediately before PRID removal. Plasma samples were assayed for prolactin.

Statistical analyses

Values given are mean ± s.d. Data in Exps 1, 2 and 3 were analysed by a paired *t* test on within-set differences. In Exp. 4 the data were analysed using a split-plot analysis of variance technique.

Results

Experiment 1: interval from parturition to first oestrus

The mean interval to the first oestrus *post partum* for the machine-milked cows (29·5 ± 12·7 days) was significantly ($P < 0·01$) shorter than that for their suckling twins (73·0 ± 18·1 days). All cows except one which was suckling continued to cycle after the first oestrus.

The progesterone profiles for all cows are presented in Text-fig. 1. They confirm the existence of a longer post-partum interval in the suckling cows. Cows M7, M8 and M10 (suckling) may have had a 'silent ovulation' preceding their first observed oestrus. The other 7 cows were all observed in oestrus before there was any significant rise in the plasma level of progesterone. Four of the milked cows (L1, L5, L7 and L8) may also have had 'silent ovulations' before their first observed oestrus. The changes in progesterone levels indicate that after the first observed oestrus, 7 of the suckling cows were either not detected in oestrus or they had 'silent heats' on a total of 9 occasions, whereas only 3 of the milked cows indicated a possible silent heat.

The mean LH levels over the first 6 weeks *post partum* were similar for the suckling (4·8 ± 1·0 ng/ml) and milked (5·8 ± 1·4 ng/ml) cows.

While there was a tendency for the milked cows to increase in weight and for the suckling cows to lose weight, there were no significant differences in liveweight at any stage of the trial.

Experiment 2: response to LH-RH given at parturition and thereafter at 14-day intervals

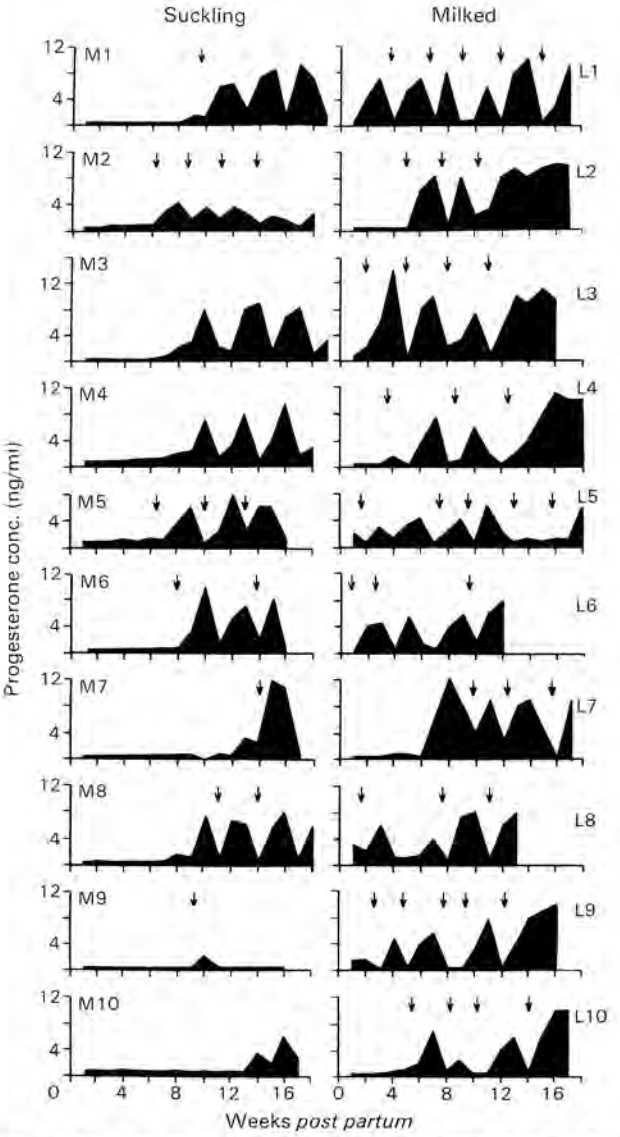
The mean interval to the first post-partum oestrus was considerably longer ($P < 0·001$) for the suckling cows (110·4 ± 15·2 days) than for those that were machine-milked twice a day (43·0 ± 22·7 days). Likewise, the suckling cows had a significantly ($P < 0·01$) longer interval from calving to first ovulation (42 ± 24 compared with 24 ± 18 days).

The interval to first oestrus was longer than that to first ovulation (except in one cow). Although the animals were ovulating in response to LH-RH, this was not related to the time of first oestrus.

The LH response to LH-RH was measured by the area under the curve (Text-fig. 2). There was a linear increase from calving to 6 weeks *post partum* in the milked cows. The development of the LH response was delayed in the suckling cows, and at 2 weeks *post partum* the response was significantly ($P < 0·01$) lower.

Experiment 3: basal concentrations of LH and prolactin and the LH response to oestradiol

In the 5 pairs of twins not treated with oestrogen, the suckling cows had a longer interval to the first oestrus *post partum* than did those in which lactation was not maintained (67 ± 16 compared with 29 ± 10 days; $P < 0·01$). Similarly, in the pairs treated with oestradiol-17β, the

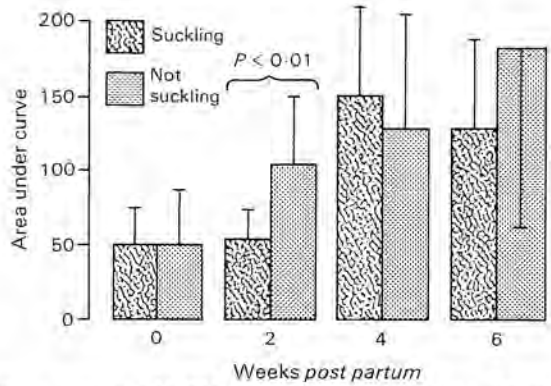


Text-fig. 1. Post-partum plasma concentrations of progesterone in cows suckling calves and their machine-milked identical twins. Arrows indicate detected oestrus.

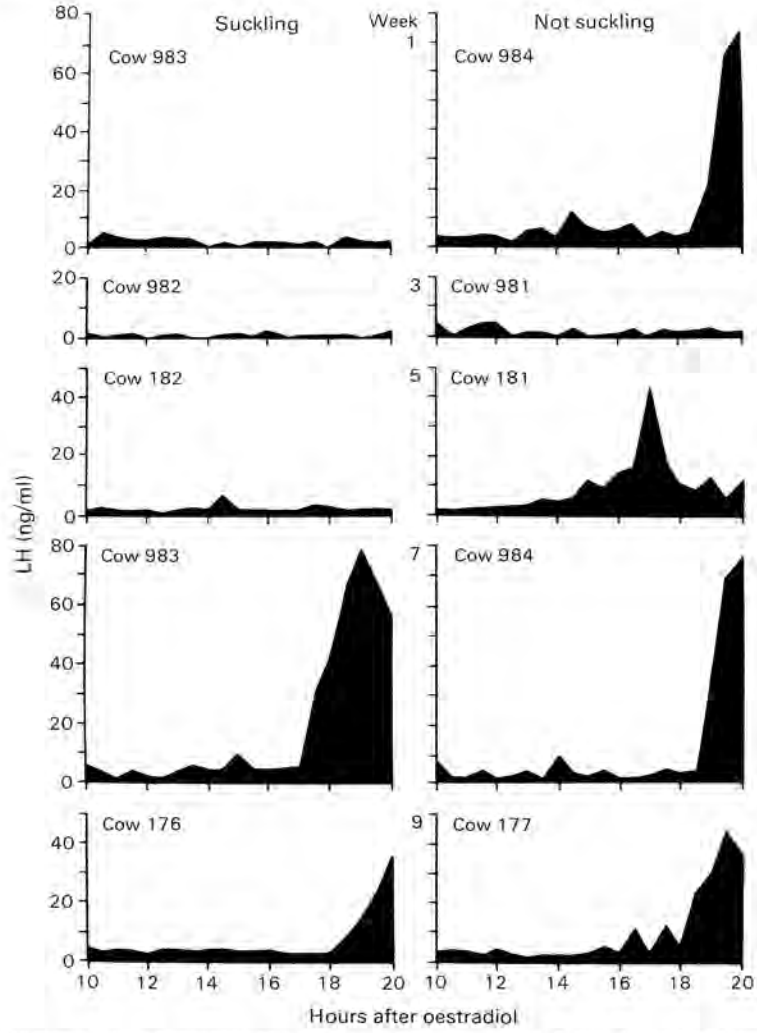
interval for the suckling cows was longer than in those not suckling (60 ± 24 compared with 24 ± 10 days; $P < 0{\cdot}01$).

Basal LH concentrations. Only blood samples collected before the first oestrus from the non-suckling cows and the corresponding samples from their suckling twins were assayed. Overall, the suckling cows tended to have more LH pulses than did the non-suckling cows but the variation between twin pairs was very marked and there were no significant differences between treatments. The basal LH values were between 0·4 and 8·0 ng/ml.

Basal prolactin concentrations. Prolactin levels for the suckling cows were higher (47 ± 37 compared with 7 ± 3 ng/ml; $P < 0{\cdot}05$) within 40 min of their removal from calves. These levels fell throughout the sampling period and were not different from those of the non-suckling cows at later times.



Text-fig. 2. Mean LH response to LH-RH in suckling and machine-milked identical twin cows at various times *post partum*. Values are mean ± s.d. for 7 animals in each group.



Text-fig. 3. Plasma LH concentrations in 5 suckling cows and 5 non-lactating identical twins after treatment with oestradiol-17β at various times *post partum*.

LH response to oestradiol-17β. Peak levels of LH up to 80 ng/ml were recorded in response to oestradiol-17β. However the timing of the peak relative to oestrogen treatment was very inconsistent. Suckling cows did not give an LH response within 24 h to oestradiol-17β until 7 weeks *post partum*, but responses in some non-suckling cows were recorded at 1 and 5 weeks *post partum* (Text-fig. 3).

Experiment 4: effect of hormone therapy

Cows treated with a PRID + PMSG showed a synchronous onset of oestrus after treatment. This resulted in these cows having a shorter interval from calving to first oestrus (57·6 ± 2·1 days) than did the cows treated with a PRID + bromocriptine (68·8 ± 19·9 days) or the control cows (68·8 ± 13·6 days; $P < 0·05$). The conception rate to first mating was also higher ($P < 0·05$) in the PRID + PMSG group (64·7%) than in the PRID + bromocriptine group (33·3%) or control group (37·5%). This was reflected by the cows treated with a PRID + PMSG having a shorter ($P < 0·05$) interval from calving to conception (67 ± 18·1 days) than did those treated with a PRID + bromocriptine (86·5 ± 30·5 days) or the controls (80·4 ± 25·2 days).

Prolactin levels. The bromocriptine treatment suppressed the prolactin concentrations from a mean concentration of 30 ± 17·6 ng/ml before treatment to mean concentrations of 6·8 ± 6·7 and 6·2 ± 5·6 ng/ml on the 3rd and 7th days of treatment.

Progesterone levels. The concentrations of progesterone before Day 47 *post partum* were low (about 0·6 ng/ml) in all cows. Those treated with a PRID (Groups B and C) showed high circulating levels of progesterone during treatment (4–6 ng/ml). These levels declined to about 0·5 ng/ml within 1 day of PRID removal. This fall was associated with oestrus in the Group B (PRID + PMSG) cows, which then showed a rapid rise in progesterone concentrations to levels of 10–40 ng/ml, reflecting the multiple ovulations produced in response to this treatment.

The progesterone levels in the Group C (PRID + bromocriptine) cows were more variable, with a wide range in the period of low levels following PRID removal. This paralleled a similar variation in the time to first oestrus. After oestrus the progesterone levels rose to 6–14 ng/ml.

The control cows showed a prolonged period of low progesterone levels, 0·2–1·0 ng/ml, before the ovarian cyclic changes reappeared.

Discussion

The interval to onset of first oestrus *post partum* was increased by suckling, with the suckling cows showing oestrus 5–9 weeks later than their non-lactating or machine-milked twins. These results are similar to those reported by Radford *et al.* (1978) and indicate that this increased interval is an effect of suckling rather than lactation *per se*. Because of the similar levels of nutrition of suckling and non-suckling cows in these experiments and the lack of any significant differences in body weight it seems unlikely that differences in the onset of oestrous activity were affected by nutrition *post partum*.

Examination of the progesterone levels observed in Exp. 1 shows that the longer interval to oestrus in the suckling cows was due to a longer period of ovarian inactivity. This supports the findings of Moller (1970) but does not agree with his observations of more frequent 'silent ovulations' before the first oestrus in the suckling cows. The increased incidence of subsequent 'silent heats', however, is in agreement with his findings.

The lack of any relationships between the interval to first oestrus and the number of calf hours that cows suckled each day in this experiment differs from other reports (Moller, 1970; Wetteman, Turman, Wyatt & Totusek, 1978) in which increased intensity of suckling (numbers of times cows suckled per day) was associated with an increase in the interval to first oestrus. It is possible, however, that cows suckling 4 calves reach a plateau in suckling stimuli and that the

variation in the interval to first oestrus amongst the suckling cows was due to factors other than suckling.

One possible explanation for the longer interval to first oestrus in suckling cows is that the suckling stimulus leads to an increased release of prolactin through the activation of serotoninergic neurones (Martin, Reichlin & Brown, 1977). Since sertonin inhibits gonadotrophin release (Cramer & Barraclough, 1978) suckling could have a depressing effect on gonadotrophin release. This is supported by Radford *et al.* (1978) who reported lower basal levels of LH in suckling than in non-suckling cows during the first 4 weeks *post partum*. However, in the present trials no significant difference in basal levels of LH was observed between the suckling and non-suckling cows.

Suckling could also increase prolactin release by an inhibitory effect on the production or release of dopamine. Treatment with bromocriptine, a dopamine agonist, lowers prolactin levels but unlike dopamine will not increase LH as it is not a precursor of noradrenaline (Hokfelt, 1978). The results of Exp. 4 agree with this hypothesis. Treatment with bromocriptine depressed the levels of prolactin, but did not reduce the post-partum interval in suckling cows. This contrasts with the findings of Schams (1975), but this difference could be related to the time of treatment *post partum*. Thus the high levels of prolactin seen in the continuously suckling cow may well be incidental to the effects of suckling on anoestrus. This hypothesis is supported by the finding that stimulation of prolactin levels by thyrotrophin-releasing hormone treatment did not affect the positive oestrogen feedback on LH production (C. D. Nancarrow & H. M. Radford, personal communication).

The differences between suckling and non-suckling cows in their responses to LH-RH and oestradiol-17β, however, indicate that suckling has in some way affected the hypothalamic–pituitary axis. The increase in the response of the non-suckling cow to LH-RH with time *post partum* was similar to other observations (Fernandes, Thatcher, Wilcox & Call, 1978). Suckling delayed the development of this increase in response by at least 2 weeks. This delay was also reflected in a difference between suckling and non-suckling cows in the time to first ovulation *post partum* but cannot explain the much greater difference in time to first oestrus. Similarly, the suckling cows failed to respond to oestradiol-17β treatment for a longer period *post partum* than did their non-lactating twins, which indicates a possible lack of positive oestrogen feedback. This is in agreement with the findings of Radford *et al.* (1978) although the responses in the non-suckling cows in Exp. 3 were variable.

In the suckling cow it appears that there is a longer delay *post partum* in the development of a positive LH feedback to oestrogen than the capability to release LH in response to LH-RH, and this may well be the dominant factor affecting anoestrus. The removal of the suckling stimulus by temporary weaning of calves for 48–56 h either on its own or at the end of a period of progesterone treatment has been shown to increase the proportion of cows exhibiting oestrus and conceiving within 3 weeks of treatment and to reduce the post-partum interval to first oestrus (Smith *et al.*, 1979; Smith & Tervit, 1980). The temporary removal of the suckling stimulus results in elevated LH concentrations within 12 h. This is due to an increase in the number and magnitude of LH peaks in cows with temporarily weaned calves compared to that observed in suckling cows (D. W. Forrest, personal communication).

The post-partum interval to oestrus and conception is reduced by treatment of cows by intravaginal administration of progesterone for 7 days, augmented by oestrogen and PMSG treatments at the beginning and end of this period respectively, and this reduction indicates the potential for this technique as a treatment for anoestrus in the suckling cow.

We thank the staff of the Physiology Section at Ruakura for their technical assistance throughout the trial; the staff of the Chemical Services Section for assistance with the hormone assays; the National Institutes of Health, Bethesda, for the provision of standards and purified material of both prolactin and luteinizing hormone; ICI (Australia) for the provision of the LH-RH; and Abbott Laboratories, Chicago, for the PRIDs.

References

Arije, G.R., Wiltbank, J.N. & Hopwood, M.L. (1974) Hormone levels in pre- and post-parturient beef cows. *J. Anim. Sci.* **39**, 338–347.

Britt, J.H., Kittok, R.J. & Harrison, D.S. (1974) Ovulation, estrus and endocrine response after GnRH in early post-partum cows. *J. Anim. Sci.* **39**, 915–919.

Cramer, O.M. & Barraclough, C.A. (1978) The actions of serotonin, norepinephrine, and epinephrine on hypothalamic processes leading to adenohypophyseal luteinizing hormone release. *Endocrinology* **103**, 694–703.

Dunn, T.G., Ingalls, J.E., Zimmerman, D.R. & Wiltbank, J.N. (1969) Reproductive performance of two-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. *J. Anim. Sci.* **29**, 719–726.

Edgerton, L.A. & Hafs, H.D. (1973) Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. *J. Dairy Sci.* **56**, 451–458.

Fairclough, R.J., Hunter, J.T. & Welch, R.A.S. (1975) Peripheral plasma progesterone and utero-ovarian prostaglandin F concentrations in the cow around parturition. *Prostaglandins* **9**, 901–914.

Fell, L.R., Beck, C., Brown, J.M., Catt, K.J., Cumming, I.A. & Goding, J.R. (1972) Solid phase radioimmunoassay of ovine prolactin in antibody coated tubes. Prolactin secretion during estradiol treatment at parturition and during milking. *Endocrinology* **91**, 1329–1336.

Fernandes, L.C., Thatcher, W.W., Wilcox, C.J. & Call, E.P. (1978) LH release in response to GnRH during the post-partum period of dairy cows. *J. Anim. Sci.* **46**, 443–448.

Graves, W.E., Lauderdale, J.W., Hauser, E.R. & Casida, L.E. (1968) Studies on the post-partum cow. *Res. Bull. Wis. agric. Exp. Stn*, No. 270.

Greenwood, F.C., Hunter, W.M. & Glover, J.S. (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**, 114–123.

Henricks, D.M., Dickey, J.F., Hill, J.R. & Johnston, W.E. (1972) Plasma estrogen and progesterone levels after mating, and during late pregnancy and post-partum in cows. *Endocrinology* **90**, 1336–1342.

Hokfelt, B. (1978) Dopaminergic transmission and dopamine agonism. *Acta endocr., Copenh., Suppl.* **216**, 9–12.

Hudson, S.J. (1977) Multiple fostering of calves on to nurse cows at birth. *Appl. Anim. Ethol.* **3**, 57–63.

Knight, T.W., Peterson, A.J. & Payne, E. (1978) The ovarian and hormonal response of the ewe to stimulation by the ram early in the breeding season. *Theriogenology* **10**, 343–353.

Koprowski, J.A. & Tucker, H.A. (1971) Failure of oxytocin to initiate prolactin or luteinising hormone release in lactating dairy cows. *J. Dairy Sci.* **54**, 1675–1680.

Langley, O.H. (1974) *The role of prolactin in the bovine estrous cycle.* M.S. thesis, Cornell University.

Macmillan, K.L. & Curnow, R.J. (1977) Tail painting—a simple form of oestrus detection in New Zealand dairy herds. *N.Z. J. exp. Agric.* **5**, 357–361.

Martin, J.B., Reichlin, S. & Brown, G.M. (1977) *Clinical Neuroendocrinology*. F.A. Davis, Philadelphia.

Moller, K. (1970) Uterine involution and ovarian activity after calving. *N.Z. vet. J.* **18**, 140–145.

Radford, H.M., Nancarrow, C.D. & Mattner, P.E. (1978) Ovarian function in suckling and non-suckling beef cows *post partum*. *J. Reprod. Fert.* **54**, 49–56.

Schams, D. (1975) Prolactin secretion and action in domestic animals. *Acta endocr., Copenh., Suppl.* **193**, 153–154.

Smith, J.F. & Tervit, H.R. (1980) The successful development of a PRID regime for oestrous synchronisation in NZ beef cattle. *Proc. N.Z. Soc. Anim. Prod.* **40**, 272–279.

Smith, M.F., Burrell, W.C., Shipp, L.D., Sprott, L.R., Songster, W.N. & Wiltbank, J.N. (1979) Hormone treatments and use of calf removal in postpartum beef cows. *J. Anim. Sci.* **48**, 1285–1294.

Wetteman, R.P., Turman, E.J., Wyatt, R.D. & Totusek, R. (1978) Influence of suckling intensity on reproductive performance of range cows. *J. Anim. Sci.* **47**, 342–346.