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Modulation of Functional Capacity of Small Ovarian Follicles in the Post-partum Cow by Prolactin

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Abstract

This study was undertaken to examine the possibility that the prolonged anovulatory period frequently experienced by the post-partum cow is due to a disruption of function at the ovarian level promoted by the high, suckling-induced, blood prolactin concentrations. Fifteen cows, less than 35 days post partum, were allocated to three groups (1, 3 and 5) and given no hormonal treatment, prostaglandin plus pregnant mare serum gonadotrophin (PMSG) treatment or injected with 2-bromo-α-ergocryptine to reduce circulating prolactin levels. Ten synchronized cyclic cows were allocated to two groups (2 and 4) and given prostaglandin or prostaglandin plus PMSG treatment. All cows were ovariectomized 1 or 2 days after treatment and Graafian follicles less than 9 mm in diameter were selected after dissection from the ovaries. The follicles were cultured for 18 h with or without prolactin (1 μg/ml) and steroid accumulation in the culture medium estimated. The follicles were then separated into theca and granulosa which were incubated for 40 min with LH (1 μg/ml) or FSH (5 μg/ml). Cyclic AMP concentrations were estimated as an indication of tissue responsiveness to gonadotrophins.

The secretion of oestradiol-17β, progesterone, testosterone or androstenedione during 18 h culture did not differ between follicles isolated from post-partum or cyclic cows. The presence of prolactin in the culture medium had no overall effect on steroid secretion although some specific effects within each group were noticed. Incubation with LH increased cyclic AMP levels in the theca but the granulosa did not respond. Likewise FSH increased cyclic AMP levels in granulosa preparations but not in theca. There were no differences in response between post-partum and cyclic cows, but exposure of the follicles to prolactin *in vitro* did significantly reduce the LH-induced increase in cyclic AMP levels in isolated theca.

We have concluded that endogenous prolactin may modify but does not inhibit the resumption of ovarian function following parturition in the beef cow.

Introduction

The aetiology of the prolonged post-partum anoestrous period in beef cattle remains enigmatic despite considerable research into the problem (Nancarrow *et al.* 1978). In women, much evidence has been produced linking high suckling-induced prolactin (PRL) levels with the delay in resumption of ovarian function post partum. In the cow, PRL has been shown to be essential for mammogenesis some days prior to parturition but reduction in PRL levels after calving with an inhibitor of PRL secretion, 2-bromo-α-ergocryptine (CB154), has little effect on lactogenesis (Karg and Schams 1974). However, it has not been established that PRL is a major determinant of prolonged post-partum anoestrus in the beef cow.

There are two levels at which impairment of reproductive function may occur. Firstly, at the hypothalamo-pituitary level, no conclusive evidence has been produced which relates high PRL levels to the impairment of the feedback relationships of oestrogen and LH despite strong evidence that the presence of the calf *per se*, perhaps in association with other stressful factors, is often associated with prolonged post-partum anoestrus. Secondly, in view of the reported regulatory action of PRL on luteal cell LH receptors (Richards and Williams 1976) and the inhibitory effect of high concentrations of PRL on progesterone secretion by cultured human granulosa cells (McNatty *et al.* 1974), it seems possible that the site for the disruption of ovarian function in cattle may lie within the ovary.

In this initial study, we have attempted to ascertain whether high suckling-induced concentrations of blood PRL or the presence of PRL *in vitro* could affect either the steroid secretion of entire follicles in culture or the response of the isolated follicular tissues to LH and FSH.

Materials and Methods

Animals, Treatments and Collection of Follicles

Fifteen cows that had been artificially inseminated following an oestrus-synchronization program were allowed to calve normally and allocated to three groups. Ten cyclic cows were treated with the synthetic luteolytic prostaglandin, Cloprostenol (ICI, Australia, Ltd), to synchronize their oestrous cycles and allocated to two groups. The groups were matched for cow breed (Hereford or Hereford cross), age and reproductive status for the day on which they were to be ovariectomized. The composition of the groups and the treatments afforded each are described in Table 1.

It was seldom that cows from this herd experienced ovulation before 40 days post partum. Hence group 1 was untreated with drugs or hormones. Cloprostenol (500 μg) was injected (s.c.) into cows in groups 2 and 4 to reduce any short-term effect of progesterone on the ovaries (an effect not present in most post-partum cows) and to standardize as far as possible the state of the ovaries when sampled (see below). Cows in group 3 also received prostaglandin as this group was to be directly compared to group 4. Stimulation of the ovaries of cows in groups 3 and 4 with 1000 i.u. of pregnant mare serum gonadotrophin (PMSG, Organon, Modern, Surrey, England) was carried out to increase the yield of non-atretic follicles (Turnbull *et al.* 1977) and to investigate any differences between post-partum anoestrous and cyclic cows in their responses to follicle stimulation as measured by *in vitro* techniques. Cows in group 5 were treated with an inhibitor of PRL secretion, CB154 (Sandoz) for 8 days (Table 1) prior to ovariectomy. The inhibition of PRL release would be sufficient (Schams *et al.* 1972) to enable us to investigate whether high endogenous levels of PRL (groups 1 and 3) affect the ability of small follicles to secrete steroids and respond to gonadotrophins *in vitro*.

Cows were ovariectomized under sterile conditions using an emasculator passed through either a lateral incision made in the wall of the vagina proximal to the cervix, or in the case of heifers, through a flank incision made after induction of a spinal block with 2% (w/v) Biotal. Ovaries were put into sterile plastic bottles containing 10 ml ice-chilled Dulbecco phosphate-buffered saline medium and placed in ice for transport to the laboratory. Ovaries were removed 24 h after prostaglandin treatment in groups 2, 3 and 4 rather than 48 h after as by this latter time the pro-oestrous surge of oestradiol was evident (Scaramuzzi *et al.* 1980). Even by 24 h large follicles with LH receptors may be synthesizing oestradiol at a greater rate than the more numerous, smaller follicles. We have assumed from the results of Scaramuzzi *et al.* (1977), that there would be few, if any, of these follicles with a high propensity to secrete oestradiol. In fact in the Cloprostenol-treated cyclic cow there were few (0·7 per cow) non-atretic class 13 follicles (Scaramuzzi *et al.* 1980). This class, arbitrarily based on doubling granulosa volumes, contains follicles greater than about 7 mm diameter and has been described as the preovulatory class. However, the final preovulatory swelling only occurs through fluid accumulation, cell division having virtually ceased with follicles of size about 10–11 mm. For consistency, follicles greater in size than 9 mm were not considered suitable for culture. However, follicles cultured in these experiments still represented the classes 9 through 13,

the period during which the mitotic index of the granulosa was decreasing with increase in follicle diameter (Scaramuzzi *et al.* 1980).

Table 1. Allocation of cows to groups, their immediate reproductive status and the treatment rendered on predetermined days

Group No.	Cow No.	Status	Treatment	Days post partum or day of cycle
1	270	Lactating, post partum	Food and water removed 36 h before ovariectomy	35
	90			24
	47			28
	101			26
	182			24
2	61	Cyclic, mid-luteal	Food and water removed 36 h and PG[A] given 24 h before ovariectomy	10
	273			10
	199			15
	281			15
	300			16
3	157	Lactating, post partum	Food and water removed 36 h, PG[A] and PMSG[B] given 24 h before ovariectomy	27
	100			23
	173			27
	167			28
	192			29
4	60	Cyclic, mid-luteal	Food and water removed 36 h, PG[A] and PMSG[B] given 24 h before ovariectomy	10
	103			15
	272			15
	303			16
	383			14
5	295	Lactating, post partum	Food and water removed 36 h, CB154[C] given 8, 6, 4 and 2 days before ovariectomy	30
	99			28
	176			32
	155			26
	104			26

[A] Prostaglandin $F_{2\alpha}$ analogue, Cloprostenol (I.C.I. Australia Ltd.); 500 μg injected subcutaneously.
[B] Folligon (Organon, Morden, Surrey, U.K.); 1000 i.u. in water injected intramuscularly.
[C] 100 mg initially then 50 mg subsequently injected intramuscularly.

Following ovariectomy as many follicles as possible were dissected free from ovarian stroma. The presence and activity of corpora lutea were recorded and the follicles measured and counted. The list of follicles given in Table 2 is not complete since loss of follicles during the gross dissection was unavoidable. However, it provides a reasonable estimate of follicle numbers, particularly of those greater than 4 mm in diameter. Grossly atretic follicles were identified visually and discarded.

Table 2. Distribution of follicles by size

Status of cow	No. of cows	Mean No. of follicles ($\pm$ s.e.m.) in size classes[A]			
		>15 mm	6–15 mm	4–6 mm	<4 mm
Post partum	15	$0{\cdot}9 \pm 0{\cdot}23$	$1{\cdot}1 \pm 0{\cdot}27$	$6{\cdot}5 \pm 1{\cdot}38$	$10{\cdot}3 \pm 1{\cdot}94$
Cyclic	10	$1{\cdot}1 \pm 0{\cdot}16$	$1{\cdot}5 \pm 0{\cdot}41$	$12{\cdot}3 \pm 1{\cdot}64$	$4{\cdot}6 \pm 1{\cdot}95$

[A] Follicle diameter (mm) determined with a vernier caliper at the commencement of the culture period. Visibly atretic follicles were not included.

The remaining follicles, which probably included healthy plus early atretic follicles, were allocated to culture dishes. The atretic classes IV and V (Carson *et al.* 1979) would mostly have been eliminated from the study.

Culture of Follicles

Once the follicles were dissected from the ovarian stroma, they were selected and matched with follicles of similar size from the same cow and then one group was incubated for 18 h in the presence of PRL (NIH-P-S12, 1 μg/ml) and the other in hormone-free medium. The culture conditions were the same as those described by Moor *et al.* (1973). In some cases, more than one follicle of similar size were incubated in the same culture dish. The size range of follicles incubated was 2·1–8·6 mm. Duplicate culture dishes were used for each prolactin treatment. After 18 h incubation, the medium was removed, frozen and stored at −80°C prior to steroid analyses.

We were only able to utilize follicles from four cows per group to investigate the responses to gonadotrophin. One cow was randomly eliminated from each group. The theca and granulosa cells were separated (Weiss *et al.* 1978) and the theca from each follicle divided between the three treatment groups. The granulosa cells from each follicle were suspended in 1·6 ml HEPES-buffered* Medium 199 (Commonwealth Serum Laboratories, Parkville, Vic.) and then divided into three 0·5-ml aliquots. Hormones (in 10 μl of HEPES) were added over a 5-min period immediately before beginning the 40-min incubation at 37°C. The final concentration of LH (NIH-LH-B10) and FSH (NIH-FSH-S10) was 1 and 5 μg/ml of medium, respectively, with control flasks containing no added hormone. The incubation was ended after 40 min by rapid freezing and the samples stored at −80°C until assayed for cyclic AMP.

Blood Sampling and Progesterone Assay of Plasma Progesterone

Cows were bled via the jugular vein into 10 ml heparinized syringes and the plasma separated by centrifugation and stored at −20°C. Samples were taken daily for 5 days before and at the time of ovariectomy. Progesterone was estimated by radioimmunoassay (Thorneycroft and Stone 1972). The sensitivity of this assay was 0·2 ng/ml and the intra- and interassay variation were respectively 9·0% (6·9–12·3%) and 7·5% (1·2–16·8%) over the range 32–512 pg.

Assay of Steroids in Culture Medium

Progesterone, oestradiol-17β, testosterone and androstenedione contents in the 18 h culture media were estimated by direct radioimmunoassay using validated procedures previously described by Janson *et al.* (1978). The limit of assay sensitivity for oestradiol and testosterone was 0·025 ng, and for progesterone and androstenedione 0·05 ng. Steroid concentration is expressed as nanograms per milligram total protein (theca + granulosa protein).

Assay of Cyclic AMP

Prior to assay, the theca and granulosa cell preparations were thawed and disrupted ultrasonically (Sonifier, B-12, Bronson Sonic Power Co., Danbury, Connecticut, U.S.A.). Protein was precipitated by the addition of 3 vol. ethanol and the protein-free supernatant assayed for cyclic AMP using the competitive protein binding assay of Brown *et al.* (1971). The minimum amount of cyclic AMP that could be detected was 0·4 pmol. The intra- and interassay coefficients of variation were 12% (n = 36) and 14% (n = 10), respectively. Protein was determined in the precipitates by the method of Lowry *et al.* (1951), using bovine serum albumin as standard. Results are expressed as picomoles of cyclic AMP per milligram of protein.

Statistical Analyses

The experimental layout consisted of a hierarchical or 'split-plot' design with the two levels of PRL (control, PRL) nested within each of the five main treatment groups. Steroid concentration was subjected to analysis of variance by using appropriate error terms for testing main effects and the group × PRL interaction, and for calculation of least significant differences for pairwise comparisons of means. Progesterone concentration was analysed following a logarithmic transformation to remove variance heterogeneity.

The relationships between the production of each steroid per follicle (Y) and follicle size (X = mean diameter of follicles within a flask) were investigated by linear regression.

*N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

In the second phase of the experiment the above design was further stratified by the introduction of three levels of hormone (control, LH, FSH) within each level of the PRL treatment for both the theca and granulosa tissues. The response of these isolated follicular tissues to incubation with hormones was analysed by examining the difference in cyclic AMP production between LH- or FSH-treated tissue and the respective controls using a hierarchical analysis of variance of logarithmic transformed differences of flask averages.

Results

State of Ovary

Four of the 15 post-partum cows had recommenced their oestrous cycles as determined by examination of the ovaries and measurement of plasma progesterone concentrations. Three of these were from group 1 and one from group 3. This was reflected in the mean concentrations of progesterone in the plasma samples taken on the day of ovariectomy, which were, for groups 1–5 respectively, $2{\cdot}5 \pm 1{\cdot}0$ (s.e.m.), $3{\cdot}1 \pm 0{\cdot}9$, $0{\cdot}5 \pm 0{\cdot}1$, $2{\cdot}7 \pm 0{\cdot}8$ and $0{\cdot}4 \pm 0{\cdot}0$ ng/ml. One cow in group 2 did not have an active corpus luteum but contained a luteinized cystic follicle.

Table 3. Effect of prolactin (1 μg/ml of culture medium) on steroid release by bovine Graafian follicles during 18 h in culture

All cow groups combined (n = 50); n.s., not significant

Steroid	Steroid released (ng/mg protein)[A]		S.e. of difference[B]
	Control	Prolactin-treated	
Oestradiol	16·8	13·2	2·7 (n.s.)
Progesterone	11·2	18·1	7·1 (n.s.)
Testosterone	744	716	33·9 (n.s.)
Androstenedione	247	238	20·9 (n.s.)

[A] Total protein contributed by theca + granulosa tissues.
[B] Progesterone data were log transformed for analysis of variance.

There was no difference in the total number of follicles isolated from the cows in each group, whether post partum or cyclic. However, the larger number of follicles in the size range of 4–6 mm and the smaller number of follicles less than 4 mm in size, isolated from the ovaries of cyclic cows, suggest that there may be a reduction in the rate of follicular development in the post-partum cows (Table 2). This trend was also evident when individual groups were compared (groups 1 *v.* 2, groups 3 *v.* 4) but no differences were seen for the predicted effects of PMSG (groups 1 *v.* 3, groups 2 *v.* 4) or CB154 (groups 1 *v.* 5) in increasing the proportion of 4–6-mm follicles.

Steroid Production

There was no overall difference in the accumulation of any of the four steroids examined in culture medium when cow follicles were incubated in the presence or absence of 1 μg of PRL per millilitre of medium (Table 3). It is assumed that this accumulation represents *de novo* synthesis and release of steroid from the follicles and is not merely a reflection of original follicular fluid concentrations as it has previously been shown that extrafollicular accumulation of progesterone continues over a

72-h culture period (Shemesh and Ailenberg 1977). When the comparisons were made for the individual steroids within each treatment group, the only differences ($P < 0{\cdot}05$) noted were that untreated follicles in group 4 secreted more testosterone than PRL-treated follicles (879 *v.* 644, L.S.D. = 203), while in group 3 PRL increased androstenedione production (355 *v.* 238, L.S.D. = 115). Also, PRL-treated follicles from the post-partum cows in group 1 released significantly more testosterone than did PRL-treated follicles from cyclic cows in group 4 (1184 *v.* 981, L.S.D. = 203).

Table 4. Effect of treatment group on steroid release by bovine Graafian follicles during 18 h in culture

Control and prolactin treatments combined ($n = 20$); n.s., not significant

Group	Steroid released (ng/mg protein)[A]			
	Oestradiol	Progesterone	Testosterone	Androstenedione
1	10·5	2·4	855	233
2	3·4	30·3	753	312
3	16·6	14·9	712	247
4	20·9	11·3	616	220
5	23·5	14·4	713	200
S.e. of difference between any two groups	14·0	14·6	153	73
Significance between groups[B]	n.s.	n.s.	n.s.	n.s.

[A] Total protein contributed by theca + granulosa tissues.
[B] Progesterone data were log transformed for analysis of variance.

There was no significant difference ($P > 0{\cdot}05$) in the production of any steroid between the treatment groups of cows (Table 4). In particular, differences between the groups 1, 3 and 5, groups 1 and 2, groups 3 and 4, and groups 2 and 4 were

Table 5. Response of theca[A] to LH (1 μg/ml) when incubated for 40 min at 37°C

Results are expressed as the natural logarithm of the number of picomoles of cyclic AMP per milligram of protein and are obtained from the difference between incubation with and without LH. Effects of group of cows and hormone pretreatment of follicles are presented. Values with identical superscripts are significantly different, $P < 0{\cdot}05$ (s.e. of difference between control and prolactin within any group is 0·23)

Group	Group means	Group × pretreatment	
		Control	Prolactin
1	2·90	3·01	2·79
2	2·40	2·92[b]	1·88[b]
3	2·33	2·67[c]	1·99[c]
4	2·11	2·12	2·10
5	2·41	2·56	2·26
S.e. of difference between any two groups	0·74	0·76	0·76

[A] Theca obtained from four to six follicles per cow and four cows per group.

considered. Despite apparently low oestradiol-17β release by group 2 and low progesterone release by group 1, the large variation between cows within groups decreased the significance of these results. Apart from the difference between groups

1 and 4 in the effect of PRL on testosterone production, follicles from post-partum animals had a similar propensity to synthesize steroids as did those from cyclic animals. In addition, treatment with PMSG or CB154 prior to ovariectomy did not significantly alter the pattern of steroid release of the isolated follicles in culture.

The relationship between the production of each steroid per follicle (Y) and mean follicle diameter (X) was as follows:

Oestradiol-17β	$Y = -192{\cdot}6 + 47{\cdot}6X$	$r = 0{\cdot}68$, $n = 94$
Testosterone	$Y = 77{\cdot}7 + 175{\cdot}2X$	$r = 0{\cdot}50$, $n = 94$
Androstenedione	$Y = 161{\cdot}9 + 23{\cdot}9X$	$r = 0{\cdot}22$, $n = 94$
Progesterone	$Y = -15{\cdot}7 + 8{\cdot}1X$	$r = 0{\cdot}16$, $n = 94$

Although these relationships were significant ($P < 0{\cdot}05$) for all steroids but progesterone, the proportions of variation accounted for by regression were only 46, 25, 5 and 3% respectively, indicating the low predictive value of follicle diameter for steroid accumulation.

Table 6. Response of granulosa[A] to FSH (5 μg/ml) when incubated for 40 min at 37°C

Results are expressed as the natural logarithm of the number of picomoles of cyclic AMP per milligram of protein and are obtained from the difference between incubation with and without FSH. Effects of group of cows and hormone pretreatment are presented. Values with identical superscripts are significantly different, $P < 0{\cdot}05$ (s.e. of difference between control and prolactin within any group is 0·37)

Group	Group means	Group × pretreatment	
		Control	Prolactin
1	2·45	2·30	2·60
2	1·83	1·49	2·16
3	3·57	4·07[b]	3·06[b]
4	1·71	1·91	1·52
5	2·66	2·90	2·42
S.e. of difference between any two groups	1·06	1·10	1·10

[A] Granulosa obtained from four to six follicles per cow and four cows per group.

Cyclic AMP Production

Thecal tissue from only one cow in each group responded to FSH and in no flask did granulosa tissue respond to LH. Consequently FSH was omitted as a level of hormone in the analysis of cyclic AMP production by theca while LH was omitted from the analysis of the response of the granulosa.

Prolactin reduced the response to LH of theca of follicles from lactating post-partum cows treated with PMSG (group 3) and from cyclic cows (group 2), $P < 0{\cdot}05$ (Table 5). Although not significant, a decrease was also observed in groups 1, 4 and 5, resulting in an overall significant decrease due to PRL ($P < 0{\cdot}01$). Response of granulosa cells to FSH (Table 6) was significantly reduced by PRL in group 3, but this difference was not reflected in the overall treatment means.

There were no significant effects of the reproductive state (post partum or cyclic) or treatment (prostaglandin and PMSG, CB154) on the response of theca or granulosa to LH or FSH.

Discussion

This study has shown that the response of follicular adenylate cyclase to gonadotrophic stimulation is not suppressed in the post-partum cow. The increases in cyclic AMP levels in the theca after LH treatment, and in the granulosa cells after FSH treatment, were not affected by the reproductive state or hormonal treatment of the animal from which the follicles were obtained. The lack of increase in cyclic AMP concentrations in isolated theca incubated with FSH, and in isolated granulosa cells incubated with LH is similar to the response observed in tissue from sheep follicles at a comparable stage of development (Weiss *et al.* 1978). In subsequent studies, we have found identical results using bovine follicles less than 7 mm diameter (Selstam and Weiss 1979) and that bovine granulosa cells gradually acquire the ability to increase the concentration of cyclic AMP in response to LH as the follicles increase in size to 15–20 mm in diameter, i.e. during the accumulation of fluid, (T. J. Weiss, G. Selstam and D. T. Armstrong, unpublished results).

These data support our decision to investigate the small (<9 mm diameter) follicles as representing a reasonably homogenous group despite the fact that follicles of 9 mm may have reached the maximum volume of granulosa tissue and are placed in the final class 13 (Scaramuzzi *et al.* 1980). Marion *et al.* (1968) have suggested that follicles of *c.* 8 mm diameter or more have the capacity to luteinize in response to LH and the studies discussed above are in fair agreement. Thus bovine granulosa tissue would appear to develop LH receptors after cell division has ceased and when further increase in follicular size was due to fluid accumulation alone.

Our observation that PRL in culture reduced the response of adenylate cyclase in the theca to LH, and in the granulosa of one experimental group when incubated with FSH, provides evidence that PRL has a modifying effect in the bovine ovary. Binding sites for PRL have been demonstrated in rat (Richards and Williams 1976), bovine and human ovarian tissue (Poindexter *et al.* 1979). It has been proposed that this peptide may regulate progesterone production by human granulosa cells (McNatty *et al.* 1974), and that the elevated levels of PRL in human follicular fluid may be sufficient to suppress granulosa cell progesterone production. No such effects could be found in the present experiments.

Prolactin can affect both LH receptor concentration and steroidogenesis in the ovary of various mammals but the sites of these actions are not certain. Veldhuis and Hammond (1980) recently found that PRL, in the presence of oestrogen, stimulates progesterone production by porcine granulosa cells after several days in culture but has little or no effect on its own. In contrast, acute exposure of these cells to PRL or oestrogen inhibits progesterone production. In addition, PRL has been shown to inhibit ovulation in perfused rabbit ovaries (Hamada *et al.* 1980), but the site of action is not clear. The follicles in the present study released little or undetectable amounts of oestradiol, probably due to their lack of LH receptors in the granulosa, perhaps permitting PRL to exert the observed inhibitory effect on other gonadotrophic actions. However, it is not clear why we did not observe any differences in response between tissues isolated from post-partum cows, which have high concentrations of plasma PRL (Karg and Schams 1974), and tissues isolated from cyclic or CB154-treated cows. Further studies are required to establish the repeatability and significance of the *in vitro* PRL-induced suppression of the cellular cyclic AMP response to gonadotrophic stimulation.

The lack of any major difference in steroid secretion by follicles isolated from post-partum and cyclic cows provides evidence that the site of the lesion during post-partum anoestrus is not at the follicular level. However, before this possibility can be eliminated, two factors must be considered. Firstly, in this study we elected to examine small follicles which differ considerably from preovulatory follicles, in terms of their size and possibly the PRL content of the follicular fluid (McNatty *et al.* 1974), their lower oestrogen synthesis and different responsiveness of their granulosa cells to gonadotrophins. Secondly, a major problem encountered in this study was the large variation in the steroid secretion by follicles of similar size and tissue protein content. This lack of homogeneity may be due to the unobserved inclusion of early atretic follicles which in sheep have been shown to exhibit a markedly different steroidogenic pattern to nonatretic follicles (Moor *et al.* 1978). However, this large variation in steroid output has also been observed for follicles which, by gross morphological criteria, appear 'normal' and non-atretic (Weiss 1979) and appears to be a problem indigenous to this type of study. As a result, any differences between the steroid production of follicles isolated from post-partum and cyclic cows may be masked. It is possible that the methodology used here is not suitable to achieve the aims of the experiments.

The somewhat smaller number of follicles in the 4–6 mm size range in the ovaries of post-partum cows compared with cyclic cows (Table 2) suggests that follicular development may be partially blocked in the post-partum period. This could be interpreted as a decrease in the mitotic index of follicles of class 10 and less (Scaramuzzi *et al.* 1980) which would result in an increase in the doubling time of the granulosa tissue. This area is worthy of further investigation. As oestradiol secretion is most highly correlated with follicle diameter, this may partially explain why Scaramuzzi *et al.* (1977) found lower concentrations of oestradiol circulating in the anoestrous post-partum cow than in the cyclic cow. Perhaps this slower development of follicles <4 mm is a reflection of lower gonadotrophin levels. The concentrations of LH during the first 30 days post partum were lower in cattle with an anoestrous period extending beyond 80 days as compared with those experiencing their first ovulation by 45 days (H. M. Radford, G. J. Faichney, C. D. Nancarrow and N. McC. Graham, unpublished observations). Various anoestrous conditions in cattle and sheep are accompanied by lower gonadotrophin levels (Nancarrow *et al.* 1978). It is probable that our results were compromised by the chance occurrence of three out of five cows in group 1 ovulating prior to 35 days post partum.

The increase of endogenous concentrations of PRL with thyrotrophin releasing hormone failed to influence the positive feedback of oestradiol on LH release and oestrous response (Nancarrow and Radford 1976). Injections of CB154 failed to induce resumption of cyclical ovarian activity in post-partum cattle (Cummins *et al.* 1977). We have been unable to demonstrate differences between cyclic and post-partum cows in the ability of their follicles to secrete steroids or respond to gonadotrophins in culture. Alteration of endogenous PRL concentrations with CB154 was without effect on these parameters as was pre-exposure to PMSG *in vivo*. The inclusion of PRL in the culture medium, although not affecting steroid accumulation over 18 h, did partially suppress the subsequent response of the isolated follicular tissues to gonadotrophins. We therefore conclude that the high endogenous levels of PRL which occur prior to and following calving may modify but not prevent the endocrinological changes leading to the revival of ovarian activity and ovula-

tion. Other more subtle mechanisms must contribute to prolonged post-partum anoestrus.

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