REPRODUCTIVE PHYSIOLOGY OF THE *POST PARTUM* COW

I. CLINICAL AND HISTOLOGICAL FINDINGS

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Summary. The mean interval to the first observed *post partum* oestrus was 14 days in milked cows. In contrast, none of eleven nursed animals showed behavioural oestrus by Day 30 *post partum*, although two ovulated before that time. Only one of seven anaemic animals (haemoglobin levels of 9 to 10 g/100 ml) showed behavioural oestrus by Day 22 *post partum*; two others ovulated, but showed no signs of oestrus. Thyroprotein-fed cows also had prolonged periods of *post partum* anoestrus.

Pregnancy corpora lutea were quite degenerate by Day 7 *post partum*. At Day 14 *post partum*, they were approximately 9.0 mm in diameter and remained at this size, at least to Day 30. There was an increase in follicular size following parturition. At Day 7, the mean diameter of the largest follicle was 9.6 mm; at Day 14, it was 11.3 mm; at Day 30, the largest follicle averaged 13.0 mm.

Neither the presence of suckling calves, nor anaemia delayed uterine involution. Caruncular tissue sloughed and was expelled from the uterus between Day 7 and Day 14 *post partum*. Regeneration of the surface epithelium over the caruncles occurred by growth from the surrounding tissue, and was complete in a majority of animals slaughtered at Day 30 *post partum*.

INTRODUCTION

Morrow, Roberts, McEntee & Gray (1966) recently reviewed the literature on the *post partum* interval before cyclic reproductive activity is re-established. Based primarily on rectal palpations and clinical observations, the time period needed for uterine involution to occur has been reported to be from 25 (Morrow *et al.*, 1966) to 50 days (Buch, Tyler & Casida, 1955). The time of first ovulation has been reported as 15 days (Morrow *et al.*, 1966), 19 days (Menge, Mares, Tyler & Casida, 1962) and 35 days *post partum* (Casida & Wisnicky, 1950). Nursing by calves delays the return to *post partum* cyclic activity, and depresses fertility according to Wiltbank & Cook (1958). Gier & Marion (1968) found that normal mucosa is re-established over the caruncles in most animals by 25 days *post partum*.

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In this study, the relationship of clinical observations to histological change in the reproductive organs has been examined.

MATERIALS AND METHODS

Animals

Forty-four animals were used, thirty-nine primipara and five pluripara; they were grade Holsteins, fed mixed hay, grain and silage. Animals were observed for signs of oestrus at least twice daily. Rectal palpations were not carried out following parturition.

Treatments

The seven experimental groups are described in Table 1. All animals were

TABLE 1
COMPOSITION OF EXPERIMENTAL GROUPS

Group	No. of animals	Days post partum at slaughter	Days of oestrous cycle at slaughter	Additional treatments
1. Day 7	5	7	Anoestrus	None
2. Day 14	6	14	Anoestrus	None
3. Day 30 milked	7	28 to 41*	17 to 19	None
4. Day 30 nursed	11	23 to 32	Anoestrus†	Nursed calves at will during the entire <i>post partum</i> period
5. Day 30 anaemic	6	30 to 41	Anoestrus‡	Bled at frequent intervals to maintain haemoglobin levels at two-thirds of level at parturition
6. Cyclic control	3	310 to 426	17 to 20	None
7. Thyroprotein fed	6	107 to 347	17 to 20§	Fed 10 g thyroprotein (100 mg equivalent L-thyroxine) during the lactation period

* Six of the animals were slaughtered between 28 and 32 days *post partum*.

† Two animals were found to have ovulated when examined at slaughter. The corpora were estimated to be 2 to 3 days of age. Oestrus was not observed in any animals in this group.

‡ No oestrus was observed in five of the six animals, but two had ovulated about 5 days before slaughter, as estimated by the size and colour of the corpora. The sixth animal came in oestrus and was slaughtered at Day 19 of the first oestrous cycle.

§ Two animals were anoestrus at the time of slaughter, and one was found to have a corpus luteum estimated as 4 days of age at slaughter. The other three animals had been observed in oestrus.

milked twice daily by machine except for Group 4, as noted in the table. Animals in Group 5 were bled sufficiently to result in haemoglobin values of 9 to 10 g%, as compared to levels of 13 to 16 g% at parturition. Group 7 consisted of six cows that were available for slaughter from a larger group that was being used for a thyroprotein-feeding experiment.

Necropsy and histological procedures

All animals were killed by stunning with a captive bolt pistol, and bled from the neck. The entire reproductive tract was removed, the uterine horns trimmed, weighed and measured at the bifurcation. The ovaries were trimmed, weighed

and cyclic corpora lutea dissected free, weighed, and 0.4 to 0.5 g samples stored frozen in 95% ethanol for subsequent progesterone assay.

Portions of uterus, cervix, vagina and the entire ovaries were placed in Bouin's fixative. After fixation, each ovary was sliced in 2-mm slices and the cross-sections of the pregnancy corpus luteum and all follicles > 7 mm diameter were measured.

After fixation, tissues were trimmed, embedded in paraffin, sectioned at 6 μ and stained with haematoxylin and eosin.

Progesterone assay

Samples of cyclic corpora lutea were assayed for progesterone by homogenization in 95% ethanol, refluxing with 95% ethanol three times, and thin layer chromatography using two-dimensional development in hexane-ethyl acetate (5:2), followed by methylene chloride-ether (5:2) (Armstrong, O'Brien & Greep, 1964). The spots were then located by ultra violet absorption, eluted with absolute ethanol and the amount of progesterone measured quantitatively in a Beckman Model DB spectrophotometer. A blank for each sample was prepared from the same plate. ^{14}C -labelled progesterone was added to the original sample to allow correction for losses during the analysis. Complete details of the method are given by Seifart & Hansel (1968).

RESULTS

The occurrence of behavioural oestrus was recorded on all *post partum* animals. The results of Groups 3, 4 and 5 are shown in Table 2. No oestrus or ovulation occurred in either Group 1 (Day 7) or Group 2 (Day 14). This may have been

TABLE 2
INCIDENCE OF POST PARTUM OVULATION AND OESTRUS

Group	No. animals	No. in oestrus	Days to first oestrus		No. ovulated	Days to first ovulation	
			Mean	Range		Mean	Range
3. Day 30 milked	7	5	14	10 to 22	6	14	10 to 22
4. Day 30 nursed	11	0	—	—	2	27.5	22 to 28
5. Day 30 anaemic	6	1	22	22	3	23	22 to 25

due, in part, to the fact that three of the six animals in Group 2 were slaughtered before Day 14 (at Days 11, 12 and 13). There was a significant difference in incidence of oestrus between the Day-30 milked group and the nursed and anaemic animals ($P < 0.005$). The interval from parturition to first ovulation for Group 3 (14 days) was much shorter than for Groups 4 (27.5 days) and 5 (23 days). Statistical analysis of these two comparisons shows that they are significant at $P < 0.10$. Two animals in both the anaemic and nursed groups ovulated, but did not show behavioural signs of oestrus. Only one animal in Group 3 ovulated without being observed in oestrus. Thus, there was a differ-

ence in occurrence of ovulation and behavioural oestrus in some animals. Both Menge *et al.* (1962) and Morrow *et al.* (1966) reported an increased incidence of 'silent' oestrus during the first *post partum* oestrus.

The occurrence of the first *post partum* oestrus was delayed in cows fed thyroprotein when compared to controls (87 days versus 57 days). However, services per conception were not greatly different (1.93 versus 1.66) for thyroprotein-fed and control cows, respectively. This result agrees with the previous report of Leech & Bailey (1953) that thyroprotein or thyroxine administration did not depress fertility, even after two successive lactations.

Mean diameter of the regressed pregnancy CL was 12.7 mm at Day 7, 9.3 mm at Day 14 and 8.5 mm at Day 30 *post partum*. The maintenance of this size is apparently due to the larger amount of vascular tissue present in these pregnancy corpora as compared to cyclic corpora. Even at Day 7 *post partum*, there were few, if any, active luteal cells present. Similarly, Labhsetwar, Collins, Tyler & Casida (1964) reported that progesterone was undetectable in pregnancy corpora on the day of parturition. The thickened, coiled blood vessels suggest that a constriction or blockage that could cause a sudden drop in progesterone secretion may occur.

TABLE 3
OVARIAN WEIGHT

Group	Left (g)	Right (g)	Gravid (g)	Non-gravid (g)
1. Day 7	6.20	4.75	7.07	3.88
2. Day 14	5.35	7.06	5.92	5.32
3. Day 30 milked	9.18	10.28	8.61	11.35
4. Day 30 nursed	6.98	8.84	7.43	8.30
5. Day 30 anaemic	7.52	8.47	7.34	8.47
Mean	7.05	7.97	7.29	7.75

The ovarian weights for the *post partum* animals (Groups 1 to 5) are given in Table 3. The average size of the largest follicle increased from 9.6 mm at 7 days *post partum* to 13.1 mm in cows slaughtered 18 to 19 days after the first *post partum* oestrus. There was no difference in follicle size at Day 30 *post partum* regardless of whether the animals were milked twice daily, nursed calves or had anaemia. The considerable follicular activity seen in the ovaries of the nursed and anaemic animals that did not ovulate suggests that adequate FSH is present in such animals. The continued presence of nearly mature follicles and the absence of ovulations indicates a possible deficiency of luteinizing hormone. The thickening and infolding of granulosa cells seen in a few follicles may represent unsuccessful attempts at luteinization of intact follicles.

Since follicles are usually present in the ovaries of suckled animals, one might be able to induce ovulation by removing the young for a short period of time. This would allow for LH release and ovulation, and might prove an effective method for synchronizing oestrous cycles. There is some evidence, however, that cows suckling young may show shortened cycles similar to those produced

by injections of exogenous oxytocin (Hansel, Donaldson, Wagner & Brunner, 1966). If such is the case, producing oestrus and ovulation by temporary removal of young might result in hypofunction of the newly formed CL.

Cyclic CL taken from animals slaughtered 5 or more days post-oestrus had progesterone contents as shown in Table 4. Only two cystic CL were observed in the *post partum* cows; one in W29 and one in W30.

Position of the first *post partum* ovulation, or the largest follicle when no CL was present, was studied in relation to site of the pregnancy CL. Since the overall incidence of activity in the right ovary was 0.4857 and in the left ovary 0.5143, expansion of these as the binomial $(a+b)^2$ would give the theoretical incidence of the various combinations, as seen in Table 5. The actual incidence was not significantly different from the theoretical.

TABLE 4
PROGESTERONE CONTENT AND CONCENTRATION OF CYCLIC CORPORA LUTEA

Group treatment	Cow no.	Day of cycle	Weight (g)	Progesterone conc. ($\mu\text{g/g}$)	Total (μg)
4. Day 30 milked	W46	17	0.90	13.92	12.53
	W37	18	4.66	31.19	145.35
	W29	18	4.30	38.14	164.00
	W29	18	4.35	38.26	166.43
	W25	19	3.70	16.25	60.13
	W30	19	3.85	5.55	21.37
Mean			3.62	26.16	94.97
5. Day 30 anaemic	W33	5	1.56	37.48	58.47
	W45	5	1.42	21.80	30.96
	W32	19	3.95	19.14	75.60
	Mean		2.31	23.81	55.01
6. Cyclic control	W17	17	8.30	58.86	488.54
	W16	19	5.65	61.60	328.04
	W21	20	6.60	44.45	293.37
	Mean		6.85	54.97	376.65
7. Thyroprotein-fed	W18	17	3.70	65.95	244.02
	W20	18	4.72	15.41	72.73
	W12	20	2.25	None detectable	None
	Mean		3.56	23.78	105.58

Saiduddin, Riesen, Tyler & Casida (1967) reported that 80% of first ovulations up to 20 days *post partum* occurred on the ovary contralateral to the previously gravid side. We have no explanation for the apparent differences in these results, but the parity of the experimental animals may be an important factor.

Involution of the uterus was not altered by nursing calves, or anaemia. At Day 30 *post partum*, the average diameter of the gravid horn at the bifurcation was 3.94 cm in milked animals compared to 4.83 cm for the nursed group. However, average weights of the gravid horn were not very different, 306 g versus 314 g. The non-gravid horn was approximately 1 cm smaller in diameter

and weighed about 100 g less than the gravid horn in all animals killed at Day 30 *post partum*.

Histologically, the process of involution showed the following progression. At Day 7, some exudate was present on the mucosal surface. A line could be seen on cross-sections of caruncles indicating the limit of the necrosis and subsequent sloughing of the caruncular surface (Pl. 1, Fig. 1) with only the base of the caruncle remaining viable.

TABLE 5
SITE OF POST PARTUM OVARIAN
ACTIVITY

<i>Sequence</i>	<i>Theoretical</i>	<i>Actual</i>
Left-Left	9	6
Left-Right	9	14
Right-Right	8	5
Right-Left	9	10
Total	35	35

The sloughing appeared to begin at a point just basal to the crypts and usually involved entire crypts. Thus, regeneration of the epithelium must normally occur from the margin of the slough. Giant multinucleated cells of unknown origin were often seen just outside the line shown in Pl. 1, Fig. 1 (D-D). The nuclei of these cells were usually peripherally orientated, and it appears likely that they were of histocytic origin, and distinct from the large binucleated trophoblastic cells often seen during pregnancy. By Days 12 to 14 *post partum*, the necrotic tissue had sloughed from the surface of the caruncles and regeneration of the epithelium was beginning. In some areas, the surface epithelium appeared to be highly proliferative and anaplastic (Pl. 1, Fig. 2). The tissue had lost its normal columnar arrangement, and was several cells thick. Large vacuoles were also present in many of the cells.

Regeneration occurred by the epithelium advancing from the edges of the caruncles. Cells in the advancing edge of the epithelium were usually flat or low cuboidal in nature (Pl. 1, Fig. 3). As the epithelization progressed the cell height of the new epithelium gradually increased to the normal columnar arrangement. Neutrophils could be seen migrating through the mucosal surface

EXPLANATION OF PLATE I

FIG. 1. Cross-section of caruncle from cow killed at Day 8 *post partum* (Group 1). The line (D-D) separates the vascular hillock (below) from foetal villi and maternal septa (above) undergoing coagulation necrosis. Blood vessels in normal base of the caruncle are thickened and constricted.

FIG. 2. Section of uterine mucosal epithelium from the inter-caruncular area of a cow killed on Day 14 *post partum* (Group 2). The mucosa is proliferative in appearance with loss of normal columnar structure. Hyaline droplets are present (H) and cytoplasmic vacuolation is occurring (V). Leucocytes are present in the submucosal tissue. $\times 375$.

FIG. 3. Section from caruncular surface undergoing regeneration of epithelium (Day 30 *post partum*, Group 3). Normal columnar epithelium is present (A) and tapers off to a thin cell layer at the upper end (B). $\times 250$.

FIG. 4. Section of uterine mucosa from cow killed at Day 14 *post partum* (Group 2). Some vacuolation is present in the mucosa and a large mass of neutrophils (N) may be seen migrating through the wall. $\times 375$.

PLATE 1

(Facing p. 498)

in some sections (Pl. 1, Fig. 4). The cells occasionally contained pigment granules in their cytoplasm. In all animals slaughtered at approximately 30 days *post partum* (Groups 3, 4 and 5), the findings were similar regardless of treatment. In a majority of animals, the epithelium appeared to have completely covered the caruncular surfaces; in the remaining animals, caruncles in one or more of the sections examined were not completely re-covered with epithelium. In some sections, where the epithelial covering was complete, there was considerable variation in the height of the epithelium. It was not unusual to see both squamous and tall columnar epithelium in the same high power field.

Five of the twenty-four animals in these three groups were showing scattered foci of lymphocytes just below the mucosa in the stratum compactum. Presence, or absence, of these foci was not related to extent of regeneration of the epithelium.

The Day-30 milked group (Group 3), generally had more fully developed endometrial glands. This fact was probably related to the higher incidence of ovulation and CL formation in this group; the progesterone from the new corpora providing stimulation for the endometrial glands.

Since the anaemic and nursing animals did not ovulate and produce CL, it appears that normal, cyclic ovarian activity is not essential to the overall process of involution. Possibly, sufficient steroids are produced by the non-cyclic ovary to cause normal involution to occur. Sharman (1966) has shown that ovariectomized guinea-pigs and rats show the normal processes of uterine involution. Goodall (1966) has shown that uterine involution in the rabbit can be blocked only if progesterone therapy is begun within 24 hr of parturition. Marion, Norwood & Gier (1968) stated that neither ovariectomy, nor physiological doses of 17β -oestradiol influenced regression of the uterus in cows. Foote & Hunter (1964) found that progesterone, with or without oestradiol, shortened the intervals to *post partum* ovulation and conception, which they interpreted to mean an early return of normal uterine function. However, Marion *et al.* (1968) found that progesterone delayed uterine regression in cattle.

The uterine mucosal epithelium was re-established in most normal cows within 30 days *post partum* in our study, confirming similar findings by Rasbech (1950) and Gier & Marion (1968). Perhaps such animals could be mated and have good fertility earlier than 60 days *post partum*, the date usually recommended for first breeding. Morrow *et al.* (1966) and Menge *et al.* (1962) stated that the first *post partum* cycle was shorter than normal, and this might suggest a deficient production of progesterone by the CL at this time. We do not feel that the differences in luteal progesterone content (Table 4) are of particular significance in this regard, since the progesterone contents of corpora decline rather sharply at the end of the cycle. At this stage of the cycle, the number of days until oestrus is of more importance than days post-oestrus in determining luteal function, and there is considerable variability in progesterone content of 18- to 20-day corpora.

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