THE CORPUS LUTEUM OF THE SHEEP: EFFECT OF UTERINE REMOVAL DURING LUTEAL REGRESSION

R. M. MOOR,* MARY F. HAY*, R. V. SHORT AND L. E. A. ROWSON*

A.R.C. Unit of Reproductive Physiology and Biochemistry*
and Department of Veterinary Clinical Studies, University of Cambridge

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Summary. The degree to which the corpus luteum is influenced by the uterus during the period of luteal regression has been investigated in two series of experiments. Ovarian vein blood was collected for progesterone analysis and the uterus was removed from forty normal sheep (Experiment 1) and eleven superovulated animals (Experiment 2) on the 15th day after oestrus. In addition, one ovary was removed from each sheep in Exp. 2 and the corpora lutea were studied histologically. At autopsy, on either Day 18, 25, 50 or 100, further samples of ovarian venous blood and luteal tissue were obtained from those animals whose corpora lutea had been maintained.

Luteal involution was arrested by hysterectomy on Day 15 even when the concentration of progesterone in the ovarian venous blood had declined to values as low as 40 μg/100 ml plasma and karyorrhexis had become widespread. It is suggested that the uterus is essential both for the initiation and for the continuation of the degenerative processes that occur during regression of the corpus luteum.

INTRODUCTION

A substantial amount of evidence indicates that the non-gravid uterus is involved in the cyclical regression of corpora lutea in many species (Melampy & Anderson, 1968). For example, removal of the uterus in sheep prolongs the life-span of the corpus luteum (Wiltbank & Casida, 1956), while the grafting of endometrial tissue into hysterectomized animals terminates luteal function (Rowson, Hay, Caldwell & Moor, unpublished observations; Melampy & Anderson, 1968). Although it has been established that the corpora lutea in each ovary are affected most markedly by the adjacent uterine horn (Moor & Rowson, 1966a), the nature of the lytic stimulus from the uterus still remains obscure.

The purpose of the present study was to ascertain more precisely the time during which the lytic influence of the uterus is necessary for complete luteal regression.

* Postal address: Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ.
A total of fifty-one non-pregnant sheep was randomly assigned to two experiments, forty to the first and eleven to the second experiment. The ewes were checked for oestrus twice daily using vasectomized rams and the day on which mating occurred was designated Day 0.

Experiment 1

A laparotomy was performed on each animal on Day 15 of the cycle and 10 ml of blood was aspirated into a heparinized syringe from the principal vein draining the ovary containing the corpus luteum. The corpus luteum was marked with animal charcoal and the uterus immediately removed. The sheep were placed in a recovery pen with a vasectomized ram and those animals that did not exhibit oestrus within 5 days of hysterectomy were examined on either Day 25, 50 or 100. A second laparotomy was performed and 10 ml of blood was withdrawn from the same vein as before. The animal was then killed under anaesthetic and a sample of the corpus luteum was fixed in Bouin's fluid for histological examination. From the corpora lutea obtained on Day 100, samples were also taken for study by histochemical methods.

Experiment 2

In order to induce corpora lutea in both ovaries, eleven sheep were treated with a subcutaneous injection of 750 i.u. pregnant mares serum gonadotrophin on Day 12 of the cycle. Fifteen days after the animals had returned to oestrus, 10-ml aliquots of ovarian venous blood were aspirated at laparotomy from the principal ovarian vein draining each ovary. The uterus and one of the ovaries were then removed; the corpus lutea in the remaining ovary were marked while those in the excised ovary were dissected out and fixed in Bouin's fluid. In three sheep in which there was a difference in the appearance of the corpora lutea between the two ovaries or luteal tissue was present in only one ovary, a biopsy was taken from one of the corpora lutea in the ovary that was left in situ. A vasectomized ram was placed in the recovery pen with the hysterectomized sheep. On Day 18 or 19, each ewe was re-anaesthetized and if she had not returned to oestrus, a further 10-ml sample of venous blood was taken from the remaining ovary. All the sheep were then killed and the corpora lutea prepared for histological examination.

After collection, each 10-ml sample of ovarian vein blood was immediately chilled, and the plasma was separated by centrifugation and stored at -20°C until required for assay. The assay procedure for progesterone was based on the method described by Short (1958) with the addition of an internal isotope standard; in those cases where there was too little progesterone to quantitate by ultraviolet spectrophotometry, a gas chromatographic technique was used (Schomberg, Coudert & Short, 1967). All results were corrected for extraction losses.

Paraffin sections of the corpora lutea were stained with Delafield's haematoxylin and chromotrope 2R, by Heidenhain's azan method and with the Martius–Scarlet–Blue method of Lendrum, Fraser, Slidders & Henderson (1962). Luteal tissue collected on Day 100 was also examined histochemically.
Hysterectomy during luteal regression

for 3β-hydroxysteroid dehydrogenase activity, and for acid phosphatase activity (Burstone's method). These tests were carried out as described previously (Deane, Hay, Moor, Rowson & Short, 1966; Dingle, Hay & Moor, 1968).

RESULTS

Experiment 1

The concentration of progesterone in the ovarian venous blood sampled at the time of hysterectomy and at slaughter is shown in Text-fig. 1.

![Text-fig. 1. Progesterone concentration in ovarian vein blood of sheep at hysterectomy on Day 15 and immediately before slaughter on Day 25, 50 or 100. O, Sheep in which the corpora lutea regressed within 3 days of hysterectomy; —, sheep in which corpora lutea were maintained following hysterectomy (sheep no. shown above —).](image)

In nine animals, the ovarian venous blood progesterone concentration at hysterectomy was over 100 μg/100 ml plasma which is similar to the progesterone concentration found in sheep between the 8th and 14th day of the cycle (Short, 1964); the corpora lutea in all nine animals were maintained as a result of the hysterectomy. The blood progesterone concentration was measured in six of them at the time of slaughter and indicated that the corpora lutea were still fully functional. Of the remaining three animals, a suitable blood sample was not obtained from one (No. 10) killed on Day 50, and the other two (Nos. 18 and 19) died 60 and 30 days after hysterectomy. The corpora lutea were also maintained in ten sheep in which the progesterone level was between 80 and 40 μg/100 ml plasma at the time of removal of the uterus. However, when
the progesterone concentration was below 30 µg at hysterectomy then complete involution of the corpora lutea invariably occurred (nineteen animals) and the sheep returned to oestrus within 3 days of the operation. Two animals had ovarian venous progesterone levels of between 30 and 40 µg; in one (No. 20) the corpus luteum was maintained until the animal died 43 days after hysterectomy while in the other, the corpus luteum regressed within 3 days of the operation.

Blood samples, in which the concentrations of progesterone at hysterectomy and at slaughter could be compared, were obtained from sixteen of the twenty animals in which the corpora lutea were maintained by removal of the uterus (see Text-fig. 1). In six animals, the progesterone levels on the two occasions were almost identical (within 10 µg); in four animals, the concentrations at hysterectomy were greater by more than 10 µg/100 ml plasma and in six animals, they were less. It would appear, therefore, that those corpora lutea whose life-span was extended by hysterectomy continued to secrete progesterone at approximately the same level as at the time of surgery.

Lutein cells of functional appearance were observed in all the maintained corpora lutea obtained at autopsy and none showed any of the usual retrogressive changes, such as karyorrhexis. However, in the corpora lutea of sheep killed on Day 25, small lutein cells with shrunken nuclei and cytoplasm that stained darkly with chromotrope and in the azan method were found interspersed among the normal lutein cells. These darkly stained cells were particularly evident in one animal (sheep No. 3) with an ovarian venous progesterone concentration of 49 µg/100 ml plasma at hysterectomy (Pl. 1, Fig. 1). In the sheep killed on Days 50 and 100, the lutein cells were more rounded and regular in shape and there were fewer connective tissue type cells than in the fully functional corpora lutea of the cycle. In most of the 100-day corpora lutea, a few of the lutein cells contained granules that stained with azocarmine; none was seen on Day 25, and they were present in only one animal examined on Day 50. These granules are possibly related to the age of the gland as they are very obvious in late pregnancy, and also 135 to 145 days after oestrus in sheep hysterectomized on Day 7, 8 or 9 of the cycle (Bjersing, Hay, Moor & Short, unpublished observations). Histochemically, all the corpora lutea that were maintained to Day 100 in the present study appeared to be fully functional: steroid dehydrogenase activity was high and sites of acid phosphatase activity were

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**EXPLANATION OF PLATE 1**

Fig. 1. Corpus luteum on Day 25 from sheep No. 3, hysterectomized on Day 15 when the progesterone concentration in the ovarian vein was 49 µg/100 ml plasma. Note large lutein cells with lightly stained cytoplasm and many smaller lutein cells with darkly stained cytoplasm and shrunken nuclei. Heidenhain's azan stain. ×635.

Fig. 2. Partially regressed corpus luteum on Day 18 from superovulated sheep No. 44, hysterectomized and unilaterally ovariectomized on Day 15. In the upper left half of the figure, the luteal tissue shows no obvious sign of regression while in the lower right half it has the structure of a corpus albicans. A and B indicate areas shown at higher magnification in Figs. 3 and 4. Delafield's haematoxylin and chromotrope 2R. ×200.

Fig. 3. Detail of area A shown in Fig. 2. Large lutein cells are present and the tissue is of functional appearance. ×635.

Fig. 4. Detail of area B shown in Fig. 2. The luteal tissue has involuted and heavily vacuolated lutein cells with fragmenting nuclei (karyorrhexis) can be seen. ×635.
small. No clear histological or histochemical differences that could be related to functional activity were detected between the corpora lutea of the seven animals killed on Day 100. The luteal tissue of sheep No. 17 appeared to be fully functional, even though the progesterone concentration in the ovarian vein blood was only 2 µg/100 ml; in this animal it seems likely that, due to adhesions, the blood was taken from a vein that was not draining the corpus luteum.

**Experiment 2**

The progesterone concentrations in the ovarian venous plasma at hysterectomy and at slaughter are shown in Table 1, together with the number of corpora lutea in each ovary. It is noteworthy that, in some animals, a considerable difference existed between the blood progesterone concentrations from the two ovaries. Table 1 also indicates whether or not the corpora lutea in the remaining ovary had regressed by Day 18 or 19.

The corpora lutea were maintained in four of the five animals in which the progesterone concentration in the blood from the ovary left in situ was over 40 µg/100 ml plasma at hysterectomy. Luteal regression occurred within 3 days in all six animals in which the steroid level was below 40 µg/100 ml plasma when the uterus was removed.

There were marked differences in the histological appearance of the corpora lutea removed on Day 15 and these corresponded well with the steroid values. For instance, in two sheep (Nos. 41 and 42), the progesterone concentration was over 100 µg/100 ml plasma, and the corpora lutea showed no histological signs of regression. In four sheep in which the progesterone value was below 20 µg/100 ml plasma, widespread cellular degeneration throughout the gland had occurred and many nuclei exhibited karyorrhexis. The degenerative

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**Table 1**

<table>
<thead>
<tr>
<th>Sheep no.</th>
<th>Progesterone concentration in ovarian venous plasma (µg/100 ml)</th>
<th>Day of slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At hysterectomy</td>
<td>At slaughter</td>
</tr>
<tr>
<td></td>
<td>Ovary subsequently removed</td>
<td>Ovary left in situ</td>
</tr>
<tr>
<td>41</td>
<td>18.4 (6)</td>
<td>180.9 (9)</td>
</tr>
<tr>
<td>42</td>
<td>108.4 (2)</td>
<td>172.8 (2)</td>
</tr>
<tr>
<td>43</td>
<td>0.9 (4)</td>
<td>77.7 (3)</td>
</tr>
<tr>
<td>44</td>
<td>18.3 (2)</td>
<td>51.8 (2)</td>
</tr>
<tr>
<td>45</td>
<td>96.9 (4)</td>
<td>47.7 (2)</td>
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<td>46</td>
<td>0 (0)</td>
<td>31.0 (2)</td>
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<tr>
<td>47</td>
<td>0 (0)</td>
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</tr>
<tr>
<td>48</td>
<td>12.9 (4)</td>
<td>0.6 (2)</td>
</tr>
<tr>
<td>49</td>
<td>— (1)</td>
<td>6.1 (2)</td>
</tr>
<tr>
<td>50</td>
<td>2.2 (1)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>51</td>
<td>2.3 (5)</td>
<td>1.7 (4)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are numbers of corpora lutea.
changes observed in this study were typical of those described and illustrated by Dingle et al. (1968).

A differential rate of luteal regression was observed between the two ovaries of one sheep (No. 43) on Day 15. In this animal, all four corpora lutea had completely regressed in the ovary that was removed at hysterectomy, while a biopsy of one of the corpora lutea in the other ovary showed only limited involutional changes. This asynchronous luteal regression was also clearly reflected in the markedly different ovarian vein blood progesterone concentrations from the two ovaries. A difference in the degree to which regression had occurred in individual corpora lutea within the same ovary was also seen in some animals: in sheep No. 45, two corpora lutea in the excised ovary had only very few degenerating cells, whilst the other two corpora lutea showed advanced regressive changes and numerous cells exhibited karyorrhexis.

Two of the hysterectomized sheep examined on Day 18 or 19 had high levels of progesterone in ovarian vein blood but in each case, although one corpus luteum appeared histologically to be relatively normal, at least one other showed advanced regression. Moreover, in both these animals (Nos. 43 and 44), differences in histological structure within a single corpus luteum were very clear (Pl. 1, Figs. 2 to 4). The corpus luteum that was biopsied on Day 15 (sheep No. 43) when it showed a little cellular degeneration had largely become a corpus albicans by Day 19, although there were still small areas of apparently healthy luteal tissue. While the ovarian venous plasma progesterone concentration was higher on Day 18 or 19 than on Day 15 in all the four sheep in which the corpora lutea were maintained, in only one of these animals was there any evidence of proliferation of luteal tissue. This was in sheep No. 44 which had only two corpora lutea in the ovary that was left in situ. The progesterone concentration in the ovarian venous effluent on Day 18 in this animal was more than double that found on Day 15, and yet, during this period, about two-thirds of the tissue in one of the corpora lutea involuted; the second corpus luteum, however, appeared healthy and contained a number of dividing cells.

DISCUSSION

The results of our experiments indicate that the involution of the sheep corpus luteum during pro-oestrus can be halted by the removal of the uterus even after the luteal tissue has undergone marked functional and morphological regression. If, however, the corpus luteum is almost non-functional at the time of hysterectomy, then luteal regression is completed despite removal of the uterus. Similar experiments in guinea-pigs and pigs have demonstrated that hysterectomy during the period of luteal regression can prolong the life of corpora lutea (Rowlands, 1961; Anderson, Butcher & Melampy, 1963). The presence of the uterus would thus appear to be essential both for the initiation and the continuation of those processes that lead to the death of the lutein cells.

No single method is at present available for accurately determining the earliest degenerative changes that occur in the corpus luteum. For example, without an accompanying accurate measurement of blood flow, any determination of the concentration of progesterone in the ovarian vein blood can be
regarded only as a relatively crude estimate of the secretory activity of the corpus luteum, a fact illustrated by the results in Exp. 2. Here, there was no obvious correlation between the number of corpora lutea and the concentration of progesterone in the ovarian vein blood. This suggests that there might have been a compensatory increase in ovarian blood flow, for with an increased number of corpora lutea it has been shown that there is a marked increase in progesterone concentration in peripheral blood (Short, 1961; Thorburn, Bassett & Smith, 1968).

To minimize surgical damage to the corpus luteum, no blood flow determinations were made in the present study. Thus, it would probably not have been possible to detect small differences in the functional activity of the corpus luteum. To compensate for this loss of sensitivity, careful histological studies were made on samples of luteal tissue both at hysterectomy and again at autopsy. It must be pointed out, however, that the use of biopsy samples from regressing corpora lutea sometimes gives results that are unrepresentative of the corpus luteum as a whole; this is particularly likely during the early stages of regression when changes may sometimes be restricted to small localized areas. This focal type of regression seems to occur much more often in superovulated sheep than in untreated animals where it is seen only very occasionally (Bjersing, Hay, Moor, Short & Deane, unpublished observations). By using both biochemical and histological techniques in the present study, we have attempted to overcome the deficiencies associated with either technique used alone.

On Day 15, relatively few animals had intermediate progesterone concentrations in the ovarian vein blood (Text-fig. 1). This suggests that steroid secretion stops very rapidly. The correlation between the biochemical and morphological parameters of luteal regression was good. Karyorrhexis was observed in at least some of the lutein cells by the time that the progesterone concentration in the ovarian venous effluent had fallen to 70 µg/100 ml plasma. As the blood progesterone level declined still further, the number of cells that showed degenerative changes, and the extent of the changes, increased. Judging from the histological evidence, it would appear that the removal of the uterus did not prevent the death of those lutein cells that had already started to involute at the time of hysterectomy. However, lutein cells which were still functional at the time of surgery appeared to be maintained in a functional state for at least a further 85 days, though some death and replacement of lutein cells are possibilities that cannot be disregarded. Cells exhibiting karyolysis were present in the maintained corpora lutea of the hysterectomized sheep; they are also commonly seen in ovine luteal tissue throughout the cycle and during pregnancy (Bjersing, Hay, Moor & Short, unpublished observations). The small darkly stained lutein cells seen on Day 25 in Exp. 1 (Pl. 1, Fig. 1) were probably dying. Such cells are not evident during normal regression at the end of the cycle; their presence may be a consequence of a slower type of death occurring in the absence of a uterine lytic stimulus.

In superovulated sheep with corpora lutea in both ovaries (Exp. 2), unilateral ovariectomy may possibly have led to a compensatory rise in progesterone concentration in the venous effluent from the remaining ovary. In the four
sheep in which the corpora lutea were maintained to slaughter 3 or 4 days after hysterectomy, the mean progesterone concentration increased two-fold.

It is interesting that the life-span of the corpus luteum is prolonged during pregnancy only when the conceptus is in the uterus by the 12th day after mating (Moor & Rowson, 1966b). Hysterectomy, on the other hand, is effective in maintaining the corpora lutea, even when performed as late as Day 15. The results in the present study indicate that the influence of the uterus on the regression of the corpus luteum is a continuous one which is immediately arrested by hysterectomy. It would appear likely, therefore, that there must be a rapid turnover of the lytic factor and this would also imply that the amount present within the endometrium at any given moment would be small, possibly a reason for the difficulties encountered in isolating such a factor from endometrial tissue or washings.

ACKNOWLEDGMENTS

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REFERENCES


