THE CORPUS LUTEUM
OF THE SHEEP: EFFECT OF THE CONCEPTUS ON
LUTEAL FUNCTION AT SEVERAL STAGES
DURING PREGNANCY

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(Received 4 September 1968)

SUMMARY

The relationship between the conceptus and the corpus luteum during the first 90 days of pregnancy was determined in sheep which were made unilaterally pregnant by egg transfer 4 or 5 days after oestrus. Additional corpora lutea were established approximately 24 hr. after an injection of human chorionic gonadotrophin given on day 20, 30, 50 or 70, and 18–24 days later the sheep were killed.

When 17 unilaterally pregnant ewes, in which new corpora lutea had been induced on or before day 31, were killed, only the corpora lutea on the same side as the gravid horn were still functional; total regression of the corpora lutea had occurred in the ovary adjacent to the non-gravid horn. In 13 out of 14 sheep in which corpora lutea had been induced on day 51 or 71, the luteal tissue was still present in both ovaries at autopsy. Thus a local effect is exerted during the first one-third of pregnancy by conceptuses confined to one uterine horn, but this effect changes to a more general one about day 50.

In five sheep, embryos were allowed to develop in an intact uterus and under these conditions no unilateral effect could be demonstrated on corpora lutea induced on day 31.

INTRODUCTION

It has been established that a local relationship exists between the embryo and the corpus luteum in some mammals. For example, in the Canadian porcupine only those accessory corpora lutea which are formed in the ovary adjacent to the gravid horn are maintained, while the corpora lutea in the opposite ovary regress (Mossman & Judas, 1949). More recently it has been demonstrated that the conceptuses exert a local effect on luteal function during early pregnancy in both sheep and pigs. In the sheep, conceptuses confined to one uterine horn during early pregnancy maintain only those corpora lutea which are on the same side as the gravid horn (Moor & Rowson, 1966a). Likewise in the pig unilateral regression of the corpora lutea can be induced by rendering a portion of one uterine horn non-gravid during the first 12–14 days of pregnancy (du Mesnil du Buisson, 1961; Dhindsa & Dziuk, 1968). After
this stage the corpora lutea in pregnant pigs are maintained in both ovaries even if the conceptuses are excluded from an entire uterine horn.

The purpose of the present study was to determine the effect exerted by the conceptus on the corpora lutea of sheep at several stages of pregnancy.

MATERIALS AND METHODS

A total of 137 mature Welsh Mountain sheep was used: 43 served as donors of embryos, and 94 as recipients.

The techniques used for the detection of oestrus (day 0), induction of super-ovulation, recovery of embryos from the donors, and the subsequent transfer of embryos to recipient sheep were as described previously (Moor & Rowson, 1966b).

<table>
<thead>
<tr>
<th>Group</th>
<th>Original no. of sheep in group</th>
<th>No. of sheep in which embryos developed</th>
<th>Interval between oestrus and HCG injection (days)*</th>
<th>No. of sheep with viable conceptuses at autopsy†</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>7</td>
<td>20</td>
<td>3</td>
<td>Single, naturally formed corpus luteum. Contralateral uterine horn isolated surgically</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>13</td>
<td>30</td>
<td>6</td>
<td>Single, naturally formed corpus luteum. No surgical isolation of uterine horn (control)</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>18</td>
<td>50</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>19</td>
<td>70</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>7</td>
<td>30</td>
<td>5</td>
<td>Naturally formed corpus luteum in both ovaries: no gonadotrophin treatment. Surgical isolation of one uterine horn.</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>8</td>
<td>—</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

* PMSG given 3 days before HCG in all cases.
† This number does not include sheep which had to be rejected for technical reasons (see text).

During the pre-implantation stages the embryonic disk and its membranes will be referred to as the ‘embryo’. After implantation the term ‘conceptus’ will be used to describe the foetus plus the maternal and foetal components of the placenta. The corpora lutea formed at ovulation on day 1 are termed the ‘naturally formed’ corpora lutea, while those formed as a result of gonadotrophic hormone injections during pregnancy are referred to as the ‘induced’ corpora lutea. The ‘ipsilateral uterine horn’ refers to the horn on the same side as the corpus luteum; the ‘contralateral uterine horn’ refers to the horn on the side opposite to the corpus luteum.

Laparotomy was performed on each recipient on the 4th or 5th day after oestrus, and the naturally formed corpus luteum was marked with animal charcoal for subsequent identification. The great majority of the recipients (84 out of 94) had only one corpus luteum, but there were ten animals with corpora lutea in both ovaries.

During laparotomy, in 74 out of the 84 sheep with a single corpus luteum, the contralateral uterine horn was ‘isolated’ from the rest of the uterus by transection between ligatures placed around the horn just above its junction with the body of the
uterine. The ligatures were always so positioned as to cause minimal damage to the uterine blood supply (see radiograph, Pl. 1, fig. 1). The remaining ten sheep which had a single corpus luteum were used as controls—that is, without ‘isolating’ one uterine horn by ligature. Before the wound was closed, two embryos were transferred to the tip of the ipsilateral uterine horn of each of the recipients. In the ten sheep with naturally formed corpora lutea in both ovaries, one uterine horn was ‘isolated’ as described above, and two embryos were transferred to the tip of the opposite horn. Transfers were carried out only when the onset of oestrus in the donor and recipient sheep was synchronized to within 24 hr.

After the embryo transfers had been completed, the sheep were allocated into groups (see Table 1). The 74 unilaterally pregnant recipients with a single naturally formed corpus luteum were randomly allocated to one of groups 1–4; group 5 comprised the ten intact control sheep; the ten sheep with naturally formed corpora lutea in both ovaries were put into group 6. The sheep in each group were run with vasectomized rams and the occurrence of any post-operative oestrous cycles was recorded.

The treatment subsequently given to each group of pregnant recipients is also shown in Table 1. Induced corpora lutea were established at various stages of pregnancy by the s.c. injection of 600 i.u. pregnant mare serum gonadotrophin (PMSG), followed 3 days later by an i.v. injection of 500 i.u. human chorionic gonadotrophin (HCG: Lutormone, Burroughs Wellcome and Co.). Ovulation occurs approximately 24 hr. after the administration of HCG.

The ovaries of all the pregnant recipients were examined on the 4th or 12th day after the HCG injection. The number and position of the induced corpora lutea were recorded together with the size and macroscopic appearance of the pregnant and sterile uterine horns. The condition of the conceptus was in some cases ascertained by visual inspection through a small incision in the wall of the gravid uterine horn. The uterine incision was sutured with fine catgut and the wound closed in the normal manner. The ovaries of each recipient were again examined at autopsy, 18–24 days after HCG injection. A heparinized syringe was used to aspirate a 10–20 ml. aliquot of blood from the major vein draining each ovary of some of the sheep; the recipients were then killed, the ovaries and uteri were inspected and appropriate samples of luteal and uterine tissue were fixed in Bouin’s fluid for histological examination. The ovarian venous blood was used for the determination of progesterone concentration; this was carried out by the u.v. spectrophotometric method (Short, 1958) except that thin-layer chromatography (diethyl-ether:benzene, 2:1) replaced paper chromatographic separation.

**RESULTS**

Of the 94 recipients used for this study, 32 were eliminated either because the transferred embryos failed to develop (22 sheep), the gonadotrophin treatment failed to induce corpora lutea in the contralateral ovary (seven sheep), or complete isolation of the contralateral horn was not achieved (three sheep). At autopsy 36 (28 in the gonadotrophin-treated groups 1–5, and eight in group 6) of the remaining 62 recipients were found to be carrying viable conceptuses (Table 1). Degenerating conceptuses were found in the other 26 sheep; luteal function in these animals will be discussed in a separate paper.
Table 2. The physiological state of corpora lutea (c.l.) in control and unilaterally pregnant recipient sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>Interval between oestrus and HCG injection (days)</th>
<th>No. of sheep in group</th>
<th>Physiological state of corpora lutea at autopsy (18–24 days after HCG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.l. on same side as pregnant horn*</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>3</td>
<td>Maintained (3)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>6</td>
<td>Maintained (6)</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>6</td>
<td>Maintained (6)</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>8</td>
<td>Maintained (8)</td>
</tr>
<tr>
<td>5 (control)</td>
<td>30</td>
<td>5</td>
<td>Maintained (5)</td>
</tr>
<tr>
<td>6</td>
<td>0†</td>
<td>8</td>
<td>Maintained (8)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are numbers of sheep.
† Naturally formed corpus luteum in both ovaries: no gonadotrophin treatment; autopsy 18–24 days after ovulation.

Table 3. Progesterone concentration in ovarian venous effluent of unilaterally pregnant sheep 18–24 days after new corpora lutea were induced by injection of HCG

<table>
<thead>
<tr>
<th>Group</th>
<th>Interval between oestrus and HCG injection (days)</th>
<th>Sheep no.</th>
<th>Progesterone in ovarian venous effluent (µg./100 ml. plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of corpora lutea</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
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<td>4</td>
<td>70</td>
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<tr>
<td>70</td>
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<td>3</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
The physiological state of the corpora lutea in the 36 unilaterally pregnant sheep at the time of autopsy is shown in Table 2. In the ovary on the same side as the pregnant horn, both the naturally formed and the induced corpora lutea were still present in all the experimental animals, and were of normal appearance for corpora lutea of pregnancy. In the control sheep in which neither horn had been isolated (group 5), the corpora lutea induced by HCG on day 30 were present in the contralateral ovary also. By contrast in the 17 unilaterally pregnant sheep in experimental groups 1, 2 and 6, all the corpora lutea in the contralateral ovary had completely regressed to corpora albicantia within 20 days of formation. However, those corpora lutea which had been induced later, i.e. on day 50 or 70, in 14 unilaterally pregnant sheep (groups 3 and 4) were maintained in the contralateral ovary in all but one animal.

The concentration of progesterone in samples of ovarian venous blood obtained from sheep in groups 3 and 4 just before autopsy showed that both the naturally formed and the induced corpora lutea were fully functional (Table 3). It is also evident from the Table that the level of progesterone in the venous blood from the ovary adjacent to the non-pregnant horn was equally as high as that in the blood from the ovary on the pregnant side.

In all the recipient sheep the histological appearance of the corpora lutea was in accordance with both the macroscopic and steroidal observations. At autopsy the corpora lutea in the ovary adjacent to the pregnant horn consisted principally of typical lutein cells. In the control sheep in which neither horn had been isolated, corpora lutea in both the ovaries were of typical functional appearance. By contrast, in the ovary on the non-pregnant side of unilaterally pregnant animals all those corpora lutea formed on or before day 31 (groups 1, 2 and 6) had completely regressed to corpora albicantia by the time of autopsy 20 days later. Corpora lutea induced on day 50 or 70 (groups 3 and 4), on the other hand, were still present in the contralateral ovary at autopsy and contained large numbers of plump lutein cells. In groups 3 and 4 no difference could be seen between the induced corpora lutea in the two ovaries. However, it was usually possible to distinguish histologically between the naturally formed and the induced corpora lutea. The latter frequently contained large numbers of leucocytes and also more small non-lutein cells than were seen in the naturally formed corpora lutea (Pl. 2, figs. 2 and 3).

Macroscopic examination of the non-pregnant uterine horn of recipients in groups 3 and 4 showed evidence of caruncular stimulation. Histologically these changes were most evident in group 4 sheep where the caruncles had become cup-shaped. The epithelium in the centre of the caruncle was attenuated or lost and the underlying tissue clearly necrotic (Pl. 2, fig. 4). Round the rim of the caruncles the epithelium was thickened and papillae or small villi were present (Pl. 2, fig. 5). This contrasts markedly with the histological appearance in cyclic non-pregnant sheep, where the caruncles are covered by a simple columnar epithelium (Pl. 2, fig. 6).

**DISCUSSION**

These experiments show that a local relationship exists between the conceptus and the corpus luteum in sheep throughout the first one-third of pregnancy. Up to about
day 50, a conceptus confined in one uterine horn is able to maintain the corpora lutea only in the adjacent ovary. Thereafter, the corpora lutea in both ovaries are maintained by a unilaterally confined conceptus. The possibility that the non-pregnant uterine horn in recipients treated with gonadotrophins on day 50 or 70 might become ‘pseudopregnant’ and so lose its ability to induce luteal regression is suggested by the appearance of the endometrium at autopsy. It might be possible to test this hypothesis by using one of the methods currently being developed for determining the ‘lytic’ effects of endometrial tissue (Caldwell, Rowson, Moor & Hay, 1969). Another possible mechanism by which the unilaterally confined conceptus might maintain the corpora lutea in both ovaries is by the production of placental luteotrophin.

It is interesting that the stage of pregnancy at which the effect of the conceptus changes from a local to a generalized one corresponds to the stage at which pregnancy can first be maintained in the absence of the ovaries (Denamur & Martinet, 1955). Whether there is any significant relationship between these two findings, however, is not at present clear.

Several advantages accrue from the use of unilaterally pregnant sheep in the study of the relationship between the conceptus, uterus and corpus luteum. Perhaps the most important of these is the small amount of surgical damage involved in making the animals unilaterally pregnant (Pl. 1, fig. 1). One can also demonstrate the local lytic effect exerted by a non-pregnant uterine horn on the corpora lutea in the adjacent ovary without recourse to hysterectomy. A further point of interest has been provided by the clear change, seen after day 50, in the non-gravid horn of unilaterally pregnant sheep. Even in the absence of any direct intervention of a conceptus, the caruncles become cup-shaped, and the changes occurring in the epithelium and underlying stroma were somewhat similar to those seen in early pregnancy. This observation is being used to investigate further the roles of the conceptus and endometrium in implantation.

We are grateful to Professor T. R. R. Mann, F.R.S., for reading and discussing the manuscript, and to Mrs P. Miles and Mr H. Strange for skilled technical assistance.

REFERENCES
DESCRIPTION OF PLATES

PLATE 1

Fig. 1. Radiograph showing the arterial blood supply to the sheep uterus, and also the position in which the ligatures were placed when one uterine horn was ‘isolated’.

PLATE 2

Paraffin sections stained with haematoxylin and chromotrope 2R.

Figs. 2, 3. Sections of corpora lutea from the ovary adjacent to the gravid horn of a unilaterally pregnant ewe treated with HCG on day 70 and autopsied 20 days later (×480). Fig. 2, Naturally formed corpus luteum. Fig. 3, Induced corpus luteum: numerous leucocytes can be seen, and there are also more small cells than in naturally formed corpora lutea (compare with fig. 2).

Figs. 4, 5. Sections through the caruncle in the non-gravid horn of a unilaterally pregnant ewe that had been treated with HCG on day 70 and autopsied 24 days later; the caruncle is cup-shaped (×154). Fig. 4 is taken from the centre of the caruncle where the epithelium is attenuated and the underlying stroma is necrotic. Fig. 5 is taken from the edge of the caruncle where the epithelium appears to have hypertrophied; papillae and short villi are present (compare with fig. 6).

Fig. 6. Section of uterine caruncle from a non-pregnant sheep on day 12 of the cycle. The epithelium is columnar and of uniform thickness. In the stroma beneath the epithelium some pigment can be seen. (×205).