Effect of exogenous oestrogen on blood flow and quantitative histology of the corpora lutea of pseudopregnant rabbits

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The effect of exogenous oestrogen on blood flow and quantitative histology of the corpus luteum were studied in pseudopregnant rabbits. Pseudopregnancy was induced with human chorionic gonadotrophin and an oestradiol capsule was implanted on day 0 of pseudopregnancy. Regression of the corpus luteum was initiated in the mid-luteal phase by removal of the capsule. Thirty-two New Zealand white rabbits were randomly allocated to four groups (eight rabbits per group) for studies of blood flow – control: oestradiol capsule not removed; – 24 h: oestradiol capsule removed 24 h before blood flow measurements; – 48 h: oestradiol capsule removed for 48 h; and – 48 h/+ 48 h: oestradiol capsule removed for 48 h and then reimplanted for an additional 48 h. All blood flow studies were performed on day 11 of pseudopregnancy with radioactive microspheres. Quantitative histology was performed on a separate group of 16 rabbits allocated to the same groups as above (four rabbits per group). Blood flow to the corpus luteum was unchanged in the – 24 h group, but declined by 44% in the – 48 h group. Blood flow returned to control values in the – 48 h/+ 48 h group. Quantitative histology revealed no changes in the – 24 h group, but a significant decrease in volume of the corpus luteum and volume of the luteal cell cytoplasm in the – 48 h group. Corpus luteum volume was fully restored and luteal cell cytoplasm volume was partially restored in the – 48 h/+ 48 h group. In contrast, the number of luteal cells, vessel space volume and capillary surface area did not change with oestrogen withdrawal. These results suggest that the structure of the corpus luteum and corpus luteum blood flow are modulated by oestrogen in pseudopregnant rabbits.

Introduction

Oestrogen is known to be the luteotrophic hormone in rabbits and corpus luteum progesterone secretion can be maintained by administration of oestradiol in the absence of pituitary hormones (Bill and Keyes, 1983). The corpus luteum has one of the highest blood flows per unit mass of any organ, but the physiological significance of this is unknown. Corpus luteum blood flow is not thought to be a primary regulator of steroidogenesis in rabbits. Luteal regression induced by prostaglandin F2α (PGF2α) infusion in pregnant rabbits is not preceded by a decline in corpus luteum blood flow (Bruce and Hillier, 1974). Furthermore, 24 h after removal of an oestradiol capsule, corpus luteum blood flow is unchanged, despite a significant fall in progesterone concentration (Wiltbank et al., 1989). This finding suggests that, at least for the first 24 h after the onset of luteal regression, luteal blood flow and steroidogenesis are not related. However, one study reports a positive correlation between ovarian progesterone secretion and corpus luteum blood flow on day 9 of pseudopregnancy (Janson et al., 1981).

Quantitative changes in the structure of the corpus luteum during growth and spontaneous regression in pseudo-pregnancy were reported by Dharmarajan et al. (1988a). However, a detailed analysis of the time during regression when these changes occur, and the possible reversibility of these changes, has not been reported.

Placement of an oestradiol capsule at the onset of pseudopregnancy results in dependence of the corpus luteum on the exogenous oestrogen source and provides a mechanism whereby steroidogenesis by the corpus luteum can be regulated by inserting or removing the capsule (Holt et al., 1975). This model was used to study corpus luteum blood flow and quantitative histology at various stages of regression and rescue in pseudopregnant rabbits. In contrast to previous studies (Wiltbank et al., 1989), blood flow was measured after a longer period (48 h) of luteal regression, and after a longer period (48 h) of luteal rescue by oestradiol. This study addressed the following questions. (i) Does corpus luteum blood flow decline after luteal regression is initiated by withdrawing exogenous oestradiol support? (ii) If there is a decline in blood flow, when does it occur and can it be reversed? (iii) Are there changes in the quantitative histology of the rabbit corpus luteum after withdrawal of exogenous oestradiol support? (iv) If there are such changes, when do they occur and can they be reversed?
Materials and Methods

Animals

Sexually mature New Zealand white rabbits with a mean mass of 3.5 kg were used. All rabbits were housed individually for a minimum of 3 weeks under controlled light and temperature and given Purina rabbit chow (Ralston-Purina Co., St Louis, MO) and water ad libitum. The housing of these animals followed the Department of Health and Human Services—National Institutes of Health Guide for the Care of Laboratory Animals. The experimental protocol was approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee.

Experimental groups

Pseudopregnancy was induced by the administration of 100 iu hCG (Schein Pharmaceutical Inc., Port Washington, NY) via the marginal ear vein. The day of hCG administration was defined as day 0 of pseudopregnancy. A single polymethylsiloxane capsule (3 cm long, 3.35 mm internal diameter; Dow-Corning Corp., Midland, MI) was implanted subcutaneously at the base of the neck under local anaesthesia with 1% (w/v) lidocaine. Each capsule contained 2 cm filled length of oestradiol (Sigma Chemical Co., St Louis, MO). Before placement of the capsule, 32 rabbits were randomly allocated to four groups (eight rabbits per group) for blood flow studies as follows – control: oestradiol capsule remained in place for the duration of the experiment; −24 h: oestradiol capsule removed 24 h (day 10) before blood flow measurements; −48 h: oestradiol capsule removed 48 h (day 9) before blood flow measurements; and −48 h / +48 h: oestradiol capsule removed for 48 h (day 7) and then reimplanted for an additional 48 h (day 9) before blood flow measurements. A fifth group of rabbits (n = 4) was not randomized but had the oestradiol capsule removed for 48 h (day 7) and then a sham oestradiol capsule implanted for an additional 48 h (day 9) before blood flow measurements (−48 h / + sham capsule). All blood flow measurements were performed on day 11 of pseudopregnancy. The selection of this day was based upon previous studies in which progesterone secretion of the corpus luteum was found to increase from day 1 to a maximum on day 11 and then to decline to values similar to those at day 1 by day 18 (Dharmarajan et al., 1988b).

For quantitative histology studies, a separate group of 16 rabbits was randomly assigned (four rabbits per group) to the control, −24 h, −48 h and −48 h / +48 h groups.

Tissue preparation for light and electron microscopy

Luteal tissue was harvested on day 11 of pseudopregnancy and perfusion fixed as described by Dharmarajan et al. (1988a). Quantitative histology studies were performed at a magnification of ×1000 using the stereological methods of Weibel (1979). The formulae used for estimating numbers of cells and surface area were described by Weibel (1979). For this study the coefficient, K, relating to uniformity of nuclear size, was given a value of 1 and the coefficient, β, which adjusts for shape of the profile counted, was given a value of 1.382 for luteal cells, since they are spherical. Corpus luteum volume was calculated by dividing the measured corpus luteum mass by the specific gravity. It was assumed that the specific gravity was not affected by treatment. For electron microscopy, one grid was examined from each corpus luteum at a magnification of ×10 000. Electron micrographs were taken randomly at the interface between luteal cell cytoplasm and the capillary to determine surface specialization.

Blood flow measurements

The rabbits were anaesthetized i.v. with sodium pentobarbital (30 mg kg$^{-1}$ body mass). Supplemental pentobarbital (1–2 mg kg$^{-1}$) was provided if arterial blood pressure became high during surgery. A tracheotomy was performed and the animals were mechanically ventilated to maintain arterial blood gases within normal limits. Arterial blood pressure, CO$_2$, O$_2$, pH and haemoglobin were monitored during the experiments. A left atrial catheter for radiolabelled microsphere injection was inserted via thoracotomy. Another catheter was advanced into the lower abdominal aorta via the other femoral artery for reference sample withdrawal. No vasopressor or inotropic drugs were used to maintain blood pressure.

Blood flow was measured with radiolabelled microspheres (15 ± 1.5 μm diameter; Dupont—New England Nuclear Products, Boston, MA) suspended in 10% (w/v) dextran with 0.01% (w/v) Tween 80 using the reference sample method (Heymann et al., 1977). DeVoto et al. (1977) reported that arterio-venous shunts were not present in the rabbit corpus luteum with the use of 15 μm spheres. Warmed microspheres were vortexed for 30 s and then injected through the left atrial catheter. This was followed by a 3 ml saline flush. The reference blood sample was withdrawn from the abdominal aorta with a Harvard pump syringe at 1.94 ml min$^{-1}$ beginning 30 s before the microsphere injection and continuing for 90 s after the saline flush. No change in arterial blood pressure was noted during or after the microsphere injection. Approximately 1.0 × 10$^6$ microspheres were injected for each blood flow measurement using $^{153}$Gd, $^{113}$Sn, $^{103}$Ru, $^{99}$Nb or 46Sc-labelled spheres. Two blood flow measurements (taken approximately 5 min apart) were obtained, each using a different randomly selected isotope. The average of these two measurements was used for all data analysis. No difference in measured blood flow between the first and second injections was found (paired t test, P = 0.2).

At the conclusion of the experiment, the animals were killed with an intracardiac injection of potassium chloride. Both ovaries were immediately harvested along with a sample of the right and left kidneys. The corpora lutea were immediately dissected from the ovarian stroma under a layer of kerosene to minimize fluid loss before weighing. The corpora lutea and ovarian stroma were then weighed separately. Radioactivity of specimens was measured in a multichannel autogamma spectrometer (Packard Instruments, Model 9042; Downers Grove, IL). Differential spectroscopy was used to correct the raw emissions for overlap of isotopes among energy windows. Blood flow (ml min$^{-1}$ (100 g)$^{-1}$) was calculated as the product of the corrected tissue counts (C_T) and the reference withdrawal rate (RBF; ml min$^{-1}$), divided by the radioactivity in reference sample (C_R) and the tissue weight (W; g):

\[
\text{Blood flow} = 100 \times \frac{(C_T \times RBF)}{(C_R \times W)}
\]
Total ovarian blood flow was calculated by adding the amounts of radioactivity and masses of the corpora lutea and the stroma.

**Steroid hormones**

Peripheral blood samples for progesterone and oestriadiol determination were obtained from the marginal ear vein at the time of capsule removal, at the time of capsule reimplantation, and at the time of blood flow measurements. After the last microsphere injection, but before the animal was killed, a midline laparotomy was performed. The anastomotic vessels to the ovary were suture ligated. The right and left ovarian veins were isolated, cannulated in situ, and a sample of blood obtained by capillary action. All blood samples were centrifuged at 1500 g for 10 min. Serum was separated from the blood and stored at -20°C until assayed for progesterone and oestriadiol.

Progesterone and oestriadiol concentrations in the peripheral and ovarian venous samples were measured in duplicate with commercial radioimmunoassay kits (Diagnostic Products Corporation; Los Angeles, CA). The sensitivity was 8 pg ml⁻¹ for the oestriadiol assay and 0.05 ng ml⁻¹ for the progesterone assay. The inter- and intra-assay coefficients of variation at the concentrations obtained in this experiment were 7.5% and 6.6% for progesterone, and 4.4% and 6.8% for oestriadiol, respectively.

**Statistical analyses**

The generalized linear interactive modelling (GLIM) statistical program (1985, Royal Statistical Society, London) was used for all statistical analyses. A histogram of the standardized residuals was used to test for normality of the data. All data were found to follow a normal distribution thereby allowing for the use of parametric statistical calculations. Analysis of variance (ANOVA) was used to evaluate differences in means among the groups. When differences were found, they were further evaluated with the Student–Newman–Keuls' test. A P value < 0.05 was considered statistically significant. All results are presented as means ± SEM.

**Results**

**Steroid hormones**

The study design required that the oestriadiol capsule be removed as early as day 7 of pseudopregnancy (~48 h group) or as late as day 10 (~24 h group). Despite removal of the oestriadiol capsule at different times during pseudopregnancy, the corpus luteum responded to the removal in a similar fashion in each group (Table 1). There was a significant decline in peripheral progesterone concentration regardless of when the capsule was removed. Therefore, at all periods studied, the corpus luteum had become dependent on the exogenous oestriadiol source, and responded to removal of oestriadiol with a decrease in progesterone production.

Periperal oestriadiol and progesterone concentrations declined 24 h after capsule removal, although the decline in the former was not significant until 48 h later. After 48 h of oestrogen deprivation, 48 h of oestrogen replacement restored peripheral oestriadiol and progesterone concentrations to control values. In contrast to peripheral oestriadiol concentrations, ovarian venous oestriadiol concentrations on day 11 of pseudopregnancy did not vary significantly among the four study groups. However, we studied a separate group of eight rabbits that received hCG alone (no capsule). Ovarian venous oestriadiol concentration on day 11 was 485 ± 51 pg ml⁻¹ (mean ± SEM), which was significantly higher than the oestriadiol concentrations in the study groups with an oestriadiol capsule. This finding indicates that endogenous oestriadiol production was suppressed by the oestriadiol capsule. Ovarian venous progesterone concentration decreased significantly 24 h after oestrogen withdrawal. Oestrogen deprivation for 48 h, followed by oestrogen replacement for 48 h restored ovarian venous progesterone concentrations to control values (Table 2).

**Histology**

The volume of the corpus luteum declined 48 h after capsule removal and was restored to control values after 48 h of capsule replacement (Table 3). The volume of the luteal cell cytoplasm decreased 48 h after capsule removal and was partially restored with capsule replacement. Neither capsule removal nor reimplantation affected the number of luteal cells. Vessel space volume and capillary surface area were unchanged with oestriadiol withdrawal, but both increased above control values when oestriadiol was readministered for 48 h. The electron micrographs of the luteal cell–capillary interface during oestriadiol withdrawal and replacement are shown (Fig. 1). Oestriadiol capsule removal for 48 h resulted in a marked diminution in the microvilli of luteal cells adjacent to capillaries. Replacement of oestriadiol for an additional 48 h restored the microvilli.

Mean corpus luteum mass was significantly decreased 24 h after oestrogen withdrawal. Oestrogen replacement restored luteal mass to control values (Table 2), but replacement of a sham oestriadiol capsule for 48 h failed to restore luteal mass (mean luteal mass = 10.4 mg after sham capsule replacement). In contrast to the change in luteal mass, mean ovarian stroma mass did not vary significantly (P = 0.17) among the groups (Table 2).

**Blood flow**

The mean number of microspheres trapped in the organ of interest with the lowest blood flow (the ovarian stroma) was 3448 ± 740. This corresponds to a less than 5% error in blood

<p>| Table 1. Peripheral progesterone concentration before and after oestriadiol capsule removal |
|-----------------------------------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>− 24 h</td>
<td>12.9</td>
<td>4.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>− 48 h</td>
<td>11.8</td>
<td>3.3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>− 48 h + 48 h</td>
<td>11</td>
<td>4*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05, paired t test for before versus after capsule removal.
Table 2. Steroid and blood flow measurements on day 11 of pseudopregnancy in rabbits

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 8)</th>
<th>- 24 h (n = 8)</th>
<th>- 48 h (n = 8)</th>
<th>- 48 h + 48 h (n = 8)</th>
<th>- 48 h/ + sham capsule (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral oestradiol (pg ml⁻¹)</td>
<td>24 ± 5ᵃ</td>
<td>14 ± 4ᵇᶜ</td>
<td>9 ± 1ᵇᶜ</td>
<td>28 ± 3ᵇ</td>
<td></td>
</tr>
<tr>
<td>Peripheral progesterone (ng ml⁻¹)</td>
<td>11.0 ± 1.3ᵇ</td>
<td>4.5 ± 1.0ᵇ</td>
<td>3.3 ± 0.6ᵇ</td>
<td>12.8 ± 1.7ᵇ</td>
<td></td>
</tr>
<tr>
<td>Ovarian venous oestradiol (pg ml⁻¹)</td>
<td>76 ± 16ᵇ</td>
<td>51 ± 28ᵇ</td>
<td>55 ± 10ᵇ</td>
<td>61 ± 16ᵇ</td>
<td></td>
</tr>
<tr>
<td>Ovarian venous progesterone (ng ml⁻¹)</td>
<td>1199 ± 264ᵃ</td>
<td>437 ± 112ᵇᶜ</td>
<td>356 ± 80ᵇ</td>
<td>1407 ± 170ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Corpus luteum mass (mg)</td>
<td>18.8 ± 1.4ᵇ</td>
<td>14.7 ± 1.0ᵇᶜ</td>
<td>13.1 ± 1.1ᵇ</td>
<td>17.6 ± 0.9ᵇ⁺</td>
<td></td>
</tr>
<tr>
<td>Ovarian stroma mass (mg)</td>
<td>352 ± 37ᵇ</td>
<td>305 ± 45ᵇ</td>
<td>251 ± 24ᵇ</td>
<td>267 ± 24ᵇ</td>
<td></td>
</tr>
<tr>
<td>Corpus luteum blood flow (ml min⁻¹ (100 g⁻¹))</td>
<td>3525 ± 379ᵃ</td>
<td>3334 ± 271ᵃ</td>
<td>1976 ± 227ᵇ</td>
<td>3292 ± 355ᵇ</td>
<td>1805 ± 206ᵇ</td>
</tr>
<tr>
<td>Ovarian stroma blood flow (ml min⁻¹ (100 g⁻¹))</td>
<td>296 ± 60ᵇ</td>
<td>271 ± 48ᵇ</td>
<td>241 ± 59ᵇ</td>
<td>171 ± 23ᵇ</td>
<td>141 ± 19ᵇ</td>
</tr>
<tr>
<td>Total ovary blood flow (ml min⁻¹ (100 g⁻¹))</td>
<td>1030 ± 84ᵇ</td>
<td>1054 ± 151ᵇ</td>
<td>641 ± 80ᵇ</td>
<td>1016 ± 114ᵇ</td>
<td>562 ± 107ᵇ</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. Numbers in the same row without a common superscript are significantly different (ANOVA, P < 0.05) from each other. Control: oestradiol capsule not removed; - 24 h: oestradiol capsule removed for 24 h; - 48 h: oestradiol capsule removed for 48 h; - 48 h + 48 h: oestradiol capsule removed for 48 h then reimplanted for an additional 48 h.

Table 3. Quantitative measurements on day 11 of pseudopregnancy in rabbits*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 4)</th>
<th>- 24 h (n = 4)</th>
<th>- 48 h (n = 4)</th>
<th>- 48 h + 48 h (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus luteum volume (µl)</td>
<td>23.13 ± 1.16ᵇ</td>
<td>23.08 ± 0.86ᵇ</td>
<td>16.43 ± 0.54ᵇ</td>
<td>20.88 ± 2.12ᵇ</td>
</tr>
<tr>
<td>Luteal cell cytoplasm volume (µl)</td>
<td>19.43 ± 0.61ᵇ</td>
<td>19.45 ± 0.66ᵇ</td>
<td>13.82 ± 0.37ᵇ</td>
<td>16.24 ± 0.29ᵇ</td>
</tr>
<tr>
<td>Number of luteal cells × 10⁸</td>
<td>4.36 ± 0.68ᵇ</td>
<td>3.32 ± 0.05ᵇ</td>
<td>3.50 ± 0.07ᵇ</td>
<td>4.00 ± 0.18ᵇ</td>
</tr>
<tr>
<td>Vessel: space volume (µl)</td>
<td>1.19 ± 0.41ᵇ</td>
<td>1.31 ± 0.55ᵇ</td>
<td>1.00 ± 0.45ᵇ</td>
<td>2.35 ± 0.15ᵇ</td>
</tr>
<tr>
<td>Capillary surface area × 10⁵ (mm² per corpus luteum)</td>
<td>8.36 ± 3.05ᵇ</td>
<td>6.41 ± 2.30ᵇ</td>
<td>5.38 ± 2.40ᵇ</td>
<td>14.22 ± 0.97ᵇ</td>
</tr>
</tbody>
</table>

*All ovaries were perfusion-fixed before stereological analysis. Values presented are means ± s.e.m. Values in the same row without a common superscript are significantly different (ANOVA, P < 0.05) from each other. Control: oestradiol capsule not removed; - 24 h: oestradiol capsule removed for 48 h; - 48 h: oestradiol capsule removed for 48 h then reimplanted for an additional 48 h.

flow measurement owing to random variability in the microsphere distribution (Buckberg et al., 1971). There was no significant difference in the mean number of corpora lutea per ovary among the groups (mean = 6.4 ± 0.4). The physiological parameters at the time of the blood flow measurements are summarized in Table 4. No significant differences between groups were found in any of the measured physiological parameters (Table 4). Renal blood flow did not vary during oestrogen withdrawal or readministration. No significant difference in blood flow was found between the right and left corpora lutea, the right and left ovarian stroma or the right and left kidneys. We also determined luteal blood flow in a group of eight rabbits that received hCG alone (no capsule). The mean luteal blood flow on day 11 was 3742 ± 414 ml min⁻¹ (100 g⁻¹), which was not significantly different from the value of 3525 ± 379 ml min⁻¹ (100 g⁻¹) obtained in the control (oestradiol capsule + hCG) group. Therefore, the presence of the oestradiol capsule did not alter luteal blood flow on day 11 of pseudopregnancy.

Mean luteal blood flow did not decline until 48 h after capsule removal, at which time there was a 44% decrease from the control (Table 2). After 48 h of oestradiol deprivation, oestradiol replacement for 48 h restored luteal blood flow to control values, while placement of a sham oestradiol capsule failed to restore blood flow. Changes in total ovarian blood flow paralleled those observed in the corpus luteum, while ovarian stroma flow was unchanged.

Discussion

The dependence of the pseudopregnant rabbit corpus luteum on exogenous oestrogen was demonstrated by the decline in peripheral and ovarian venous progesterone concentrations after removal of the oestradiol capsule. The restoration of peripheral and ovarian venous progesterone concentrations with oestrogen readministration indicates that the corpus luteum remains responsive to oestrogen for at least 48 h after the initiation of luteal regression.

The decrease in corpus luteum volume 48 h after capsule removal was accompanied by a similar decrease in volume of cytoplasm in luteal cells and corpus luteum mass. Reimplantation of the capsule for 48 h restored corpus luteum mass and volume and partially restored the cytoplasm volume of luteal cells. The change in the volume of cytoplasm of luteal cells may reflect a change in the volume of intracellular organelles such as mitochondria and smooth endoplasmic reticulum. A decrease in the surface area of the smooth endoplasmic
changes in luteal mass, volume and volume of cytoplasm of luteal cells in the study reported here were accompanied by similar changes in peripheral and ovarian venous concentrations of progesterone. It is possible that these changes in the corpus luteum are a reflection of alterations in the steroidogenic processes of the luteal cells. The ovarian stroma, which is relatively inactive compared with the hormonally active luteal tissue, did not show any significant change in mass.

The absence of a change in vessel space volume or capillary surface area with oestradiol withdrawal suggests that, at least during the first 48 h of luteal regression, the structure of the luteal vasculature is unaltered. As a consequence, any changes in luteal blood flow during this period may be reversible. Although other studies have evaluated the quantitative histological changes during corpus luteum growth and regression (Dharmarajan et al., 1988a), the study reported here defines the period during which these changes occur and demonstrates that some of these changes are reversible.

The electron micrographs demonstrated a decrease in luteal cell microvilli during luteal regression. The microvilli appear to be restored when the corpus luteum is rescued by oestradiol replacement. The presence of microvilli increases the effective surface area available for luteal cell contact with capillaries (Dharmarajan et al., 1991). In addition to its effect on luteal steroidogenesis, oestradiol may also affect the transport of substances from the luteal cells to the capillaries, and vice versa.

The most notable finding in this study was the change in luteal blood flow that occurred in response to oestradiol withdrawal and readministration. Luteal blood flow did not decline until 48 h after oestradiol withdrawal despite a decline in progesterone concentration by 24 h. These observations provide additional evidence that blood flow of the corpus luteum is not a primary regulator of luteal steroidogenesis.

The vasculature of the rabbit corpus luteum lacks a smooth muscle investment and therefore functions as a low resistance sinusoidal network that is incapable of autoregulation (Wiltbank et al., 1990). Because of this lack of autoregulation, there is a positive correlation between mean arterial pressure and luteal blood flow in anaesthetized rabbits (Janson et al., 1981). Differences in luteal blood flow in our experiment cannot be explained by differences in mean arterial pressure between the groups. The change in luteal blood flow is either a direct or indirect consequence of the change in peripheral oestradiol concentration. Although oestradiol receptors have been identified in the rabbit corpus luteum (Lee et al., 1971), it is unlikely that the observed changes in blood flow are due to a direct effect of oestradiol on luteal or extra-luteal vasculature. Changes in oestradiol concentrations would be expected to alter blood flow acutely, if oestradiol was acting directly on the ovarian vasculature. The results of this study do not support this hypothesis.

The rescue of luteal blood flow after readministration of oestradiol has not previously been reported. Corpus luteum blood flow declines during advanced gestation in pregnant rabbits (Abdul-Karim and Bruce, 1973) and this is presumed to be associated with structural regression of the corpus luteum. However, the continued presence of an abundant intact vasculature in the regressing corpus luteum has been described (Koering and Thor, 1978). An increase in RNA synthesis in the endothelial and interstitial cells of the regressing corpus luteum is described by Han et al. (1977), who suggest that this increase

Fig. 1. Transmission electron micrographs of the luteal cell–capillary interface in pseudopregnant rabbits. (a) Control. Normal microvilli (MV). (b) Oestradiol capsule removed for 48 h. Note the marked decrease in microvilli. (c) Oestradiol capsule removed for 48 h then reimplanted for an additional 48 h. Microvilli partially restored to control. Scale bars represent 1 μm.
Table 4. Physiological parameters at the time of corpus luteum blood flow measurements on day 11 of pseudopregnancy in rabbits\*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>−24 h</th>
<th>−48 h</th>
<th>−48 h + 48 h</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g dl (^{-1}))</td>
<td>11.0 ± 0.3</td>
<td>11.0 ± 0.3</td>
<td>11.2 ± 0.3</td>
<td>11.1 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>84 ± 3</td>
<td>84 ± 4</td>
<td>85 ± 3</td>
<td>86 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>pO(_2) (mm Hg)</td>
<td>169 ± 22</td>
<td>163 ± 16</td>
<td>150 ± 10</td>
<td>162 ± 33</td>
<td>NS</td>
</tr>
<tr>
<td>pCO(_2) (mm Hg)</td>
<td>38 ± 2</td>
<td>38 ± 1</td>
<td>37 ± 1</td>
<td>40 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.42 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Renal blood flow (ml min (^{-1}) (100 g (^{-1}))</td>
<td>404 ± 36</td>
<td>391 ± 24</td>
<td>396 ± 43</td>
<td>473 ± 35</td>
<td>NS</td>
</tr>
</tbody>
</table>

\*Blood samples were obtained from the abdominal aorta. Values presented are means ± SEM. Statistical comparison of the four treatment groups was with ANOVA. NS: not statistically significant (P>0.05). Control: oestradiol capsule not removed; −24 h: oestradiol capsule removed for 24 h; −48 h: oestradiol capsule removed for 48 h; −48 h + 48 h: oestradiol capsule removed for 48 h then reimplanted for an additional 48 h.

in RNA synthesis is indicative of a reorganization of the structural framework of the corpus luteum in response to the decrease in luteal cell size that accompanies regression. Most evidence indicates that the vascularization of the regressing corpus luteum remains intact in pseudopregnant rabbits. Because the changes in blood flow observed in this study were reversible, it is unlikely they were due to structural regression of the luteal vasculature. Our quantitative histological analysis also supports this hypothesis.

On the basis of the model of ovarian and luteal blood flow suggested by Wiltbank et al. (1990), it is proposed that oestrogen (either directly or indirectly) alters resistance to the capsular arteries within the ovary that supply the corpus luteum. Alternatively, oestrogen may be acting outside the ovary, i.e. at the main ovarian artery. Because the stromal vessels can autoregulate (Wiltbank et al., 1990), they could vasconstrict or vasodilate in response to vasodilatation or vasoconstriction, respectively, of the main ovarian artery. In this model, stromal blood flow would remain constant, whereas corpus luteum blood flow would change in response to the vascular tone of the ovarian artery.

In summary, the period after the onset of luteal regression during which changes in luteal blood flow and quantitative histology occur have been defined. Changes in luteal blood flow and quantitative histology can be reversed when luteal regression is reversed with oestriadiol administration. The results of this experiment provide evidence that oestrogen is a mediator of luteal blood flow in pseudopregnant rabbits. On the basis of the electron microscope analysis of luteal cell microvilli, it is proposed that oestrogen may also alter the surface area available for transport of substances between the luteal cells and adjacent capillaries.

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