Luteal blood flow and plasma steroids in rats
with corpora lutea of different ages

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Abstract. Ovarian and luteal blood flow rates were measured at different stages of luteal development in anaesthetized rats using 15 ± 5 µm radioactive microspheres. Ovulations were induced by injection of 8 IU of PMSG at 28 days of age. Steroid concentrations in peripheral plasma were determined using radioimmunoassays. The highest luteal blood flow was found in the youngest corpora lutea and decreased gradually with luteal age. Luteal flow on days 13 and 14 was significantly lower than that of days 2, 4, and 6. Ovarian stromal blood flow remained unchanged throughout the lifespan of the corpus luteum. Peripheral progesterone concentrations rose significantly on days 4, 6, and 8 with a maximum on day 8, as compared to day 2, and fell significantly after day 8. The levels of 20α-OH-progesterone increased sharply on day 10, indicating functional luteolysis. The progesterone levels fell significantly between day 8 and 10 without a concomitant significant decline in luteal blood flow. Since a fall in progesterone production seems to precede a drop in luteal blood flow, the initiation of luteolysis does not appear to be caused by a vascular mechanism.

In rabbits (Novy & Cook 1973; Janson & Albrecht 1975) and sheep (Bruce & Moor 1978) luteal blood flow has been found to be extremely high, exceeding that of the surrounding interstitial tissue by more than ten times. It has been suggested by Niswender et al. (1976) that blood flow is an important regulator of ovarian function and hypotheses have been put forward of vascular mecha-

nisms underlying the initiation of luteolysis. Experiments in rabbits (Janson et al. 1975; Bruce & Hillier 1974) sheep (McCracken et al. 1979) and rats (Pang & Behrman 1979) do not support these ideas since the decline in luteal progesterone secretion appeared to precede the fall in luteal blood flow. However, experiments in dogs (Romanoff et al. 1962), sheep (Niswender et al. 1976), and recently in rabbits (Janson et al., in press), indicated a positive correlation between luteal blood flow and progesterone secretion of the fully functional corpus luteum. It is therefore of interest to assess the relationship between corpus luteum blood flow and progesterone secretion throughout the life span of the corpus luteum. In the present study plasma progesterone was measured simultaneously with luteal blood flow at different ages of the corpus luteum in rats, in which ovulations had been induced by pregnant mare serum gonadotrophin (PMSG). In addition, plasma concentrations of 20α-OH-progesterone and oestradiol-17β were measured to characterize the functional stage of the ovary at various ages of the corpus luteum.

Material and Methods

Animals

Eighty pre-pubertal rats of the Sprague-Dawley strain received 8 IU of PMSG sc in the morning of day 28 of age. Previous studies in our laboratories have shown that the animals ovulate between 03.00 and 05.00 h in the morning of day 31, after an endogenous LH peak in the
afternoon of day 30 (Herlitz et al. 1976). The animals were divided into 8 groups for experiments at day 2, 4, 6, 8, 10, 12, 13, and 14 of lutal age.

**Measurement of ovarian blood flow**

The animals were anaesthetized with sodium pentobarbitone (Nembutal, Abbot, UK) in a dose of 40 mg/kg ip and placed in a supine position on a heating pad. The right common carotid artery was cannulated with a PE 50 catheter, the tip of which was advanced into the ascending aorta. The arterial blood pressure was recorded with a Statham P23 AC transducer connected to a Grass Model 7 Polygraph. Immediately after the measurement of blood pressure a mechanically mixed suspension of approximately 20 000 Cerium-141-labelled microspheres (specific activity 9.37 mCi/g, 14.8 ± 1.9 μm, range) was flushed into the aorta over a 30 s period. The spheres were suspended in 0.9 ml 0.15 m NaCl. Blood from the cut right common carotid artery was collected and centrifuged for 10 min at 2000 r.p.m. The plasma was stored at –20°C until analysis of steroid hormones. One minute after the infusion of microspheres the animal was killed by exsanguination. Both ovaries were excised and all corpora lutea (CL) were dissected under the microscope. The CL and stroma were weighed and counted separately for 5 min in a well scintillation and counter (Packard Autogamma) set to cover the main gamma energy peak of Ce-141. A standard sample with a known number of microspheres from the actual batch was counted along with the tissue samples. The numbers of spheres lodging in the CL and stroma, respectively, were calculated. The total number of microspheres given to the animals was determined by counting the radioactivity of the whole body of the animal and the standard sample in a Packard Model 440 Armac Detector consisting of a counting chamber (volume 1800 ml) surrounded by a scintillation detector. Calculations of the number of microspheres in the whole bodies were based on calibration measurements where a certain activity of Ce-141 was measured first as a point source and then diluted in a number of water phantoms corresponding to rat bodies of different sizes. Blood flows to the CL and stroma were expressed as flow indices:

\[
\text{Blood flow index} = \frac{\text{Number of spheres in tissue} \times 100}{\text{Total number of spheres injected} \times \text{mg tissue wet weight}}
\]

**Steroid determinations**

The plasma concentrations of progesterone (P), 20α-OH-progesterone (20-OH-P) and oestradiol-17β (Oe2) were measured using radioimmunoassays. Plasma samples (100 μl P and 20-OH-P; 500 μl Oe2) were aliquoted into glass tubes and diluted to a final volume of 1 ml with distilled water. Purified tritiated steroids (New England Nuclear, approximately 2000 DPM) were added to all samples in order to monitor procedural losses. Each sample was extracted once with 10 ml diethyl ether (Merck, UVASOL). The extracts were dried under nitrogen at 40°C. For P and Oe2 further purifications were obtained by chromatography on celite microcolumns as described by Brenner et al. (1973); 20-OH-P was assayed directly, omitting a chromatographic step.

The progesterone antibody was purchased from Endocrine Sciences Inc., Tarzana, Cal., USA, and diluted according to the manufacturer’s instruction. The 20-OH-P antibody was a gift from Dr. H. Lindner and S. Bauminger, Weizmann Institute of Science, Israel. The cross-reaction to P was less than 0.1% and the antibody was used in a final dilution of 1:500 000. The antibody to Oe2 was obtained from Miles-Yeda Ltd., Rehovot, Israel and diluted as recommended by the manufacturer. The dilutions of antibodies were made in borate buffer (0.06 M; pH 8.0) containing human albumin (1.5 mg/ml) human gamma globulin (0.05 mg/ml) and tritiated steroids (2900 DPM/ml for P, 9000 DPM/ml for 20-OH-P and 19 000 DPM/ml for Oe2).

Two hundred μl of the antibody solution was added to the dried eluate (P and Oe2) or diethyl ether fraction (20-OH-P) and was incubated at 4°C overnight. Standard steroid samples, ranging from 0 to 400 pg and experimental samples were assayed in duplicate. After incubation with antibody 200 μl of saturated ammonium sulphate was added and samples centrifuged (15 min × 2500 r.p.m.). Two hundred μl of the supernatant was then aliquoted into polyethylene vials (NEN) and 10 μl of a toluene solution containing 5.5 g/l Permablend (Packard, Scintillation Grade) and the vials were counted for radioactivity. The steroid concentration in the unknown samples was calculated with a computer program based on the ‘logit-log’ method (Rodhard & Lewald 1970) for the transformation of the standard curve. The amounts of steroid recorded from the standard curve were corrected for blank values and extractional losses (see below).

Data of the accuracy and precision of the radioimmunoassays are shown in Table 1. The sensitivity of the standard curve was 5 pg for Oe2 and 10 pg for P and 20-OH-P, when applying the 95% confidence limit. The overall procedural recovery for P was 73.4 ± 6.9 (sd) %, for 20-OH-P 97.9 ± 8.8 (sd) % and for Oe2 88.0 ± 10.9 (sd) %. Method blanks were close to zero.

**Statistics**

Comparisons between groups were performed using Wilcoxon’s nonparametric test based on range (Siegel 1956).

**Results**

The mean arterial pressure during blood flow measurements was 97 ± 14 mmHg (mean ± sd, n = 51). The infusion of microspheres did not
Table 1.

Accuracy and precision for radioimmunoassays of oestradiol-17β, 20α-OH-progesterone and progesterone.

<table>
<thead>
<tr>
<th>Picogram steroid added to plasma¹</th>
<th>Oestradiol-17β</th>
<th>Amount of steroid recovered</th>
<th>20α-OH-progesterone</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Picogram</td>
<td>Coefficient variation²</td>
<td>Picogram</td>
<td>Coefficient variation</td>
</tr>
<tr>
<td>25</td>
<td>29.5 ± 2.1³</td>
<td>7.3</td>
<td>30.5 ± 2.7</td>
<td>8.9</td>
</tr>
<tr>
<td>50</td>
<td>48.5 ± 8.3</td>
<td>17.1</td>
<td>51.2 ± 8.2</td>
<td>16.0</td>
</tr>
<tr>
<td>100</td>
<td>108.3 ± 18.4</td>
<td>16.0</td>
<td>110.0 ± 25.1</td>
<td>22.8</td>
</tr>
<tr>
<td>Plasma pool (picogram/ml plasma)</td>
<td>15.5 ± 5.8</td>
<td>11.5²</td>
<td>2070 ± 215</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3750 ± 680</td>
<td>18.1</td>
</tr>
</tbody>
</table>

Values represent M ± SEM of five determinations.

¹ Known amount of steroid added to plasma and processed as described in text.
² sd × 100 mean
³ Total amount measured minus basal plasma level.
⁴ Between assay coefficient of variation.

The number of corpora lutea per rat ranged between 7 and 19 with a mean value of 14.6. There were no difference in weight between 4 to 14 days old corpora lutea while the weights of 2 days old corpora lutea were smaller confirming Herlitz et al.

- Blood flow index in the corpus luteum (stipped bars) and stroma (open bars) at various luteal ages. Corpora lutea were induced by injecting prepubertal rats with an ovulatory dose of PMSG. The blood flow was measured with radioactive microspheres; blood flow index = No. of spheres in tissue × 100/total No. of spheres injected × mg wet weight of the tissue. The number of spheres were determined in the corpora lutea and ovarian stroma separately. Each value represents M ± SEM of 6 or 7 rats. The levels of luteal blood flow on days 13 (P < 0.01) and 14 (P < 0.05) were significantly smaller than those of days 2, 4, and 6. No significant differences were found between different luteal ages for stromal tissue.
The mean number of microspheres in the corpora lutea in the different age-groups varied between 1178 and 6164, which corresponds to an accuracy in the blood flow measurements of 5.7 and 2.5%, respectively (Buckberg et al. 1971). The accuracy of stromal blood flow measurements varied between 7.1 and 3.9%.

In Fig. 1 the blood flow index is illustrated for the corpus luteum and stromal tissue in relation to luteal age. There was a decrease of luteal blood flow with luteal age. The correlation coefficient calculated for blood flows in relation to luteal age was \(-0.58\), which was significant \((P < 0.01)\). The luteal blood flow on day 2, 4, and 6 was significantly higher than blood flows on days 13 and 14. No significant changes in stromal blood flow was observed.

Fig. 2 depicts the plasma levels of progesterone and 20α-OH progesterone at different luteal ages. There was a significant rise in progesterone concentration on day 4, 6, and 8 with a maximum on day 6 and 8, as compared to day 2. After day 8 a significant fall was observed and on day 12, 13, and 14 levels comparable to those of day 2 were reached. The concentration of 20α-OH-progesterone remained constant until day 10. A sharp rise was observed between day 10 and 12 and the levels remained elevated on days 13 and 14.

Plasma levels of oestradiol-17β are shown in Fig. 3. Two maxima of oestradiol-17β were observed on days 8 and 14, respectively.

**Discussion**

The results of the present study demonstrate a high rate of blood flow through the young corpus...
luteum and a gradual decline in flow with increasing age. Simultaneous measurements of stromal blood flow showed no changes throughout the lifespan of the corpus luteum. The use of radioactive microspheres for measuring gonadal blood flow in small laboratory animals has been extensively investigated in this laboratory (Janson & Selstam 1975; Damber & Janson 1978) and it has been found that this method is the best one available measuring intraovarian blood flow distribution. With the modification used in the present study it was however, not feasible to measure blood flow in absolute values. Under similar experimental conditions using anaesthetized rats of similar size, the cardiac output has been shown to be fairly constant, 20–25 ml/min (Pang & Behrman 1979; Lundgren, personal communication). The blood flow indices found in the present study correspond to luteal blood flow values of 200–1000 ml/100 g × min, which is in agreement with values reported for the rat by Pang & Behrman (1979).

A striking finding in the present study was that the mean value of luteal blood flow was highest in 2 and 4 days old corpora lutea, i.e. before they had been fully developed. Gospadorowicz & Thakral (1978) have reported an extremely rapid vascularization of the newly formed corpus luteum and suggested the existence of a corpus luteum angiogenic factor. These morphologic findings are in agreement with the high flow rates found in the present study. During the early phase of the luteal development blood flow remains at a high level when peripheral plasma levels of progesterone increase progressively. Thus, no obvious relationship appears to exist between luteal blood flow and progesterone production at this stage of development. However, it is possible that a high luteal blood flow is of importance for cell growth, cell differentiation and development of hormone receptors.

The fall in plasma progesterone concentration after day 8 was followed by an increase in 20α-OH-progesterone, indicating functional luteolysis (Tørjesen et al. 1978; Lambrecht et al. 1975; Hall & Robinson 1978). During this phase there was no significant reduction in luteal blood flow indicating that luteolysis is not primarily caused by a vascular mechanism. This finding is in accordance with
recent observations in anaesthetized pseudopregnant rats (Pang & Behrman 1979) and cycling guinea-pigs (Hossain et al. 1979).

A possible role of oestrogens in the luteolytic process in certain species, e.g. sheep (Akbar et al. 1972), cow (Williams & Marsh 1978), monkey (Karsch et al. 1973), and human (Williams et al. 1979), has been discussed. In this context, the finding in the present study of a significant increase of oestradiol-17β preceding the fall in plasma progesterone is an interesting observation. However, further studies are needed to investigate any possible role of oestrogens in the luteolytic process of the rat.

Acknowledgments

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References


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