Comparison of flow rates and composition of ovarian lymph and blood in the Day-16 pregnant rat*

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Summary. Rats (5) at Day 16 of pregnancy were anaesthetized and a modification of a venous outflow technique was used to collect ovarian venous blood and lymph for 2 h. Both fluids were analysed for progesterone, 20α-dihydroprogesterone, total protein, transferrin and albumin concentrations. In addition SDS gel electrophoresis was carried out to obtain an initial indication of permeability of capillaries to the various protein fractions. The concentrations of progesterone and 20α-dihydroprogesterone in ovarian lymph were only 37% and 48% respectively of the corresponding concentrations in the venous plasma. Total protein concentration in the lymph was 53% of the venous plasma. The albumin and transferrin concentrations were similarly lower in lymph than plasma but the difference was only significant for transferrin. This study confirms that the rate of lymph flow, per unit mass of tissue, is high for the ovary and represents about 1.1% of plasma flow. It shows also that of the total progestagens secreted only around 0.5% leave by the lymphatic route. The finding of relatively low progestagen concentrations in lymph questions the view that progestagens are transported by simple diffusion from the luteal cell to blood and raises the possibility of a counter-current flow between fluid in the interstitial space and blood.

Introduction

The mammalian ovary has a remarkably rich blood vascular system (Dharmarajan, Bruce & Meyer, 1985) and a well developed set of lymph drainage vessels (Morris & Sass, 1966; Staples, Fleet & Heap, 1982). The rate of blood flow through the ovary, and particularly that directed to the corpus luteum when expressed per unit mass of tissue (relative flow), is amongst the highest in the body (Bruce & Moor, 1975). From the only study available on the relative flow rate of ovarian lymph (Lindner, Sass & Morris, 1964) it appears that this too, is high compared to most other organs. Little is known, however, about the biological significance of the ovarian lymph drainage or indeed of the composition of lymph relative to ovarian venous blood. Such comparisons are important since they provide information on the composition of fluids in the interstitial spaces within the ovary and on transport processes between luteal cells and adjacent capillaries within the corpus luteum (Dharmarajan et al., 1985).

In the present work, rates of ovarian lymph and blood flow were examined in rats at Day 16 of gestation, a time of maximal progesterone secretion (Uchida, Kadowaki, Nomura, Miyata & Miyake, 1970).

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Nulliparous albino Wistar rats, 3–5 months old, were used. The 5 rats were kept in an environmentally-controlled building (17–23°C, relative humidity 50–70%, lights from 07:00 to 21:00 h). Food and water were freely available. The rats were mated during darkness and the morning on which spermatozoa were found in a vaginal smear was called Day 1 of gestation. Rats from this colony normally carry a litter on the morning of Day 23.

Surgery and measurement procedures

The surgical preparation was carried out as two phases; the first was to establish a venous outflow circuit and the second was to identify and then cannulate the common lymphatic vessel draining the ovary.

The ovarian venous outflow technique closely followed that previously described (Bruce & Meyer, 1981). Rats were anaesthetized with an intraperitoneal injection of pentobarbitone sodium (40 mg/kg body weight) and this was supplemented with further injections (subcutaneous, 10 mg/kg) each hour. Incisions were made with an electrocautery unit to minimize blood loss. The trachea was cannulated to assist breathing and a cannula was inserted into the dorsal aorta through the left common carotid artery to monitor arterial pressure and take arterial blood samples for progestagen and protein analysis. One ovary was prepared for venous outflow collection by inserting a cannula into the parametrial vein and tying ligatures around all other veins draining the ovary. Blood leaving the ovary was directed through a drop counter for assessment of flow rate and returned to the rat through a peristaltic pump and jugular cannula. In this way, and by using donor blood to replace that taken for progestagen and protein analysis, the volume of blood in the circulation was maintained to within about ±0.2 ml.

Samples were taken of arterial and venous blood at 20–40 min intervals to measure the secretion rates of progestagens, and protein and progestagen concentrations. The rat was maintained in dorsal recumbency but was not placed in a warm saline bath, as in previous studies, since this would have interfered with the delicate placement of the lymphatic cannula and might have affected the constituents of the lymph fluids due to transport across the thin lymphatic walls. Plastic film was placed over the operation site to minimize fluid loss by evaporation. Body temperature was maintained at ~37°C by keeping the rat on an adjustable electric blanket and carefully monitoring rectal temperature.

The lymphatic cannulation presented considerable difficulty. Several very thin-walled vessels leave the ovary at the hilum and pass down to join with the lymphatics draining the uterus. The ovarian lymphatics intertwine amongst the veins of the ovarian venous plexus and are closely applied to them. The initial problem was to visualize these vessels: at best they appeared as clear channels running through surrounding adipose tissue but even with the use of a dissecting microscope many of the lymphatics could not be identified. This problem was solved by cannulating the parametrial artery (Dharmarajan, Meyer & Bruce, 1983) and injecting 0.1–0.2 ml of 2.5% patent blue V (Guerbet, Cedex, France) in 0.9% (w/v) NaCl. This dye passed directly into the ovary and most was cleared rapidly via the venous drainage. Some dye passed through the capillary walls, however, and thus accumulated in the lymphatic vessels within 5–10 min. These vessels were most easily demarcated after 15 min. All anastomotic lymphatic vessels from the uterus and oviduct were then tied off and the common ovarian lymphatic vessel, running parallel with the ovarian artery and vein, was identified and carefully isolated for a distance of 2–5 mm. Care was taken not to occlude the ovarian veins or handle the ovary, since extravasated red cells or their breakdown products can readily enter the ovarian lymph. Two ligatures were positioned but not tied around the lymphatic vessel. Tension was applied to the ligature distal to the ovary, to occlude drainage temporarily and thus dilate the vessel, usually to a diameter of about 0.5 mm. A polyethylene cannula (0.5 mm i.d., 0.8 mm o.d.) with an intravascular tip fashioned from a section
of a pointed 30-gauge needle was carefully inserted into the dilated vessel towards the ovary for a distance of about 2 mm and then tied in place by the proximal ligature. Further ligatures were positioned to anchor the cannula and prevent the tip from piercing the delicate lymphatic wall. A negative hydrostatic pressure was created in the cannula by filling its distal portion with saline and holding the open end about 50 cm below the animal. This caused the lymph vessel near the tip of the cannula to collapse and ensured that lymph pressure was maintained at about 0 mmHg. The first lymph flow into the cannula was relatively rapid due to the slight pooling in the lymphatics during the cannulation procedure. When the flow rate appeared to have stabilized, usually within 10–15 min, a mark was made on the cannula at the lymph:air interface. At the end of the collection period (2 h) all lymph that had passed the mark was weighed and an average flow rate was calculated assuming a relative density for lymph of 1.0.

Progestagen and protein concentrations were determined from the single pool of lymph fluid. Four samples of arterial and ovarian venous blood were taken from each rat during the collection period. Since two-way analyses of variance revealed no significant changes with time in ovarian blood flow, arterial and ovarian venous progestagen concentrations and progestagen secretion rates, average values were calculated for each rat. Total proteins were measured in arterial and ovarian venous samples obtained near the beginning and the end of the collection period. There was no apparent difference due to time of sampling (paired t test) and so averages of the two measurements were used in the results. Albumin and transferrin measurements and electrophoresis gel examinations were carried out on arterial and ovarian venous samples selected at random from each rat.

Radioimmunoassay of progestagens

Plasma concentrations of progesterone were measured by radioimmunoassay (RIA) as previously described (Meyer & Bruce, 1979). All samples were measured in a single assay; the intra-assay coefficient of variation was 12%. The recovery of progesterone from 5 estimates for lymph and plasma was similar (94.8 ± 1.3 and 97.3 ± 1.1% respectively; mean ± s.e.m.). The procedure for RIA of 20α-dihydroprogesterone was similar to that used for progesterone assay. The antibody was obtained from Endocrine Sciences (Tarzana, CA, U.S.A.), and used in accordance with the manufacturer's method, except that sample extracts were not purified before assay. This purification was found to be unnecessary since the concentration of 20α-dihydroprogesterone measured in 30 blood samples obtained from pregnant rats was similar before and after thin-layer chromatography (57.9 and 57.3 ng/ml, respectively). The minimum quantity of 20α-dihydroprogesterone detectable in the assay was 10 pg, and the intra-assay coefficient of variation was 5%. The recovery of 20α-dihydroprogesterone from 5 estimates for lymph and plasma was similar (96.6 ± 1.3 and 99.0 ± 0.4% respectively; mean ± s.e.m.).

Estimation of protein concentration

Protein concentrations were measured by an adaptation of the Lowry method (Maddy & Spooner, 1970) using bovine serum albumin as a standard. Albumin and transferrin concentrations were measured by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965; McArule & Morgan, 1982) using rabbit anti-rat transferrin or serum albumin as appropriate, and homologous purified protein for standards.

Gel electrophoresis

For samples from 3 of the 5 rats examined, SDS polyacrylamide gel electrophoresis was performed on 7% gels using the method of Laemmli (1970). Protein (100 μg) was applied to each lane, alternating between plasma and lymph and the gels were run at 18 mA/gel for about 5 h. The gels
were stained in 0·05% Coomassie brilliant blue in acetic acid:methanol:water (1:5:5 by vol.) and de-stained with the same solution without Coomassie blue. The gels were scanned at 540 nm in a Beckman DU8 spectrophotometer using the peak pick and area estimation programs supplied. Low molecular weight standards (10 000–100 000, Bio-Rad Laboratories Ltd, CA, U.S.A.) were run concurrently with the samples, which allowed identification of serum proteins such as transferrin and albumin.

Since single concentration gels do not give linear resolution over the entire molecular weight range of serum proteins, other high molecular weight proteins, such as IgG or macroglobulin, could not be identified unequivocally. However, the relative concentrations and the relative mobilities of the proteins could be compared directly from the gel scans.

Results

General

The weight of the 5 rats at mating was 226 ± 10 g (mean ± s.e.m.) and their weight gain over Days 1–16 was 54 ± 6 g. There were 7·0 ± 0·2 live fetuses in the uterine horn on the side of the ovary used for lymph collection. The total weight of the ovary from which blood/lymph samples were taken, and the weights of its luteal and stromal tissues were 48·2 ± 7·0, 30·9 ± 3·4 and 17·3 ± 4·0 mg respectively. The mean number of corpora lutea (CL) in this ovary was 8·0 ± 0·3.

Various physiological data were recorded at each of the four blood sample times. Two-way analyses of variance revealed no significant differences with time and so average values were determined for each rat. The group mean values were: arterial pressure, 115 ± 5 mmHg; arterial haematocrit, 41·1 ± 1·0; ovarian venous haematocrit, 42·0 ± 0·7; and heart rate, 355 ± 8 per min. These values were comparable with those previously reported from this laboratory (Bruce & Meyer, 1981).

Rates of ovarian lymph and blood flow, progestagen concentrations and secretion rates

The results are shown in Table 1. Two-way analyses of variance revealed no significant differences between the four sampling times in these values. There was, however, evidence of a relationship between rate of ovarian blood flow and progestagen secretion. When all 20 measurements (4 samples from each of 5 rats) were examined by analysis of covariance, the common correlation coefficients between rate of blood flow and progesterone and 20α-dihydroprogesterone secretion rates were $r = 0·60\ (P < 0·05)$ and $r = 0·71\ (P < 0·01)$ respectively.

After the initial stabilization period the rate of lymph flow appeared steady and remained so to the end of the 2 h collection period. During this time the rate of lymph flow was 1·1% of that of ovarian venous plasma flow. The concentrations of progesterone and 20α-dihydroprogesterone in ovarian lymph were only 37% and 48% respectively of the concentrations in the ovarian venous plasma. Calculated from these concentrations and the respective rates of plasma and lymph flow, the percentage of each progestagen secreted through the lymphatic route was only 0·44% for progesterone and 0·48% for 20α-dihydroprogesterone.

Protein concentration of ovarian lymph and plasma

As shown in Table 1, total protein concentration was similar in arterial and ovarian venous plasma, but its concentration in lymph was only 53% of the latter. The albumin and transferrin concentrations were similarly lower in lymph than in plasma although this difference was only significant for transferrin.

Partitioning of the proteins on the SDS gels indicated general similarity both in the relative mobilities and the relative proportions of proteins present. There was a suggestion in 2 of the 3 rats
The relative flow rate of lymph from the ovary is generally regarded as high but Morris & Sass (1966) have provided the only quantitative estimate relating specifically to flow of ovarian lymph when anastomoses with uterine lymphatics were excluded. These workers reported a flow rate of 100–600 ml/h per 100 g of ovarian tissue for conscious sheep with an active corpus luteum. The present results, although obtained from anaesthetized animals and involving the cannulation of extremely small lymph vessels, fell within this range (137 ml/h per 100 g). Total lymph flow through the cisterna chyli of the rat is in the order of 0.2 ml/h per 100 g tissue (Reinhardt, 1945; Nix, Mann, Bolman, Grindlay & Flock, 1951) and thus the flow per unit weight from the rat ovary is around 700-fold greater than the average rate from all other tissues of the body.

The rate of lymph flow is a function of total blood flow through an organ and the net percentage of the blood volume which escapes from the blood capillaries into the lymphatics. The high relative flow rate of lymph from the ovary, is presumably related, at least in part, to the rich blood vascular supply. Cardiac output in the rat is in the order of 16 ml/per 100 g body weight, whereas the rate of ovarian blood flow is around 1600 ml/min per 100 g tissue (Bruce, Meyer & Dharmarajan, 1984). If the percentage of blood volume escaping from all capillaries is uniform, then this difference in blood flow would account for only a 100-fold difference between lymph flow from the ovary and that from other organs in the body. In addition, however, there is some evidence that fenestrated capillaries are more permeable to plasma proteins than continuous capillaries (Courtice, 1971) and the ovary, in common with some other endocrine organs contains mostly fenestrated capillaries.

Table 1. Ovarian plasma flow, lymph flow and composition of ovarian plasma and lymph in rats

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Lymph</th>
<th>Lymph/plasma (%)</th>
</tr>
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<tbody>
<tr>
<td>Flow rate (μl/min)</td>
<td>99 ± 2</td>
<td>1.1 ± 0.2</td>
<td>1.1**</td>
</tr>
<tr>
<td>Progestagen concentration (ng/ml)</td>
<td></td>
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<td></td>
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<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>88 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous</td>
<td>2824 ± 469</td>
<td>1049 ± 114</td>
<td>37*</td>
</tr>
<tr>
<td>20α-Dihydroprogesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>36 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous</td>
<td>1107 ± 70</td>
<td>530 ± 118</td>
<td>48*</td>
</tr>
<tr>
<td>Protein constituents (mg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
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<tr>
<td>Arterial</td>
<td>94.5 ± 6.4</td>
<td>45.0 ± 7.4</td>
<td>53**</td>
</tr>
<tr>
<td>Venous</td>
<td>85.4 ± 6.0</td>
<td>45.0 ± 7.4</td>
<td>53**</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous or Arterial</td>
<td>37.2 ± 2.7</td>
<td>28.1 ± 6.5</td>
<td>75</td>
</tr>
<tr>
<td>Transferrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous or Arterial</td>
<td>4.8 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>51*</td>
</tr>
<tr>
<td>Progestagen secretion (μg/h per ovary)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>15.9 ± 3.2</td>
<td>0.07 ± 0.008</td>
<td>0.44**</td>
</tr>
<tr>
<td>20α-Dihydroprogesterone</td>
<td>6.3 ± 1.1</td>
<td>0.03 ± 0.005</td>
<td>0.48**</td>
</tr>
</tbody>
</table>

The values given are mean ± s.e.m. derived from a single mean value calculated for each rat (N = 5).

Plasma value significantly different from lymph value: *P < 0.05; **P < 0.01 (paired t test).

Discussion

The relative flow rate of lymph from the ovary is generally regarded as high but Morris & Sass (1966) have provided the only quantitative estimate relating specifically to flow of ovarian lymph when anastomoses with uterine lymphatics were excluded. These workers reported a flow rate of 100–600 ml/h per 100 g of ovarian tissue for conscious sheep with an active corpus luteum. The present results, although obtained from anaesthetized animals and involving the cannulation of extremely small lymph vessels, fell within this range (137 ml/h per 100 g). Total lymph flow through the cisterna chyli of the rat is in the order of 0.2 ml/h per 100 g tissue (Reinhardt, 1945; Nix, Mann, Bolman, Grindlay & Flock, 1951) and thus the flow per unit weight from the rat ovary is around 700-fold greater than the average rate from all other tissues of the body.

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Fig. 1. Representative gel scans of plasma (a) and lymph (b) samples run on SDS gels as described in 'Materials and Methods'. Scans were run at 540 nm on Coomassie blue-stained gels (scan speed: 1 cm/cm chart/min). Arrows refer to relative mobility of molecular weight standards.
S, start of gel; F, dye front.
*Area of the high molecular weight protein absent in 2 of the 3 lymph samples.

(Morris & Sass, 1966). From the above figures it would follow that net percentage of blood volume escaping from the ovarian capillaries is around 7-fold greater than the average of that from capillaries in other body tissues.

The analysis of protein composition of lymph showed that total protein concentration was $53 \pm 7\%$ of that in venous plasma. Morris & Sass (1966) specifically examined ovarian lymph in conscious sheep and reported lymph/plasma protein ratios in the range of 59 to 90%. In addition, Staples et al. (1982) examined utero-ovarian lymph and reported lymph/plasma protein ratios in the order of 80–90%. The lower percentage in the present work could be due to species differences, reproductive state or technique. With regard to technique, the lower ratios in our work could be explained by a higher intracapillary pressure or a lower pressure within the interstitial space. It is
possible that interstitial pressure was lower since considerable care was taken to ensure that lymph pressure remained at about 0 mmHg. With the conscious sheep preparations (Morris & Sass, 1966) the lymph flow may have been retarded by the demands of the procedures for chronic cannulation. Further studies involving manipulation of arterial and venous pressures, such as those reported for the rat tail (Aukland & Wiig, 1984; Aukland, Kramer & Renkin, 1984), and lymphatic pressures would be needed to determine normal lymph:plasma ratios definitively.

The question of whether the various proteins or protein fractions are differentially permeable to the capillary wall in the ovary has not previously been examined. In this study it appeared that albumin crossed the capillary wall more readily than did transferrin, as based on lymph:plasma concentration ratios. However, this difference was not statistically significant, nor was there any statistically significant evidence for differences in lymph:plasma ratios between total proteins, transferrin or albumin. Examination of the SDS gels revealed that all major protein fractions identified in plasma were apparent in lymph in at least qualitatively similar proportions, with the possible exception of the heaviest molecular weight fraction. The latter was identified in all three plasma gels but only unequivocally identified in one of the three lymph gels. This region of the gel is in the area of the high density lipoproteins and, given its possible relevance to cholesterol transport to the luteal cell (Christie, Strauss & Flickinger, 1979), we intend to carry out further investigations of high molecular weight protein transport by using specifically-labelled protein fractions.

The percentage of blood plasma escaping from capillaries to be returned via lymphatics was 1.1%. However, since the progesterone concentration in lymph was only 37% that of venous plasma, the resultant final fraction of the total progesterone secreted by the ovary was about 0.42% and so can be ignored in any assessment of total progesterone secretion. Lindner et al. (1964) had previously reported that no more than one-tenth of the progesterone output appears to be carried from the sheep ovary by the lymphatic route.

Ovarian blood flow in this work was lower than previously recorded from venous outflow studies in this laboratory (Bruce & Meyer, 1981). In the present study, however, the rat was not maintained in a water bath and the parametral artery was ligated to accept a cannula for injection of the dye to visualize the lymphatics and this may also have retarded blood flow. The reduced flow did not appear to affect average progesterone secretion but the significant positive correlation between blood flow and secretion of both progestagens suggests that at times thresholds were reached when progesterone secretion was compromised.

The most surprising result was the finding that the concentrations of progestagens in lymph were substantially less than those in the venous plasma. We expected that progestagen concentrations in lymph would reflect those in the interstitial space (Courtice, 1971) and that the latter would lie somewhere between concentrations in the luteal cells and the ovarian venous plasma. We estimated that progesterone concentration in the luteal cell would be in the order of 20 000 ng/ml (based on a value for concentration in the total CL of 15 724 ± 2852 ng/ml; n = 5; R. T. Swann & N. W. Bruce, unpublished observations; arterial and venous plasma concentrations of 90 ng/ml and 2800 ng/ml respectively, from the present work; and that luteal cells occupy about 65% of the CL and blood volume about 22%; Dharmarajan et al., 1985). The finding that progesterone concentration in lymphatic fluid was below that in ovarian venous plasma shows that there is not a simple diffusion gradient between luteal cell cytoplasm and plasma within the blood capillaries. We are currently investigating the possibility that the flow of lymph in the CL is counter-current to capillary blood flow and/or that selective mechanisms operate in transport of progesterone across interstitial space.

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