Rate of blood flow and growth of the corpora lutea of pregnancy and of previous cycles throughout pregnancy in the rat

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Summary. The rate and distribution of ovarian blood flow was measured with radioactive microspheres in nulliparous rats at Days 4, 7, 10, 13, 16 and 22 of gestation; term is Day 23. Ovaries were dissected into large corpora lutea (or CL of pregnancy), small CL, assumed to be those of previous cycles, and the ovarian stroma which included follicles and additional small CL. Total ovarian blood flow was similar over Days 4–10, but then increased progressively to reach $916 \pm 69 \mu\text{l/min}$ at Day 22. The main cause of this increase was a 786% rise in blood flow to the large CL over Days 10–22. Weight of the CL of previous cycles increased by 289% over Days 10–16 and their rate of blood flow and histological appearance were similar to those of the large CL. These results indicate that neither reduced blood flow nor vascular degeneration, as determined by light microscopic examination, initiates the reported fall in progesterone secretion near term. At least some of the CL of previous cycles are maintained and indeed grow during gestation and have a blood circulation and histological appearance characteristic of steroidogenic tissue.

Introduction

The corpora lutea (CL) of most mammals receive a high rate of blood flow but, despite numerous investigations, the biological significance of this high rate of flow remains unknown. Several studies have suggested that a change in the rate of blood flow precedes and perhaps initiates a change in the rate of progesterone secretion (Romanoff, Deshpande & Pincus, 1962; Niswender, Reimers, Diekman & Nett, 1976). In others, however, changes in progesterone secretion or in plasma concentrations of progesterone have preceded any change in blood flow (McCracken, Glew & Scaramuzzi, 1970; Bruce & Hillier, 1974; Janson, Albrecht & Ahrén, 1975; Pang & Behrman, 1979; Damber *et al.*, 1981). In the rat, the corpora lutea of pregnancy go through a major growth phase over Days 10–16 gestation (Meyer & Bruce, 1979). This phase is accompanied by an increase in the rate of progesterone secretion and is followed by a rapid decline in secretion near term (Uchida, Kadowaki, Nomura, Miyata & Miyake, 1970). The present work was carried out to determine whether the rate and distribution of ovarian blood flow during pregnancy was related to the patterns of growth and hormone secretion by the CL. In addition, data were collected on growth and blood flow to the CL of previous cycles. The CL of normal cycles have been well characterized (Neill & Smith, 1974) but their fate and functional significance during subsequent pregnancy have received little attention.

Materials and Methods

Nulliparous albino Wistar rats, 3–5 months old, were used. The rats were kept in an environmentally controlled building (17–23°C, relative humidity 50–70%, lights from 07:00 to

21:00h). Food and water were freely available. The 39 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 litter on the morning of Day 23.

Blood flows were measured with radioactive microspheres. The procedures used were similar to those described previously (Bruce, 1976). The rats were examined on Days 4, 7, 10, 13, 16 or 22 of gestation. Each rat was anaesthetized with an i.p. injection of 40 mg pentobarbitone sodium/kg and a tracheal tube was inserted to assist breathing. Cannulae (0.5 mm o.d., 0.3 mm i.d.) were inserted into the left and right femoral arteries, and the left ventricle via the right common carotid artery. The cannula in the left femoral artery was used to monitor arterial pressure and pulse rate, and the cannula in the right femoral artery was used to take a reference sample of blood during injection of microspheres. Surgery took 30–45 min then the rat was allowed to stabilize for about 15 min. The reference sample of blood was then withdrawn at a rate of 0.4 ml/min. After 10 sec, the microspheres (75 000–150 000 in a volume of 0.2–0.3 ml) were steadily injected over a period of 20 sec into the left ventricle. Blood withdrawal was continued throughout the injection of microspheres and for a further 30 sec. The rat was killed with an overdose of pentobarbitone sodium and its organs were dissected out, weighed and measured for radioactivity. Cardiac output was calculated by multiplying the radioactivity of the total dose of microspheres injected by the rate of blood withdrawal in the reference sample and dividing by the radioactivity of the reference sample. Organ blood flow was calculated similarly by substituting organ radioactivity for radioactivity of the injection dose.

The major change from the procedure adopted previously (Bruce, 1976) was that 'TS' microspheres (Pharmacia, Tracer Sephadex; 15 μ m diameter and labelled with ^{46}Sc) were used rather than '3M' microspheres (Minnesota Mining and Manufacturing Co.; 25 μ m diameter and labelled with ^{46}Sc). The TS microspheres were radioactively labelled in the laboratory and were validated along the lines previously described (Bruce & Abdul-Karim, 1973; Bruce, 1976). The mean $\pm$ s.d. diameter of the TS microspheres was 13.9 ± 2.0 μ m ($n = 30$); the radioactivity of the medium, a 10% Ficoll solution, was less than 0.5% of that of the microspheres and, with the counting conditions used, there were 7–15 counts per min per microsphere. The TS microspheres have a lower specific gravity than the 3M microspheres and do not settle out of the suspension so quickly. There were no significant changes in heart rate or blood pressure during or after injection of TS microspheres into the left ventricle. In 4 rats, TS microspheres were injected and reference blood samples were withdrawn simultaneously from the right carotid and left femoral arteries. The correlation coefficient of the radioactivity per ml of blood sampled from the two sites at a rate of about 0.5 ml/min was 0.99 and the difference between the two sites ranged from only -4 to $+5\%$. These results indicate an even bilateral and longitudinal distribution of microspheres in the general circulation. The fraction of the cardiac output apparently distributed to the lungs was $2.1 \pm 1.0\%$ (mean $\pm$ s.d.) indicating that less than that amount of the cardiac output passed through terminal vessels of greater than 15 μ m diameter. Flow rates in this paper refer only to flow through vessels of less than 15 μ m diameter. Changes were made also to the techniques used to inject radioactive microspheres and withdraw the reference blood sample. The injection dose of microspheres was taken up into a glass tube (injection tube) fashioned from a Pasteur pipette with a 3 cm wide portion and two ends tapered smoothly down to a diameter of about 1.0 mm. One end was connected to a 1 ml plastic syringe filled with air and the other end to the ventricular cannula. The injection tube was more easily agitated to mix the microspheres before injection and, with its smooth glass walls, the tube retained a substantially smaller proportion of the injection dose than did the plastic syringes used previously. The reference blood sample was collected into a similar glass tube connected at one end to the cannula in the right femoral artery and at the other end to a Pharmacia P3 roller pump. The system was filled with saline (9 g NaCl/l) and the tube was held vertically so that the blood withdrawn entered the bottom of the tube but did not go on to the pump. Fluid leaving the other end of the pump tubing was weighed to the nearest mg so that rate of blood withdrawal could be

accurately assessed. Radioactivity was monitored on an automatic well counter (Packard Auto-Gamma Spectrometer 5320) and was corrected for the effect on count rate of geometrical height of the sample in the vial. Total activity of the injected dose of microspheres was determined by subtracting the activity of the empty glass tube after injection from the activity of the full tube before injection. These counts were made with a Total Body Gamma Counter (designed and validated by the Department of Physiology, University of Western Australia) because the radioactivity of the injection tube exceeded the capacity of the well counter. Count rates were adjusted by the use of a standard vial which was counted in each system.

Considerable care was taken over the dissection of ovarian tissues. Each ovary was immersed in 10% formol-saline as soon as it was taken from the rat. This hardened the tissues, made them easier to dissect and prevented them from drying out. Further dissection was carried out the same day with the help of a dissection microscope, at about $\times 10$ magnification. The tissues were still immersed in formol-saline and were kept in a watch glass. The oviduct, ovarian bursa and fat were removed, the ovary was quickly weighed and returned to the watch glass. The CL of pregnancy (large CL) were dissected free with sharpened jewellers forceps. As many as possible of the CL of previous cycles (small CL) were dissected free also. The three subdivisions of the ovary (large CL, small CL, and the remaining stroma which included the follicles) were each rolled along a plastic surface to remove excess fluid and weighed to the nearest 0.01 mg. They were then placed in counting vials and their radioactivity measured. The radioactivity of the fluid remaining in the watch glass was also measured. This never exceeded 5% of the stromal radioactivity but was added to it since it was assumed to be due to microspheres dislodged during the dissection procedure. For the final calculation and presentation of the results the weight and blood flow values for the small CL were added to the stromal tissue values to conform with earlier reports.

Histological sections of small and large CL were prepared from one rat on each of Days 10, 16 and 22 of gestation. The rat was anaesthetized with pentobarbitone sodium and one ovary was exposed through a ventral midline incision. Ligatures were quickly tied around the ovarian pedicle, so as to maintain blood vessels in a dilated state (Dharmarajan, Meyer & Bruce, 1983) and the ovary was excised and immersed in a 2.5% glutaraldehyde solution for 6 h. The ovary was then dissected into small and large CL and these were processed, sectioned for light microscopy and stained as described previously (Meyer & Bruce, 1979).

Variation in results between groups of rats was tested by one-way analysis of variance and, when the F test was significant, differences between any two groups were tested by the least significant difference statistic (Snedecor & Cochran, 1967).

Results

General

Maternal weight at mating did not differ significantly between the 6 groups (217 ± 4 g; mean $\pm$ s.e.m.; $N = 39$). The weight gained between mating and the time of examination on Days 4, 7, 10, 13, 16 and 22, respectively, was 1.4 ± 1.3 , 9.2 ± 2.1 , 17.3 ± 1.8 , 30.3 ± 2.1 , 37.1 ± 1.8 and 102.8 ± 6.6 g (F test significant, $P < 0.01$; L.S.D., $P < 0.05$ for 9.0 g, $P < 0.01$ for 12.1 g). Cardiac output increased in parallel with maternal weight, the values on Days 4, 7, 10, 13, 16 and 22, respectively, being 29.8 ± 2.4 , 32.3 ± 3.0 , 41.1 ± 3.7 , 45.1 ± 5.0 , 46.8 ± 4.1 and 50.9 ± 5.1 ml/min (F test significant, $P < 0.01$; L.S.D., $P < 0.05$ for 11.7 ml/min, $P < 0.01$ for 15.8 ml/min). There were no significant changes during gestation in pulse rate (378 ± 8 beats/min; $N = 39$), respiration rate (61 ± 2 breaths/min; $N = 32$, records not obtained from 7 rats) or mean arterial blood pressure (121 ± 3 mmHg; $N = 39$). The number of implantation sites per rat did not differ between groups (12.8 ± 0.4 ; $N = 32$; values could not be obtained for rats at Day 4 of gestation).

Ovarian weights and blood flows

Large CL were easily identified and dissected out and, except for 2 rats, the number obtained equalled or exceeded the number of implantation sites. Small CL were less than one third of the weight of large CL and were also easy to identify. They were harder to dissect out, particularly at Day 22, since they were more deeply embedded in stromal tissue. Only those small CL that could be dissected out intact were included for weight and blood flow measurements.

Total ovarian weight showed little change in Days 4, 7 and 10 (74 ± 4 , 75 ± 5 and 79 ± 6 mg) but was heavier on Days 13, 16 and 22 of gestation (102 ± 6 , 112 ± 6 and 104 ± 5 mg). The increase in total ovarian weight was mostly due to a 293% increase in weight of the large CL over Days 10 to 16 (Table 1). The weight of the small CL similarly increased (by 289%) over Days 10 to 16 but then fell by 66% over the next 6 days. There was no significant change in the weight of ovarian stroma (which included follicles and some small CL).

Total ovarian blood flow was similar on Days 4, 7 and 10 (178 ± 36 , 150 ± 42 and 223 ± 44 μ l/min, respectively) but then increased to 416 ± 46 , 675 ± 46 and 916 ± 69 μ l/min on Days 13, 16 and 22 of gestation, respectively. The main cause of this increase was a 786% rise in blood flow to the large CL over Days 10 to 22. The proportion of the total ovarian blood flow distributed to the large CL increased from around 50% on Days 4, 7 and 10, to 85% on Days 16 and 22 of gestation. Blood flow to the small CL also increased rapidly over Days 10 to 22, but there was no significant change in blood flow to the ovarian stroma (Table 1).

Table 1. Ovarian tissue weights and blood flows throughout gestation in rats

	Day 4	Day 7	Day 10	Day 13	Day 16	Day 22	L.S.D. values*	
							$P < 0.05$	$P < 0.01$
No. of rats	7	7	6	6	7	6		
Stroma								
Weight (mg)	55.2 ± 3.2	53.8 ± 3.5	59.6 ± 6.4	62.4 ± 3.4	39.0 ± 6.4	45.6 ± 1.1	12.4	16.7
Blood flow (μ l/min)	101 ± 20	64 ± 22	127 ± 29	103 ± 14	82 ± 23	126 ± 13		
Large CL tissue								
Weight (mg)	18.2 ± 0.6	21.0 ± 1.6	19.8 ± 2.0	39.8 ± 2.4	60.0 ± 2.2	58.4 ± 4.2	6.9	9.3
Blood flow (μ l/min)	74 ± 16	82 ± 24	92 ± 16	297 ± 34	580 ± 28	780 ± 73	106	142
Large corpus luteum								
No. per rat	12.6 ± 0.5	13.3 ± 0.7	12.5 ± 0.6	14.2 ± 1.6	13.6 ± 0.7	13.9 ± 0.8		
Weight (mg)	1.43 ± 0.07	1.50 ± 0.04	1.53 ± 0.11	2.85 ± 0.17	4.48 ± 0.10	4.23 ± 0.19	0.35	0.48
Blood flow (μ l/min)	6.0 ± 1.4	5.9 ± 1.4	7.2 ± 1.5	21.4 ± 2.2	43.8 ± 2.2	56.6 ± 4.1	6.7	9.1
Small corpus luteum								
No. per rat	9.8 ± 0.5	9.7 ± 0.4	9.2 ± 0.9	8.2 ± 0.8	5.3 ± 1.1	3.3 ± 0.9	3.2	4.3
Weight (mg)	0.53 ± 0.06	0.48 ± 0.05	0.55 ± 0.09	0.82 ± 0.06	1.59 ± 0.29	0.53 ± 0.06	0.42	0.57
Blood flow (μ l/min)	1.4 ± 0.4	1.2 ± 0.3	2.0 ± 0.6	4.7 ± 0.8	10.7 ± 1.9	12.0 ± 2.0	3.6	4.8

* L.S.D. values are given at the $P < 0.05$ and $P < 0.01$ levels where the variance between groups was significant at the $P < 0.01$ level. All other values are means $\pm$ s.e.m. for the no. of rats indicated. Stroma and large luteal tissue values refer to the sum of the two ovaries from each rat. Large and small corpus luteum values refer to a single corpus luteum.

PLATE 1

Figs 1-6. Light photomicrographs of 0.5 μ m sections of luteal tissue from Day 10, Day 16, and Day 22 CL of pregnancy (Figs 1, 2 and 3 respectively) and CL of previous cycles (Figs 4, 5 and 6 respectively). Histological features appear similar between large CL of pregnancy and small CL of previous cycles. The luteal cells (L) contain a near spherical nucleus with one or two prominent nucleoli. Some luteal cells contain aggregations of lipid droplets; the tissue has an extensive capillary network (C) with some endothelial cell nuclei (E) visible adjacent to the capillary lumen. There are red blood cells within the lumen of most capillaries. $\times 600$.

(Facing p. 448)

Text-fig. 1. Blood flow to the ovarian stroma and the corpora lutea of pregnancy and of previous cycles throughout pregnancy in rats.

The increase in blood flow to the large and small CL generally exceeded the increase in weight of these organs. Blood flow, expressed per unit weight of tissue, therefore increased from Day 4 to Day 22 by about 338% for the large CL and 1143% for the small CL (Text-fig. 1). In contrast, there was little change in blood flow per unit weight of ovarian stroma.

Histology of the large and small CL

Corpora lutea were examined on Days 10, 16 and 22 of gestation to cover the phase of major growth of the CL and their functional regression; typical histology of these CL is shown in Pl. 1, Figs 1–6. The large CL had a dense network of large, sinusoidal-like capillaries on each of the 3 days examined. There was no evidence, at the light microscope level, of capillary degeneration or of debris within or outside the vessels at any stage. The luteal cells were large and polyhedral and contained nearly spherical nuclei with a prominent nucleolus. The cytoplasm contained numerous lipid droplets irregularly distributed within each cell and between the cells. There was no evidence of cell death or of changes in luteal cell nuclei or cytoplasm suggestive of luteolysis at any stage. The histological features of the small CL were very similar to those of the large CL. Again there was no evidence of capillary or luteal cell degeneration at any stage.

Discussion

The possibility that a reduction in ovarian blood flow initiates luteal regression has been investigated by many workers (sheep: McCracken *et al.*, 1970; Baird, 1974; Niswender *et al.*, 1976; rabbit: Bruce & Hillier, 1974; guinea-pig: Hossain, Lee, Clarke & O'Shea, 1979; rat: Pang & Behrman, 1979; dog: Romanoff *et al.*, 1962) and different models of regression have been investigated, including natural regression at the end of the oestrous cycle, pregnancy and pseudo-pregnancy as well as experimentally induced regression with prostaglandins, cycloheximide and other agents. The results and interpretations reported have been at variance and luteal regression is often ill defined. It is generally assumed to have structural and functional components and to involve an irreversible reduction in progesterone secretion by the ovary. In the rat it has been suggested that the first sign of regression is an increase in 20α -hydroxysteroid dehydrogenase

activity which results in a decrease in progesterone secretion which may be partly balanced by an increase in 20α -dihydroprogesterone secretion (Wiest, Kidwell & Balogh, 1968). In the rabbit, sheep and goat, the total steroid hormone secretion is reduced substantially as determined directly by venous outflow technique or indirectly by measuring hormone concentrations in the peripheral plasma (Thorburn & Mattner, 1971; Thorburn & Schneider, 1972; Hilliard, 1973). These functional changes generally precede or accompany structural degeneration of luteal tissue which can include subtle changes in luteal cells at the ultrastructural level (Deane, Hay, Moor, Rowson & Short, 1966; O'Shea, Cran & Hay, 1979) or more gross changes including luteal and endothelial cell breakdown and invasion by macrophages (Gemmell, Stacy & Thorburn, 1976; Stacy, Gemmell & Thorburn, 1976). For the present discussion luteal regression is taken to mean any evidence of an irreversible reduction in progesterone secretion, and luteolysis to mean any degenerative structural change in the luteal cells or their blood vessels.

In the rat CL regression at the end of pseudopregnancy, is not preceded by any change in ovarian or CL blood flow (Pang & Behrman, 1979). Although earlier reports suggested that prostaglandin-induced CL regression resulted from reduced blood flow (Pharriss, 1970) more recent work, in which the time course of events was followed more closely (Pang & Behrman, 1981), this was not found to be so. Regression of the CL of pregnancy in the rat has received little attention although we have reported that total ovarian blood flow on Day 22, when progesterone secretion has fallen to low levels, is still very high (Bruce, 1976). The present results support this finding and show that total ovarian blood flow increased 35% over Days 16–22. The results also discount any possibility that a redistribution of ovarian blood flow away from the CL initiates regression; blood flow per unit weight of CL increased by 37% over the same period. Similarly, there was no evidence, at least at the light microscope level, of vascular degeneration or debris accumulation in or outside the capillaries of the CL that might interfere with the normal transport processes between the blood and the luteal cell, as has been reported to occur in the guinea-pig (Hossain & O'Shea, 1977) at the time of regression of the cyclic CL.

The pattern of regression of the CL of pregnancy in the rat contrasts greatly with that in the rabbit. In the rat over Days 16 and 22 plasma progesterone concentrations decline rapidly (Uchida *et al.*, 1970), the weight of the CL remains relatively constant and the rate of blood flow to the CL is increased by around 37%. In the rabbit, the decline in progesterone concentrations appears more gradual over Days 20 to 28 (Mikhail, Noall & Allen, 1961; Hilliard, Spies & Sawyer, 1968). Furthermore, this decline is almost paralleled by a decline in CL weight and blood flow (Abdul-Karim & Bruce, 1973). Whether the decline in blood flow precedes CL regression, or luteolysis, has not been determined with sufficient precision. However, Bruce & Hillier (1974) have reported that, at least in prostaglandin-induced regression, CL blood flow is maintained until after progesterone concentrations have started to decline. Whatever the cause of CL regression in the pregnant rabbit, it is clear that the whole process of regression and luteolysis is different from that in the rat and generalizations of the possible role of reduced blood flow in these events are unfounded.

Little has been published on the fate of CL of previous cycles during pregnancy. It is assumed generally that they degenerate and cease to have any functional role early in pregnancy. Pang & Behrman (1979) noted their existence, however, and suggested that they may have contributed to their estimates of blood flows and weights of the CL of pregnancy. It was assumed here that the small CL were indeed the CL of cycles preceding pregnancy. They fell into a distinct weight category, the heaviest small CL was less than half the weight of the lightest large CL; they were more deeply embedded in stromal tissue; and the close correlation between the number of implantation sites and large CL suggested that the small CL had no role in the production of oocytes for that pregnancy. We suspect that most of the small CL examined were derived from the immediately preceding cycle, the number that we could dissect out confidently never exceeded the number of ovulations expected in any one cycle. As pointed out in the results, there were other still smaller structures which may have been luteal remnants of still earlier cycles but these were included as part of the stromal tissue calculations.

The present results offer a new perspective of the fate of the CL of previous cycles. It is now apparent that, firstly, the small CL are not only maintained but grow substantially over Days 10–16 as do the large CL, and, secondly, that they have a high rate of blood flow, comparable to, and indeed near term exceeding, that of the large CL. During the oestrous cycle, if mating does not take place, plasma progesterone concentrations rise after ovulation to reach a peak about 2 days later. Concentrations then fall to very low levels before the next ovulation (Neill & Smith, 1974) and we had assumed that this was due to luteolysis. Now, however, it seems that a fertile mating rescues the CL after about 4 days so that they are maintained and induced to grow well into the second half of pregnancy, longer than the functional time span achieved by the CL of pseudopregnancy (Fajer & Barraclough, 1967; Hashimoto, Henricks, Anderson & Melampy, 1968). Furthermore their blood flow is maintained for a substantially longer period than is that to CL of pseudopregnancy (Pang & Behrman, 1979). Their potential size, however, is reduced in that they only reach about a third of maximum weight of the large CL. They also behave differently in that they diminish in size over the last 6 days of pregnancy whereas the weight of the large CL remains relatively constant over this period.

The presence of the small CL would have affected the estimates of stromal blood flow, particularly over the last third of gestation. In the present study weight and blood flow values for small CL were added to the stromal tissue values to conform with earlier reports. If, however, they had not been included, stromal blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$) would have been 116 rather than 166 at Day 13, 83 rather than 210 at Day 16 and 195 rather than 276 at Day 22. The true rate of blood flow to the ovarian stroma, including follicles, might have been lower again if all of the luteal tissue of previous cycles had been removed, but the practical difficulty of dissection precludes a more accurate assessment at this stage.

We are unaware of any previous description of the small CL of pregnancy in rats. In this study at the light microscope level, the small CL bore a close resemblance to the large CL. They were richly vascularized with large, sinusoidal-like capillaries. The luteal cell had a near spherical nucleus, often with a prominent nucleolus. The cytoplasm had numerous lipid droplets and small electron-dense bodies which, on a preliminary electron microscopic examination, were found to be mitochondria. By these criteria, the small CL fit the description of classic steroid hormone-secreting tissue (Enders, 1962; Long, 1973). Again in preliminary studies, we have found that they have a tissue progesterone concentration in the order of 1000–6000 ng/ml, values slightly lower than those of the large CL (R. T. Swann & N. W. Bruce, unpublished). This, together with their histological appearance, suggests that the small CL might still actively secrete steroid hormones, or provide a functional reserve of steroidogenic tissue. Whatever their function, the present results discount the view that rat CL regress if they are not stimulated by the act of mating soon after their formation and so bring into question the importance of critical timing of luteotrophic stimuli at this stage.

From the blood flow, growth and histological observations of the present study we could not identify any clear change preceding the reported decline in ovarian progesterone secretion near term. At least in the pregnant rat, the latter decline is likely to have a more subtle, enzymic cause.

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