Ovarian blood flow and progesterone secretion in anaesthetized rats at Day 16 of gestation, and the effects of haemorrhage

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Summary. A venous outflow technique was used to estimate the rate of progesterone secretion from the ovary of rats on Day 16 of gestation. In 9 normal rats the rate of progesterone secretion was 13.7 $\mu\text{g/h}$ per ovary and ovarian blood flow was 0.48 ml/min. In 6 rats in which haemorrhage had occurred the respective values were 38.3 $\mu\text{g/h}$ per ovary and 0.53 ml/min. Comparison with earlier reports suggests that ovarian blood flow can be substantially reduced for short periods without affecting progesterone secretion.

Introduction

The control of progesterone secretion by the ovary during pregnancy is poorly understood. One factor which may be of significance is the rate of ovarian blood flow (Niswender, Reimers, Dickman & Nett, 1976). In the rat, a standard method for estimating the rate of progesterone secretion has been to collect the ovarian venous outflow and to multiply the rate of collection, or blood flow, by the concentration of hormone in the blood. However, estimates of flow rates have varied greatly between workers (see Piacsek & Huth, 1971) and have generally been less than 25% of that measured with radioactive microspheres or with an improved venous outflow technique (Bruce & Dimmitt, 1977). It is therefore possible that secretion rates have been substantially underestimated or that secretion is largely independent of the rate of blood flow.

These possibilities were tested by using the ovarian venous outflow technique, as modified by Bruce & Dimmitt (1977), and comparing estimated progesterone secretion rates with those previously reported. Measurements were made on rats at Day 16 of pregnancy when progesterone secretion rates are highest (Uchida, Kadowaki, Nomura, Miyata & Miyake, 1970). The effect of accidental haemorrhage during surgery on progesterone secretion was also examined since haemorrhage has been reported to elevate gonadotrophin levels in female rats (Seyler & Reichlin, 1973).

Materials and Methods

Mature, nulliparous, albino Wistar rats were used. Housing and maintenance of animals and the technique used for collecting ovarian venous blood and determining ovarian hormone secretion rates have already been described (Bruce & Dimmitt, 1977; Bruce, Meyer & Dimmitt, 1980), but the main features were as follows. Rats were examined on Day 16 of pregnancy: Day 1 is the day that spermatozoa were found in a vaginal smear. Each rat was anaesthetized with pentobarbitone sodium and after tracheal cannulation a cannula was inserted into the dorsal

aorta via the left common carotid artery to monitor arterial pressure and take arterial blood samples for progesterone assay. 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. Heparin, 500 i.u., was administered to prevent clotting and blood leaving the ovary was directed through a drop counter and then returned to that rat via an infusion pump and jugular cannula. During the measurement period the abdomen and exposed viscera of the rat were immersed in a saline bath at 37°C which maintained body heat and tended to stabilize arterial pressure and ovarian blood flow. There was a small degree of blood loss during surgery in all rats. To compensate, 1.0 ml blood from a donor rat was administered at the end of the operation. Six rats lost a further 1.5–3.0 ml blood during the ovarian cannulation procedure. These rats, classed as 'haemorrhaged', were given an appropriately increased volume of donor blood after surgery. Only dioestrous rats were selected as donors to minimize luteotrophic activity in the replacement blood.

At about 20 min after the venous outflow circuit had been established and when arterial pressure pulse rate and ovarian blood flow had reached reasonably stable levels, 3 measurements of these variables were made at 20 min intervals. Blood samples, 0.3 ml, were also taken from both the arterial and the venous drainage cannulae for later measurement of progesterone. Further donor blood, 0.6 ml, was administered to compensate for that lost by sampling. The ovarian arterio-venous differences in plasma progesterone concentrations (A–Vp difference) were calculated and so too were the rates of progesterone secretion by the ovary (A–Vp difference $\times$ the product of ovarian blood flow and the percentage of plasma in whole blood, 58%).

Of the 15 rats used 6 were classed as 'haemorrhaged' and 9 as controls. The results from the 3 measurements made on each rat were first averaged and then the means of each group were compared by standard *t* tests. Where variances were heterogeneous, Wilcoxon two-sample tests were used rather than *t* tests (Sokal & Rohlf, 1969). Within the control group, relationships between ovarian blood flow, hormone secretion, blood pressure and the mass of luteal tissue were determined by simple and multiple linear correlation analyses (Snedecor & Cochran, 1967).

Results

Surgery was relatively uneventful in the control rats and little blood loss resulted. The 6 'haemorrhaged' rats became briefly hypotensive, as indicated by a 25% reduction in minimum arterial pressure recorded during surgery, but blood pressure, heart rate and haematocrit returned to values similar to those of the controls after receiving donor blood.

The results for the two groups of rats are shown in Table 1. The main finding was that progesterone secretion in the 'haemorrhaged' rats was 180% higher than that in the controls. This was due to a 174% increase in the A–Vp difference: the rate of ovarian blood flow was not affected. Simple linear correlation analyses showed that the rate of progesterone secretion in control rats was significantly related to the mass of luteal tissue present ($r = 0.786$; $P < 0.05$) but not to the rate of ovarian blood flow ($r = 0.517$; N.S.). Since there was a positive, although non-significant, relationship between luteal mass and ovarian blood flow ($r = 0.545$), partial correlation coefficients were calculated to examine these relationships further. With ovarian blood flow held constant, progesterone secretion and luteal mass were still significantly related ($r = 0.702$; $P < 0.05$), but with luteal mass held constant the relationship between progesterone secretion and ovarian blood flow was again non-significant ($r = 0.170$). No significant relationships were revealed by simple or multiple correlation analyses between ovarian blood flow, luteal mass and arterial pressure.

Table 1. Ovarian blood flow and progesterone secretion in control and 'haemorrhaged' rats at Day 16 of gestation

	Control rats	'Haemorrhaged' rats
No. of rats	9	6
Body wt at mating (g)	211 ± 5	216 ± 9
Mean arterial pressure (mmHg)		
Minimum during pregnancy	96 ± 3	72 ± 6*
At measurement	130 ± 4	118 ± 5
Heart rate (per min)	394 ± 4	396 ± 8
Haematocrit (%)	42.3 ± 0.6	43.1 ± 0.8
No. of live fetuses in collected horn	4.9 ± 0.6	5.7 ± 0.4
No. of CL in collected ovary	6.1 ± 0.6	7.0 ± 0.6
Collected ovary		
Total wt (mg)	36.7 ± 3.9	45.8 ± 3.2
CL wt (mg)	21.3 ± 2.1	23.2 ± 2.3
Stroma and follicle wt (mg)	17.4 ± 2.1	22.6 ± 1.7
Ovarian blood flow		
ml/min	0.48 ± 0.03	0.53 ± 0.08
ml/min per 100 g ovary	1308 ± 93	1170 ± 183
Arterial plasma progesterone (ng/ml)	80 ± 3	97 ± 4*
Ovarian venous plasma progesterone (ng/ml)	914 ± 66	2380 ± 413**
Arterio-venous plasma progesterone difference	833 ± 65	2282 ± 412**
Ovarian progesterone secretion rate		
µg/h per ovary	13.7 ± 1.2	38.3 ± 5.8**
µg/h per mg luteal tissue	0.66 ± 0.04	1.71 ± 0.26**

Values are mean ± s.e.m. for the number of rats indicated. Ovarian blood flow and progesterone values are the means of the average of 3 measurements taken from each rat.

Values significantly different from those of control rats: * $P < 0.01$, unpaired t test;

** $P < 0.01$, Wilcoxon two-sample test.

Discussion

The major question asked was whether progesterone secretion by the rat ovary is related to the rate of ovarian blood flow and, specifically, whether previous estimates of progesterone secretion could have been influenced by low rates of flow. In Table 2, the present results are compared with those of other workers who examined rats at a similar stage of gestation and who provided sufficient details for reasonable estimates to be made of both progesterone secretion and blood flow. In the present work the rate of ovarian blood flow was about 4 times greater than that reported in the other publications. Despite this, the estimated rate of progesterone secretion was no greater, indeed it was the second lowest reported. It follows that the concentration of progesterone in the ovarian venous plasma (a value similar to the A-Vp difference) must have been substantially higher in all of the previous studies (see Table 2).

There is good evidence to suggest that the normal rate of blood flow to the ovary at Day 16 of pregnancy is similar to that reported in the present work, i.e. about 0.4 ml/min. It is in accord with reported rates of blood flow to CL-bearing ovaries in a variety of species when the stage of gestation and differences in ovarian mass are taken into account (see Bruce & Moor, 1975). It is also in accord with measurements made with radioactive microspheres in conscious and anaesthetized rats at Day 16 of pregnancy (mean ± s.e.m. values 0.56 ± 0.11 , $n = 7$, and 0.34 ± 0.02 ml/min, $n = 7$, respectively; N. W. Bruce & G. T. Meyer, unpublished). It would therefore seem that, at least in the short term, a reduction to about 25% of the normal rate of ovarian blood flow has little or no effect on progesterone secretion rate. This extends our previous findings that within individual rats changes in ovarian blood flow have little influence over progesterone secretion rate but are inversely related to the A-Vp difference. Still further

Table 2. Comparisons of values for ovarian blood flow and progesterone secretion in rats at Day 15 or 16 of gestation

Reference	Day examined	Blood flow (ml/min)	Progesterone secretion ($\mu\text{g/h}$ per ovary)	Ovarian venous progesterone conc. (ng/ml)
Fajer & Barraclough (1967)	16	<0.1	20.5	
Hashimoto <i>et al.</i> (1968)	15	0.1	22.9	
Uchida <i>et al.</i> (1970)	15	≈ 0.1	11.2	
Ichikawa <i>et al.</i> (1974)	16	≈ 0.15		6000
Sanyal (1978)	15	0.03–0.10	15.6	5509
Present work	16	0.48	13.7	914

support for this view comes from the multiple correlation analyses in the present work in which it was found that the rate of progesterone secretion was closely related to the mass of luteal tissue present but was not related to the rate of ovarian blood flow.

The finding that progesterone secretion rate was greatly increased in rats in which haemorrhage had occurred during surgery was particularly interesting. At the time of measurement, these rats had similar blood pressures, heart rates, haematocrits and rates of ovarian blood flow as the controls, although they had been hypotensive for a brief period during surgery. A possible explanation is that haemorrhage induced the release of luteotrophins from the pituitary which subsequently increased the rate of progesterone secretion by the ovary. A similar mechanism has been reported in non-pregnant rats (Seyler & Reichlin, 1973). It is also possible that placental luteotrophins (which have a significant role in the control of progesterone secretion later in gestation; Kato, Morishige & Rothchild, 1979), have a role in this effect. But whatever the mechanism, it is likely that haemorrhage or hypotension may have helped to maintain or even enhance the rate of ovarian progesterone secretion reported by previous workers. Of the 5 studies listed in Table 2, only Ichikawa, Sawada, Nakamura & Morioka (1974) reported replacement of blood lost at sampling. The latter generally amounted to 2–4 ml which represents a substantial degree of haemorrhage and may well have influenced pituitary or placental luteotrophin secretion. Accidental haemorrhage might also explain the high rate of progesterone secretion that we have previously reported (Bruce *et al.*, 1980). It is now clear that more attention should be paid to maintaining rats in good physiological condition not only during the venous outflow measurements but also during the preparative surgery.

In conclusion, it would seem that low rates of ovarian blood flow are compatible with normal rates of progesterone secretion in the rat. Furthermore, following haemorrhage, progesterone secretion can increase by 180% without any change in the rate of ovarian blood flow. These results suggest that either there is a large physiological reserve of blood flow or that the rate of blood flow is merely a reflection of some other factor, causally related to progesterone secretion. We are currently investigating the possibility that surface area of the ovarian vasculature, rather than the rate of ovarian blood flow, is a more significant determinant of the capacity of the ovary to secrete progesterone.

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