Ovarian arterial blood velocity measured with Doppler ultrasonic transducers in conscious ewes

B. W. Brown, M. J. Emery and P. E. Mattner

C.S.I.R.O., Division of Animal Production, P.O. Box 239, Blacktown, New South Wales 2148, Australia

Summary. Changes in the mean velocity of ovarian arterial blood flow during the oestrous cycle were monitored in 5 ewes by the use of Doppler ultrasonic transducers chronically implanted around the ovarian arteries. In arteries supplying ovaries with a corpus luteum (CL), the velocity was minimal from Day –1 to Day 2 inclusive (Day 0 = day of oestrus), increased steadily until Day 13 (–4) and then declined precipitously. In contralateral arteries supplying ovaries without a CL, the velocity remained at a consistently low level throughout the oestrous cycle.

In each ewe, arterial blood velocity to the ovary with a CL and progesterone levels in peripheral plasma were highly correlated; within-individual correlations falling between $r = 0.830$ ($P < 0.001$, $n = 15$) and $r = 0.936$ ($P < 0.001$, $n = 15$). The changes in the velocity of the arterial supply to the ovulatory ovary and the plasma progesterone levels during the luteal stage of the cycle followed a similar pattern to that for weight of luteal tissue in similar ewes.

Introduction

Quantitative changes in ovarian blood flow have been implicated in the control of ovarian function (Pharriss, Cornette & Gutknecht, 1970). The advent of Doppler ultrasonic transducers which can be implanted around blood vessels has afforded a practical means of monitoring ovarian arterial blood flow in unanaesthetized animals. Studies of this nature in conscious sheep have been made using transducers with a luminal diameter of 3.0 mm (Niswender, Moore, Akbar, Nett & Diekman, 1975; Niswender, Reimers, Diekman & Nett, 1976) and 1.5 or 2.0 mm (Brown, Emery & Mattner, 1976). However, a complete description of the changes in ovarian arterial flow over the whole of the oestrous cycle in sheep has not been reported.

In our preliminary studies, poor signal-to-noise ratio and consequent variability between replicate estimates of blood velocity appeared to be a greater problem with transducers of relatively large diameter than with those that fitted closely around the artery. Accordingly, only transducers with a luminal diameter of 1.5 mm were used in the present study on the relationships between ovarian arterial flow velocity, plasma progesterone levels and corpus luteum weight.

Materials and Methods

Animals

Five mature Merino ewes from a low ovulation-rate flock (1.06 ova/ewe/oestrous cycle) were used to study changes in ovarian arterial blood flow during the oestrous cycle. In 6 groups

of 4 ewes from the same flock, the ovaries were removed on Day 4, 6, 9, 12, 13 or 14 (Day 0 = day of oestrus) and the corpus luteum (CL) was weighed. The occurrence of oestrus was determined by using the chloride smear technique (Turnbull, Shutt & Braden, 1967) and vasectomized rams. All animals were accustomed to the experimental area and to frequent handling for several weeks before the commencement of the study.

Ultrasonic blood flow transducers

Ultrasonic cuff-type transducers with a luminal diameter of 1.5 mm and 2 opposing crystals set at 45° to the longitudinal axis of the lumen were constructed using methods similar to those described by White, Angus, McRitchie & Porges (1974). The mean integrated difference frequency (difference between transmitted and reflected sound frequencies) was recorded with a Doppler zero-crossing ultrasonic flow meter (Type 806: Parks Electronic Laboratory, Beaverton, Oregon, U.S.A.).

To establish the relationship between estimates of mean blood velocity calculated from difference frequency readings and estimates of blood velocity calculated from measured flow rate, each transducer was positioned around Silastic tubing (i.d. 0.6 mm, o.d. 1.5 mm: Dow Corning Corporation, Michigan, U.S.A.) with acoustical coupling jelly filling the space between the crystals and the tubing. A Harvard perfusion pump was used to pump heparinized blood through the tubing at known flow rates which provided blood velocities of 2.0, 5.1, 10.3, 15.1, 19.8 and 30.1 cm/sec. Velocity values were calculated from difference frequency values according to the Doppler equation:

$$\Delta F = \frac{2Fev \cos \theta}{c}$$

Where, ΔF = difference frequency; v = mean velocity of blood; θ = angle between acoustical axis of sound and the direction of blood flow (45°); Fe = oscillator frequency (8.7 MHz); and c = velocity of sound in blood (1.5×10^5 cm/sec).

Velocity data were analysed using linear regression tests.

The inner diameter of the Silastic tubing used was chosen to approximate the luminal diameter of the ovarian artery of the sheep to be used in subsequent experiments. At the conclusion of a preliminary experiment on ewes (Brown *et al.*, 1976), in which transducers had been located around each ovarian artery for several months, 2 animals at Day 12 of the oestrous cycle were anaesthetized and ligatures were tied simultaneously around the artery on either side of each transducer. The transducers and ligated portions of the arteries were excised and fixed in Serra's fixative for 2 weeks. The transducers were then sectioned and the mean inner diameter for each enclosed artery was measured. The values were 0.55 mm for the arteries supplying the 2 non-ovulatory ovaries and 0.73 mm for those supplying the ovulatory ovaries.

Implantation of transducers

With the ewes under halothane anaesthesia, a 2 cm length of each ovarian artery near the middle of the vascular cone region was separated from the ovarian veins and connective tissue. The transducer cuffs which had been sterilized in a 1% solution of Zephiran (Winthrop Laboratories, Sydney, Australia) and filled with sterile 1.5% nutrient agar were tied around the freed portions of the arteries and anchored by sutures to surrounding connective tissue. If an ovarian artery was bifurcated within the vascular cone, the transducer cuff was placed proximal to the bifurcation. In each case, arterial branches supplying the oviduct and the anterior end of the uterine horn were ligated. The leads from each transducer were exteriorized through a stab wound in the flank; the incision was closed and the animals allowed to recover.

Monitoring procedure

An oscilloscope was routinely used in conjunction with the blood flow meter to assist in tuning out random noise associated with the received signal and to determine the signal-to-noise ratio of each transducer at the time of frequency measurement. At 10 days after implantation, each transducer was tested for similarity of alignment of the 2 crystals to the axis of the direction of blood flow. In each case, the frequency readings obtained when either crystal was used as the transmitter or the receiver were identical, indicating that the vessel had remained centrally aligned within the transducer lumen.

Initially, several readings from the transducers in each ewe were taken over a 4–5-min period each hour for 8 h daily for several days. Because there was little or no variation between frequency readings in the short term or over an 8 h period for individual arteries, subsequent monitoring consisted of a once daily measurement period each morning at 10:00 h. Daily monitoring of ovarian arterial blood velocity in each ewe was continued over several oestrous cycles. After each reading, 10 ml jugular venous blood were removed by venepuncture into heparinized syringes and stored on ice until centrifugation about 15 min later.

Progesterone in 0.5 ml plasma was measured by a protein-binding assay which was a modification (Thorburn & Schneider, 1972) of the method of Thorburn, Bassett & Smith (1969). The results were not corrected for recovery, which varied from 85 to 90% over the range 0.3–4.0 ng/ml. Water blanks were <0.1 ng/tube and sensitivity of the assay, defined as twice the s.d. of the blank values, was 0.1 ng progesterone/tube. The within- and between-assay coefficients of variation were <16% over the range 0.3–4.0 ng/ml. Corticosteroids were removed from the n-hexane extracts with a distilled water wash (1 ml). Interference from other steroids in the plasma was not significant in the assay system (Thorburn *et al.*, 1969).

Results

Tests *in vitro* showed that within a range of flow velocities from 2.0 to 30.1 cm/sec there was, for each transducer, a straight-line relationship described by $y = 0.86 + 0.95x$ ($r = 0.999$, $P < 0.001$) between velocity values calculated from frequency readings and velocity values calculated from measured flow rates and diameter of the Silastic tubing used. With each transducer, variation between velocity values ($n = 9$) derived from the frequency readings at any particular flow rate was extremely small (s.e.m. <1.2%).

Oestrous cycle data

Chronic implantation of the transducers appeared to have no effect on ovarian function. All of the ewes experienced regular oestrous cycles of normal length and plasma progesterone values over the oestrous cycle were within the normal range previously reported by Thorburn *et al.* (1969).

Until 7–10 days after the implantation of the transducers, the time required for adequate fixation of the devices to the vessel by granulation tissue (White *et al.*, 1974), low signal-to-noise ratios rendered estimates of difference frequency (and velocity) variable and thus unreliable. Consequently, only data obtained 10 or more days after implantation were included in the study. Over the experimental period (2 complete oestrous cycles per ewe) the signal-to-noise ratio was 25:1 (range, 16:1 to 40:1).

The grand mean for individual ewe means for blood velocities in the ovarian arteries and for plasma progesterone levels in the same animals during the oestrous cycle are shown in Text-fig. 1. Observations at laparotomy performed on each ewe during the luteal stage of the 2 oestrous cycles following the experimental periods, showed that ovulation had occurred from alternate ovaries in 4 of 5 ewes and that, in each instance, flow velocity was much greater in the artery

supplying an ovary bearing a CL than in that supplying the contralateral ovary without a CL. In the latter vessel, flow velocity remained at a low value (grand mean $\pm$ s.e.m.) of 4.8 ± 0.1 cm/sec ($n = 17$) with little between-day variation, throughout the oestrous cycle. In the artery supplying the ovary bearing the CL, flow velocities increased slowly between Days 2 and 4, rose

Text-fig. 1. Grand means ($\pm$ s.e.m.) for ovarian arterial blood velocity to ovaries with CL (○—○) and ovaries without CL (●—●) and for progesterone concentrations (▲—▲) in peripheral plasma of ewes ($N = 5$) during the oestrous cycle.

Text-fig. 2. Relationship between the grand mean ($\pm$ s.e.m., $N = 5$) for ovarian arterial blood velocity to the ovary with a CL (○—○) and for plasma progesterone concentrations (▲—▲) in the same ewes and mean ($\pm$ s.e.m., $N = 4$) luteal weight (□) in similar ewes during the luteal stage of the oestrous cycle.

rapidly between Days 4 and 6 (6.3 ± 0.8 and 10.9 ± 1.3 cm/sec, $n = 5$, respectively) and reached peak values at Day 13 (17.9 ± 2.5 cm/sec, $n = 5$). They then fell precipitously to near base level over the next 3 days. At all times other than at Days -1, 0 and 1, the values were significantly different ($P < 0.05$) from those for the contralateral artery.

The velocity of blood flow to the ovulatory ovary and the concentration of progesterone in peripheral plasma over the oestrous cycle were highly correlated, within-individual correlations lying between $r = 0.830$ ($P < 0.001$, $n = 15$) and $r = 0.936$ ($P < 0.001$, $n = 15$). Further, a comparison of the data presented in Text-fig. 2 shows that the velocity of the arterial blood to the ovary with a CL, the plasma progesterone levels and the weight of luteal tissue in comparable ewes followed similar patterns of change over the luteal stage of the oestrous cycle.

Discussion

The present data are in accord with previous findings which indicated that the velocity of the arterial blood supply to the ovary with a CL, but not of that to the contralateral ovary without a CL, is markedly increased during the luteal stage of the oestrous cycle in sheep (Niswender *et al.*, 1975). There were, however, some differences between the findings obtained in the 2 studies. Difference frequency readings reported by Niswender *et al.* (1975) indicated that there was little difference between the flow velocities in the arteries to the "CL ovary" and the "non-CL ovary" for a much longer period, namely from Day -3 to Day 5 of consecutive oestrous cycles. In the same study the relationship between the velocity of flow to the ovulatory ovary and circulatory progesterone levels was poor ($r = 0.2543$).

It is unlikely that breed of sheep contributed significantly to the above differences. Rather, because the signal-to-noise ratio of Doppler transducers greatly influences the accuracy with which the difference frequency (hence the velocity) can be determined (Reneman, Clarke, Simmons & Spencer, 1973; White *et al.*, 1974), it is possible that the experimental protocol adopted in the present study may have been largely responsible. Firstly, data obtained during the first 10 days after implantation of the transducers (the period required for satisfactory fixation of the transducers to the vessels by fibrotic tissue) were excluded to eliminate artefacts arising from movement of the transducers and changes in vessel diameter during that time (Vatner, Franklin & Van Citters, 1970; White *et al.*, 1974). Secondly, the signal-to-noise ratio of all of the implanted transducers regularly exceeded that recommended for good differentiation of flow signal (16:1, Reneman *et al.*, 1973; 10:1, Franklin, 1974). Additionally, the use of small diameter (1.5 mm) transducers which fitted closely around the arteries ensured proper alignment of the enclosed vessels within the transducers such that the resultant angle between the transducer crystals and the direction of blood flow was, in all instances, the same as the designed angle used in the calculation of flow velocity. Such accuracy of alignment of the arteries within the transducers may not be obtained when the luminal diameter of the transducers is relatively large (e.g. 3 mm) and even small differences between the resultant and designed angle could markedly influence estimates of blood velocity obtained.

Normally, the internal diameters of ovine ovarian arteries do not remain constant because the diameter is greater in the artery supplying the ovary bearing a CL (Niswender *et al.*, 1976). While fixation of the arterial wall to the transducers by granulation tissue may eliminate major changes in the internal diameter of the enclosed portions of the vessel (White *et al.*, 1974) even small variations in diameter, such as slight ballooning in response to increased flow and pressure, could markedly influence estimates of total blood flow obtained. In the light of this and the absence of methods for simultaneous monitoring of lumen diameter and blood velocity at the same site, quantification of the data into flow rates may not be strictly valid. From the mean internal diameter of the ovarian arteries obtained from 2 of the ewes and blood velocities, it is possible only to suggest that ovarian blood flow may have risen from about 1.0–2.0 ml/min

near oestrus to peak values of about 4.2–7.5 ml/min during the late luteal phase of the oestrous cycle.

Measurements of ovarian venous outflow in anaesthetized ewes indicated that blood flow through both ovulatory and non-ovulatory ovaries increased during the luteal stage of the oestrous cycle and that ovarian blood flow is controlled by humoral mechanisms (Mattner & Thorburn, 1969). However it was subsequently shown that venous cannulation and anaesthesia can markedly influence rates of ovarian blood flow (Mattner, Stacy & Brown, 1976). The present findings indicate that blood flow to the two ovaries is under separate and local control. As seen in Text-fig. 2, there was an extremely good relationship between blood velocity in the artery supplying an ovary with a CL, plasma progesterone levels and weight of luteal tissue. Thus, the cyclic changes in blood supply to the ovary with a CL may be determined primarily by changes in ovarian vascular resistance associated with the growth and subsequent regression of the capillary beds within the CL.

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