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Response of Ovine Uterine Blood Flow to Epinephrine and Norepinephrine[1] (37941)

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Killam *et al.* have recently investigated the reactivity of the ovine uterine vascular bed to estrogen (1). During the course of these experiments, a method was developed which allowed simultaneous flowmeter readout of blood flow in each of the two uterine arteries. The method also allowed the infusion of estrogens directly into the lumen of either uterine artery via an indwelling catheter without impeding uterine artery flow.

This preparation appeared potentially useful in a more general study of uterine blood flow because: (a) the animals can be studied in the unanesthetized state after full recovery from surgery; (b) the intra-arterial injection of drugs permits the study of local uterine responses in the absence of systemic effects; (c) small doses of vasoactive substances injected into one of the two uterine arteries evoke responses which are not shared by the contralateral artery; (d) the two uterine arteries carry most of the total ovine uterine blood flow (2); and (e) the quantity of the drug injected via the arterial catheter is diluted by a known blood flow. It is thus possible to calculate the arterial concentration of the drug and to evaluate the physiologic response as a function of this concentration. In this paper we describe the response of the uterine blood flow to the intra-arterial injection of epinephrine and noreprinephrine in nonpregnant, oophorectomized ewes before and after estrogen stimulation and in pregnant sheep.

Materials and Methods. Five sheep were used in the study. Prior to surgery, the ewes were starved for 48 hr, sedated with iv pentobarbital (5 mg/kg), and then gives a 6–8 mg hyperbaric tetracaine subarachnoid block. The animal was placed supine on an operating table, shaved, and surgically scrubbed with betadine soap. The ewe was draped with sterile sheets and a lower abdominal midline incision was made. The nonpregnant animals were oöphorectomized; the uterine arteries in both broad ligaments were identified, and square wave electromagnetic flowprobes (Micron Instruments Co., Los Angeles, CA) were placed about the uterine arteries, as previously described (1). A branch of the uterine artery, 1 cm distal to the probe, was tied and catheterized with a polyvinyl tubing (i.d. 0.58 mm, o.d. 1.2 mm). The tip of the catheter was positioned by palpation to within 1 cm of the main uterine artery. Polyvinyl catheters (i.d. 0.9 mm, o.d. 1.2 mm) were inserted into the femoral artery and femoral vein for the purpose of blood pressure measurements and systemic injections. The femoral vein catheter was advanced 40 cm up to the inferior vena cava. This catheter position is below the hepatic bed, and was confirmed at autopsy after the animal was sacrificed. No uterine vein catheters were used in the preparation. All the catheters and flowmeter wires were led subcutaneously to a flank incision. All incisions were closed with silk ligatures and steel skin clips. The free ends of the catheters were plugged with finishing nails and the catheters were flushed daily with heparin (1000 USP U/ml).

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When not in use, the flowmeter leads and the catheters were protected by a pouch constructed from adhesive tape and fastened to the ewe's skin with steel pins. The ewes received penicillin, 6,000,000 U intramuscularly, and streptomycin, 0.5 mg intramuscularly, for three days following surgery. Four to seven days were allowed to pass while the ewe was recovering from surgery. When the animal was ready for experimentation, flow probes were connected to an electromagnetic flowmeter (Micron Instruments RL 1000). Arterial and venous pressures were monitored by pressure transducers (Clark model 4-327-0109) throughout the experiment utilizing the femoral arterial and venous catheters. Arterial blood pH was measured on several occasions and found to be in the normal range for unanesthetized and unstressed sheep (3). Each flow and pressure signal was electronically integrated and mean values recorded on charts running at the speed of 1 mm/min, which allowed easy visualization of the experimental data over several hours (Gould Brush 220 recorders). Catecholamines of a known concentration were infused into the uterine artery catheter with a Harvard pump (model 600-954). Concentration of the drug in the arterial blood perfusing the uterus was calculated according to the equation:

$$\text{Arterial drug concentration } (\mu g/ml) = \frac{\text{rate of drug infusion } (\mu g/min)}{\text{arterial blood flow (ml/min)}} \cdot \quad (1)$$

The data for this calculation were obtained after the system had reached a new steady state, i.e., after the infusion had cleared the dead space of the catheter and the flow had maintained its stable value for 1 min. It should be noted that Eq. (1) calculates the concentration of the exogenous hormone, which is added to the endogenous concentration already present in the arterial blood. Drugs used for this study were norepinephrine (Levophed–Winthrop) and epinephrine (Parke–Davis). Norepinephrine concentrations were expressed in nanograms of norepinephrine base. The drugs to be infused were diluted to concentrations of 4 μg/ml with a sterile solution of 5% dextrose in 0.9% sodium chloride. This solution is recommended by the Winthrop Co. to prevent oxidation of the norepinephrine. As a precaution, these solutions were used for a maximum of 2 hr and then discarded. Infusions of the diluent alone directly into the uterine artery did not cause any change in uterine artery flow. Estrogen stimulation of the nonpregnant, oöphorectomized sheep was achieved by the systemic intravenous injection over a 2-min period of 1 μg/kg estradiol-17β (E_2) in a 10% alcohol solution. The estradiol-17β was injected into the femoral venous catheter described in the Methods section. As shown by Killam *et al.* (1), the estrogen effect on uterine blood flow appears after a 30-min delay and reaches its maximum at 2 hr.

Results. Three groups of animals were used for these experiments: pregnant ewes of various gestational ages, castrated ewes, and castrated ewes stimulated with intravenous (systemic) estradiol-17β. Estradiol-17β increased uterine blood flows in nonpregnant animals to levels encountered in the midtrimester of pregnancy. The estrogen effects followed a 30-min delay after systemic intravenous injection, reached a maximum plateau in 2 hr, and lasted for several more hours (1). The animals studied here received either epinephrine or norepinephrine infusion. In many instances, infusions of both drugs were used in the same animals, but the infusions were performed on separate days. The total number of experiments performed and the animals used for this communication are summarized in Table I.

1. *Epinephrine.* Figure 1 represents a typical experiment done during 40 min of recording the blood flows to the right and left uterine artery of a pregnant sheep, at 130 days gestation. This experiment is listed as Expt 10, Table I. The left artery received four epinephrine infusions in the periods indicated by A, B, C, and D. The right artery was kept as a control. Numerical data derived from this typical experiment are presented in Table II.

The blood flow in the left uterine artery, before the epinephrine infusion, is recorded

TABLE I. Summary of the Experiments Performed. The Baseline Blood Flow Shown Here Represents Blood Flow through the Uterine Artery Studied During Each Experiment Before Catecholamine Infusion. The Estrogenic Effect is Clearly Demonstrated in Expts 2, 3, 5, and 6.

Animal No.	Experiment	Physiological data	Baseline flow (ml/min)	Mean arterial pressure (mm Hg)	Drug used[a]
72-024-00	1	Nonpregnant, oöphorectomized	38	103	NE, E
72-024-00	2	Nonpregnant, oöphorectomized, E_2 stimulated[c]	280	92	E
72-024-00	3	Nonpregnant, oophorectomized, E_2 stimulated	275	100	NE
72-011-00	4	Nonpregnant, oöphorectomized	21	112	NE, E
72-011-00	5	Nonpregnant, oöphorectomized, E_2 stimulated	188	100	NE
72-011-00	6	Nonpregnant, oöphorectomized, E_2 stimulated	190	100	E
71-126-2	7	Twin pregnancy, 98 days	500	90	NE
72-007-1	8	Single pregnancy, 110 days	350[b]	93	NE
72-007-1	9	Single pregnancy, 111 days	370[b]	93	NE
72-050-1	10	Single pregnancy, 130 days	483	92	E
72-050-1	11	Single pregnancy, 145 days	500	100	E

[a] NE (norepinephrine), E (epinephrine).

[b] Blood flows through the right (Expt No. 8) and left (Expt No. 9) uterine arteries, respectively, of the same animal.

[c] E_2 stimulated means approximately 2 hr after the intravenous injection of estradiol-17β, 1 μg/kg.

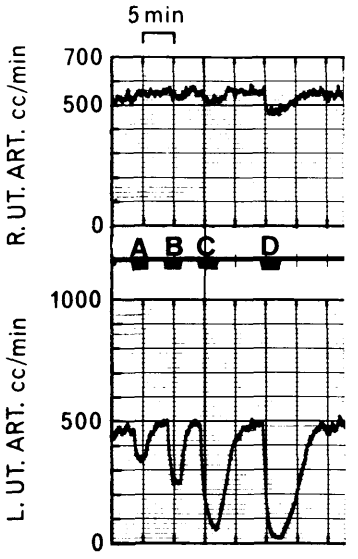

FIG. 1. A section of recording representing 40 min of experimental time. A, B, C, and D represent infusion speeds of 0.197, 0.388, 0.970, and 1.940 ml/min of 4 μg/ml epinephrine into the lumen of the left uterine artery of a pregnant animal. This experiment is also listed as Expt 10, Table I.

in column 5 (Table II); the flow in the left uterine artery, after a steady lower flow had been reached, is recorded in column 6 (Table II). These lower flows are the mean flows measured in the last minute of epinephrine infusion. The arterial concentration of exogenous epinephrine added to the left uterine artery was then calculated utilizing Eq. (1), and recorded in column 8 (Table II).

Epinephrine was infused in four separate experiments in three sheep (Expts 2, 6, 10, and 11, Table I). Epinephrine infusions were performed with a single pregnant sheep at 130 and 145 days gestation, and with two separate castrated but estrogen-stimulated ewes. The base uterine blood flow values before epinephrine infusion for these and all other experiments are shown in Table I. The data from all experiments were tabulated in the same fashion as shown in Table II. These data were used for the construction of the dose-response curves shown in Fig. 2. In every case (25 separate epinephrine infusions), the local

TABLE II. Numerical Data Derived from a Typical Experiment of Epinephine (E) Infusion as Presented in Figure 1.

Event	Infusion rate (ml/min)	Conc. of E in infusate (μg/ml)	Dose of E infused (μg/min)	Left artery baseline blood flow (ml/min)	Left artery experimental blood flow (ml/min)	100 × Exp. flow baseline flow (%)	Conc. of E in arterial flow[a] (ng/ml)
A	0.197	4.0	0.79	460	340	74	2.3
B	0.388	4.0	1.55	480	240	50	6.5
C	0.970	4.0	3.90	490	60	12	65.0
D	1.940	4.0	7.80	490	0	0	

[a] Calculated from Eq. (1).

flow response was not accompanied by significant changes in either systemic arterial blood pressure or inferior vena cava pressure as measured through the catheter inserted via the femoral vein. In addition, the ewes did not exhibit any blood flow change in the contralateral artery except with doses that were very large. As shown in Fig. 1, slight decreases in flow are seen in the contralateral artery with the two largest doses.

Interpolation of the dose-response curves, shown in Fig. 2, shows that a 50% decrease in uterine arterial flow was caused by concentrations of exogenous epinephrine in the arterial blood ranging from 3 to 13 ng/ml of blood.

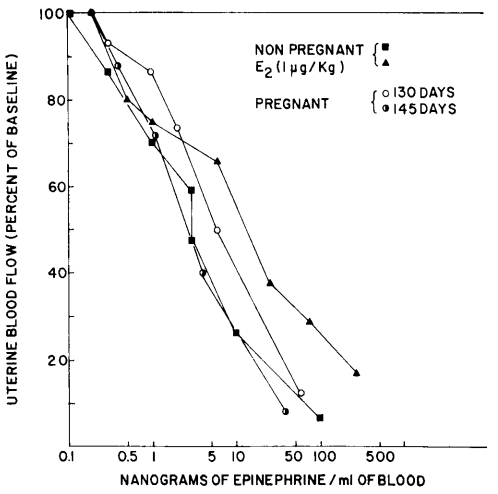

FIG. 2. Response curves of a single uterine arterial blood flow to the concentration of exogenous epinephrine in uterine arterial blood. Data from the four epinephrine experiments are plotted in the same fashion as the norepinephrine experiments in Fig. 5. The position and the slope of these curves and those in Fig. 5 are similar. Note that these data were obtained from Expts 2, 6, 10, and 11 in Table I.

Epinephrine was also infused into two castrated, nonpregnant sheep (Table I) (Expts 1 and 4, Table I). In the nonpregnant uterus without estrogen stimulation, very low infusion rates of epinephrine produced only vasoconstriction, whereas higher infusion rates produced vasoconstriction followed by vasodilation after the cessation of the infusion. This biphasic response was observed with epinephrine infusion rates greater than 20 ng/min. The phenomenon is illustrated in Fig. 3. This figure shows repetitive infusions of epinephrine, 0.4 μg/min, into the left uterine artery of a nonpregnant castrate ewe, while the right uterine artery serves as a control. An increase of flow over baseline level occurs after cessation of the infusion. Both uterine arteries show no response to 6 cc of 10% ethanol (used as a diluent for the estradiol) infused systemically into the femoral vein catheter but do respond to the systemic infusion of the hormone itself (estradiol-17β, 1 μg/kg). As the uterine blood flow increases in response to estrogen stimulation, the postinfusion vasodilation can no longer be seen. We observed, in 17 different epinephrine infusions performed in 2 castrate ewes without estrogen stimulation, that the increase of flow after the cessation of epinephrine infusion is proportional to the original infusion rate.

Propranolol (Inderal, Ayerst), 1.2 mg, was infused into the uterine arteries of the

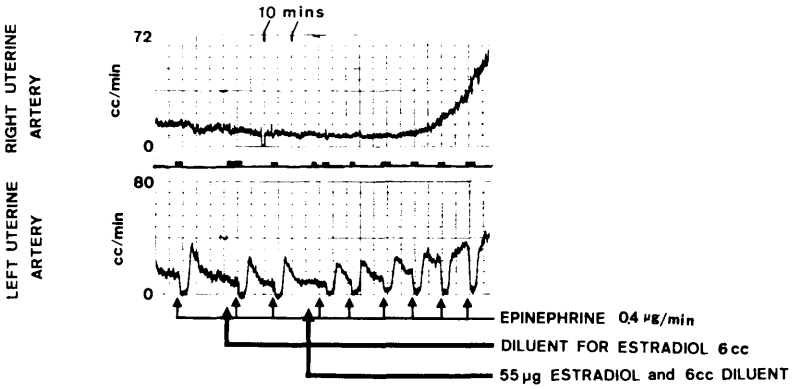

FIG. 3. This is a representative tracing from experiments designed to show the effect of intra-arterial epinephrine on the blood flow to the castrate uterus. Repetitive infusions of epinephrine at 0.4 µg/min in the left uterine artery reproduce the biphasic response described in the text. The systemic injection of 10% ethanol, the diluent for estradiol-17β, had no effect on uterine blood flow. The large increase of flow produced by the systemic injection of estradiol-17β makes the vasodilatory effect of epinephrine increasingly difficult to detect.

two nonpregnant, oöphorectomized animals studied. After this infusion, the increase of the uterine arterial flow following the cessation of epinephrine infusion was no longer seen. By itself, propranolol had no detectable effect on uterine blood flow.

2. *Norepinephrine.* This drug was infused into 2 nonpregnant (Expts 1 and 4) castrated animals, 2 nonpregnant, castrated, and estrogen-stimulated animals (Expts 3 and 5), and 2 pregnant animals (Expts 7–9). One of these two pregnant animals (72-007-1) had infusion catheters installed in both right and left uterine arteries, the right artery was studied 1 day before the left. This allowed a total of 7 experiments and a total of 39 separate infusions to be performed (Table I). In all instances norepinephrine produced uterine artery vasoconstriction only.

The dose-response curves, calculated with the rate of norepinephrine infusion in nanograms/minute, are shown in Fig. 4. These data were recalculated utilizing Eq. (1). The dose-response curves thus calculated for uterine arterial concentration of exogenous norepinephrine are shown in Fig. 5. When the uterine arterial flow responses, expressed as a percentage of baseline, are plotted against exogenous norepinephrine concentrations in the uterine arterial blood (Fig. 5), the response is similar for all three groups of animals. Vasoconstriction appeared with arterial concentrations of norepinephrine ranging from 0.1 to 1 ng/ml. Interpolation of the dose response curves in

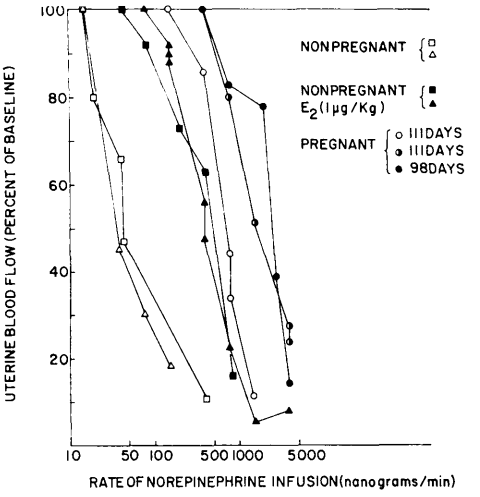

FIG. 4. Response curves of uterine blood flow to the intra-arterial infusion of various doses of norepinephrine. The points were calculated from mean flow during the last minute of infusion. When a certain norepinephrine infusion produced no response, the infusion rate was located as producing 100% control flow. Nonpregnant animals without estrogen stimulation appear to be more sensitive to norepinephrine infusion than pregnant animals and castrated animals with estrogen stimulation.

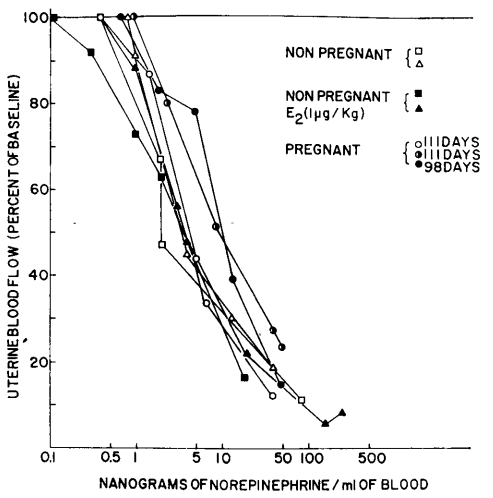

FIG. 5. Response curves of uterine blood flow to the concentration of exogenous norepinephrine in arterial blood. The data presented in Fig. 4 have been recalculated using Eq. (1) in the text. Note that the response of all animals to a given concentration of norepinephrine falls into a narrow range and that the apparent sensitivity of nonpregnant animals to norepinephrine can no longer be seen.

Fig. 5 shows that a 50% decrease of uterine blood flow was achieved by norepinephrine concentrations of 2–10 ng/ml of blood. There was no postinfusion increase in flow following cessation of norepinephrine infusion in the estrogen-stimulated uterus or in the pregnant uterus.

Discussion. The results of these experiments bring up three questions: (a) What is the relationship of the blood concentration to the plasma concentration of catecholamines? (b) Do the blood levels of exogenous catecholamines that produce the observed changes in uterine blood flow fall into the physiologic range? (c) Is the adrenergic reactivity of the uterine vascular bed demonstrated here consistent with previous investigations?

Catecholamine concentrations in these experiments are expressed as nanograms per milliliter of arterial blood. Although catecholamines added to blood are taken up rapidly by erythrocytes (4), incomplete equilibration of plasma and red cell concentrations may have occurred under our experimental conditions. The sheep hematocrit value is approximately 35% cells (3). Thus, if one assumes that catecholamines did not enter the erythrocytes, the actual concentrations in plasma may have been 1.5 times those expressed as the concentration of catecholamines/ml of arterial blood:

$$\frac{\text{Plasma concentration}}{\text{Blood concentration}} = \frac{100}{100 - 35} = 1.54. \quad (2)$$

Plasma concentrations of catecholamines have been determined under a variety of experimental conditions and by different methods. Catecholamines have been determined in sheep under thiopental anesthesia and minor surgery (5), but no values are available for unstressed ewes. In unanesthetized, unstressed man, Engleman and Portnoy (6) found norepinephrine plasma levels of 0.2 ± 0.08 ng/ml and epinephrine plasma levels of 0.05 ± 0.03 ng/ml. Significantly higher levels have been observed in patients with anxiety (7), during surgery (8), and in shock (9, 10). As an example, anesthetized baboons in shock 4 hr after hemorrhage exhibited epinephrine concentrations of 2.77 ± 2.7 ng/ml plasma (9). Hanquet *et al.* (10) demonstrated the same order of magnitude of catecholamine concentrations in severe shock in man. The highest values recorded by Hanquet *et al.* for epinephrine were 11.9 ng/ml plasma for norepinephrine 46.2 ng/ml plasma in a patient with intraperitoneal hemorrhage secondary to trauma.

The reader will note that exogenous catecholamines, added to uterine arterial blood, produce vasoconstriction when added in concentrations similar to endogenous catecholamine concentrations found in various physiologic states. This does not imply that circulating catecholamines are wholly or even partly responsible for changes in uterine blood flow that might occur with anxiety, stress, or shock, but rather that the catecholamine concentrations recorded here fall in the physiological range.

The present study is the first quantitative investigation of the adrenergic reactivity of uterine blood vessels in unanesthetized, unstressed animals.

Greiss (11) was able to demonstrate beta-receptors in the uterine vasculature with

isoproternol infusion in the nonpregnant ewe. He also demonstrated the vasodilatating properties of epinephrine in nonpregnant sheep uteri, but only after alpha-receptor blockade with phenoxybenzamine (12). In this study we have shown post-vasoconstriction vasodilatation following an epinephrine infusion without alpha blockade, but only in the oöphorectomized ewe without estrogen stimulation.

Estrogens are an extremely potent vasodilator of the uterine circulation. The post-vasoconstriction vasodilation seen with epinephrine could not be demonstrated when estrogen was present. The vasodilation after cessation of epinephrine infusion might be attributed to reactive hyperemia or to β-receptor stimulation. However, the phenomenon was not seen after noreprinephrine infusion in the castrate ewe without estrogen and was blocked with propranolol. Thus, these observations suggest that β-receptor stimulation was responsible for the phenomenon. Uterine vasoconstriction, mediated by α-receptors, was previously demonstrated in anesthetized, nonpregnant, and pregnant sheep by Greiss and Pick (13) and by Ladner *et al.* (14). Uterine vasodilation, from whatever cause, should only be studied in animals where the estrogen level can be controlled.

Two general concepts have been advanced concerning the actions of catecholamines on the uterus. The first is that the adrenergic reactivity of the myometrium varies with the hormonal background, primarily estrogen and progesterone. The second concept is that the placental vascular bed is less affected by catecholamines than the myoendometrial vascular beds. The first concept, derived from experiments on the contractile state of the myometrium, has been recently summarized by Axelsson (15): ". . . it appears that in the uterus the alpha and beta receptors come and go according to the hormonal state of the animal." By analogy, one might expect a similar variability in the adrenergic response of the uterine vasculature. Indeed, previous investigators of ovine uterine blood flow (14) are "tempted to postulate a diminished alpha receptor activity during pregnancy" because they found that for a given dose of norepinephrine the nonpregnant uterus showed a greater percentage reduction in blood flow than the pregnant uterus. In apparent agreement with this finding, Fig. 4 shows the nonpregnant uterus to be more sensitive to a given intra-arterial dose of norepinephrine than the estrogen-stimulated or pregnant uterus. However, when the dose-response curves are recalculated utilizing Eq. (1) (Fig. 5), the blood flow response of all the animals studied falls into a narrow range. Therefore, we have been unable to substantiate the concept of a great variability of vascular catecholamine response secondary to a variation in the hormonal state of the animal.

The concept that the myoendometrial circulation is affected more by catecholamines than is the placental vasculature has been advanced by Greiss (12). This concept is based on his observation that the pregnant uterus requires approximately 80 times the dose of catecholamines necessary to produce the same degree of vasoconstriction in the nonpregnant uterus or in a uterine horn carried by a pregnant animal but devoid of any placental tissue. It should be noted that Greiss' observations were obtained by means of intraarterial injections proximal to the trifurcation of the abdominal aorta. Thus, the apparent discrepancy of response to the same dose of catecholamine could have been, a least in part, the result of differences in the magnitude of the flows diluting the hormone. It would seem that in order to demonstrate a clear difference in the adrenergic reactivity of myometrial, endometrial, and placental circulations, a direct approach to the problem, such as the determination of uterine flow distribution in the pregnant animal by the microsphere technique (16), is necessary.

Summary. The effect of the infusion of epinephrine and norepinephrine directly into the lumen of either uterine artery was studied in unanesthetized, unstressed sheep in both the nonpregnant and pregnant state. The vasoconstrictive response to those catecholamines was found to lie within a narrow range; a 50% reduction of flow was

caused by a 3–13 ng/ml arterial blood concentration of epinephrine and 2–10 ng/ml of arterial blood concentration of norepinephrine. This method of *in vivo* uterine artery infusion plus uterine blood flow measurement appears well-suited for precise quantitative analysis of the reaction of the uterine vascular bed to pharmacologic agents.

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