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Transplacental Diffusion in a Bicornuate Uterus: Comparison of Uterine Blood Flow and Oxygen Uptake between Horns (42334)

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Abstract. The purpose of this study was to determine whether samples from the veins of the pregnant and the nonpregnant horn of the uterus lead to similar estimates of uterine blood flow and oxygen consumption. To accomplish this, a comparison of uterine blood flow, arteriovenous differences of oxygen content, and oxygen consumption measured by sampling the venous drainages of the two uterine horns was performed on eight pregnant sheep during the last 20 days of pregnancy. Each sheep carried a single fetus. Umbilical and uterine blood flows were measured with the test substances ethanol and antipyrine by application of the steady-state diffusion method. Twenty-three measurements of uterine blood flow comparing the two horns were not significantly different ($P > 0.1$), and were highly correlated ($r = 0.98$). The ratio of the oxygen content arteriovenous difference in the pregnant to that in the nonpregnant horn and the ratio of the uterine blood flow in the nonpregnant to that in the pregnant horn were significantly correlated ($r = 0.7$). As a consequence, paired calculations of oxygen consumption for the whole pregnant uterus had a small coefficient of variation (±3.7%). These results demonstrate that the use of highly diffusible test substances for the measurement of uterine blood flow in pregnant sheep can provide accurate data for the calculation of uterine oxygen uptake, in part because the oxygen and test substance molecules are similarly affected by local variations in placental perfusion. © 1986 Society for Experimental Biology and Medicine.

The placental transfer of highly diffusible molecules has been investigated using both non-steady-state (1, 2) and steady-state methods (3–5). The steady-state method, which utilizes the constant infusion of test molecules into the fetus, is particularly useful for studying problems of placental perfusion (6, 7) and for studying metabolic exchange across the placenta (8–10).

Because fetal umbilical venous blood can be sampled from a common umbilical vein (11), application of the Fick principle to the umbilical circulation is straightforward. However, central to the application of the Fick principle to the estimate of uterine blood flow and metabolic substrate uptakes in the sheep placenta (a cotyledonary variety of epitheliochorial placenta distributed in an uterus bicornis) is the assumption that test molecule and substrate concentrations in the venous drainage of one uterine horn are representative of concentrations in the venous output of the entire uterine circulation (10). If the ratio of cotyledonary to noncotyledonary uterine blood flow is different between the two uterine horns, or if a difference in the ratio of umbilical to maternal placental blood flow exists between the cotyledons in the two uterine horns, this assumption would not be correct (12).

The purpose of the present study was to compare two separate calculations of uterine blood flow and of uterine oxygen uptake as determined by sampling simultaneously the venous drainage of the two uterine horns in pregnant sheep carrying a single fetus, thereby providing direct verification of an assumption basic to the study of placental transfer and metabolism *in vivo*. In this presentation, the horn containing a singleton fetus is designated the pregnant horn while the other, less developed horn is designated the nonpregnant horn.

Theoretical Model. A schematic illustration of the maternal and the umbilical blood flow to a bicornuate uterus containing a singleton fetus and placental tissue in both horns can be used to demonstrate the placental transfer of a highly diffusable test substance under steady-state conditions (Fig. 1). In this model, the test substance used is ethanol, which is known to diffuse across the placenta quickly, is metabolized minimally by the fetus and negligibly by the placenta, and does not accumulate in either the maternal or fetal tissues appreciably when infused at rates less than 20

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$mg \cdot min^{-1}$ (4). As a numerical example, assume that the uterine blood flow is 1500 $ml \cdot min^{-1}$, the umbilical blood flow is 600 $ml \cdot min^{-1}$, and the test substance is infused into the fetus at a constant rate equal to 12 $mg \cdot min^{-1}$. Furthermore, assume that the placental tissue, maternal uterine blood flow, and umbilical blood flow are divided between the pregnant horn and the nonpregnant horn such that 60% of the maternal and the umbilical blood flow serves the placental tissue in the pregnant horn and the remaining 40% serves the placental tissue in the nonpregnant horn. Finally, assume that the concentration of ethanol in the maternal uterine artery equals 2 $mg \cdot dl^{-1}$, a value which is determined by the rate of ethanol clearance by the mother. Based upon these assumptions and an experimentally derived equation for the relationship between the placental clearance of ethanol to uterine and umbilical blood flows (4), the concentrations of the test substance can be calculated for blood sampled from the umbilical artery, the common umbilical vein, the uterine vein draining the pregnant horn, and the uterine vein draining the nonpregnant horn; these values are indicated by brackets in Fig. 1. The values of placental blood flow are determined by the assumptions of the model, cannot be measured by the steady-state diffusion method, and are therefore indicated by parentheses in Fig. 1.

Application of the Fick principle to the umbilical circulation where uptake of the test substance by the placental tissue equals the rate of infusion of the test substance and where the arteriovenous concentration difference of the test substance can be determined allows for calculation of the umbilical blood flow. Application of the Fick principle to the maternal uterine circulation is complicated by the anatomic absence of a common uterine vein and is therefore dependent on the assumption that a unilateral uterine venous blood sample is representative of a blood sample from the hypothetical common uterine vein, thereby allowing an estimate of total uterine blood flow to be made in a manner similar to that for the estimate of umbilical blood flow. Under conditions in which the uterine/umbilical blood flow ratio is the same in both horns, the value of total uterine blood flow calculated from the venous effluent of either horn will be an accurate estimate of the total uterine blood flow (Fig. 1A).

If, however, the ratio of uterine to umbilical blood flow at the site of placental exchange is different between the two horns for reasons intrinsic to the site of placental exchange (e.g., less developed maternal vascularization of the placental cotyledons on one side of the uterus) or extrinsic (e.g., increased noncotyledonary blood flow in one horn relative to the other horn), the estimates of uterine blood flow determined from the separate uterine venous effluents will differ and will bracket the true total uterine blood flow value (Fig. 1B). To the extent that the estimates of uterine blood flow differ between the horns for such biologic reasons, a limitation exists with respect to the unbiased estimate of total uterine blood flow by the steady-state diffusion method.

Materials and Methods. *Surgery and animal care.* Eight mixed-breed (Rambouillet–Columbia) ewes in the last month of pregnancy and carrying a single fetus were fasted for 48 hr before surgery. The ewes were sedated with intravenous pentobarbital and 1% tetracaine hydrochloride spinal anesthesia (10–12 mg). After cotyledons in both uterine horns were palpated, polyvinyl catheters (0.054 in o.d.) for blood sampling were placed in a fetal hindlimb artery with the catheter tip positioned in the abdominal aorta, in an umbilical vein with the catheter tip positioned in the common umbilical vein, in a maternal femoral artery with the catheter tip positioned in the external iliac artery, in the uterine vein draining the horn containing the fetus, and in the uterine vein draining the nonpregnant horn. With respect to the two uterine vein catheters, a small vein toward the tip of each horn with a straight course through the broad ligament to the base of the uterus was cannulated; the tip of each catheter was advanced to a position in each of the uterine veins approximately 4 cm below the level of the ovary. A polyvinyl catheter (0.054 in o.d.) for infusion was placed in a fetal hindlimb vein with the catheter tip positioned in the inferior vena cava. A catheter was also placed in the amniotic fluid cavity for instillation of antibiotic. In 5 animals, a 6-mm cuff-type balloon occluder (*In vivo* Metric Systems, Healdsburg, Calif. 95448) was positioned around the fetal descending aorta below the renal arteries and in one animal a similar

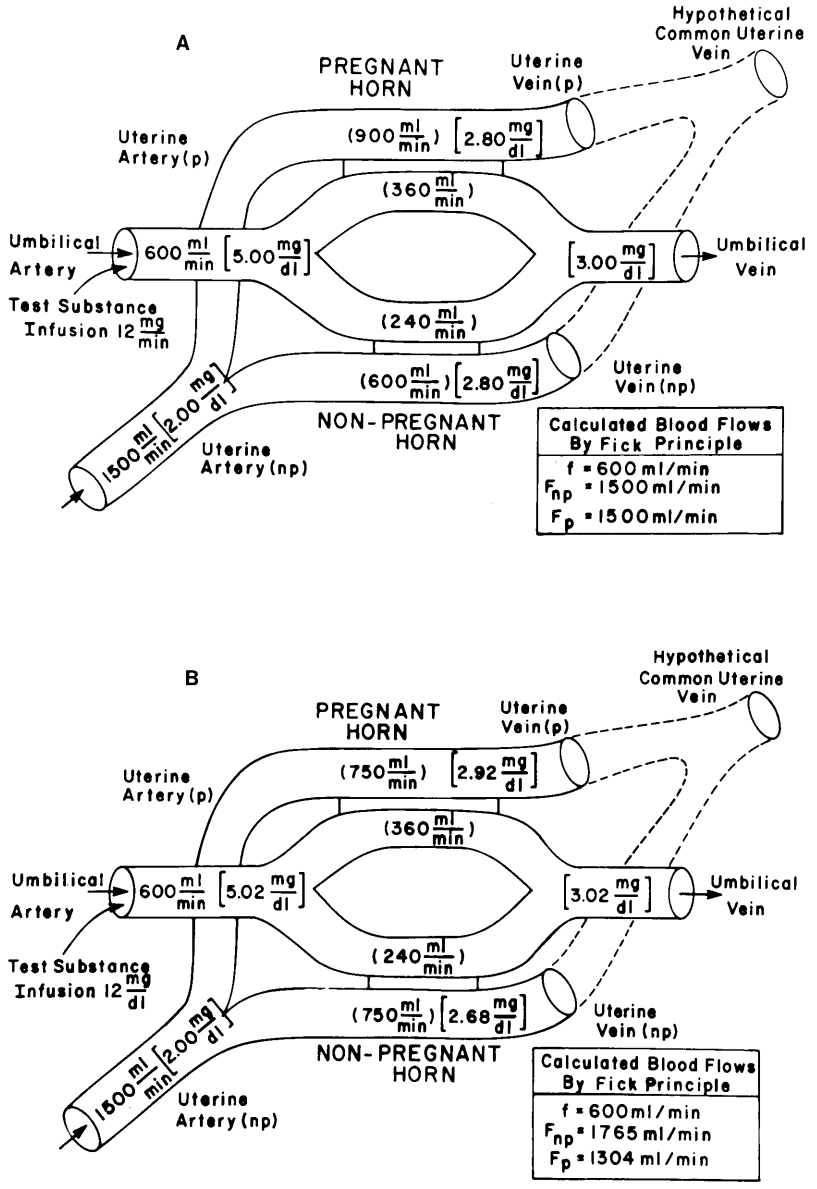

FIG. 1. Model of steady-state placental diffusion in the ovine bicornuate uterus. Comparison of umbilical blood flow (f) and uterine blood flows determined across the venous drainage of the pregnant horn (F_p) and nonpregnant horn (F_{np}) by application of the Fick principle when the fetal to maternal placental blood flow ratio in the pregnant horn equals that in the nonpregnant horn (A), and when the fetal to maternal placental blood flow ratio in the pregnant horn is different from that in the nonpregnant horn (B). See text for discussion.

12-mm occluder was positioned around the maternal terminal aorta below the external iliac arteries for alteration of fetal umbilical and uterine blood flow, respectively. In the remaining two animals, no occlusive devices were applied. All catheters were led subcutaneously to an external flank pouch.

The animals recovered promptly from surgery and were standing and feeding in their individual pens within 6 hr. Antibiotic was

administered and catheters were maintained as previously described (6). The ewes were allowed water and food (their standard alfalfa pellet diet) *ad libitum*.

Study design. The animals were allowed 5–7 days recovery from operative stress prior to study. On each study day, a solution of antipyrine and ethanol in normal saline was delivered to the fetus via the hindlimb venous catheter at a constant rate (0.1 ml · min^{-1}) by means of a syringe pump. The ethanol and antipyrine concentrations were adjusted based on estimates of fetal size and placental clearance to give a fetal arterial concentration not exceeding 10 mg · dl^{-1} at the conclusion of the experiment.

Approximately 60 min after the start of the fetal ethanol and antipyrine infusion, steady state was reached with respect to both test molecules. Blood samples for antipyrine and ethanol concentrations (1.7 ml in EDTA-coated syringes) and for hemoglobin concentration and oxygen saturation (0.5 ml into heparin-coated capillaries) were drawn simultaneously from the maternal arterial catheter, both uterine venous catheters, the fetal arterial catheter, and the umbilical venous catheter. Four such sample sets were obtained at 15-min intervals. In the six animals prepared with vascular occluders, blood flow was reduced by partial inflation and, following an additional 40-min equilibration period, the sampling procedure was repeated. The blood pressure in the artery distal to the occluder was monitored continuously and, during the period of partial occlusion, was kept constant by adjustments in the inflation of the occluder.

Each animal was studied for 1 to 4 days, with a control level of blood flow each day. The six animals prepared with a vascular occluder were studied additionally at a reduced level of blood flow following the control study. On the final day of study, each animal was killed by injection of an euthanasia solution, at which time the condition and placement of the catheters were verified and fetal, uterine, and placental weights were measured.

Physiological measurements. Umbilical blood flow and uterine blood flow were calculated separately for each test molecule by application of the Fick principle to steady-state transplacental diffusion (10). For each test molecule, uterine blood flow was calculated using the arteriovenous difference from the pregnant horn and from the nonpregnant horn. The blood flow values calculated for each of the two test molecules were averaged. Antipyrine was measured in duplicate by an automated version (4) of the method of Brodie *et al.* (13). Ethanol was measured in triplicate (14) using the enzymatic conversion of ethanol to acetaldehyde (Sigma Reagents).

Blood hemoglobin concentration and oxygen saturation were measured colorimetrically in duplicate by a radiometer hemoximeter. Blood oxygen content was calculated as the product of hemoglobin concentration and oxygen saturation.

At each level of blood flow studied, the oxygen content value was determined as the mean of the four blood samples obtained during the steady-state interval. Oxygen uptake by the pregnant uterus was calculated by application of the Fick principle to the uterine circulation determined from the arteriovenous difference of the pregnant horn and compared to that similarly determined from the nonpregnant horn.

Results. A total of 23 sets of umbilical blood flow, uterine blood flow calculated from the venous drainage of the pregnant horn and uterine blood flow calculated from the venous drainage of the nonpregnant horn were obtained in eight ewes. All animals tolerated the experimental procedures well.

Uterine blood flow: Pregnant versus nonpregnant horn. Each of the blood flow values presented in Table I is the average of two estimates using antipyrine and ethanol as the test molecules. As previously demonstrated with respect to the measurement of umbilical blood flow, the antipyrine estimates were not significantly different from the ethanol estimates (paired t test, $P > 0.3$), and in the measurement of uterine blood flow, the values calculated from the ethanol and antipyrine data were well correlated ($r = 0.96$) (4).

The uterine blood flow rates measured from the separate venous drainage of the two horns are presented in Table I and Fig. 2A. The measurements of uterine blood flow comparing the pregnant to the nonpregnant horn were not significantly different (paired t test, $P > 0.1$) and were highly correlated by linear regression analysis ($r = 0.98$).

Oxygen consumption: Pregnant versus non-

TABLE I. UMBILICAL AND UTERINE BLOOD FLOWS, O_2 CONTENT ARTERIOVENOUS DIFFERENCE, AND O_2 CONSUMPTION IN EIGHT SHEEP: PREGNANT VERSUS NONPREGNANT HORN

Animal number	Fetal weight (kg)	Umb[a] ($ml \cdot min^{-1}$)	Pregnant horn[b]			Nonpregnant horn[c]		
			F_p ($ml \cdot min^{-1}$)	$([O_2]_A - [O_2]_V)_p$ (mM)	$\dot{V}O_{2p}$ ($mM \cdot min^{-1}$)	F_{np} ($ml \cdot min^{-1}$)	$([O_2]_A - [O_2]_V)_{np}$ (mM)	$\dot{V}O_{2np}$ ($mM \cdot min^{-1}$)
1		495	1542	0.97	1.50	1473	1.03	1.52
	2.48	305	1529	0.86	1.31	1320	0.97	1.28
2		459	2222	0.64	1.42	2616	0.59	1.54
		302	2812	0.58	1.63	2752	0.52	1.43
		624	2232	0.65	1.45	2405	0.58	1.39
	3.06	331	2684	0.42	1.13	2688	0.41	1.10
3		508	802	1.88	1.51	910	1.72	1.57
	2.24	313	677	1.66	1.12	761	1.53	1.16
4		349	937	1.41	1.32	1032	1.39	1.43
	2.15	94	1065	0.80	0.85	1111	0.66	0.73
5		594	1280	1.39	1.78	1258	1.41	1.77
	3.22	274	1171	1.23	1.44	1208	1.18	1.43
6	3.16	646	1180	1.55	1.83	1350	1.34	1.81
7		821	1305	1.53	2.11	1337	1.45	2.14
	3.70	693	1380	1.44	1.87	1474	1.35	1.80
8		655	1514	1.30	1.97	1563	1.25	1.95
		651	604	3.13	1.89	733	2.71	1.98
		590	913			945		
		661	537			525		
		656	912			907		
		678	732			653		
		637	1175			1165		
	3.17	666	738			656		

[a] Umb: umbilical.

[b] F_p, $([O_2]_A - [O_2]_V)_p$, $\dot{V}O_{2p}$: uterine blood flow, O_2 content arteriovenous difference, and O_2 consumption, respectively, as calculated across the venous drainage of the pregnant horn.

[c] F_{np}, $([O_2]_A - [O_2]_V)_{np}$, $\dot{V}O_{2np}$: uterine blood flow, O_2 content arteriovenous difference, and O_2 consumption, respectively, as calculated across the venous drainage of the nonpregnant horn.

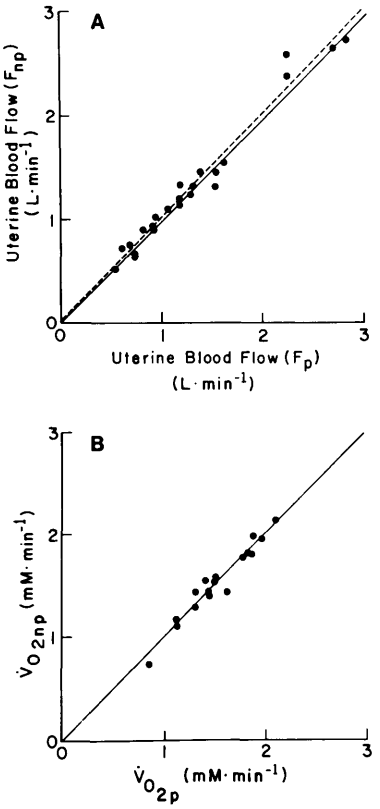

FIG. 2. Comparison of results determined across the venous drainage of the pregnant horn (x axis) versus the nonpregnant horn (y axis) for uterine blood flow (A) and oxygen uptake by the pregnant uterus (B). The line of identity (—) is the solid line in both panels. For uterine blood flow (A), the calculated line of regression (---) fits the equation, $y = 1.02\ (x) + 8$, where y = uterine blood flow, nonpregnant horn, and x = uterine blood flow, pregnant horn. Abbreviations are defined in Table I.

pregnant horn. The oxygen consumption of the pregnant uterus was calculated from the venous drainage of the pregnant horn by Eq. [1] and from the venous drainage of the nonpregnant horn by Eq. [2]:

$$\dot{V}O_{2p} = F_p([O_2]A - [O_2]v)_p \qquad [1]$$

and

$$\dot{V}O_{2np} = F_{np}([O_2]_A - [O_2]v)_{np} \qquad [2]$$

where $\dot{V}O_{2p}$, F_p, $([O_2]_A - [O_2]v)_p$, $\dot{V}O_{2np}$, F_{np}, and $([O_2]_A - [O_2]v)_{np}$ are as defined in Table I. The calculated oxygen consumptions of the pregnant uterus were not significantly different between the two horns (paired t test, $P > 0.7$) and were highly correlated ($r = 0.97$) as illustrated in Fig. 2B. The calculated regression equation ($y = 1.02(x) - 0.04$, where y = oxygen consumption by the pregnant uterus as calculated across the venous drainage of the pregnant horn and x = oxygen consumption by the pregnant uterus as calculated across the venous drainage of the nonpregnant horn) fits well with a zero-intercept line of identity.

The coefficient of variation of the differences between the paired observations [(SD/mean) × 100] was higher for the estimates of uterine blood flow than for the $\dot{V}O_2$ observations (±6.5% vs ±3.7%). This was due to a significant relation of the flow ratio to the oxygen content arteriovenous difference ratio (Fig. 3). Note that for a constant value of the calculated oxygen consumption, the ratio of the flows equals the ratio of the oxygen content arteriovenous differences (Eq. [3]):

$$\frac{F_{np}}{F_p} = \frac{([O_2]_A - [O_2]v)_p}{([O_2]_A - [O_2]v)_{np}}. \qquad [3]$$

The calculated regression for the data points in Fig. 3 was $y = 0.92(x) + 0.08$ ($r = 0.7$). The flow ratio was not significantly different from the ratio of the oxygen content arteriovenous differences by paired t test ($P > 0.9$), demonstrating the x intercept of the regression equation was not different from zero. The slope of the regression equation was not significantly different from 1 ($P > 0.7$) and, with the intercept not significantly different from 0, thus demonstrates a covariant relationship as defined by Eq. [3].

Discussion. It has been noted previously that an inequality of test substance concentration in the venous outputs of the pregnant uterus might limit the application of the Fick principle with respect to the estimation of uterine blood flow by the steady-state diffusion technique (12, 15). The data presented in this study demonstrate that, under the circumstances of these experiments, there was no statistically significant difference in the estimate of total uterine blood flow between the two uterine horns (Fig. 2A). This result provides the first direct evidence in support of a previously unverified assumption of the steady-state diffusion method by demonstrating that the concentration of the flow indicator substance in the venous drainage of a single uter-

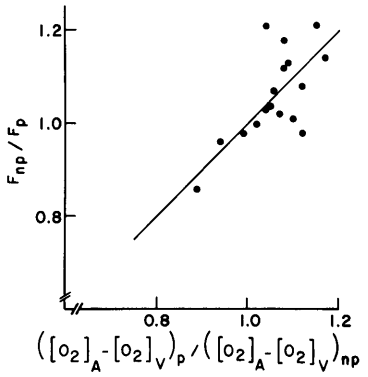

FIG. 3. Relationship of the ratio (F_{np}/F_p) to the ratio $([O_2]_A - [O_2]_V)_p/([O_2]_A - ([O_2]_V)_{np}$. The solid line (—) is the line of identity. Abbreviations are defined in Table I.

ine horn can be considered representative of the venous output of the entire ovine uterine circulation.

Previous evidence in agreement with this finding has been indirect. The calculation of transplacental clearance with the steady-state method is independent of any venous measurement; therefore, the consistent relation between the clearance of highly diffusible molecules and the uterine and umbilical blood flows determined by the steady state method gives some confidence that unilateral uterine venous sampling is representative of the whole venous output (4, 10). Furthermore, there does not appear to be a systematic difference between the two horns in the maternal/fetal–placental blood flow ratios as determined by a microsphere method (16).

It is important to note, however, that there is histologic evidence consistent with a slower development of the placenta in the nonpregnant horn of a single pregnancy at less than 70 days gestation (17). While the discrepancy disappears thereafter, such a delay might result in a persistent difference between the horns with respect to the umbilical to maternal blood flow ratio. Furthermore, given the complex venous drainage of the ovine uterus (18) and variations in placental development, it is likely that differences exist between animals and between the horns of the same animal with respect to the placental mass drained by the catheterized uterine vein. That the estimates of uterine blood flow between the two horns were not significantly different suggests that normally such biologic variability is exceeded by the sources of error attributable to the steady-state diffusion methodology, the magnitudes of which have been discussed and compared to other methodologies previously (19).

The data in this study also demonstrate a covariant relationship between the transplacental diffusion of oxygen and the test substances, ethanol and antipyrine (Fig. 3). The two uterine blood flow estimates were seldom identical in any single animal (Table I). However, with respect to the horn giving the higher estimate of uterine blood flow, that horn also demonstrated a lower arteriovenous difference of oxygen, resulting in comparable estimates of oxygen uptake (Fig. 2B). It is apparent that when the placental diffusion of the test substance is uneven between the two uterine horns, thereby causing an overestimation of the uterine blood flow in one horn, the placental uptake of oxygen is limited similarly, the result of which is a narrowing of the arteriovenous concentration difference for both the test and the oxygen molecules. The calculation of oxygen uptake by the Fick principle across a single uterine horn under these circumstances compares favorably with the calculation across the other horn. This finding provides support for the sampling of a single uterine vein catheter for determination of substrate uptakes by the pregnant uterus under similar steady-state conditions. As a practical matter in previous studies, this single catheter has been placed in the venous drainage of the pregnant horn because the pregnant horn generally contains more cotyledons than the nonpregnant horn (20). The data presented here demonstrate this bias to be unnecessary, as long as both horns contain cotyledons, but does suggest that for studies of placental perfusion, additional information might be gained by placement of uterine vein catheters in the drainage of both horns.

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