Uterine Blood Flow in Young and Aged Rabbits (36717)

L. L. LARSON AND R. H. FOOTE
(Introduced by W. Hansel)
Department of Animal Science, Cornell University, Ithaca, New York 14850

Reproductive capacity in older females decreases with advancing age at a rate characteristic of the species (1). A major cause of this decline in reproductive performance has been reported to be uterine aging (2, 3). Limitation of blood flow to one horn of the mouse uterus by electrocoagulation increased early embryonic death (4). The objective of the present study was to compare uterine blood flow rate in young does with aged does whose reproductive efficiency had declined (5), using the inert gas "wash-out" technique.

This technique has been shown to be valid with a variety of tissues such as brain (6), kidney (7), myocardium (8), skeletal muscle (9), testis (10) and myometrium (11, 12). In a preliminary study with rabbits we found that clearance of ^{85}krypton (^{85}Kr) was stopped by compressing the miduterine vessels. Upon release of the vessels clearance proceeded at the preocclusion rate, indicating that the wash-out of ^{85}Kr from the uterus was a function of blood flow.

Materials and Methods. Thirty-five female rabbits were used. Does were assigned by age and reproductive history to the following five treatment groups: (A) prepuberal nulliparous, (B) postpuberal nulliparous, (C) postpuberal uniparous, (D) mature multiparous, and (E) aged polyparous. There were five estrual does per group in groups A to D. Treatment group E consisted of 8 pregnant and 7 nonpregnant aged does, randomly selected from control does or does given supplemental progesterone, estrogen or progresterone plus estrogen (5). Blood flow in the 15 aged does was examined 12 days postcoitum (pc). Does were lightly anesthetized with sodium pentobarbital and maintained with ether as needed. A midventral incision was made so that a small loop of either uterine horn could be exposed. Blood flow rates were estimated by a slight modification of the inert gas wash-out technique described by Carter, Nilsen and Bengtsson (11). In the present study two estimates of uterine blood flow were obtained by an injection of ^{85}Kr into the uterine lumen of one uterine horn (intra-uterine) and into the myometrial tissue (myometrial) of the opposite uterine horn of does in groups A to D. The side and location of the first injection were randomly selected. In group E does, both injections were into the myometrium; in pregnant does it was between the implantation sites.

Injections were made with a 0.5-ml syringe attached to a 26-gauge needle. Approximately 0.05 to 0.10 ml of saline solution containing 100 to 200 μCi ^{85}Kr was injected. All injections were made with the doe in a ventilation hood to remove the expired ^{85}Kr. A Nuclear-Chicago scintillation probe with a 2.5 cm sodium iodide crystal was used to monitor the wash-out of the radionuclide for 20 min or until the activity had reached background level. The output from the probe was connected to a linear rate meter, and continuously recorded on a potentiometric recorder. After correcting for background activity the counts per min (cpm) were plotted against the time postinjection on semilogarithmic graph paper. A straight line drawn through these points was extrapolated to zero time. The biological half-life of ^{85}Kr was determined from the curve. The partition coefficient for ^{85}Kr between uterine tissue and blood was assumed to be one. Blood flow rate (ml/100 g tissue/min) was determined by the method described by Thorburn *et al.* (7).

Results and Discussion. There was no significant difference ($p > .05$) in capillary blood flow rates estimated by intra-uterine

TABLE I. Age Effect on Capillary Blood Flow Rate in the Rabbit Uterus.

Treatment group	No. of does	Age (mo)	Reproductive history	Blood flow (ml/100 g tissue/min)[a]	
				Intra-uterine	Myometrial
A	5	3	Nulliparous	35.3	37.7
B	5	6	Nulliparous	49.4	43.9
C	5	6	Uniparous	30.8	40.1
D	5	30	Multiparous	28.3	29.6
E	15	45	Multiparous	—	18.6[b]

[a] Estimated from intra-uterine and myometrial injections of ^{85}Kr.

[b] Group mean derived from average of two myometrial estimates/aged doe.

versus myometrial injection of ^{85}Kr in groups A to D (Table I). The correlation coefficient between the two blood flow estimates in each doe was $r = 0.75$. Although the mean blood flow rate in group B appears to be higher than the rest, the difference among treatment groups A to D was not significant ($p > .05$) due to considerable animal variation and small numbers per group. There was no difference ($p > .05$) between the aged pregnant and nonpregnant does in group E (20.3 and 16.4 ml/100 g tissue/min, respectively). Likewise there were no significant differences among hormone treated does (5), so all aged does were combined into a single group (Table I, group E). Therefore, the uterine blood flow rates estimated by myometrial injections in the 20 younger does in group A to D were combined and compared to the 15 aged does in group E. The average uterine blood flow rate of 37.8 ml/100 g tissue/min in the younger does was significantly greater ($p < .005$) than the rate of 18.6 ml/100 g tissue/min observed in the aged does.

Cotter, Blechner and Prystowsky (13) using the antipyrine method, reported uterine blood flow rates of 67, 53, 19 and 9 ml/100 g/min in nonpregnant, 11- to 19-, 23- to 27- and 28- to 32-day pregnant rabbits, respectively. Duncan and Lewis (14) using an electromagnetic flowmeter and Duncan (15) using isotope labeled microspheres reported total uterine blood flow rates of 11 and 7 ml/100 g/min and myometrial rates of 27 and 14 ml/100 g/min, respectively, in does 27 to 29 days pregnant. Carter, Nilsen and Bengtsson (11) reported that using the ^{133}xenon clearance technique following a myometrial injection the blood flow rate at 26 days after mating was 24 ml/100 g/min. It is not possible to strictly compare the results in different studies because of the different methods used. Does in the present study were either nonpregnant or 12 days pregnant. Since uterine blood flow previously reported for nonpregnant animals and those in early pregnancy was similar (13), and since the does in the present study were measured at the same time and with same technique, the difference in blood flow between the young and aged does is thought to be physiologically significant.

Reproductive performance of the aged does was poor, with an average of 3.4 implants present 12 days pc and only 1.5 young kindled/doe bred (5). However, there was no effect of implants on blood flow measured between the implantation sites, as the correlation between the number of implants and blood flow was $r = .29$ ($p > .05$) The lower blood flow rates in aged does might result in a reduction in general metabolic function in the aged uterus and in slower transport of hormones and metabolites to the target tissues. Johnson (16) suggested that maternal capacity in the rabbit was limited by a systemic factor and that the vascular system was most likely involved since it is the primary supplier of nutrients to the fetus. Limitation of blood flow to the mouse uterus resulted in fewer implants (4).

It is concluded that the reduced uterine blood flow rate in aged rabbits likely is one of the uterine factors limiting reproductive performance in aged does.

Summary. Uterine blood flow rate in 15 aged does average 18.6 ml/100 g tissue/min,

and this was significantly lower than the average rate of 37.8 ml/100 g tissue/min in 20 younger does. It is concluded that reduced uterine blood flow rate is one component of the aging uterus which may decrease reproductive performance in aged rabbits.

The authors are indebted to Dr. R. A. Wentworth for assistance with the radionuclide measurements. The work was supported in part by NIH Grant HD-03471.

1. Talbert, G. B., Amer. J. Obstet. Gynecol. **102**, 451 (1968).

2. Adams, C. E., J. Reprod. Fert. Suppl. **12**, 1 (1970).

3. Biggers, J. D., J. Reprod. Fert. Suppl. **8**, 27 (1969).

4. Senger, P. L., Lose, E. D., and Ulberg, L. C., J. Exp. Zool. **165**, 337 (1967).

5. Larson, L. L., Spilman, C. H., Dunn, H. O., and Foote, R. H., J. Reprod. Fert. in press.

6. Glass, H. I., Harper, A. M., and Glover, M. M., Phys. Med. Biol. **6**, 65 (1961).

7. Thorburn, G. D., Kopald, H. H., Herd, J. A., Hollenberg, M., O'Morchoe, C. C. C., and Barger, A. C., Circ. Res. **13**, 290 (1963).

8. Herd, J. A., Hollenberg, M., Thorburn, G. D., Kopald, H. H., and Barger, A. C., Amer. J. Physiol. **203**, 122 (1962).

9. Tønnesen, K. H., Scand. J. Clin. Lab. Invest. **21**, 65 (1968).

10. Setchell, B. P., Waites, G. M. H., and Thorburn, G. D., Circ. Res. **18**, 755 (1966).

11. Carter, A. M., Nilsen, R., and Bengtsson, L. P., Acta Pharmacol. **26**, 29 (1968).

12. Nyström, C., Forssman, L., and Roos, B., Acta Radiol. **8**, 193 (1969).

13. Cotter, J. R., Blechner, J. N., and Prystowsky, H., Amer. J. Obstet. Gynecol. **107**, 411 (1970).

14. Duncan, S. L. B., and Lewis, B. V., J. Physiol. (London) **202**, 471 (1969).

15. Duncan, S. L. B., J. Physiol. (London) **204**, 421 (1969).

16. Johnson, A. D., J. Anim. Sci. **30**, 978 (1970).

Received May 23, 1972. P.S.E.B.M., 1972, Vol. 141.