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Search for Arteriovenous Shunts in the Genital Tract of the Pseudopregnant Rabbit (39809)

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Introduction. The blood flow within an endocrine gland plays an essential role in the control of its physiological activities. Some of the complex hemodynamics changes of the ovary have been described during the preovulatory period (1), pseudopregnancy (2, 3), and pregnancy (4). The mechanisms that regulate these changes are not clear, but hormones (5, 6) and amines have been implicated in circulatory control (7). The luteolytic process is characterized by a remarkable decline in ovarian blood flow (4) and progesterone secretion (8). The drop in blood flow to the corpus luteum has been attributed to the presence of arteriovenous shunts which would divert blood flow from the corpus luteum (9). In this report we present evidence indicating that, in the pseudopregnant rabbit, no demonstrable arteriovenous shunts are found in the ovary.

Materials and methods. Mature New Zealand White rabbits, 3–4 kg in weight, were kept in individual cages for 3 weeks prior to use. Blood flow determinations were carried out 6, 12, and 18 days after iv administration of 100 IU of hCG. Analgesia was induced with Innovar (0.2 mg/kg) administered intramuscularly. Both femoral arteries were catheterized with a polyvinyl tube (PE-60, Clay-Adams, N.J.). The right catheter was advanced until its tip was about 3 cm below the diaphragm. This catheter was used for the administration of radioactive microspheres. The left femoral catheter was advanced about 2 cm into the artery and connected to a Harvard pump. Each animal received approximately 1,500,000 microspheres, 15 ± 5-μm diameter, labeled with St^{85} and 400,000 microspheres, 50 ± 5-μm diameter, labeled with CE^{141} (3M Company, St. Paul, Minn.). The spheres were suspended in 0.8 ml of 10% dextran in physiologic saline, mixed well, and injected immediately through the right femoral catheter. With this procedure each ovary received 1500 to 2000 microspheres of 50-μm diameter and 5000 to 7000 microspheres of 15-μm diameter. Withdrawal of blood from the other femoral catheter was started 15 sec before the injection of microspheres. Blood flow calculations were carried out as described previously (10). Statistical analyses of blood flow using different sized microspheres at different times of pseudopregnancy were made by comparing the mean values with the Student t test. Paired t tests were used to evaluate the statistical difference between the paired organs.

Results. Blood flow was distributed evenly to all paired organs investigated (ovaries, oviducts, uterine horns) throughout pseudopregnancy. Representative data are seen in Fig. 1, which illustrates the perfusion rate of luteal and extraluteal ovarian compartments from the right and left ovaries of mid pseudopregnancy.

The perfusion rate of luteal and extraluteal tissue during pseudopregnancy is shown in Fig. 2. Identical ovarian perfusion rates were obtained when both microspheres were used. In mid pseudopregnancy the perfusion rate to the corpus luteum was significantly higher than in early or late pseudopregnancy ($P < 0.05$) and accounted for 95% of the total ovarian perfusion rate. In contrast, the extraluteal perfusion rate was unchanged throughout the period of pseudopregnancy studied.

An increase in the perfusion rate of the oviduct and uterus was seen in late pseudopregnancy (Fig. 3). In contrast to all other organs studied, a significant difference in perfusion rates obtained by the two differ-

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ent microspheres was found only in the uteri of late pseudopregnancy ($P < 0.05$).

Discussion. Blood perfusion rate to the corpus luteum changes during pregnancy and pseudopregnancy. In rabbits, the increased luteal blood flow observed in mid pseudopregnancy is followed by a progressive decline toward the end of pseudopregnancy. The regulatory mechanisms for these hemodynamic changes are not well understood. It has been postulated that arteriovenous shuntings in the ovary itself could control the intraorganic blood flow distribution in this organ. However, the search for the presence of arteriovenous shunts is not without difficulties; thus, conflicting results have been obtained. Niswender *et al.* (9), using a technique similar to the one employed in the present investigation, were able to demonstrate the presence of ovarian arteriovenous shunts during luteolysis in the ewe. They indicated that the unique anatomical relationship between the ovarian arteries and the dorsal aorta of sheep could result in peripheral preferential distribution of the larger microspheres (11). Hence, the blood

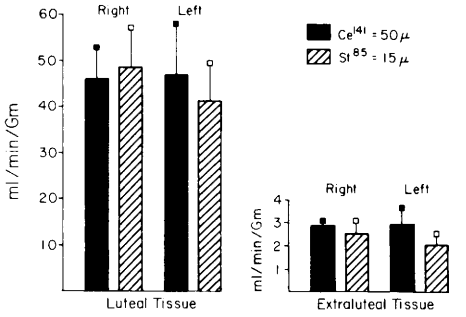

FIG. 1. Blood perfusion rates to corpora lutea and extraluteal tissue of right and left ovaries from 12-days pseudopregnant rabbits, using 15- and 50-μm diameter microspheres ($P > 0.05$, paired t test). $n = 8$ animals.

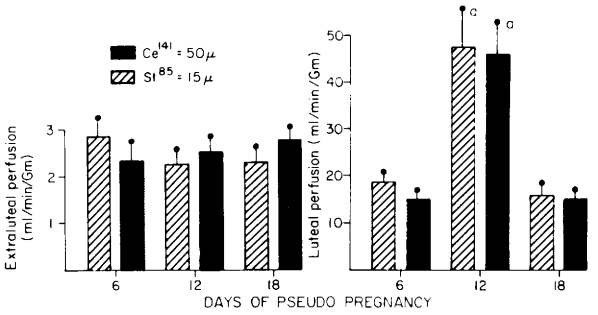

FIG. 2. Luteal and extraluteal tissue perfusion during pseudopregnancy, using 15- and 50-μm diameter microspheres. a = Significantly higher than early and late pseudopregnancy ($P < 0.05$, Student t test). Day 6, n = 6; Day 12, $n = 8$; Day 18, $n = 6$.

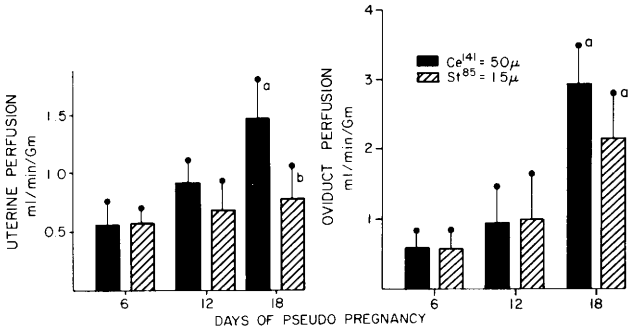

FIG. 3. Uterine and oviductal perfusions during rabbit pseudopregnancy, using 15- and 50-μm diameter microspheres. a = Significantly higher than early and mid pseudopregnancy ($P < 0.05$, Student t test). b = Significantly different (15- vs 50-μm diameter microspheres) ($P < 0.05$, Student t test). Day 12, $n = 8$; Day 18, n = 6.

flow results calculated with the larger microspheres could spuriously be elevated when compared with blood flow calculated with the smaller microspheres. It is well known that even distribution of spheres is essential when using the microsphere technique for blood flow determination. Our observation of similar blood flow to all genital organs, with the two different-diameter microspheres, except to the uterine horns of late pseudopregnancy, attests to the adequacy of the mixing and the transport of all the spheres. It permits one to conclude that there are no shunts between 15- and 50-μm diameter in the rabbit ovary. Similarly, Ahren *et al.* (12), using an *in situ* perfusion technique, were unable to demonstrate arteriovenous shunts in the corpora lutea of pseudopregnant rabbits. Reynolds (13), on the other hand, presented histologic evidence for the presence of arteriovenous shunts in the corpora lutea of these animals. He employed *in vitro* perfusion of radioopaque material. The techniques used to document the presence of arteriovenous shunts may not represent the normal physiologic conditions. The microsphere technique has the advantage of minimal animal manipulation, although it will only detect shunts between diameters of different-sized spheres. Furthermore, for comparative studies, consideration must be given to the type of anesthesia utilized. Thus, while the results of Niswender *et al.* were obtained from conscious animals, our work was accomplished under light anesthesia.

Similar blood flow to several organs, using different-sized microspheres, has been reported (14). Equal blood flow to right and left ovaries supports the concept of symmetrical blood flow to these organs, as suggested by Hilliard *et al.* (15). The only evidence of shunting was seen in the uterine horns of late pseudopregnant rabbits, where entrapment of larger spheres was greater than that of smaller ones. The presence of shunting of less than 25-μm diameter has been shown in the uterine horns of pregnant ewes (16). However, the location of these shunts was placental; myometrial shunting has not been demonstrated in these or other animals.

The increase in luteal blood flow with an unchanged rate of flow to extraluteal tissues, which we observed in pseudopregnancy, has also been observed in pregnancy (4), suggesting that intraovarian mechanisms regulate ovarian blood flow, perhaps mediated through prostaglandins (3, 17), estrogens (5), amines (7), or LH receptor (18).

Luteal perfusion decreases toward the end of pseudopregnancy, although uterine and oviductal perfusion reach maximal values at this time. Estradiol has been shown to increase blood flow to the uterus and oviducts in ewes (5). It is unlikely that plasma estradiol in the rabbit is responsible for the increase in blood flow to the uterus and tubes in this study, because plasma levels and uterine tissue concentrations of estradiol are not significantly different during the various periods of pseudopregnancy (19). Progesterone decreased sharply (8) toward the end of pseudopregnancy, suggesting that change in the ratio of E_2/P may play a role in the regulation of blood flow to the reproductive tract.

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