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Ovarian Blood Flow in the Rat: Association with Body Weight, the Estrous Cycle, and Pseudopregnancy[1] (41725)

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Abstract. The relationships of ovarian blood flow (OBF) to ovarian function, body weight, cyclic state, and age of the corpus luteum were studied in aging rats with acutely implanted electromagnetic blood flow probes. Ovarian function was monitored by estimation of serum 17-β-estradiol (E_2) and progesterone (P) levels during (i) different stages of body maturation as indexed by body weight, and (ii) various stages of the estrous and pseudopregnancy (PSP) cycles. A distinct elevation in basal OBF rates was observed to occur in relation to body weight/age. Basal OBF rates and body weight exhibited a near linear correlation ($r = 0.985$; $P \leq 0.001$) between 200 and 260 g, with a rise in mean basal OBF rates from 1.1 to 3.2 ml/min occurring in Day-8 PSP rats (Day 0 = ovulation). In weight-regulated (≥250 g) cycle rats, OBF increased in parallel with serum E_2 levels during the proestrus–estrus period in association with ovulation, and subsequently declined to basal levels on diestrus. In PSP rats, OBF remained elevated between Days 1 and 8, during the period of peak luteal function. Between Days 8 and 12, both OBF and luteal function declined in a parallel manner as the ovary prepared for the subsequent ovulatory period. These results indicate that during the estrous cycle, OBF rates and serum E_2 levels rise in a parallel manner, whereas during PSP, serum P levels and OBF are positively correlated. The parallel decline in OBF and serum P during luteolysis suggest that both parameters are functionally related and may be regulated by either a systemic or intraovarian controlling mechanism.

Ovarian blood flow (OBF) has been implicated in the regulation of growth, differentiation and endocrine function of the ovary (1, 2). Cyclic and growth-related changes in ovarian vascularization parallel biologial parameters, such as follicular maturation, corpus luteum formation, and luteolysis (2–8). Several reports have indicated that changes in OBF during the luteal phase of the reproductive cycle have a direct, regulatory role in determining the duration of luteal progesterone secretion (2, 5, 6, 8, 9). Ovarian hyperemia has been suggested to be supportive of luteal function and that a decline in OBF precedes, and may initiate, luteolysis (2). In conflict with these findings are reports indicating that luteal function declines prior to a detectable fall in OBF (6, 10, 11). However, most reports do indicate a positive correlation between elevated OBF rates and serum progesterone (P) levels during the luteal phase of the reproductive cycle (2, 5, 6). Thus, while causal relationships between OBF and luteal function during peak functional periods have been established, the role of OBF in initiating and terminating cyclic phases remains unresolved.

Previous studies have indicated that systemic blood volume increases with age in the rat (12). In females, a positive relationship between body weight/age and reproductive performance has also been established (13). Preliminary studies have indicated that OBF is affected by each of these parameters (14), and a general elevation in basal OBF rates and vascularization (15) during the midluteal phase of the reproductive cycle has been demonstrated to be directly related to the maturation of ovarian function. Thus, the alterations in basal OBF rates as related to changes in age/weight of an animal could influence the interpretation of the measurements. With respect to the conflicting reports concerning these parameters, it was deemed essential to examine the relationship between ovarian function and vascular dynamics in animals with an established OBF rate.

The present studies were undertaken in order to determine (i) the relationship between OBF and body weight during the midluteal period of pseudopregnancy in the rat, (ii) the

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relationship between OBF and cyclic state in normal and pseudopregnant rats of established age/weight, and (iii) the relationship between OBF and serum estradiol and progesterone levels in rats with established and stable OBF rates.

Materials and Methods. *Animals.* Adult, female Sprague–Dawley rats weighing between 160 and 340 g were utilized in these studies. All animals were maintained under controlled environmental conditions, with a photoperiod of 12 hr/day (light on: 0600 hr). Food and water were available *ad libitum* and body weights were recorded on a weekly basis.

Each animal was inspected daily for vaginal cyclicity, and all females exhibited at least two normal estrous cycles prior to use. Pseudopregnancy (PSP) was induced by cervical stimulation on the afternoon of proestrus and the morning of estrus. Day 0 of PSP was denoted as the last day of vaginal cornification.

Experimental procedures. All surgical procedures were performed using aseptic techniques with methoxyflurane and/or Innovar (Parke-Davis) employed as anesthetics.

The ovaries were exposed through a midventral laparotomy with care taken not to disturb the vascular supply to the reproductive tract. The ovarian branch of the uteroovarian artery was isolated by blunt dissection from the surrounding fat pad at a point just proximal to its entry into the ovarian hilus. In instances where an ovarian plexus of vessels entered the hilus, the most distal point along the artery where an identifiable segment could be visualized was isolated. In each case, OBF was subsequently monitored as previously described for uterine blood flow (16, 17). In brief, a properly sized, precalibrated (0–10 ml/min) blood flow probe was placed around the exposed arterial segment. The probe was connected to a precalibrated, electromagnetic blood flow monitor (Carolina Medical Electronics) corrected for blood hematocrit. Each animal was electrically grounded. The monitor was connected to a potentiometric recorder with a 1:1 outout ratio to the monitor. In this manner, a 10- to 20-min recording or monitor reading of OBF was collected, with the mean flow rate expressed as milliliters per minute. Failure of vascular clamping to induce an immediate drop to baseline on the monitor or failure of the probe to recalibrate to within 0.1 ml/min of the initial reading were used as criteria for discarding a measurement from analysis.

Subsequent to OBF measurements, blood samples were collected by intracardiac puncture or decapitation. The ovaries were removed, cleaned, blotted, and weighed to the nearest 0.1 mg.

Hormone analysis. Blood samples were allowed to clot at 5°C and serum collected following centrifugation and stored at −20°C until assayed. Serum progesterone (P) and estradiol (E_2) levels were estimated as previously described (16, 18), except that pooled, ethyl ether-extracted E_2 samples were not subjected to column chromatography. Intraassay variability approximated 10% in both cases, and all values were corrected for procedural loss.

Experimental protocol. In order to examine the relationship between body weight/age and basal OBF rates as they relate to reproductive performance, Day 7–8 PSP rats between 160 and 300 g were utilized for OBF measurements. Each animal was anesthetized, laparotomized, and OBF monitored during this specific period of the luteal phase when endocrine fluctuations are minimal and luteal function is maximal. Subsequently, OBF rates were correlated with body weight changes during this specific stage of PSP to determine when OBF rates become stable (i.e., $\geq$250 g). All subsequent studies were performed with rats of this body weight range.

Mature, cyclic rats weighing $\geq$250 g were subsequently used to determine the relationship between basal OBF rates and ovarian condition/function. All studies were performed between 0800 hr and 1100 hr on the respective day of the reproductive cycle or PSP. Cyclic rats were subjected to OBF measurements on either the day of estrus, metestrus, proestrus or diestrus. Serum samples were analyzed for P and E_2 levels and ovarian weights recorded. In a similar manner, PSP rats were monitored for OBF on either Day 1, 2, 4, 6, 8, 10, or 12. Serum samples were analyzed for steroid concentrations and ovarian weights were collected.

Statistical analysis. All values were expressed as group means (±SEM) according to the respective reproductive state. Intergroup differences were analyzed using multiple *t* test analysis, with a $P \leq 0.05$ established as sig-

nificant. Regression analysis was used to establish correlation coefficients.

Results. *Relationship between basal OBF rates and body weight in the PSP rat.* Changes in rat body weight had a direct influence on basal OBF rates during the midluteal phase in PSP rats. Body weights below 200 g were associated with OBF rates in the range 0.8–1.1 ml/min (Fig. 1). However, between 200 and 250 g, basal OBF rats rose from approximately 1.0 ml/min to peak rates of approximately 3.0 ml/min. Little change in this basal OBF rate was observed in rats weighing more than 250 g (Fig. 1). The relationship between body weight of the rat and basal OBF rates was correlated and regarded as a reflection of general body maturation (Table I). All subsequent studies utilized rats weighing $\geq$250 g.

Relationship between OBF and ovarian cyclic state. Basal OBF rates fluctuated during the rat estrous cycle (Fig. 2). On diestrus, OBF was low but rose to peak levels on proestrus. Flow rates remained relatively constant between proestrus and estrus before declining to lowest levels on metestrus. No significant changes in ovarian weight were observed to parallel the cyclic fluctuations in OBF (Fig. 2). Serum E_2 levels tended to parallel the OBF changes, with a peak in E_2 observed between proestrus–estrus and basal levels observed on diestrus. Serum P levels were significantly ($P \leq 0.05$) elevated on diestrus as compared to the relatively constant levels estimated for the remainder of the cycle.

Relationship between OBF and luteal function in the PSP rat. Marked changes in basal OBF rates were observed to occur in relation to luteal function in PSP rats (Fig. 3). Between Days 1 and 8 of PSP, OBF rates were at maximal levels. Subseqently, OBF levels dropped significantly to basal levels on Day 12 just before reinitiation of the next estrous cycle. Ovarian weight remained relatively constant throughout PSP. Serum P levels rose and remained elevated between Days 1 and 8 of PSP before declining rapidly through Days 10 and 12 to basal levels. Serum E_2 levels exhibited a characteristic midluteal elevation between Days 4 and 6, but remained constant between Days 1–4 and 8–10 before rising on Day 12 in association with the subsequent ovulatory cycle.

TABLE I. CORRELATION BETWEEN OVARIAN AND BODY WEIGHT AND OVARIAN BLOOD FLOW (OBF) ON DAY 8 OF PSEUDOPREGNANCY IN THE RAT

Comparisons	Correlation r	Significance
Ovarian wt vs body wt	0.6021	$P \leq 0.05$
Ovarian wt vs No. of corpora lutea	0.9858	$P \leq 0.001$
Ovarian wt vs OBF	0.5087	NS
Body wt (172–318) vs OBF	0.9466	$P \leq 0.01$
Body wt (200–270) vs OBF	0.9858	$P \leq 0.001$

Note. All values are represented as group means for ten to fourteen determinations, each grouped to the nearest 5 g of body weight on Day 8 of PSP.

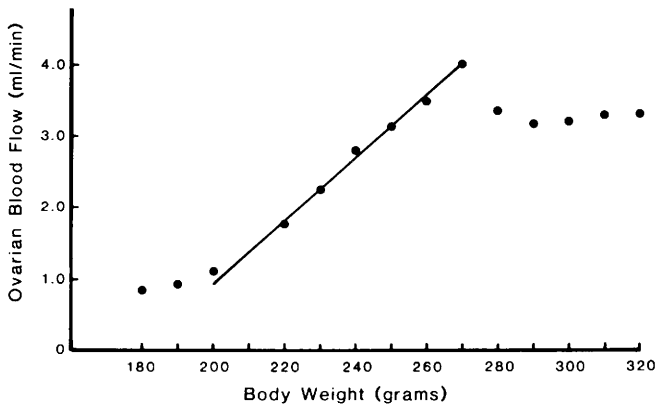

FIG. 1. Correlation between rat body weight (g) and ovarian blood flow on Day 8 of PSP. A correlation coefficient of $r = 0.9858$ was calculated for these parameters in rats weighing 200–270 g (solid line).

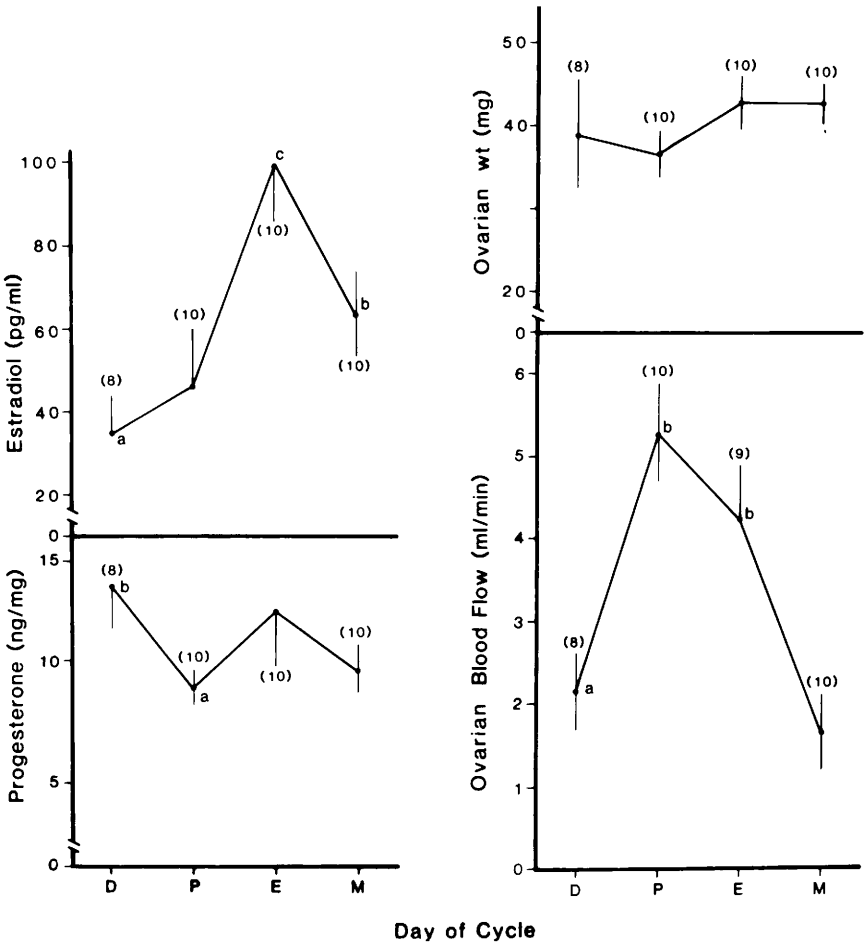

FIG. 2. Relationship between serum E_2 and P levels and ovarian weight and blood flow during the estrous cycle. All values are expressed as group means (±SEM). Diestrus (D), proestrus (P), estrus (E), and metestrus (M) periods are denoted. a vs b, $P \leq 0.05$; a vs c, $P \leq 0.01$.

Discussion. The present studies detail the temporal relationships which exist between OBF rates in rats adjusted for body weight (maturation) influences and ovarian function during the estrous cycle and PSP. While a close association between OBF and serum E_2 levels was observed in cyclic rats, a parallelism between OBF and P levels appeared during PSP. In each case, OBF rates began to decline before the respective systemic drop in either steroid was observed. The obvious association between ovarian hyperemia and the timing of ovulation and luteal formation in the present study, coupled with the gradual decline in OBF accompaning luteal regression (Days 10–12), suggest a causal relationship exists between these ovarian parameters in the rat.

Previous reports have indicated that total body blood volume changes with age in the rat (12) and that OBF rates and reproductive performance change along a similar time course (14, 19). The use of animals in which basal OBF rates were recognized to be constant aided in eliminating experimental variability which could have compromised the result. Although all measurements were collected in anesthetized animals, the obvious correlations existing between reproductive state and OBF rates indicated that the fluctuations were a reflection of ovarian steroid influences and allowed for cycle-related comparisons to be made. The absolute OBF rates are in close agreement with previous reports in the PSP rat (6) in which the microsphere method for

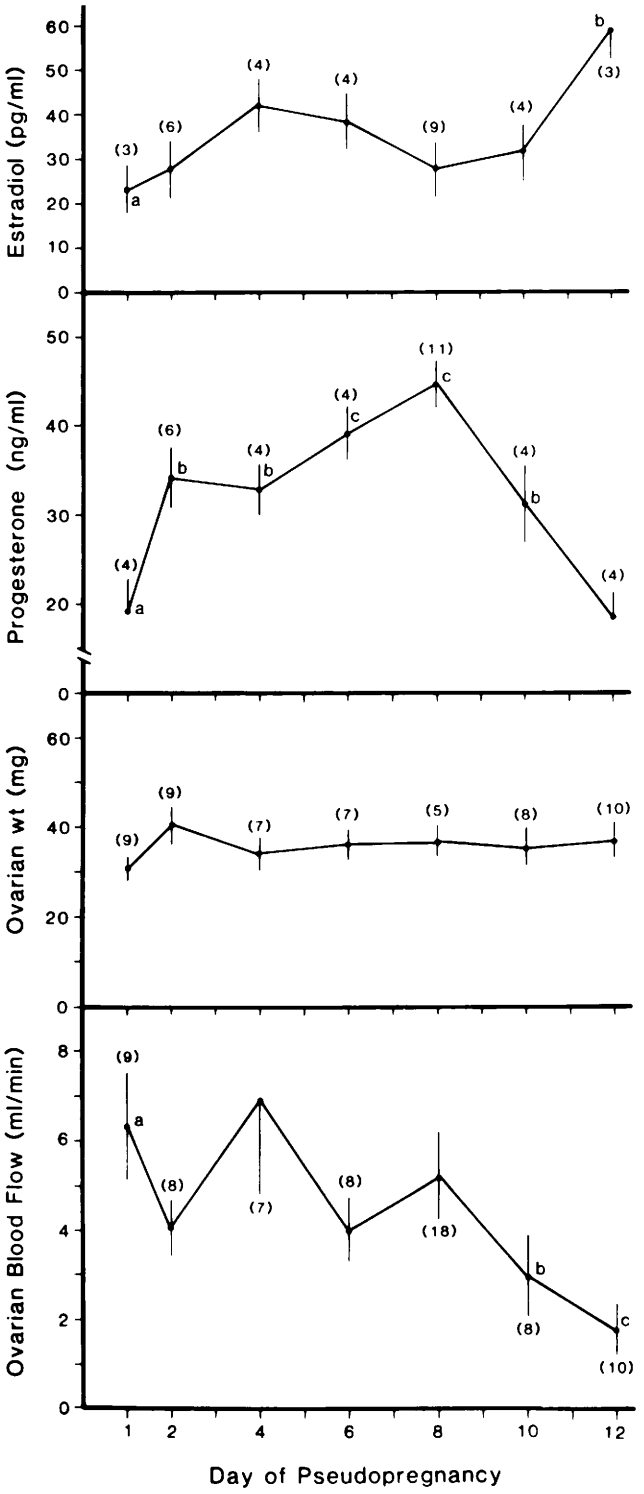

FIG. 3. Relationships between OBF, ovarian weight, and serum P and E_2 levels during PSP in the rat. All values are represented as group means (±SEM). a vs b, $P \leq 0.05$; a vs c, $P \leq 0.01$.

OBF estimation was used. The use of the non-invasive blood flow probes has several advantages over this other technique, several of which have been previously expressed (2). Most important, however, is the fact that a longer monitoring time of OBF is possible with flow probes than with microspheres which reduces transient, vascular fluctuations from interfering with the results. In addition, the ability to monitor electrode functioning *in situ* and to establish constant BF rates without concern for particle size (20) or distribution dynamics (21) has distinct advantages over other techniques. Since total OBF was monitored in the present study, compartmental distribution was not analyzed. However, it has previously been documented that the maturing follicular compartment receives and accounts for the changes in OBF during the estrous cycle (4, 7, 22) and the luteal compartment accounts for the hyperemia which occurs during PSP (6) or pregnancy (23, 24). Thus, the cyclic and luteal fluctuations observed in the present study may be regarded as reflecting the changes in vascular dynamics associated with ovulation and luteal maintenance in the rat.

The results of the present study are in temporal and quantitative agreement for OBF fluctuations during the rat estrous cycle with other reports, when corrected for body weight (7, 25). The present studies extend these observations by indicating the temporal association between elevated E_2 levels during the proestrus period and OBF rates. Although the recognized peak in E_2 levels associated with ovulation in the rat was not measured in the present studies, the elevation in E_2 levels between proestrus and estrus periods and the parallel rise in OBF suggest a causal relationship between these events. In the mouse, OBF rates were suggested to decrease slightly just prior to ovulation (9); however, studies using rabbits indicate that an ovulation-associated ovarian hyperemia (26, 27) occurs as it does in the rat. In the ewe, OBF rises rapidly through the proestrus period (22) and may be associated with the process of ovulation. In a similar manner, the primate ovary exhibits a marked increase in vascularization around the preovulatory follicles, an event which has been postulated to preferentially bathe these sites with elevated gonadotrophin concentrations (4). While a similar phenomenon may be occurring in the rat, the observation (Garris, unpublished) that OBF does not increase in unilateral-ovariectomized rats, even though the number of ovulation sites increase, suggests that this mechanism is not active in this species.

During PSP, elevated OBF rates are in temporal relation to elevated serum P levels through Day 8 in the rat. Subsequently, a gradual decline in OBF is accompanied by diminished luteal function. Similar observations have been forwarded in the ewe (2, 8, 28), mouse (9), rabbit (3), and pig (29). Progesterone secretion has been demonstrated to be positively correlated to OBF rates in the PSP rat (6). However, the role of OBF in the termination of luteal function has been disputed (2, 5, 11). In sheep, OBF rates decline prior to luteolysis (2) whereas studies in PMSG-treated, immature rats (5) and adult, PSP rats (6) suggest that OBF declines subsequent to the onset of luteolysis. The present studies indicate that OBF and serum P levels actually decline in a relatively parallel manner between Days 8 and 12 of PSP, suggesting that both may be directly regulated by either a systemic or intraovarian mechanism (30). The parallel decline in OBF and luteal function mimic the similar decline in uterine blood flow rates and related uterine parameters in the PSP rat (16, 17).

In conclusion, the present studies indicate that temporal relationships exist between OBF and ovarian function in both cyclic and PSP rats. The similar elevations in OBF and serum E_2 levels during the preovulatory period suggest a functional relationship exists between follicular maturation and ovarian vascular dynamics. In addition, the elevated OBF rates observed during the period of peak luteal function suggests a dependency by the corpus luteum on the intrinsic vascular supply. The decline in OBF and luteal function over a similar time course at the end of PSP suggest that a systemic or intraovarian regulatory mechanism (30) directs ovarian preparation for the subsequent ovulatory cycle.

1. Mossman HW, Duke DL. Comparative morphology of the Mammalian Ovary. Madison, Univ. Wisconsin Press, p65, 1973.
2. Niswender GD, Reimers TJ, Diekman MA, Nett TM.

Blood flow: A mediator of ovarian function. Biol Reprod **14**:64–81, 1976.
3. Lee W, Novy MJ. Effects of luteinizing hormone and indomethacin on blood flow and steroidogenesis in the rabbit ovary. Biol Reprod **18**:799–807, 1978.
4. Zeleznik AJ, Schuler HM, Reichert LE Jr. Gonadotropin-binding sites in the rhesus monkey ovary: Role of the vasculature in the selective distribution of human chorionic gonadotropin to the preovulatory follicle. Endocrinology **109**:356–362, 1981.
5. Damber JE, Janson PO, Axen C, Selstam G, Cederblad A, Ahren K. Luteal blood flow and plasma steroids in rats with corpora lutea of different ages. Acta Endocrinol **98**:99–105, 1981.
6. Pang CY, Behrman HR. Relationship of luteal blood flow and corpus luteum function in pseudopregnant rats. Amer J Physiol **237**:E30–E34, 1979.
7. Harvey CA, Owen DAA. Changes in uterine and ovarian blood flow during the oestrous cycle in rats. J Endocrinol **71**:367–369, 1976.
8. Niswender GD, Moore RT, Akbar AM, Nett AM, Diekman AM. Flow of blood to the ovaries of ewes throughout the estrous cycle. Biol Reprod **13**:381–388, 1975.
9. Bindon BM. Blood flow in the reproductive organs of the mouse after hypophysectomy, after gonadotrophin treatment, during the oestrous cycle and during early pregnancy. J Endocrinol **44**:523–536, 1969.
10. Bruce NW, Hiller K. The effect of $PGF_{2\alpha}$ on ovarian blood flow and corpora lutea regression in the rabbit. Nature (London) **249**:176–178, 1974.
11. Janson PO, Albrecht I, Arhen K. Effects of $PGF_{2\alpha}$ on ovarian blood flow and vascular resistance in the pseudopregnant rabbit. Acta Endocrinol **79**:337–350, 1975.
12. Bijsterbosch MK, Duursma AM, Bowma JMW, Gruber M. The plasma volume of the Wistar rat in relation to the body weight. Experientia **37**:381–382, 1981.
13. Altman PL, Katz DD. (eds.) Reproduction. In: Inbred and Genetically Defined Strains of Laboratory Animals, pt 1, Mouse and Rat. Bethesda, Md, Fed Amer Soc Exp Biol p273. 1979.
14. Curry TE, Garris DR, Lawrence I Jr. Correlations between ovarian weight, body weight and ovarian blood flow in the pseudopregnant rat. Anat Rec **202**:143, 1982.
15. Meyer GT, Bruce NW. Quantitative cell changes and vascularization in the early corpus luteum of the pregnant rat. Anat. Rec **197**:369–374, 1980.
16. Garris DR. Temporal relationships between uterine weight, uterine blood flow, and serum progesterone and estradiol levels in the pseudopregnant rat. Proc Soc Exp Biol Med **169**:334–336, 1982.
17. Garris DR, Fair JH, Whitehead DS, Muse PD. Relationship between deciduoma formation and uterine blood flow in pseudopregnant rats. Gynecol Obstet Invest **15**:84–92, 1983.
18. Garris DR. The luteotrophic effects of estradiol and prolactin in the absence of LH in the hysterectomized, pseudopregnant rat. Proc Soc Exp Biol Med **170**:203–208, 1982.
19. Garris DR, Lawrence I Jr. Body weight related changes in ovarian blood flow in the pseudopregnant rat. IRCS Med Sci **9**:1005–1006, 1981.
20. Mattner PE, Brown BW, Hales JRS. Evidence for functional arterio-venous anastomoses in the ovaries of sheep. J Reprod Fertil **63**:279–284, 1981.
21. Tegler L, Gillquist J, Anderberg B, Jacobson G, Lundstrom B, Roos P. Human thyroid blood flow response to endogenous, exogenous human, and bovine thyrotrophin measured by electromagnetic flowmetry, Acta Endocrinol **98**:540–548, 1981.
22. Bruce NW, Moor RM. Capillary blood flow to ovarian follicles, stroma and corpora lutea of anesthetized sheep. J Reprod Fertil **46**:299–304, 1976.
23. Abdul-Karim RW, Bruce N. Blood flow to the ovary and corpus luteum at different stages of gestation in the rabbit. Fertil Steril **24**:44–47, 1973.
24. Bruce NW. The distribution of blood flow to the reproductive organs of rats near term. J Reprod Fertil **46**:359–362, 1976.
25. Batta SK, Martini L. Influence of the estrous cycle and prostaglandins on utero-ovarian vein blood flow in the rat. Prostaglandins **10**:469–477, 1975.
26. Blasco L, Wu CH, Flickinger GL, Wheeler J, Mikhail G. Blood flow to the reproductive organs of the estrus rabbit. Gynecol Invest **4**:270–276, 1973.
27. Blasco L, Wu CH, Flickinger GL, Pearlmutter D, Mikhail G. Cardiac output and genital blood flow distribution during the preovulatory period in rabbits. Biol Reprod **13**:581–586, 1975.
28. Brown BW, Emery MJ, Mattner PE. Ovarian arterial blood velocity measured with Doppler ultrasonic transducers in conscious ewes. J Reprod Fertil **58**:295–300, 1980.
29. Rathmacher RP, Anderson LL. Blood flow progesterone levels in the ovary of cycling and pregnant pigs. Amer J Physiol **214**:1014–1018, 1968.
30. Pang CY, Behrman HR. Acute effects of prostaglandin $F_{2\alpha}$ on ovarian and luteal blood flow, luteal gonadotropin uptake *in vivo,* and gonadotropin binding *in vitro.* Endocrinology **108**:2239–2244, 1981.

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