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Temporal Relationships between Uterine Weight, Uterine Blood Flow, and Serum Progesterone and Estradiol Levels in the Pseudopregnant Rat (41353)

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Abstract. Changes in rat uterine weight and uterine blood flow (UBF) between Days 4 and 12 of pseudopregnancy (PSP: Day 0 = ovulation) were monitored and correlated with the respective fluctuations in serum progesterone (P) and estradiol (E) levels. Rats were monitored for UBF using a square-wave electromagnetic blood flow monitor. Serum P and E levels were estimated by radioimmunoassay. Parallel changes in uterine weight and UBF were observed between Days 4 and 9 of PSP, each exhibiting a peak on Day 6 before declining to basal levels on Day 9. Serum E levels remained relatively constant throughout PSP, ranging between 5 and 7.5 pg/ml. In contrast, serum P levels exhibited peaks (Days 8–9) when UBF and uterine weights were lowest, and lower levels when the uterine parameters were elevated (Days 6–7). These data suggest that fluctuations in the ratio of E to P (rather than the respective, absolute levels of each) induce the changes observed in uterine weight and UBF during PSP in the rat.

Changes in uterine weight are known to occur as a result of cyclic fluctuations in circulating estrogen (E) and progesterone (P) levels in the rat (1). Similar correlations (2) have been observed during pseudopregnancy (PSP). In addition, both E and P are known to alter uterine blood flow (UBF), E inducing uterine hyperemia while P maintains UBF at constant, basal levels (3–5). Although the modulation by ovarian steroids of these uterine parameters is known, information concerning the changes in uterine weight with respect to UBF during PSP is scarce. Since ovarian steroids influence each uterine aspect, the changes which occur in each parameter may themselves be causally related.

The present study was undertaken in order to determine the temporal relationships which occur in uterine weight and UBF with respect to E and P levels during mid-PSP in the rat.

Materials and Methods. *Animals.* Adult, female rats (Sprague–Dawley) weighing 200–300 g were housed at 23° under a controlled photoperiod of 14 hr light/day (lights on: 0600 hr). Purina lab chow and water were available *ad libitum*. Each rat exhibited at least two normal, 4- or 5-day estrous cycles prior to use. Pseudopregnancy was induced by mechanical stimulation of the cervix on the afternoon of proestrus and morning of estrus. The last day of vaginal cornification was denoted Day 0 of PSP.

Blood sampling and assay of serum P and E levels. Blood samples (0.5 ml) were collected under ether anesthesia by jugular puncture between 1000 and 1200 hr on Days 4–9 and 12 of PSP. Each sample was allowed to clot at 5°, serum separated by centrifugation, collected and stored at −20° until assayed. Serum P and E levels were estimated by radioimmunoassay in duplicate as previously described (6–9). Assay sensitivity was 5 and 10 pg for serum estradiol and progesterone measurements, respectively. All samples were run in single assays, with an intracoefficient of variability of approximately 8–10%. All values are expressed corrected for procedural loss.

Uterine blood flow measurements and tissue collection. Rats were anesthetized with Innovar (0.5 mg/kg im; Pitman-Moore) and the uterus exposed via a midventral laparotomy. Each uterine artery was located and isolated from the adhering mesometrial fat pad by blunt dissection. A 1-cm portion of the artery was stripped, the diameter measured with calipers, and an appropriately sized blood flow probe placed around the exposed segment. The animal was electrically grounded and UBF monitored using a square-wave electromagnetic

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blood flow monitor connected to a potentiometric recorder. In each case a stable 30- to 45-min recording of UBF was collected, interrupted midway through for an electrode calibration check. Failure of the electrode to recalibrate to within 0.1 ml/min was used as the basis for discarding a recording from analysis. All usable records were analyzed for mean UBF rates (ml/min). The uterus was subsequently removed, cleaned, blotted, and weighed to the nearest 0.1 mg.

Statistical analysis. All values are expressed as group mean (±SEM) and intergroup differences evaluated by Student's *t* test and ANOVA.

Experimental protocol. Rats were randomly assigned to groups for UBF measurements on Days 4–9 and 12 of PSP. Blood samples were collected at 24-hr intervals, except for the 24 hr prior to UBF measurements when no sample was collected. On the selected day, UBF was monitored, uterine weight collected, and a blood sample collected for analysis of serum E and P levels.

Results. As depicted in Fig. 1, UBF and uterine weight fluctuated in parallel between Days 4 and 9 of PSP. Both uterine parameters demonstrated slight peaks between Days 5 and 7 and subsequently declined to basal levels between Days 7 and 9. On Day 12, just preceeding the termination of PSP, uterine weight increased while UBF remained steady.

Serum E levels remained relatively constant throughout PSP (Fig. 1). In contrast, serum P levels exhibited fluctuations opposite to those of UBF and uterine weight. While significant changes in serum P levels were not observed until Day 12, changes in the E to P ratio did fluctuate in an identical manner to UBF between Days 4 and 8 of PSP (Table I). By Day 9, the E to P ratio had increased without affecting uterine weight or UBF.

Discussion. The results of the present study indicate that parallel changes in uterine weight and UBF which occur between Days 4 and 8 of PSP are temporally related to fluctuations in serum E/P ratios. These data support the results of several

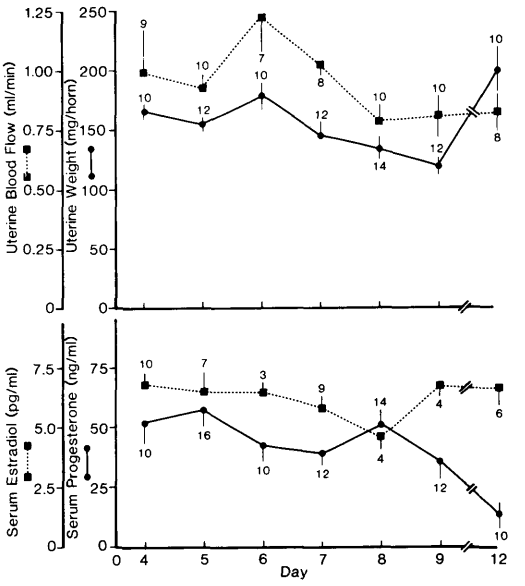

FIG. 1. Changes in uterine weight, UBF, and serum E and P levels between Days 4 and 12 of PSP in the rat. All values are expressed as group means (±SEM) and numbers denote rats/group (UBF: Days 6–7 vs Days 4–5 and 8–12, $P \leq 0.05$.) Uterine weight: Day 6 vs Days 4–5 and 7–12, $P \leq 0.05$.

studies in which ovarian steroids were demonstrated to alter both uterine parameters in ovariectomized animals (1, 3–5). However, the present results indicate that the change in the E to P ratio is more critical for the control of uterine weight and UBF than are absolute levels in the intact rat.

The Day 5–7 rise in uterine weight and UBF is temporally related to the expected time of blastocyst implantation in the rat. Previous reports have indicated that uterine blood volume is increased at the site of blastocyst implantation in the rat (9) and that UBF increases during this time in the guinea pig (10). These changes are under ovarian control, with E increasing and P suppressing uterine hyperemia. The suggestion of an increase in UBF in the present study on Day 6 suggests that the PSP uterus undergoes similar vascular changes and may serve as a useful model for the study of the endometrial-vascular changes participating in the initiation of nidation.

Whether or not the cyclic fluctuations in

TABLE I. TEMPORAL RELATIONSHIPS BETWEEN THE GROUP MEANS OF UTERINE WEIGHT, UTERINE BLOOD FLOW (UBF) AND ESTRADIOL (E) AND PROGESTERONE (P) RATIOS DURING PSEUDOPREGNANCY IN THE RAT

Parameter	Day of pseudopregnancy					
	4	5	6	7	8	9
1. Uterine weight (mg/horn)	167.2	156.1	179.2	146.5	144.5	138.2
2. UBF (ml/min)	0.99	0.84	1.2	1.1	0.80	0.87
3. E to P Ratio (pg × 10^{-4})	1.05	1.03	1.41	1.38	0.79	2.1

E/P ratios affect UBF directly by inducing changes in the uterine vascular bed (11, 12), or if they act indirectly by modulating local factors controlling uterine microcirculation (13–15) remains to be elucidated. Detailed studies focusing on estrogen regulation of uterine vascular responsiveness to local metabolites are presently in progress.

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