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Role of the Preimplantation Embryo in the Timing of LH-Dependent Progesterone Secretion from the Rat Corpus Luteum (41456)

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Abstract. The effect of the progestational uterus and preimplantation embryo on the time of appearance of LH-dependent progesterone secretion from pregnant and pseudopregnant (PSP) rat corpora lutea (CL) was studied. Two groups of experiments were performed: (1) pregnant and PSP rats were hysterectomized between Days 3 and 5 (Day 1 = vaginal estrus) and subsequently monitored for changes in serum progesterone levels following an injection of LH antiserum (LHAS) or normal horse serum (NHS) vehicle on Day 9 and, (2) Day 4 embryos were transferred to Day 3 PSP uteri; each rat was subsequently hysterectomized on Day 4 of PSP and treated with LHAS or NHS on Day 9. Progesterone levels were monitored as an index of luteal vitality. Progesterone secretion was not altered by NHS treatment in any group. LHAS treatment did not induce luteolysis in Day 3 pregnant rats or in PSP rats hysterectomized on Days 3 or 4. However, in rats hysterectomized on Day 4 or 5 of pregnancy, or hysterectomized on Day 5 of PSP, luteolysis occurred following LHAS treatment. In rats where embryo transfer occurred on Day 3 of PSP and hysterectomy on Day 4, a Day 9 injection of LHAS induced luteolysis. The results of these studies demonstrate that the embryo (i.e., morula to early blastocyst) is capable of influencing the rat CL to become critically dependent on LH for progesterone secretion. The presence of the progestational uterus in the PSP rat also has this ability, but the PSP uterus must remain *in situ* until Day 5, whereas an embryo-containing uterus exercises its influence by Day 4. It is hypothesized that the embryo may have an indirect effect on the induction of LH-dependent progesterone secretion from the rat CL.

While prolactin (PRL) is an essential luteotrophin in the rat during early pregnancy and throughout pseudopregnancy (PSP) (1, 2), the maintenance of luteal function after Day 8 of pregnancy (3), or Day 9 of PSP (4), requires the coaction of LH. In combination, these hormones support progesterone secretion from the rat corpus luteum (CL) between Days 8 and 12 of pregnancy (3) and after Day 9 of PSP (4). Withdrawal of LH from circulation by the systemic administration of an LH antiserum (LHAS) results in luteolysis, as noted by decreased progesterone levels and a shortening of the diestrus interval of pregnancy and PSP (3–7).

The factors involved in the development of LH-dependent progesterone secretion remain obscure. Previous studies have demonstrated that the presence of a progestational uterus accelerates the time of appearance of LH dependency in the PSP rat (6–8). In addition, experimentally induced deciduoma tissue (DT) enhances the critical need for LH, as was evidenced by the uniformity in LHAS-induced luteolysis in this model (4, 6). However, even in the DT-PSP rat, LH dependency does not appear until Day 9, approximately 24 hr later than it occurs in the pregnant rat (3, 4). Although the two systems are endocrinologically similar (9), the influence of the preimplantation embryo is absent in the PSP model. Since the rat blastocyst has been reported to be luteotrophic (10), this study was undertaken in order to elucidate the role of the preimplantation embryo on the timing of the appearance of LH-dependent progesterone secretion from the rat corpus luteum.

Materials and Methods. *Animals.* Adult, female Sprague–Dawley (Holtzman Co.) rats weighing 220–260 g were housed under a controlled photoperiod (12 hr light/day: lights on 0600 hr) with access to rat chow and water *ad libitum*. Each animal

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exhibited at least two normal, 4- or 5-day, estrous cycles prior to use. Pregnancy was achieved by placing a proestrus female with a fertile male. Day 1 of pregnancy was denoted by a sperm-positive vaginal smear. Pseudopregnancy was induced by cervical stimulation with a glass rod on the afternoon of proestrus and morning of estrus. The last day of vaginal cornification was denoted as Day 1 of PSP in order to standardize the dating of pregnant and PSP rats.

Surgical procedures. All surgery was performed under ether anesthesia using aseptic techniques. Hysterectomy was performed by midventral laparotomy as previously described (7, 11). Uterine exposure for embryo transfer experiments was performed through bilateral dorsal flank incisions. Laparotomy and exposure of the uterus without manipulation served as a control (sham) procedure in all experiments.

The collection of preimplantation embryos (i.e., late morula to early blastocyst stage) from the uteri of donor rats was done late on Day 4 of pregnancy by flushing each uterine horn and tubal oviduct with 0.2 ml of isotonic saline. The embryos from each uterus were collected into 0.5 ml of the flushing solution. Embryo transfer was accomplished using a 20-gauge needle and 1-ml syringe, with five or six embryos transferred into the tubal end of each recipient uterine horn in approximately 0.1 ml of collection solution. Injections of 0.1 ml of collection media alone served as the control procedure.

Blood sampling and hormone analysis. At specified intervals, 0.5 ml of blood was collected by direct jugular puncture from ether-anesthetized rats. Blood samples were allowed to clot, the serum collected, and subsequently stored at −20° until assayed.

Serum progesterone levels were estimated by radioimmunoassay as previously described (12). Assay sensitivity was 5 ng/ml and extraction recovery averaged 94%. All values are expressed uncorrected for procedural loss.

LH antiserum treatment. Between 0900 and 1200 hr on Day 9, rats received a 0.5 ml sc injection of either normal horse serum (NHS) or LHAS. The immunologic characteristics and biological effects of the antiserum have been described (3, 13).

Statistical analysis. All group values are expressed as the mean ± SEM and intergroup differences were determined by Student's t test with an acceptable significance level set at $P < 0.05$.

Experimental protocol. The first study was undertaken to determine if hysterectomy on Day 4 in pregnant rats would prevent LHAS-induced luteolysis on Day 9. Intact PSP and pregnant rats were compared with rats hysterectomized on Days 3, 4, or 5 of PSP or pregnancy for LH dependency on Day 9. In each case NHS or LHAS was administered sc on Day 9 and blood samples collected at 0, 24, and 72 hr postinjection to evaluate luteal function as indexed by serum progesterone levels.

A second study was undertaken to determine the effects of the preimplantation embryo on the development of LH-dependent, progesterone secretion. This was accomplished by collecting embryos from rats on Day 4 of pregnancy and transferring a complement of five or six embryos to each uterine horn of PSP rats on Day 3. An intraluminal instillation of the transfer medium on Day 3 served as the control procedure. All rats were then hysterectomized on Day 4 (i.e., 24 hr post-transfer) and received either an NHS or LHAS injection on Day 9. Blood sample collection was exactly as described above and serum progesterone levels were used as an index of luteal activity.

Results. *Effects of the timing of hysterectomy on the expression of LH-dependent progesterone secretion in PSP and pregnant rats.* Administration of NHS to PSP or pregnant rats on Days 3, 4, or 5 failed to induce luteolysis (Figs. 1A, B). Similarly, the CL of PSP rats (which were hysterectomized on Days 3 or 4) or pregnant rats (hysterectomized on Day 3) were not affected by LHAS treatment on Day 9. However, rats hysterectomized on Day 4 or 5 of pregnancy underwent luteolysis following an LHAS injection on Day 9. The PSP rats hysterectomized on Day 5 also ex-

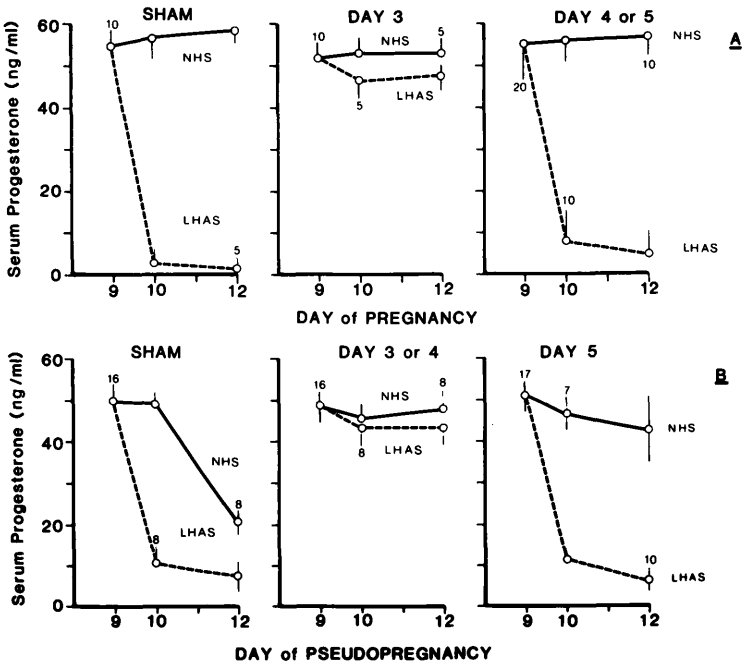

FIG. 1. Effects of NHS or LHAS treatment on Day 9 of pregnancy or PSP in either sham-operated rats or pregnant (A) or PSP (B) rats hysterectomized on Days 3, 4, or 5 (as indicated). Values are expressed as the group mean ± SEM. Numbers denote rats per group. Statistical analysis on Day 12: Pregnant: Sham/NHS vs sham/LHAS, $P < 0.001$; Day 3/NHS vs Day 3/LHAS, $P > 0.05$; Day 4 or 5/NHS vs Day 4 or 5/LHAS, $P < 0.01$. PSP: Sham/NHS vs Sham/LHAS, $P < 0.05$; Day 3 or 4/NHS vs Day 3 or 4/LHAS, $P > 0.05$; Day 5/NHS vs Day 5/LHAS, $P < 0.01$.

hibited a decline in serum progesterone levels following a Day 9 injection of LHAS. Thus, the presence of the pregnant uterus after Day 3, or the PSP uterus after Day 4, assured that LH-dependent progesterone secretion from the rat CL appeared on Day 9. It was of interest to determine what caused the 24-hr difference in the appearance of LH-dependent progesterone secretion between the two model systems.

Effects of the preimplantation embryo on the temporal aspects of LH-dependent progesterone secretion. The transfer of Day 4 preimplantation embryos to recipient uteri on Day 3 of PSP, and the effects on luteal sensitivity to LHAS treatment on Day 9 are depicted in Fig. 2. The LHAS treatment on Day 9 did not induce luteolysis in PSP rats which had received an intrauterine injection of the embryo transfer solution on Day 3 and were subsequently hysterectomized on Day 4. Similarly, NHS treatment had no effect on serum progesterone levels. However, rats which had received a full complement of embryos on Day 3 of PSP and were subsequently hysterectomized 24 hr later, underwent luteolysis following LHAS treatment on Day 9. Thus, the presence of the embryos within the uterus between Days 3 and 4 of PSP induced the early appearance of LH-dependent progesterone secretion.

Discussion. The results of the present study clarify some of the temporal differences in the time of appearance of LH-dependent progesterone secretion in pregnant and PSP rats. While the PSP rat does not become sensitive to LH withdrawal until Day 9 (4, 6, 7, 14), the pregnant rat becomes dependent on LH for luteal support by Day 8 (3). The present study indicates that the presence of the embryos in a PSP uterus can advance the time of appearance of LH-dependent progesterone secretion by

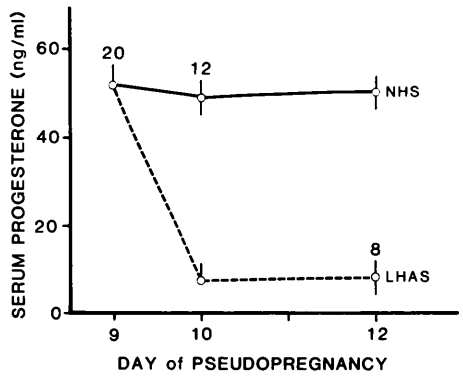

FIG. 2. Effect of transferring Day 4 embryos to Day 3 PSP uteri on luteal function following a Day 9 NHS (solid line) or LHAS (dashed line) injection. Since no statistical differences were found between NHS-treated PSP rats with or without embryo transfer, the data are expressed as one group. All rats were hysterectomized on Day 4 of PSP. Progesterone levels are expressed as the group mean ± SEM. Numbers denote rats per group (NHS vs LHAS, $P < 0.01$ on Day 12).

approximately 24 hr. It is postulated that the normal presence of preimplantation embryos in the pregnant uterus accounts for the appearance of LH-dependent progesterone secretion on Day 8 in this animal model.

Exactly how these pregnancy-related changes in the luteotrophic complex of the rat occur remains obscure. The decidualized uterus, as well as the progestational uterus, are both capable of influencing the sensitivity and timing of LH-dependent progesterone secretion in the rat (6). However, in the absence of either, LH dependency still develops but the appearance is delayed until Day 12 (7, 8). While the reasons for this remain to be clarified, the fact that uterine removal can influence the duration of the PRL surges in the rat (15) suggests that the need for LH is delayed while the luteotrophic properties of PRL are prolonged. Support for this concept comes from the fact that LH-dependent progesterone secretion can be delayed by the experimental elevation of circulating PRL levels (7, 8). Under these circumstances, the uterus must remain *in situ* longer (i.e., beyond Day 7) in order to counteract the effects of elevated PRL levels. Thus, in the hysterectomized rat, the time of appearance of LH-dependent progesterone secretion depends upon both the influence of the uterus and pituitary gland. In addition, in the pregnant rat, the preimplantation embryo is apparently also capable of advancing the timing of LH-dependent progesterone secretion and, thus, is another factor which differentiates the pregnant and PSP luteotrophic complexes. While the process of implantation has also been implicated in this system (16), it remains to be determined if it is an actual blastocyst–endometrium-induced response or merely an influence of the blastocyst or decidual tissue alone.

The lack of effect of the intraluminal injection of transfer medium on Day 3 of PSP on the subsequent development of LH dependency strongly suggests that blastocyst–uterine interaction has a direct influence on luteal function. Several reports have recently described various effects of the preimplantation blastocyst on ovarian function. In the rabbit (17–20), the conceptus exerts a luteotrophic effect and is reportedly capable of increasing peripheral progesterone concentrations as compared to those of PSP animals. However, several reports are in conflict with these findings (21–23). Similar luteotrophic properties have been described in the ewe (24), but no relationship to LH or PRL activity was demonstrated (23). While direct evidence of such activity has not been demonstrated in the rat, the rat blastocyst has been described as being luteotrophic since it can prolong the CL of PSP (10). Exactly how the blastocyst can influence the rat CL is unclear, but its potential for steroidogenesis (25), protein synthesis (26), and the ability of blastocyst fluid to stimulate progesterone secretion and follicular luteinization (27) suggest that a "blastocyst factor" may serve to influence luteal function.

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