Evidence for Maternal Regulation of Progesterone Production at Midpregnancy in the Mouse (43056)

M. BARKLEY
Department of Animal Physiology, University of California, Davis, California 95616

Abstract. **Mice with major differences in embryo survival and progesterone secretion were used to study the relative roles of maternal and embryonic genotype in the control of progesterone production at midpregnancy. Reciprocal transfer of embryos from strains with high (Line S1) or low (Line G) embryo survival resulted in progesterone concentrations consistent with maternal genotype in females carrying the same number of fetuses. This suggests that maternal rather than fetal gene expression determines the setpoint for progesterone production during the period of transition from maternal to fetal-placental control of ovarian steroidogenesis.** [P.S.E.B.M. 1990, Vol 194]

The endocrine transition from pituitary to fetal-placental regulation of progesterone production has been studied extensively because this represents a phenomenon with wide application to several species. In rodents, the corpora lutea of pregnancy initially secrete progesterone in response to luteotropic stimulation provided by the maternal pituitary. This endocrine gland can be removed at midpregnancy without consequence because luteotropic products secreted by the conceptus are then able to maintain luteal function (1, 2). In the midpregnant rat, progesterone production is maintained by the actions of intraluteal estrogen (3–5). In the absence of the pituitary, estrogen has no stimulatory effect on progesterone synthesis unless prolactin (PRL) is administered or decidual tissue is present *in situ* (6). Both PRL and a decidual luteotropin seem to regulate the actions of estrogen by modulating luteal estrogen receptor concentration at midpregnancy (7).

Decidual tissue represents the maternal portion of the placenta and is derived from endometrium (8). The remainder of the rodent placenta, including trophoblast, is derived from the embryo (9). The embryonic portion of the placenta produces PRL-like hormones commonly known as placental lactogens. Large molecular weight rat placental lactogen-I and mouse midpregnancy lactogen-I are highest in the circulation at midgestation (10, 11). The likely source of these placental hormones is the trophoblast giant cell in both the rat (10) and mouse (11). The decidual tissue of rats secretes a substance that has luteotropic activity (12, 13) and resembles but is not identical to pituitary prolactin (14, 15). The decidual product is highest in midpregnancy serum but has disappeared by late gestation, at which time only one form of a PRL-like hormone, small molecular weight rat placental lactogen-II, predominates in the maternal circulation (10).

Rat decidual luteotropin is immunologically and chemically distinct from either PRL, rat placental lactogen-I, or rat placental lactogen-II and, combined with estrogen, is able to maintain progesterone production in the absence of the maternal pituitary (6). This ability of rat decidual luteotropin and the lack of information about mouse decidual hormones prompted an investigation of progesterone production following reciprocal embryo transfer in strains of mice that differ in maternal steroid secretion at midpregnancy. A progesterone concentration consistent with the genotype of the dam would suggest maternal, i.e., decidual regulation of luteal function at this time, whereas progesterone levels reflecting the genotype of the fetus would implicate trophoblast secretions in the control of ovarian steroidogenesis.

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Materials and Methods

Animals. The mice used in this study consisted of two selected strains (S1 and G) derived from the same base population produced by crossing four inbred lines

(C57BL/6, AKR, C3H, and DBA/2). Lines S1 and G were selected for large litters and rapid postweaning weight gain, respectively (16, 17). A randomly bred control line (C) was maintained throughout the selection of Lines S1 and G. As a correlated response to selection for different traits, both lines have responded with an increased ovulation rate, but litter size is higher in Line S1 because prenatal survival is reduced by almost 50% in Line G (Table I). The reduced prenatal survival in Line G is the result of both pre- and postimplantation embryo loss (17). Lines S1 and G also differ in maternal progesterone production throughout pregnancy (19), with the greatest difference in progesterone levels found during the period of transition from pituitary to fetal-placental regulation of ovarian progesterone production. Both lines were housed in the same animal room maintained at 21–23°C and illuminated 14 hr/day (lights on from 0500 to 1900 hr). Purina Rodent Chow (#5001) and water were supplied *ad libitum*. Access to the animal room was limited to those individuals directly involved in the project.

Embryo Transfer. Virgin females approximately 8 weeks of age from Lines S1 and G were used as embryo donors and recipients. Donors were mated to fertile males of the same line, and recipients were mated to vasectomized males. Females were checked daily for copulatory plugs, and the day of plug detection was designated as Day 0 of pregnancy or pseudopregnancy. After a plug was detected, the bred female was housed individually or with other females of the same line that had mated during the same 24-hr period.

The embryo transfer procedure used was similar to that described by Dickmann (20). Embryos were collected from donors early in the afternoon on Day 3 by excision of the uteri and subsequent flushing with a sterile 3% solution of bovine serum albumin, (Calbiochem-Behring Corp., La Jolla, CA) in phosphate-buffered saline. Following collection in a small glass pipette, embryos were surgically transferred to a 2.5-day recipient female. Recipients received 5–10 embryos/uterine horn. Pregnancy rates for the dams averaged 40% for all transfer groups. Pregnant animals were subjected to sham transfer following anesthetization when fluid alone was injected into the uterus.

Sample Collection. Blood samples were obtained following rapid decapitation between 1000 and 1200 hr on Days 8, 9, and 10 of pregnancy. On the morning of the day preceding decapitation, the females were housed individually and were not disturbed until the time of sample collection. Trunk blood was collected over heparinized funnels. Following refrigerated centrifugation (20 min at 1000*g*), the plasma was recovered and stored at −20°C until assayed for progesterone. Immediately after decapitation, the reproductive tract was removed and examined under a dissecting microscope. The number of implants was recorded along with implant size. The latter was determined with the use of a micrometer (American Scientific) according to the procedure described previously which includes measurement of implant diameter and length from embryonic to abembryonic pole (21). Animals in which distinct implantation sites could not be detected were excluded from the study.

Radioimmunoassay. Plasma progesterone was quantified using the method of Bosu *et al.* (22). Plasma was extracted with nanograde petroleum ether. The extracts were immersed in an acetone/solid CO_2 bath, the ether phase was placed in a culture tube, and the extracts were evaporated to dryness. This method extracted 85–90% of the plasma progesterone. The dried extracts were resuspended in phosphate-buffered saline. Separation of the free from the bound steroid was performed using a 1-ml mixture of Dextran T70 (0.25 g/liter) and charcoal (2.5 g/liter). The antiserum (AB FO 22.5) used in the assay was produced in sheep immunized against 11β-hydroxyprogesterone hemisuccinate-bovine serum albumin and was a gift from Dr. L-E. Edqvist. The antiprogesterone serum was used at a dilution of 1/6000 which produced 40% binding of [1,2,6,7,21-^{3}H]progesterone (sp act, 165.0 Ci/m*M*, NET-724; New England Nuclear). Progesterone for standards was obtained from Calbiochem-Behring Corp. The lower limit of sensitivity of the standard curve was 0.15 ng. Water and buffer blanks yielded values of 1.55 ± 0.2 ng/ml. The intra- and interassay coefficients of variation were 8.3 and 11.8%, respectively. All sample determinations were performed in the same assay.

Data Analysis. Analysis of variance followed by Duncan's new multiple range test was used to evaluate treatment effects. Only differences between means at $P \leq 0.05$ were considered significant.

Results

As expected, Line S1 had more conceptuses (Day 9 = 14.3 ± 1.1) than Line G (Day 9 = 7.3 ± 1.4) or animals subjected to embryo transfer which did not differ in conceptus number, averaging four implants on Day 9 regardless of the strain of embryo or host (S1 dam + S1 embryo = 4.0 ± 0.6; G dam + G embryo = 4.2 ± 1.0; S1 dam + G embryo = 4.4 ± .9; G dam + S1 embryo = 4.3 ± 1.5). This effect of embryo transfer on the reduction of implant number has been observed previously in other strains of mice (23). No effect of genetic strain on conceptus size was evident, but embryo growth increased with the stage of gestation.

Progesterone concentrations in females subjected to sham-embryo transfer are shown in Figure 1. On Day 8 of pregnancy, Lines S1 and G had equivalent levels of circulating progesterone, but on Days 9 and 10, Line S1 females secreted significantly ($P < 0.05$) more progesterone than Line G females.

Table I. Ovulation Rate, Prenatal Survival, and Litter Size of Two Selected Lines and an Unselected Control Line Developed from a Common Base Population[a]

Line	Selection criterion	Mean ± SE		Prenatal survival (*n*/corpora lutea)
		Ova or corpora lutea[b]	Normal fetuses at 16 days or number born (*n*)[c]	
C	Unselected	10.1 ± 0.29	7.8 ± 0.21	0.77
S1	Large litters	17.4 ± 0.43	15.3 ± 0.31	0.88
G	Rapid 21- to 42-day gain	16.0 ± 0.55	8.1 ± 0.34	0.51

[a] Composite data from Barkley and Bradford (18).
[b] In females mated at 8–10 weeks of age; $n = 24$–58 animals/line.
[c] Based on approximately 100 females/line also mated at 8–10 weeks of age.

Following reciprocal embryo transfer, progesterone levels were always consistent with the genotype of the dam (Fig. 2). Transfer of Line G embryos to Line S1 females resulted in maternal progesterone levels on Day 9 that resembled those found in S1 controls (sham transfer) or S1 recipients of S1 embryos (intraline transfer). Conversely, when Line S1 embryos were transferred to Line G females, the concentration of progesterone found in G recipients was similar to that produced by Line G controls (sham operated) or females that received Line G embryos (intraline transfer).

Discussion

The mouse is a polytocous species in which pregnancy cannot be supported without the continuous production of progesterone and estrogen by the ovaries (24, 25). Beginning on Day 1 and until Day 5 of gestation, prolactin stimulates progesterone biosynthesis, but on Days 6–9, luteinizing hormone is required for ovarian progesterone production (26). This transient period of luteal dependency on luteinizing hormone is associated with increased androgen production (6, 27, 28) and the elaboration of placental products with demonstrated hormonal function (2, 15). These products are known to be of decidual (12, 13) as well as trophoblastic origin (10, 11).

Although the rat decidua secretes a decidual luteotropin that resembles prolactin (15), the trophoblast of both the rat and mouse produces other PRL-like hormones known as placental lactogens (10, 11). The embryonic portion of the rodent placenta is also reported to secrete an LH-like hormone (29–31) capable of stimulating androgen production (32), although evidence for an human chorionic gonadotropin-like gene in rat genomic DNA is lacking (33, 34).

In the present study, reciprocal transfer of embryos from strains of mice that differ in progesterone production was used to partition decidual from embryonic stimulation of ovarian progesterone biosynthesis at midpregnancy. This model allowed for an examination of the contribution of maternal and embryonic genotype to luteotropic control of the maternal ovaries. The results indicate that genetic factors that influence progesterone production during the period of transition from maternal pituitary to fetal-placental control seem to be regulated by the maternal rather than embryonic genotype. In mice receiving interstrain embryo transfers, progesterone concentration was correlated with maternal genotype in females carrying the same number of fetuses, at least at midpregnancy. This suggests little fetal involvement in the production of progesterone at this time. The observations that neither corpora lutea number (35) nor fetal number greater than one (36) are related to plasma progesterone concentration during mouse pregnancy provide indirect support for this concept.

Since the major source of progesterone during mouse pregnancy is the ovary (37), and by midpregnancy ovarian progesterone secretion becomes dependent on placental factors (5, 6), it was expected that varying the genotype of the embryo might result in an elevation or reduction of trophoblastic factors which in turn may alter maternal levels of progesterone. A concurrent elevation or reduction of maternal progesterone levels consistent with the genotype of the fetus would suggest fetal-placental regulation of progesterone secretion. Such a finding would lend support for the existence of a mouse chorionic gonadotropin, the activity of which is elevated by Day 9 of pregnancy (32–34). If this putative placental hormone does play a luteotropic role during gestation, it must act after midpregnancy as may be the case in the rat (38). Since mouse midpregnancy lactogen-I is also elevated at midgestation (11), it seemed possible that this PRL-like hormone was involved in stimulation of the maternal ovaries, but the present results are consistent with the report that mouse midpregnancy lactogen-I, of trophoblastic origin (11), is not a luteotropic hormone (39). In fact, it appears that the maternally derived portion of the placenta, i.e., the decidua, may produce the placental factor(s) that controls ovarian progesterone secretion at midpregnancy. The identity of this decidual component(s) is likely to resemble rat decidual luteotropin which, combined with estrogen, is able to maintain progesterone

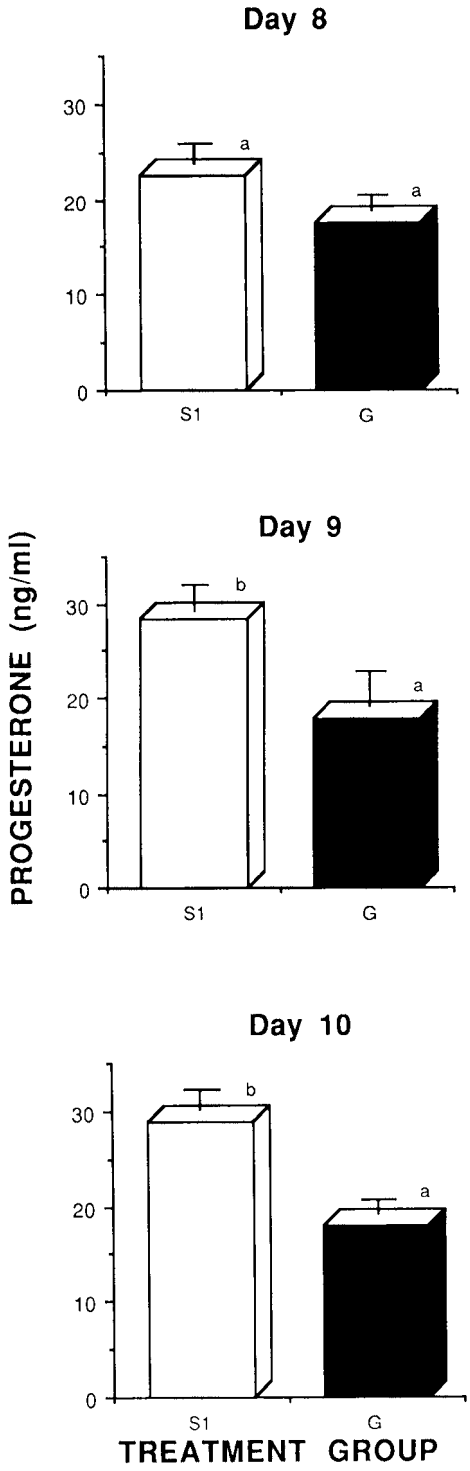

Figure 1. Plasma progesterone concentrations on Days 8–10 of pregnancy in S1 and G females following sham-embryo transfer. Bars represent the mean ± SE of seven to eight mice/line/day. Means not sharing a common letter are significantly different ($P \leq 0.05$).

production in the absence of the maternal pituitary (6). At present, it would appear that trophoblastic secretions do not direct progesterone production at midpregnancy in the mouse.

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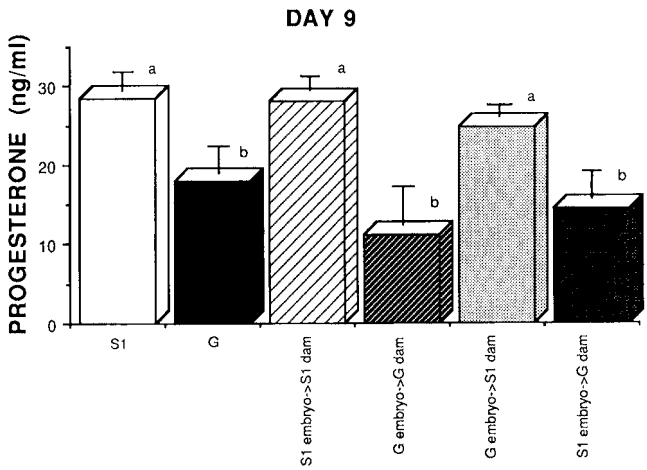

Figure 2. Plasma progesterone concentrations on Day 9 of pregnancy in S1 and G females following reciprocal embryo transfer or sham surgery. Bars represent the mean ± SE for seven to eight mice/treatment group. Means not sharing a common letter are significantly different ($P \leq 0.05$).

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