The Influence of Fetal Number on Maternal Concentrations of Progesterone and Testosterone in the Mouse (43263)

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Abstract. **Mice selected for large litters were used to study the relationship between fetal number and maternal progesterone and testosterone concentration. Experimental adjustment of conceptus number demonstrated that the presence of one fetus was sufficient to elevate maternal progesterone concentration above that found in females from which all conceptuses were removed. When one of more fetuses occupied the uterus, maternal progesterone concentration did not increase with increasing conceptus number. In contrast, maternal testosterone concentration was positively correlated with the number of fetal-placental units.** [P.S.E.B.M. 1991, Vol 197]

Progesterone production by the corpora lutea (CL) of rodent pregnancy is controlled by a number of hormones. At the outset of mouse gestation and until Day 5, prolactin stimulates progesterone biosynthesis, but on Days 6–9, luteinizing hormone is required for progesterone production (1, 2). This dependency on luteinizing hormone is transient and terminates at midpregnancy when placental production of luteotropic hormones is sufficient to maintain luteal function (3, 4).

The endocrine transition from pituitary to fetal-placental regulation of progesterone production is associated with intraluteal aromatization of androgen to estrogen, the estrogen then acting as a luteotropic hormone (5–7). Androgen production is initially maintained by luteinizing hormone (7), but if hypophysectomy is performed after midgestation, placental products can support androgen biosynthesis (8). The identity of these products is unknown, but they have demonstrated human chorionic gonadotropin-like activity (9–11). During the second half of rodent pregnancy, androgen biosynthesis occurs predominantly in the placenta (12, 13), unlike the production of estrogen and progesterone, which are produced by the ovaries throughout gestation (14, 15). These placental androgens provide substrate for ovarian estrogen production and the maintenance of luteal progesterone secretion (16, 17), thereby forming a fetal-placental-ovarian unit in the rodent (18).

The endocrine unit comprised of the conceptus and maternal ovaries seems to be influenced by genetic factors. Conceptus number is positively associated with progesterone secretion in C3H mice (19), but in the Rockland-Swiss strain, both fetal and CL number are independent of maternal progesterone concentration (20). Similarly, maternal progesterone secretion is not related to the amount of luteal tissue in randomly bred albino mice, nor is it associated with fetal number when more than one conceptus is present (21). In contrast, maternal testosterone concentration increases with increasing litter size in this randomly bred strain (21).

The observed genetic variation between fetal-placental number and progesterone production, coupled with the paucity of information about the relationship of conceptus number to androgen secretion, prompted the present investigation. Mice selected for large litter size (Line S1) were chosen for study. The results indicate that progesterone biosynthesis is not influenced by the number of viable conceptuses even when only one fetal-placental-ovarian unit is present. Unlike progesterone production, testosterone biosynthesis is increased when greater than 10 conceptuses are carried.

Materials and Methods

Animals. The mice used in this study were developed from a cross of four inbred strains (C57BL/6,

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AKR/J, C3H/J, and DBA/2). A randomly bred control line was maintained throughout the selection of various lines for reproductive traits. Line S1 was selected for large litters (22) and carried as a closed, noninvolved population for over 60 generations since separation from a common base population. As a correlated response to selection for large litter size, Line S1 has responded with an increased ovulation rate (Table I) and increased maternal concentrations of progesterone and testosterone (24).

At approximately 8 weeks of age, virgin females of Line S1 were placed three per cage with an S1 male. Females were checked daily for the presence of a copulatory plug (Day 0 of pregnancy). After a plug was detected, the bred female was housed individually or with other females that had mated during the same period. Animals were maintained at 21–23°C and illuminated for 14 hr/day (lights on from 0500–1900 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.

Adjustment of Conceptus Number. On Day 7 of pregnancy, females with at least 10 conceptuses were subjected to litter size manipulation. Under anesthesia with Avertin (0.1 ml/5 g body wt), the uterus was accessed via a midventral laparotomy. A small incision was then made on the antimesometrial surface of a section of uterus corresponding to an implantation site. The implantation site was opened by piercing to a depth of 2 mm followed by expansion with fine needle-nosed forceps. The conceptus was then removed and a small amount of sterile K-Y jelly was applied to the incision site to retard adhesion formation. Animals in which all products of conception were removed at the time of surgery served as a control group for females permitted to carry 1, 2–4, 5–9, or ≥10 conceptuses.

Sample Collection. Blood samples were obtained following rapid decapitation between 1000 and 1200 hr on Day 17 of pregnancy. This is the time when maternal concentrations of both progesterone and testosterone are maximal in Line S1 (24). Trunk blood was collected over heparinized funnels. After refrigerated centrifugation (20 min at 1000 *g*), the plasma was recovered and stored at −20°C until assayed for progesterone and testosterone. Immediately after decapitation, the reproductive tract was removed, the number of conceptuses recorded, and all products of conception submersed in ice until frozen prior to being discarded.

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 number ± SE		Prenatal survival (N/CL)
		Ova or corpora lutea[b] (CL)	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

[a] Composite data from Barkley and Bradford (23).
[b] In females mated at 8–10 weeks of age; *n* = 24–58 animals per line.
[c] Based on approximately 100 females per line also mated at 8–10 weeks of age.

Radioimmunoassay. Plasma progesterone was quantified using the method of Bosu *et al.* (25). 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. 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*; New England Nuclear, Boston, MA). Progesterone for standards was obtained from Calbiochem-Behring Corp. (La Jolla, CA). Separation of the free from the bound progesterone was performed using a 1-ml mixture of Dextran T70 (0.25 g/liter) and charcoal (2.5 g/liter). 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.

Radioimmunoassay of testosterone was performed on 0.5-ml aliquots of plasma from individual animals according to the procedure described previously (26). Samples were extracted with anhydrous diethyl ether (freshly opened) and the extracts were immersed in an acetone/solid CO_2 bath, the ether phase was decanted into a culture tube, and the extracts were evaporated to dryness. This method consistently extracted 90–95% of the plasma testosterone. The dried extracts were suspended in phosphate-buffered salines. The antiserum to testosterone (S250) was obtained from Dr. G. D. Niswender. Cross-reaction of S250 with 5α-dihydrotestosterone and androstenedione is 50% and 1.0%, respectively (27). The concentration of 5α-dihydrotestosterone in female mouse plasma is negligible during pregnancy (26). For this reason, chromatographic separation of androgens in plasma was not performed. The antiserum was used at a dilution of 1/130,000 which consistently bound 40% of [1,2,6,7,16,21-^{3}H]testosterone (sp act 135 Ci/m*M*; New England Nuclear). Testosterone used as standard was purchased from Calbiochem-Behring Corp. Separation of the free from the

bound testosterone was performed using a 1-ml mixture of Dextran T70 (0.25 g/liter) and charcoal (2.5 g/liter). The standard curve ranged from 5 to 100 pg/tube. Assay blanks (phosphate-buffered saline) averaged 5 pg. All sample determinations were performed in the same assay.

Data Analysis. Data were subjected to analysis of variance and post hoc comparisons were conducted with Tukey's Studentized range test. Only differences between means at $P \leq 0.05$ were considered significant. Correlations between plasma hormone concentrations and fetal number were determined by regression analysis.

Results

Progesterone concentrations in females bearing different numbers of viable fetuses are shown in Figure 1. As would be expected, progesterone concentration in control animals from which all conceptuses were removed (complete conceptectomy) was significantly ($P \leq 0.01$) lower than in animals bearing one or more fetuses. No other differences in progesterone secretion were found. Regardless of fetal-placental number, progesterone concentration on Day 17 was similar in females carrying from 1 to ≥10 fetuses, i.e., the regression of plasma progesterone on fetal number in pregnant animals was 0.36 ± 0.31 ng/ml ($r = 0.20$, not significant).

Testosterone concentration in Line S1 females following manipulation of conceptus number is shown in Figure 2. When complete conceptectomy was performed, testosterone concentration was significantly ($P \leq 0.01$) lower than when two or more fetuses were left to develop until Day 17. When only one fetus was present, systemic levels of testosterone did not differ from those of nonpregnant controls or females carrying two to nine viable fetuses. However, the presence of ≥10 fetuses resulted in a maternal testosterone concentration significantly ($P \leq 0.01$) elevated above that found in animals with only one conceptus. The regression of plasma testosterone on fetal number during pregnancy was 6.6 + 1.9 pg/ml ($r = 0.53$, $P \leq 0.01$).

Discussion

Experimental adjustment of conceptus number has demonstrated that progesterone biosynthesis is inde-

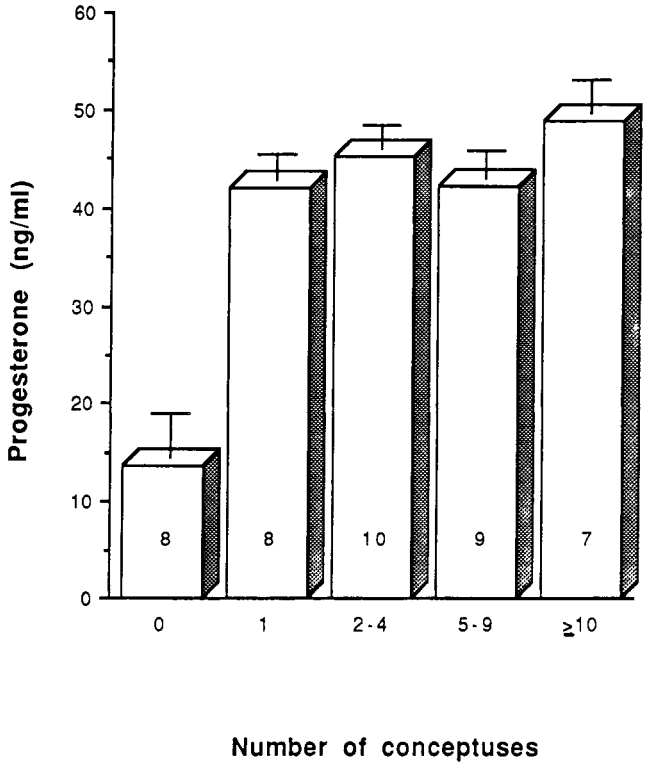

Figure 1. Maternal progesterone concentration following experimental manipulation of conceptus number in Line S1 mice. The columns and vertical bars represent the mean and SE, respectively, for each treatment group. The number of mice is given within each column. Complete conceptectomy reduced progesterone concentration ($P \leq 0.001$). The regression of plasma progesterone on fetal number = 0.36 ± 0.31 ng/ml ($r = 0.20$, not significant).

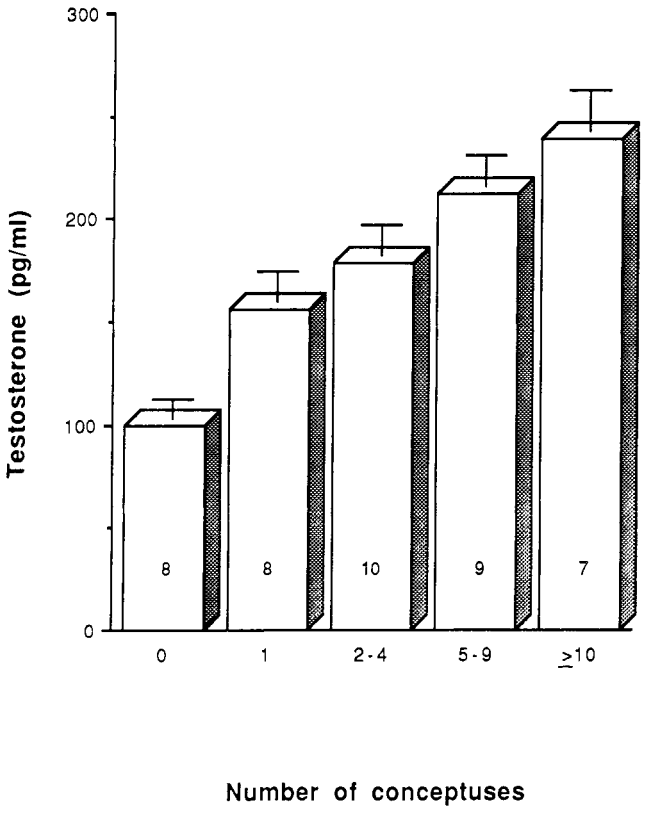

Figure 2. Maternal testosterone concentration following experimental manipulation of conceptus number in Line S1 mice. The columns and vertical bars represent the mean and SE, respectively, for each treatment group. The number of mice is given within each column. Testosterone levels were reduced following complete conceptectomy ($P \leq 0.01$). When ≥10 fetuses were carried, testosterone concentration was higher than when only one fetus was present in the uterus ($P \leq 0.01$). The regression of plasma testosterone on fetal number = 6.6 ± 1.9 pg/ml ($r = 0.53$, $P \leq 0.01$).

pendent of fetal number in mice selected for large litter size (Line S1). This suggests that, during the second half of pregnancy, the set point for ovarian progesterone production is not simply a function of the amount of placental luteotropic stimulation. In contrast, testosterone production is correlated with the number of viable conceptuses present in the uterus during late gestation.

The lack of association between progesterone production and fetal number in Line S1 was also found in Rockland-Swiss mice bearing 7–15 conceptuses (19) and in albino mice carrying 2–14 fetuses following embryo transfer (21). In the latter study, 1–18 embryos were transferred unilaterally into one uterine horn per pseudopregnant recipient (21). This animal model resulted in a reduced maternal progesterone concentration when only one fetus was present. In the present study, conceptus number was manipulated on Day 7 following the establishment of a normal pregnancy. In this case, progesterone production was not reduced if only one conceptus was allowed to develop undisturbed.

The manipulation of fetal number using embryo transfer provided a means of varying litter size independently of corpora lutea number. In agreement with an earlier report (19), CL number was not related to progesterone concentration following embryo transfer (21). Testosterone production was also independent of CL number, but androgen level was positively associated with litter size (21). With some important differences, a similar relationship between androgen production and fetal number was found in mice selected for large litters (Line S1). The presence of at least two viable conceptuses was required to elevate maternal testosterone concentration to a level distinguishable from that of a nonpregnant Line S1 female. Furthermore, androgen concentration increased as litter size increased, consistent with recent observations in the rat (28). This may be explained in part by the ability of the rat placenta to secrete an increased amount of androgen following litter size reduction (8). The effect of litter size on androgen concentration could also reflect the increased placental mass of large litters, androgen production taking place in the giant cells of the mouse trophoblast (29). It is also possible that fetal-placental substances that specifically influence placental androgen production are involved, e.g., conceptus number may be associated with the amount of stimulation provided by putative human chorionic gonadotropin-like product(s) (9–11).

The dissociation of progesterone from testosterone production during late pregnancy is not surprising given the different sources of these hormones after midgestation. Although the ovaries secrete most of the progesterone found in the circulation (15), after Day 12 of rat pregnancy the placenta preferentially produces androgen and becomes the predominant site of androgen biosynthesis (13). The impairment of luteal androgen production at this time is of no consequence because placental androgens are utilized by the ovaries as a precursor for estradiol biosynthesis (30). Estradiol in turn can influence the formation of placental androgen, resulting in the regulation of substrate availability (31).

Ovarian progesterone production during pregnancy is regulated at many levels. After midgestation, placental hormones, notably rat placental lactogen-I (rPL-I), are required to support the actions of estradiol, presumably by providing prolactin-like maintenance/formation of estradiol receptors in luteal cells (30). Prolactin in combination with testosterone or estradiol, also increases both mRNA and protein content for luteal aromatase by Day 15 of rat pregnancy (32). In contrast, both cholesterol side chain cleavage (P450scc) mRNA and protein content are constitutively maintained throughout rat pregnancy despite changes in placental production of luteotropic hormones and serum progesterone concentrations (33). The overall rate of progesterone production during pregnancy thus appears to depend on the availability of cholesterol substrate and its transport to the active site of P450scc in the inner mitochondrial membrane, both of which are influenced by estradiol (30). Indirectly then, maintenance of luteal estradiol receptor content by rat placental lactogen-I and induction of P450arom mRNA and protein by rat placental lactogen-I and placental androgen influence luteal progesterone production by affecting the availability and actions of estradiol.

In a previous study of S1 mice, maternal rather than fetal gene expression determined the set point for luteal progesterone production during the period of transition from maternal pituitary to fetal-placental control of ovarian steroidogenesis (34). Whether the same or similar genetic controls influence progesterone production during late gestation has not been determined. The inconsistent relationship between conceptus number and maternal progesterone concentration in C3H versus other strains of mice may reflect genetic variation in the regulation of ovarian steroidogenesis. Mice selected for small litter size (Line CN-) have lower gestational levels of progesterone than a randomly bred control line (35), yet maternal progesterone concentration during pregnancy is similar in CN- and S1 mice despite substantial differences in litter size and a common origin from a four-way cross of inbred strains (24). Furthermore, restriction fragment length polymorphisms have been identified in these and other inbred strains at both the P450scc (36) and the *P450arom* locus (36, 37), structural gene loci encoding two of the cytochrome P-450 enzymes on which steroid production is dependent. Since several strains of mice have different alleles at the 450scc and *P450arom* loci, our laboratory is investigating the role of these alleles in the genetic control of steroidogenic function.

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