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REGULATION OF TESTOSTERONE SYNTHESIS IN THE FETAL MOUSE TESTIS

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SUMMARY

Plasma testosterone (T) levels were determined by radioimmunoassay in male and female mouse fetuses from day 13 (1 day after sexual differentiation) to day 18 (1 day before parturition). T levels in plasma of male fetuses were significantly higher than those of female. In male fetuses, plasma T levels rose from day 13 to a peak level at 16–17 days, before declining on day 18. On the other hand, large amount of progesterone (P) was present in blood of pregnant mice over the observed period. This finding may indicate that progesterone of maternal origin is available as a potential precursor for T biosynthesis by the fetal testis. However, the dissociation between maternal P and fetal T levels between day 15 and day 16 of gestational age may suggest a more complex regulation of fetal T synthesis.

INTRODUCTION

According to Jost[1] the differentiation of the Wolffian duct, urogenital and genital tubercle could be induced by androgens secreted by the fetal testis. It has been demonstrated that the fetal testis of rodents could synthetize testosterone *in vitro* [2, 3], but only few experiments have been carried out to evaluate the levels of this androgen in the fetal blood.

Moreover, despite an *in vitro* approach, *in vivo* gonadotropic regulation of fetal testosterone synthesis is presently unclear. Although testosterone production could be controlled by gonadotrophins from fetal pituitary gland and/or from placental tissue [4–6] during the latter stages of male development, there is compelling evidence that the initiation of androgens synthesis would be independent of hormonal control [7–9].

In the present work, we attempted to determine the temporal changes in the levels of maternal progesterone and fetal testosterone in the mouse from day 13 (first day when fetal testes are recognizable) to day 18 (one day before parturition) in order to investigate a possible contribution of maternal progesterone in fetal testosterone synthesis.

MATERIAL AND METHODS

Animals

Albino Swiss female and male mice (five females and two males) were mated for one night. Fertilization was verified by the presence of vaginal plugs on the following morning (this day was designated as day 0.5 of gestational age). From day 13 to day 18 of pregnancy, groups of mice (five females per group) were killed by decapitation. Blood was collected into heparinized tubes, centrifuged and plasma was stored at −20°C until processed.

Subsequently, fetuses were exteriorized. Blood of male and female fetuses was respectively collected, after determining the gonadal sex of each fetus by dissection under a magnifying glass.

Radioimmunoassay of progesterone

Progesterone levels in pregnant mice peripheral blood were determined by an RIA method developed in our laboratory [10]. Progesterone was directly assayed in 50 μl of plasma by means of an antiserum raised against progesterone-11-NH-bovine serum albumin (gift from laboratoires Roussel-Uclaf). The cross-section with 5α-dihydroprogesterone, 20β-hydroxyprogesterone and 20α-hydroxyprogesterone was 44, 0.2 and 1.2% respectively.

Radioimmunoassay of testosterone

Radioimmunoassay of testosterone was performed on pools of fetal plasma as indicated in Table 1. The method used for radioimmunological analysis of testosterone has been described previously [11]. The antiserum was obtained from rabbits by injection of testosterone-3-(O-carboxymethyl)-oxime bovine serum albumin.

The significance of difference between means was calculated using unpaired *t*-test, with a P value of $\leqslant 0.05$ regarded as significant.

RESULTS

Plasma progesterone levels in pregnant mice

The levels of plasma progesterone in pregnant mice

Table 1. Experimental protocol for measurement of foetal plasma testosterone

Fetal age in days	Sex	Minimal and maximal number of fetuses used for assay	Number of pools measured
13	M	111–153	2
	F	136	1
14	M	55–132	5
	F	77–121	3
15	M	28–56	4
	F	53	1
16	M	33–73	4
	F	37–63	2
17	M	20–48	5
	F	34–43	3
18	M	18–40	3
	F	15–33	2

over the observed period are presented in Fig. 1. Progesterone levels showed a slight and statistically non significant increase from day 13 to day 15, and thereafter decreased significantly on day 16 ($P < 0.05$), before rising sharply on day 17. At this time, progesterone levels reached the values observed on day 15. By day 18 (1 day before parturition) plasma progesterone concentrations fall significantly ($P < 0.001$). The large standard error of the mean calculated for each gestational age, may reflect the difficulty to obtain age matched pregnant mice into each group. For this reason, it is conceivable that slight changes in progesterone levels, such as the increase observed from day 13 to day 15, might be partially masked.

Plasma testosterone levels in male and female fetuses

Testosterone levels in male and female fetuses are shown in Fig. 2. Male fetal plasma contained higher concentrations of testosterone than that of the female from day 13 to day 18 of fetal age. In female fetuses, testosterone levels remained low, without significant change during the period studied. In contrast in male fetuses, testosterone levels showed a significant increase between the 13th and 17th day, and then dropped markedly on the last day of fetal life ($P < 0.001$).

DISCUSSION

Our results demonstrate that during genital organogenesis the male mouse fetus contains high circulating testosterone levels. These observations are in agreement with those reported in human [12], rhesus monkey [13], pig [14], but differ from those obtained in rabbit [15]. The observed sex difference in the quantities of testosterone in peripheral blood of fetuses confirm our preliminary results [16], suggesting that the fetal testis is obviously the source of the high levels of testosterone measured in the male.

Our data indicates also that high levels of progesterone are secreted into the systemic circulation during gestation in the mouse. Similar results have

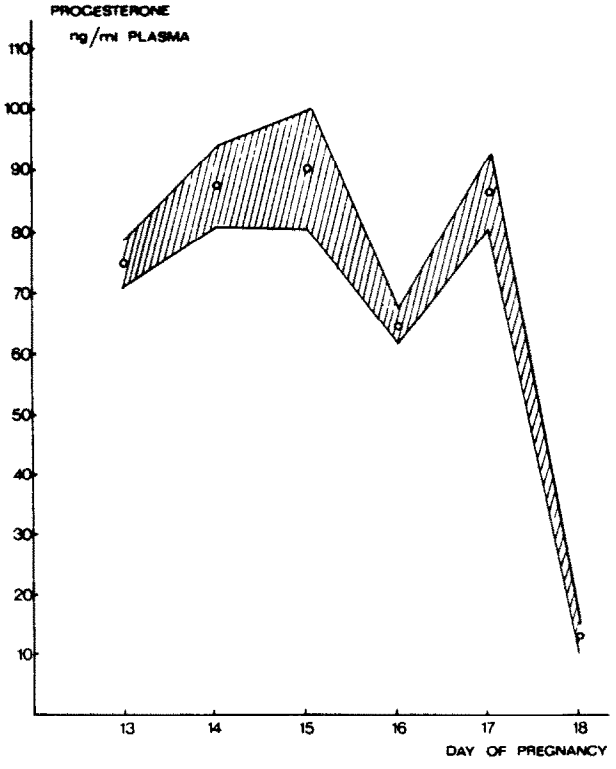

Fig. 1. Progesterone levels, expressed as means (circles) ± standard errors of the mean (shaded areas) in plasma of mice from day 13 to day 18 of pregnancy.

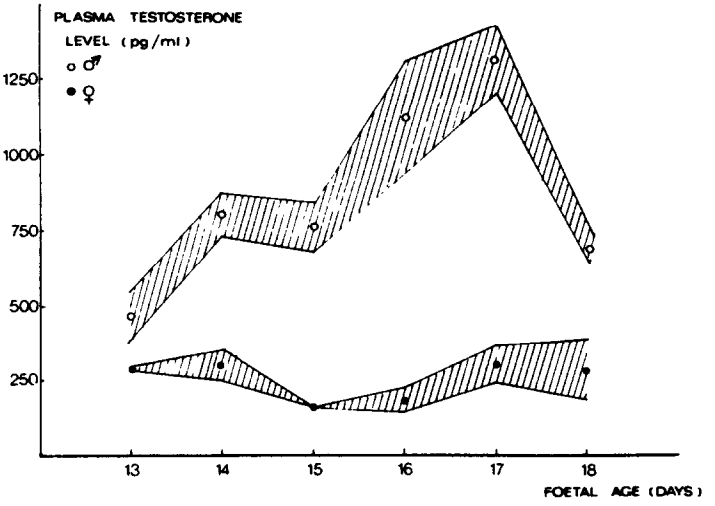

Fig. 2. Mean plasma testosterone levels in male (○) and female (●) mouse foetuses from day 13 to day 18 of gestation. Shaded areas indicate standard error of the mean.

been previously reported by other workers [17] and were confirmed in the present study. Since large quantities of maternal progesterone may reach the fetal compartment at this time, it seems reasonable to assume that the fetal testis has the capacity to synthetize testosterone from this C21 steroid. This is strongly supported by the fact that the fetal mouse testis acquires the essential enzymes necessary for testosterone synthesis just prior or at the onset of sex duct differentiation [18]. It is also clear that the fetal male gonad is capable to metabolize exogenous progesterone into testosterone *in vitro* as well as *in vivo* when progesterone is administered to pregnant mice [16]. These previous results are in agreement with the possibility that maternal progesterone serve as a precursor for fetal testosterone synthesis during the first steps of sexual differentiation. However, the testosterone levels increase between day 15 and day 16 of fetal life, although maternal progesterone levels drop, may indicate a more complex regulation of testosterone synthesis during this period. It is not clear at present whether other potential sources of testosterone are available for testosterone formation by fetal testes. But there is considerable evidence that testicular function may be gonadotrophin dependent during late fetal life. This hypothesis is strongly supported by the presence of LH in both plasma and pituitary glands of mouse fetuses [16].

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