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Androgen Level in the Sheep Fetus During Gestation[1] (38818)

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The knowledge of endocrine function in the fetus is less complete than that for the adult, yet it is known that several fetal endocrine systems operate in a similar fashion to that of adults (1). It has been noted that hypophysectomy of the fetus impairs testicular growth (2) and that Gn-RH will release LH from the pituitaries of sheep fetuses (3). Furthermore, the testis of the fetal rat or rabbit is capable of synthesizing testosterone (4, 5), and testosterone is present in the gonad of the fetal ram lamb (6). Thus, several components of the CNS–pituitary–gonad axis may operate in the fetal lamb.

In an effort to characterize further this endocrine control system, we have measured the testosterone content of various fetal compartments. An attempt has been made to understand these findings in relation to the known changes in fetal lamb plasma and pituitary LH during gestation (7, 8).

Materials and Methods. Singleton fetuses were obtained from 107 ewes of mixed breeding within 30 min of slaughter. Samples of amniotic fluid, plasma, (cardiac puncture and umbilical drainage), and gonads were obtained and frozen on dry ice. The crown–rump length of each fetus was used as an index of fetal age (9). Each animal was assigned to one of eight stages of fetal development, corresponding to 4-cm increments of length. Testosterone content of plasma and amniotic fluid were measured by radioimmunoassay using this laboratory's modifications (10) of the technique reported by Bartke *et al.* (11). This assay has been validated for sheep plasma by Sephadex LH-20 column chromatography and purification had little effect upon the amount of plasma testosterone measured. Thus, the plasma values reported here were derived from unchromatographed samples. After weighing, gonads were dispersed in phosphate-buffered saline by ultrasound. Ethyl ether extracts of this sonicate were used for subsequent androgen analysis. LH-20 chromatography of selected testicular samples showed an unpredictable discrepancy of varying magnitude (78–123%) compared with the nonpurified replicate. The reason for this nonidentity is not yet certain and to reflect this we have expressed our results as mass of testosterone-5α-dihydrotestosterone (T-DHT) in aliquots of unchromatographed testicular homogenates. All samples were assayed in duplicate and the results interpolated by computer. Recovery of added testosterone exceeded 90%. The intra-assay coefficient of variation (CV) was 6%, the interassay CV was 11%, and the limit of sensitivity was 13 pg of testosterone.

Results. Ovaries of fetal lambs of any gestational age were totally devoid of activity in the T-DHT assay. Amniotic fluid from either sex contained levels of androgen below the sensitivity of the assay; indicating that less than 13 pg/ml was present. The changes of testicular weight and T-DHT content are shown in Fig. 1. The total testosterone/testis declined during the last two-thirds of gestation (fetal length >15 cm). The combined effects of increasing testicular weight and decreasing androgen content led to a marked depression of testicular T-DHT concentration during the last 20 days of gestation ($P < 0.01$).

Testosterone was detected in the plasma of male or female fetuses and the findings are indicated in Fig. 2. In spite of a great variation in the testosterone values for individuals of similar gestational age, certain trends are evident. Testosterone concentration in the plasma of females increases about 4-fold during the last two trimesters ($P <$

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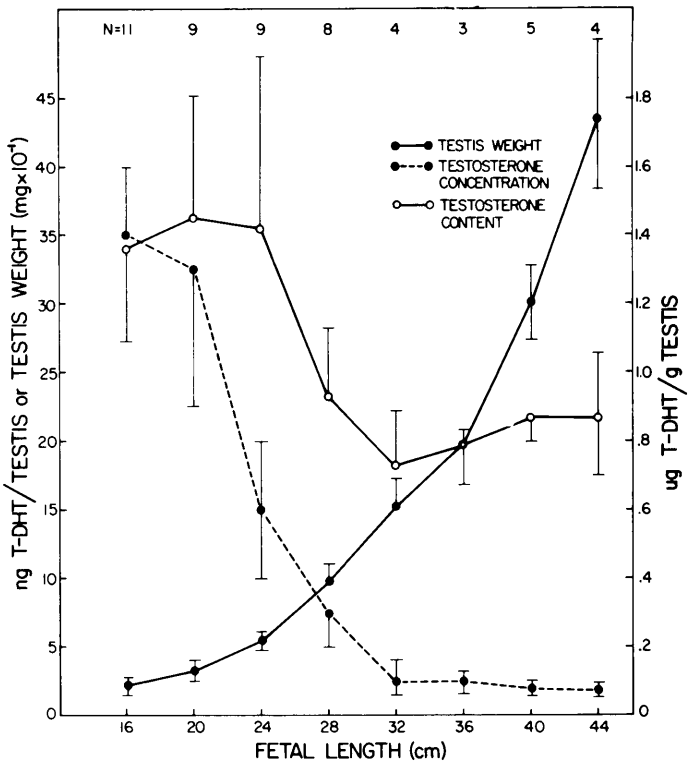

FIG. 1. Changes in testicular weight and androgen content in the male sheep fetus during the last 80 days of gestation. Fetal lengths of 16 and 50 cm correspond to approximately 70 and 150 days of gestation. Each point is the mean ± SE of number of individuals ± 2 cm of the designated length.

0.05) as fetal length increased from 18 to 50 cm. The situation in the male is contrasted by rather high plasma testosterone concentrations at the onset of the second trimester (658 ± 145 pg/ml). These values then fall to 279 ± 51 pg/ml, comparable to those observed in the female. The subsequent testosterone values then rise in a pattern parallel to, but of greater magnitude ($P < 0.05$) than those of the female. The plasma testosterone concentrations of near-term males (>42 cm) were not different from those of males of about 80 days of gestational age (16 cm).

Discussion. One milliliter of amniotic fluid contained barely detectable amounts of androgen and thus, no sex difference could be ascertained. This does not appear to be a reliable way of predicting fetal sex.

The testicular weights reported here agree with those reported by Attal (6) and Foster *et al.* (7). The report by Attal also included some measurements of gonadal testosterone, which fell within the ranges observed here. However, his sample was small, characterized by great variation, making interpretation of the data difficult. The increased number of samples measured in the present study revealed certain clear patterns. A combination of increasing gonadal weight and decreasing testicular T-DHT content yielded a marked decline of testicular androgen concentration as gestation proceeded. The reason for this phenomenon is obscure, but perhaps the known decrease in circulating fetal LH during the last half of pregnancy is responsible (7, 8). No information is available on FSH levels.

The relatively elevated concentrations of plasma testosterone in the 16-cm male is compatible with the notion that the sexual differentiation of the brain may take place quite early in fetal life (12). Apparently the secretory activity of the Leydig cells wanes after this time in a manner similar to that described by Murphy and Diez D'Aux (13)

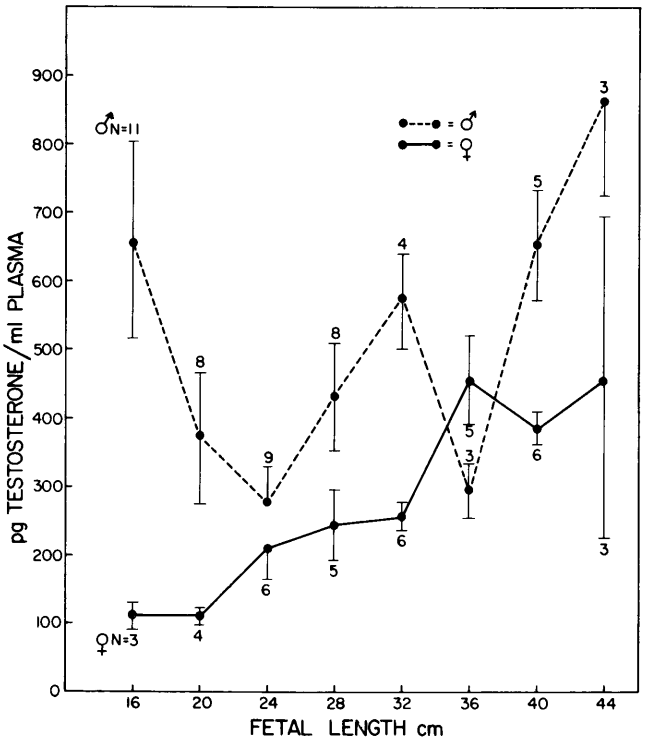

FIG. 2. Changes in plasma testosterone of male and female sheep fetuses during the last 80 days of gestation. Estimates of fetal age as in Fig. 1. Each point represents mean ± SE of number of individuals.

for the human testis. After this testosterone decline, secretion apparently recovers and climbs steadily through the remainder of gestation. At no time during gestation did fetal plasma testosterone approach the 1 ng to 20 ng/ml levels found in the adult ram (14, 15). The testosterone pattern during gestation is different from that observed in the fetal calf (16) in which androgen concentration in plasma was constant or decreasing during gestation.

The origin of fetal plasma testosterone is uncertain. Although the consistently higher levels of testosterone in males compared to females argues for testicular secretion, such a thesis assumes equal nongonadal testosterone secretion. This latter assumption is unproved. That nongonadal testosterone may be an important source of the plasma pool is implied by three observations. Because the fetal female ovary never contains testosterone during gestation, the adrenals of the female fetus or the placenta probably secrete testosterone at steadily increasing rates to account for the blood levels observed in the present study. Secondly, LH infusion into male or female fetuses does not alter circulating androgen levels (Pomerantz, Foxcroft, and Nalbandov, unpublished observations). Thirdly, testicular testosterone decreases markedly during gestation, not being consistent with rising plasma levels. Thus, it is possible that only a minimal level of gonadal testosterone secretion occurs during the later portions of intrauterine life.

Considering both the magnitude and pattern of change of androgen levels in both sexes during gestation, the following may be suggested. The testis secretes androgen vigorously early in fetal life, this activity diminishes by midgestation to a low, but finite, secretory rate until puberty. Nongonadal androgen secretion becomes progressively greater in the fetoplacental unit of both sexes as gestation proceeds. The fetal adrenal might be the source of this androgen.

The physiologic relationship between the

androgen levels reported here and earlier work on fetal LH (7, 8) is at best tenuous. A biphasic pattern in circulating LH was apparent in males and females. A zenith was noted at about 100 days of gestation with the lowest values at 55 days and 150 days. The pattern for LH is out of phase with circulating testosterone in the ram lambs and could indicate a negative feedback effect of testosterone upon LH secretion. However, the same biphasic LH pattern was observed in female fetuses that show steadily increasing testosterone levels during gestation. Therefore, there may be sex differences in feedback effect of testosterone in males and females or the changes of the two hormones may be unrelated. Additional experimental work is necessary before the observations of normal fetal hormone levels can be correlated and the physiologic significance of those findings appreciated.

Summary. The androgen content of amniotic fluid, plasma, and gonads from 107 fetal lambs was determined by radioimmunoassay in an attempt to understand the ontogeny of gonadal function. Testosterone (T) was too low to be reliably measured in the amniotic fluid from fetuses of either sex. Ovaries were without T activity at an ofy the stages of gestation studied. Testicular T-5α-dihydrotestosterone (T-DHT) concentration steadily decreased from 1.4 $\pm$ 0.2 to 0.08 $\pm$ 0.01 μg T-DHT/g testis during the last 80 days of gestation. This was due to both increasing gonadal size as well as a decline in the absolute amount of T-DHT/testis. T was detected in the plasma of fetuses of both sexes. In females, levels rose steadily from 112 $\pm$ 20 to 459 $\pm$ 223 pg/ml during the last 80 days of fetal development. The pattern in the male was different in that plasma T at 70 days of gestation was 658 $\pm$ 145 pg/ml; then fell to 279 $\pm$ 51 pg/ml at about 100 days of gestation. Plasma T then rose to 866 $\pm$ 141 pg/ml near term. It is suggested that nongonadal testosterone production increases during fetal life and that T secretion by the fetal testis may contribute steadily less to the plasma pool of T as gestation proceeds.

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