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# Endocrine function and regulation of the fetal and neonatal testis

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ABSTRACT. An interesting sex difference prevails in the early development of endocrine functions in the ovary and testis. The testis actively produces androgens already *in utero* whereas the physiologically important steroid hormone production of the ovary does not start until puberty. Likewise, the different components of the hypothalamic-pituitary-gonadal axis seem to mature earlier in the male. The hormonal regulation of the fetal testes differs in many respects from that of the adult, and these differences make it possible for the fetal testes to function in the intrauterine endocrine milieu. The purpose of this review is to summarize our findings on the development, special functional characteristics and physiological role of the fetal and neonatal pituitary-gonadal axis.

KEY WORDS: *testis, anterior pituitary, gonadotropins, testosterone, gonadotropin-releasing hormone*

## Introduction

Endocrine function of the testis begins already *in utero*. Depending on the species, the testes start producing testosterone during the first (e.g. the human), or the second half of pregnancy (e.g. rodents). A clear sex difference in this respect is discernible since quantitatively important steroid production of the ovary does not start until puberty (for reviews, see Forest *et al.*, 1976; Wilson *et al.*, 1981). The fetal testis plays an active role in induction of male genital differentiation whereas the female differentiation occurs in a more autonomous fashion, independent of the role of the ovarian hormone production.

In the human, early testicular activity is stimulated by chorionic gonadotropin; in the rodent it is under pituitary regulation. Birth brings about dramatic changes in the pituitary-gonadal axis through elimination of placental hormones, and a new phase of gonadal endocrine activity is seen in the newborn boy. The physiological meaning of the fetal testicular steroidogenesis is clear (see above) but that of the postnatal testicular activity has so far remained obscure. The sequence of changes in the pituitary-testicular activity is very similar in different mammalian species (Fig. 1), and observations of different animals therefore supplement each other. In this review, we describe some of our previous and recent findings on human, monkey and rat pituitary-testicular function in the fetal and neonatal period. Special attention is paid to the development of the hypothalamic-pituitary-gonadal interactions and to understanding the physiological role of early testicular activity.

### *Steroid production of the fetal testis*

Testicular androgen production starts in the human fetus around weeks 7-8 of gestation (Siiteri and Wilson, 1974; Tapanainen *et al.*, 1981a). We initially identified testosterone and some of its precursors in a pool of human fetal testes using gas chromatography-mass spectrometry (Huhtaniemi *et al.*, 1970). Subsequently, using more sensitive radioimmunoassay methods, we detected testosterone and a number of its unconjugated precursor steroids in individual samples of human fetal testes (Fig. 2) (Tapanainen *et al.*, 1981a). It was clearly seen that androgen production was activated between weeks 6-8 of pregnancy by a preferential increase in concentrations of 3-keto-4-ene steroids. Thus, activation of the 3$\beta$-hydroxysteroid dehydrogenase enzyme seems to be the key event in the onset of androgen production. The androgen production started slowly declining after week 14, and in this case the most conspicuous change was the decrease in the $C_{19}$ steroids, indicating decreased activity of the 17-hydroxylase/C17-20 lyase enzyme activity. At the same time, we noticed sporadic high levels of estradiol in the fetal testes. This suggests that this period of development may be the time when the testis tissue attains its ability to be negatively regulated by estrogen, a prominent feature of the adult testis (Nozu *et al.*, 1981). Despite the clear decline of testosterone production at mid-gestation, it is obvious that the remaining levels of this androgen during the latter part of gestation are still physiologically significant. A similar pattern of steroidogenic activation can be observed in testis tissue of the fetal rat (Tapanainen *et al.*, 1984; Warren *et al.*, 1984). Only the timing in relation to birth differs; testosterone levels peak during the last third of pregnancy and thereafter gradually decline towards day 15 of postnatal life.

### *Development of tropic regulation of the fetal testis*

The coincidence of peaks in early fetal testicular activity and placental gonadotropin (hCG) secretion implies that this placental hormone is possibly the tropic stimulus of testicular steroidogenesis. This is even more likely since the quantitatively significant production of fetal pituitary gonadotropins does not start until the middle of pregnancy (Kaplan *et al.*, 1976; Mulchaney *et al.*, 1987). The prerequisite for responsiveness to the tropic regulation is the presence of gonadotropin receptors in the fetal gonad. Indeed, we were able to measure high-affinity binding sites of hCG in the human fetal testis during early and mid-pregnancy (Huhtaniemi *et al.*, 1977a). Moreover, physiological concentrations of hCG were able to stimulate testosterone production in fetal testicular minces in incubation. The same was shown in perfusion experiments using mid-term placental tissue and fetal testes (Huhtaniemi and Lautala, 1979). Also the testes of late-gestation rhesus monkey fetuses res-

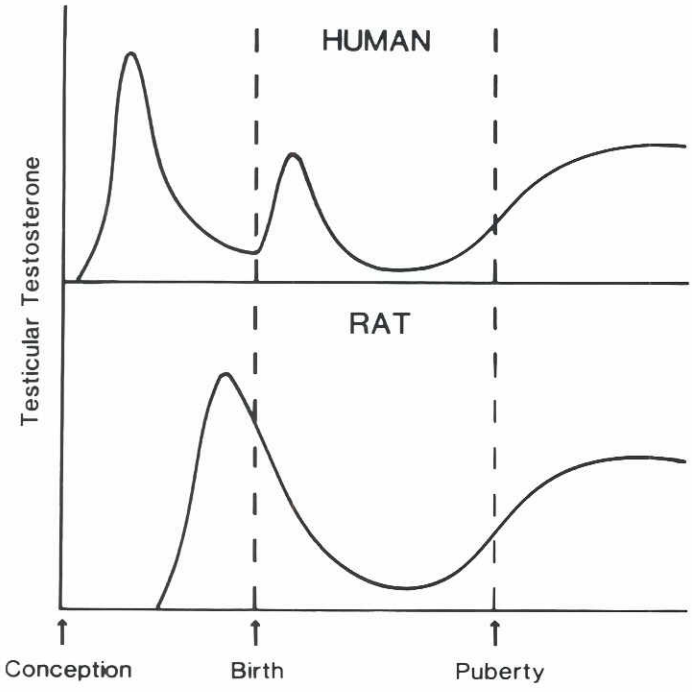


**Fig. 1.** *Schematic presentation of testicular testosterone levels in the male human (upper panel) and rat (lower panel) between conception and adult life.*

ponded to hCG stimulation *in vitro* and *in vivo* (Huhtaniemi *et al.*, 1977b).

Subsequently, we showed that the fetal rat testes also contain high levels of LH receptors which parallel the steroidogenic activity (Fig. 3) (Warren *et al.*, 1984). In this species, the fetal pituitary gonadotropin secretion is responsible for the tropic stimulus since the rat placenta does not have a chorionic gonadotropin (Carr and Chin, 1985). The fetal rat ovary is devoid of LH and FSH receptors (Huhtaniemi and Catt, 1981; Sokka and Huhtaniemi, unpublished observation), and the appearance of LH/hCG receptors in the fetal testis, making the tropic stimulation of steroidogenesis possible, is a crucial event in endocrinology of sexual differentiation.

Much less is known about the role of the other gonadotropin, FSH, in fetal development. Fetal rodent testes have FSH receptors (Warren *et al.*, 1984), but so far there is no reliable information showing whether these binding sites are functional. Even less is known about the prenatal role of FSH in the gonadal development of the human and other primates. Anencephalic fetuses have hypoplastic testicular tubules, but the ovaries seem to develop normally until at least 32 weeks of gestation (Baker and Scrimgeour, 1980).

We were able to demonstrate the presence of high-affinity FSH binding sites in the human fetal testes before mid-gestation, but none in the ovary (Huhtaniemi *et al.*, 1987). The latter part of pregnancy was monitored in rhesus monkeys, and specific FSH binding could be shown both in the fetal ovary and testis during the last third of gestation (Table 1). Hence, the FSH responsiveness also, in analogy with that of LH, seems to appear earlier in the fetal testis. In the ovary, at least, the appearance of FSH binding preceeded that of LH. We do not know yet whether the induction of receptors to these two gonadotropins is somehow interrelated.

The functional response to FSH stimulation was addressed by measuring FSH-stimulated cAMP production in human and monkey fetal gonadal minces (Huhtaniemi *et al.*, 1987). Although they showed clearly measurable FSH binding, none of them responded to FSH with increased cAMP production. The reason for the absence of the cAMP response is still obscure but some of our recent findings suggest the presence of an endogenous inhibitor of FSH-stimulated cAMP production in the testis tissue. In the adult rat testis, we were able to demonstrate an inhibitor that acts through the inhibitory Gi-protein at the site of the transmembrane signal transmission (Huhtaniemi *et al.*, 1989). It is therefore likely that the FSH action upon gonads is modulated by an endogenous inhibitor, and both of these activities undergo distinct changes during sexual maturation.

### *Development of the feedback regulation of gonadotropin secretion*

The ontogenesis of the gonadal negative feedback regulation to the hypothalamic-pituitary level is still largely unknown. The male fetus has lower gonadotropin levels than the female at mid-gestation, suggesting that the testis-pituitary axis functions at this time (Kaplan *et al.*, 1976; Mulchaney *et al.*, 1987). Detailed appearance of these functions may remain obscure in the human.

The pituitary-gonadal axis of fetal rhesus monkeys responds *in utero*, during the last third of gestation, to GnRH stimulation with increased LH and testosterone secretion (Huhtaniemi *et al.*, 1977b). Thus, at least the hypothalamic-pituitary-gonadal axis is functional at this time. Whether the feedback regulation of the hypothalamic and pituitary secretion by the testes is also functional is not yet clear. There is circumstancial evidence showing that the fetal pituitary-gonadal function is under tonic inhibition. Immediately after birth, there is an increase in gonadotropin and testicular steroid levels which is probably due to the elimination of the suppressive effect of placental steroids on pituitary secretion.

We have studied the development of gonadal negative feedback effects on the pituitary using the neonatal rat as experimental model. Obviously, the information obtained is not dir-

TABLE 1

**LH AND FSH RECEPTORS IN HUMAN AND MONKEY FETAL GONADS AT DIFFERENT STAGES OF GESTATION (Huhtaniemi *et al.*, 1977a, b, 1985a, 1987)**

| Gonad | LH binding (fmol/g tissue) | FSH binding (fmol/g tissue) |
|---|---|---|
| Early and mid-term human fetus | | |
| testis | 673-1110 | 10-37 |
| ovary | 0 | 0 |
| Late-term monkey fetus | | |
| testis | 815-2700 | 13-324 |
| ovary | 0 | 20-57 |
| Adult human | | |
| testis | 180 | 37 |

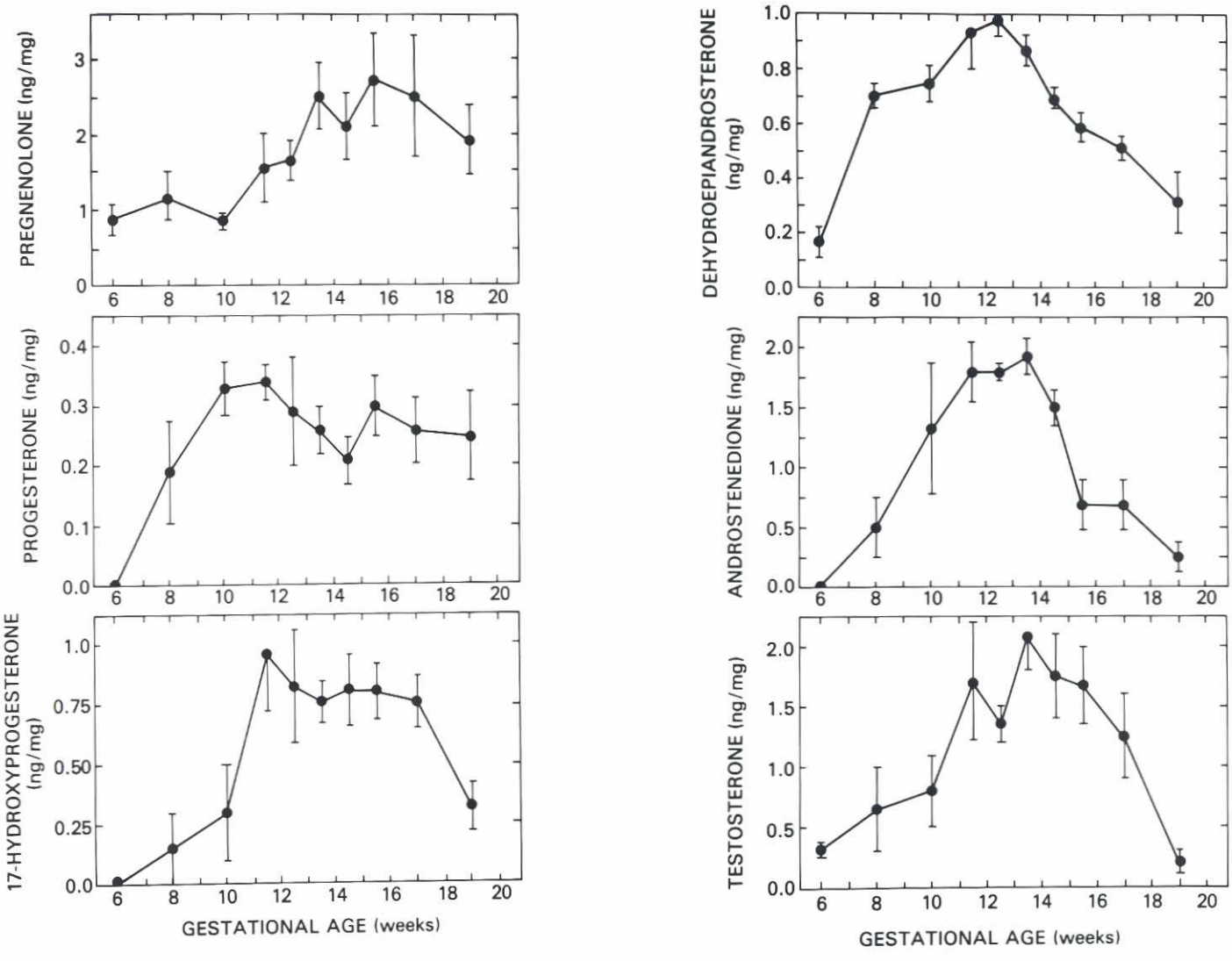


**Fig. 2.** *Concentration of pregnenolone, progesterone, 17-hydroxyprogesterone, dehydroepiandrosterone, androstenedione and testosterone in the testis tissue of human fetuses of 6-20 weeks' gestation. Each point is the mean ± SEM of 2-16 individual tissue samples, and the results were arranged in groups of 2 weeks (between 6-11 and 16.1-20 weeks) or of 1 week (between 11.1-16 weeks) (Tapanainen* et al., *1981a).*

ectly applicable to the human, but the sequence of events in the development of pituitary-gonadal functions seems to be similar in different species. Based on previous evidence our hypothesis was that the pituitary feedback regulation develops earlier in the male animals. Furthermore, as the rat is born in a relatively less mature state than the primate, the first postnatal days in the rat may well be developmentally equal to human mid-gestation, the likely period of onset of the pituitary-gonadal feedback regulation (Kaplan *et al.*, 1986; Mulchaney *et al.*, 1987).

We gonadectomized 3-day-old male and female rats and treated the males with testosterone and the females with estrogen (diethylstilbestrol) (Pakarinen and Huhtaniemi, 1988). These animals, and appropriate controls, were killed 8 days later, and their serum and pituitary gonadotropin levels, and pituitary mRNA levels for the common α-subunit and the β-chains of LH and FSH were measured. Clear post-castration increases of gonadotropins and their mRNA levels were found only in the male rats (Fig. 4). In addition, the effect of gonadectomy was reversed by testosterone in the males. The estrogen treatment was able to suppress gonadotropin secretion and gene expression in the females, indicating that the pituitary is responsive to steroids but the endogenous steroid production of the neonatal ovaries is probably too low to be effective. Although not directly applicable to the human, these observations support the hypothesis that the gonadal negative feedback effects on gonadotropin secretion appear earlier in the male.

### *Special functional characteristics of the fetal population of Leydig cells*

Two distinct growth periods of Leydig cells, termed the fetal and adult populations, are responsible for the fetal-neonatal and adult phases of testicular steroidogenesis (Roosen-Runge and Anderson, 1959; Lording and de Kretser, 1972; Tapanainen *et al.*, 1984). The fetal Leydig cell population develops and

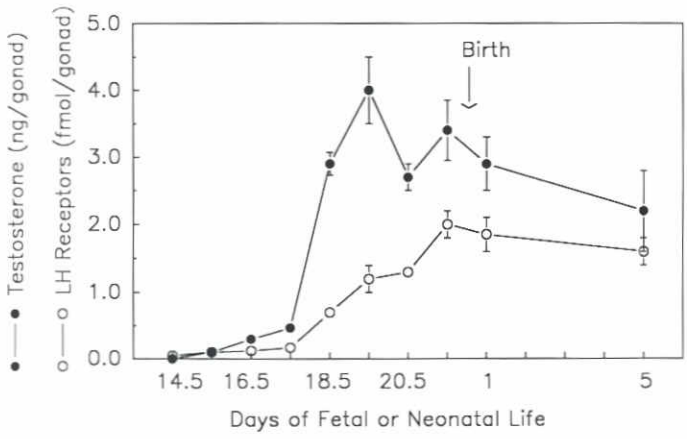


**Fig. 3.** *Contents of LH receptors and testosterone in the rat testis tissue between day 14.5 of fetal life and 5* post partum. *Each* point *is the mean ± SEM of 6 replicate determinations (Warren* et al., *1984).*

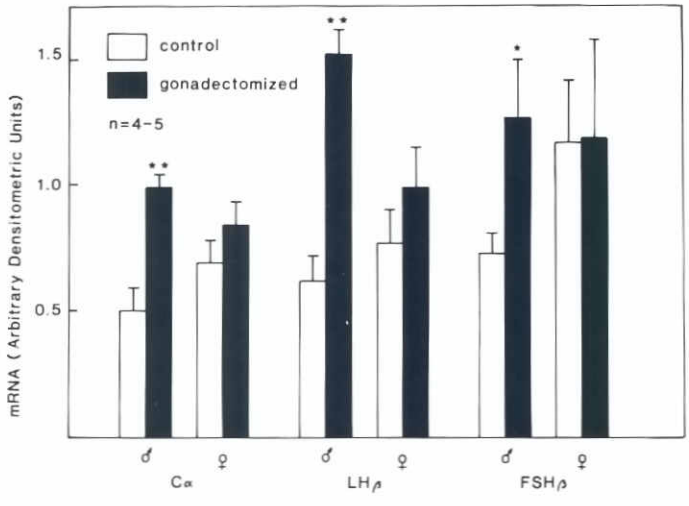


**Fig. 4.** *Messenger RNA levels of the common α-subunit (cα), and LH and FSH β-subunit in male and female rats gonadectomized at the age of 3 days and killed at the age of 11 days. The* asterisks *indicate significant increases in the mRNA levels in comparison to the respective control levels. The* bars *indicate mean ± SEM of 4-5 measurements (Pakarinen and Huhtaniemi, unpublished observations).*

functions in a very different hormonal environment from that of the adult since the fetus is exposed to high concentrations of feto-placental and maternal hormones. This is especially clear in the human where the fetus is exposed to high levels of chorionic gonadotropin, progesterone and estrogens (Huhtaniemi, 1974). Of these hormones, particularly hCG and estrogens are active regulators of the adult tesis. These actions are partly inhibitory, including LH receptor down-regulation and lesions in the androgen biosynthetic pathway (Catt *et al.*, 1980; Nozu *et al.*, 1981). If the adult testis were exposed to high intrauterine hCG and estrogen levels, Leydig cell androgen production would undoubtedly be blocked. Nevertheless, the fetal testes are steroidogenically active, and produce even more testosterone per Leydig cell than the adult testis (Tapanainen *et al.*, 1984). Therefore, the fetal testicular response to the high intrauterine hormone levels must be different from that of the adult testis in a similar hormonal environment. To test this hypothesis, we subjected neonatal (5-day old) male rats to various hormonal treatments and compared the responses to those obtained with similar treatment in the adult. This age was chosen because at that time the Leydig cells are still derived from the fetal growth phase (Lording and de Kretser, 1972; Tapanainen *et al.*, 1984).

When the rats were injected with a large dose of hCG (600 IU/kg B.W.), LH receptor occupancy and a very short (1-2 days) period of loss of free LH receptors were observed (Fig. 5) (Huhtaniemi *et al.*, 1981). However, the loss of binding was totally different from that observed in the adult where the same treatment decreased LH receptors for 2 weeks. When the occupancy and net loss of receptors (down-regulation) were examined in more detail, it was found that down-regulation of LH receptors was nearly totally missing in the neonatal testis.

Another response of the adult testis to high gonadotropic stimulation is the so-called steroidogenic desensitization (Catt *et al.*, 1980). In this case, high-dose gonadotropin treatment introduces a blockade in the $C_{21}$ steroid side chain cleavage step with loss of androgen production and a compensatory increase in $C_{21}$-type precursor steroids. This response is evidently mediated by further conversion of androgens to estrogens and may be a protective mechanism against too high tropic stimulation. When the neonatal testicular steroids were measured following a similar gonadotropin treatment, no evidence for steroidogenic lesions was seen (Huhtaniemi *et al.*, 1981, 1982b). In contrast, there was an increase of androgen formation and no change in the $C_{21}/C_{19}$ steroid ratio, which is a hallmark of the steroidogenic lesion in the adult testis. Unlike the adult, the neonatal testicular androgen production was also resistant to the deleterious effect of estrogen (Huhtaniemi *et al.*, 1985b).

One can speculate about the physiological significance of these functional differences between the fetal and adult Leydig

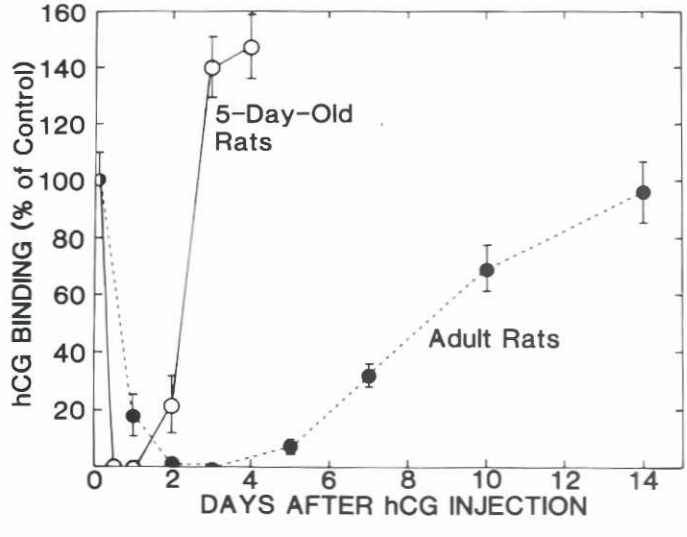


**Fig. 5.** *Comparison of effects of 600 IU/Kg injection of hCG on free LH receptors (= hCG binding) in 5-day old (0---0) and adult (●---●) rat testes. Each* point *is the mean ± SEM of measurement from 5-7 animals (Huhtaniemi* et al., *1981).*

cells. The adult testis is protected against excessive gonadotropin stimulation by two mechanisms, LH receptor down-regulation and steroidogenic lesions. The possible negative effects of high androgen production are not known. Whatever they may be, such protective mechanisms seem to be missing in the fetus and neonate. The importance of this functional feature is easier to understand. The early testicular androgen production is of critical importance for male sexual differentiation (Jost *et al.*, 1973) and this is the mechanism by which sufficient androgen production may be assured.

### *Perinatal changes in pituitary-testicular activity*

Birth brings about a dramatic shift in the hormonal environment of the developing individual. Concerning reproductive endocrinology, the most striking difference is the elimination of placental peptide and steroid hormones. Many of the placental hormones undoubtedly influence the development and function of the fetal hypothalamic-pituitary-gonadal axis. Such effects are the stimulation of testicular steroidogenesis by hCG and the likely inhibition of testicular and pituitary functions by placental estrogens. Therefore it is not surprising that a dramatic change occurs at birth in the pituitary-gonadal function. After a brief increase immediately after birth (Forest *et al.*, 1976) the serum levels of testosterone decrease during the first week of life, obviously due to the elimination of hCG. Thereafter they start increasing in parallel with an increase in gonadotropin levels, and peak at 1-3 months of age at nearly adult male levels. Thereafter the levels start declining towards the low prepubertal levels that are reached by about 6 months of age (Forest *et al.*, 1976).

Some of our previous findings elucidate the changes taking place in pituitary-gonadal functions around the time of birth. First, the last prenatal months seem to be a period when the hypothalamic-pituitary level is becoming gradually more sensitive to the negative feedback control of the gonads (and probably of placenta) or the as yet unidentified extragonadal inhibition (Plant, 1988). Whenever the inhibitory steroid feedback begins, the hypothalamus and/or pituitary are initially rather insensitive to the effect. This is indicated by our finding that the postnatal increase in gonadotropin levels is much more pronounced in prematurely born infants (Fig. 6) (Tapanainen *et al.*, 1981b). This phenomenon is clear in female infants whereas in the males, testicular androgen production obviously inhibits the pronounced postnatal rise in gonadotropins. The fact that some inhibition is eliminated at birth is shown by our experiments on the stimulation of fetal and neonatal monkeys by GnRH (Huhtaniemi *et al.*, 1977b, 1979). The same maximally-stimulating dose of the peptide elicited similar responses at the pituitary level but the gonadal responses were more pronounced in the neonate. This suggests that the intrauterine inhibitor of pituitary-gonadal function affects mainly the testis.

In addition to the elimination of inhibition, other explanations also exist for the postnatal increase of testosterone levels. It may partly occur because of cessation of transplacental passage of steroids. This possibly explains the short increase that is seen immediately after birth in serum testosterone (Forest *et al.*, 1976; Huhtaniemi *et al.*, 1982a). The 1-3 months' postnatal testosterone peak may also be only a «pseudopeak» since serum levels of the sex-hormone binding globulin (SHBG) increase at the same time (Chaussain *et al.*, 1978) and thus the bioactive free testosterone levels may not increase at all after birth. In fact, we were unable to show the 1-3 month peak in salivary testosterone levels at the same time (Fig. 7) (Huhtaniemi *et al.*, 1986a). Salivary steroid measurements reflect the plasma levels of non- protein-bound, i.e. biologically active, testosterone. In this respect, it may be erroneous to look for a physiological function for the postnatal testosterone peak which after all may only represent an adaptation phenomenon of the pituitary-testicular axis to the increased protein binding of circulating testosterone. The postnatal levels of salivary testosterone were highest immediately after birth, and therefore the physiologically most important period of perinatal testosterone secretion may be at birth or even before.

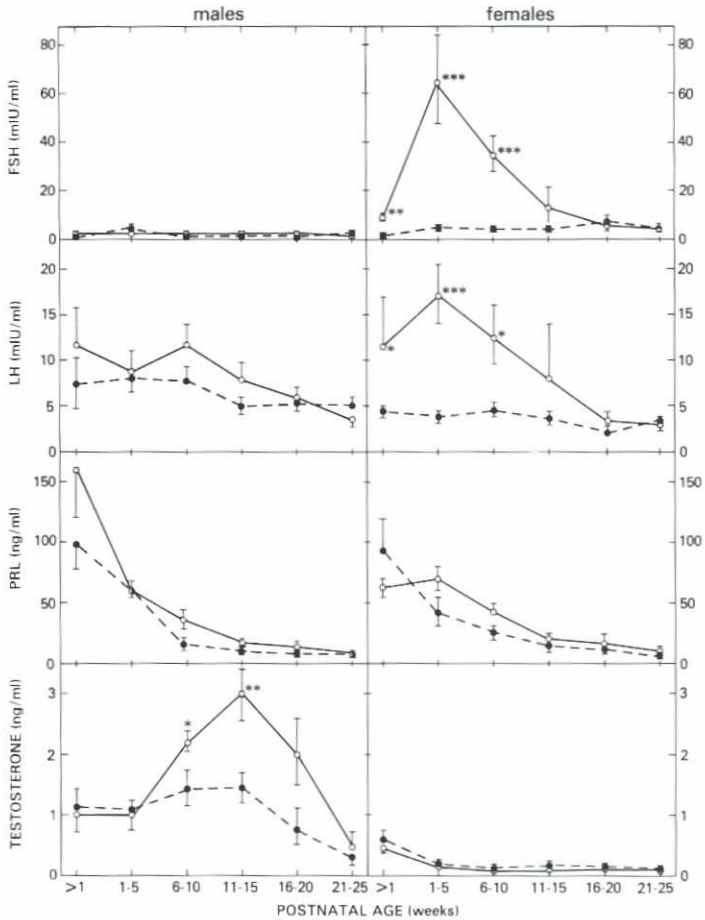


**Fig. 6.** *Serum FSH, LH, prolactin (PRL) and testosterone in full-term gestational age, 40 ± 1.0 weeks (mean ± SD);* ●---● *and premature (gestational age, 32 ± 2.3 weeks; 0---0) infants. The levels of male infants are depicted in the* left panels, *and those of the females are shown in the* right panels. *The results are grouped according to postnatal age (in weeks), as indicated at the* bottom *of each figure. Each point represents the logarithmic mean ± SEM. Statistical significances were calculated for the differences between hormone levels in premature and fullterm infants having the same postnatal age (*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$) (Tapanainen* et al., *1981b).*

### *Physiological role of postnatal androgen secretion*

Fetal testicular steroidogenesis continues after the period of genital masculinization. The physiological significance of the latter part of early testicular activity is obvious although still unknown. We have addressed this question in studies where

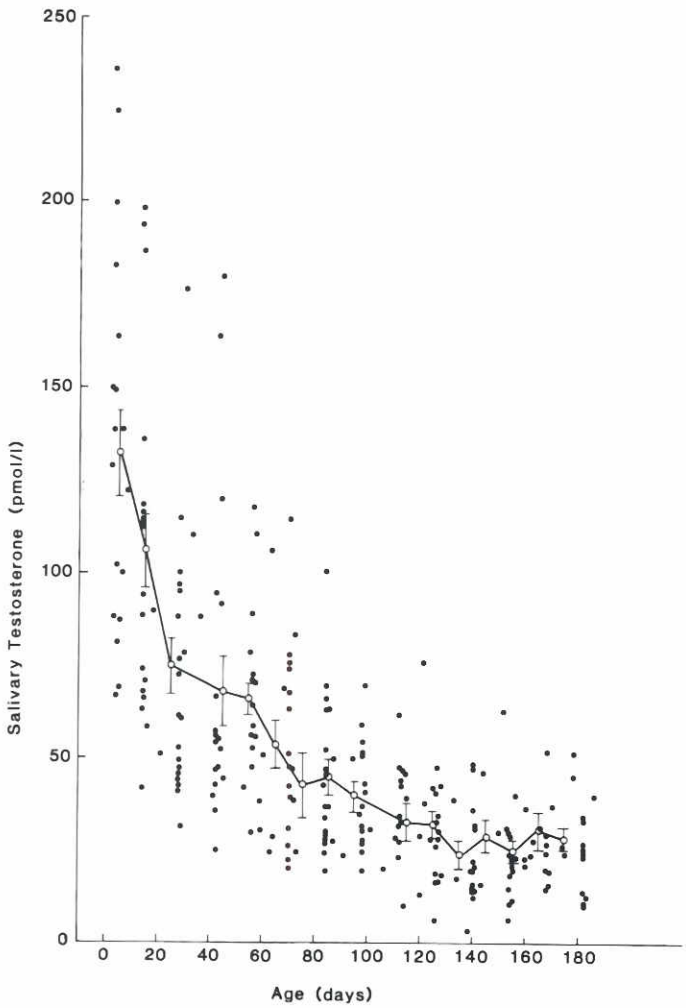


**Fig. 7.** *Distribution of individual levels of testosterone measured in salivary samples collected from 22 healthy male infants between 1-180 days of age. The open* symbols *connected by* line *indicate the mean (±SEM) levels for 10-day age periods (Huhtaniemi* et al., *1986a).*

the activity of the pituitary-testicular axis has been abolished in male rats during the first weeks of postnatal life. The animals were treated with potent antagonists of the gonadotropin-releasing hormone (GnRH) which effectively inhibit pituitary gonadotropin secretion (Huhtaniemi *et al.*, 1984, 1986b; Kolho *et al.*, 1988).

The hypothalamic-pituitary-testicular function of the treated animals was followed both acutely (following a 5-15 day period of postnatal GnRH antagonist treatment), at puberty and in adult life. Some of these findings are presented in Table 2. Immediately after cessation of the treatment, the pituitary and/or serum levels of LH and FSH were significantly suppressed. Testis weights decreased by 70%, as did those of the accessory sex glands. Also the intratesticular and serum levels of testosterone decreased.

When the animals were studied at adult age (90-150 days), a number of interesting differences persisted (Table 2). The sexual maturation of the animals was followed by the balano-preputial separation (BPS), an external sign of puberty in the male rat (Korenbrot *et al.*, 1977). The antagonist treatment clearly delayed BPS, the testis weights of the animals remained low, but those of the accesory sex glands recovered. The serum level of FSH was consistently high, those of LH and testosterone were either normal or somewhat elevated. Interestingly, the pituitary content of the GnRH receptors and the pituitary response to acute GnRH stimulation had increased (Kolho and Huhtaniemi, unpublished observations). Taken together, the findings indicated a permanent shift in the testis-pituitary feedback balance, possibly due to the neonatal disturbance of maturation of the Sertoli and Leydig cells.

About 90% of the neonatally GnRH antagonist-treated animals were infertile at the adult age of 150 days. The infertility seemed to occur in the presence of grossly normal spermatogenesis and normal sexual interest of the males in the female. However, upon tests of sexual behavior, the treated animals displayed a clear inability to ejaculate (Kolho and Huhtaniemi, unpublished observation).

The above data emphasize the importance of the latter part of the fetal-neonatal pituitary-testicular activity in programming the pubertal development of the male sexual functions. The functions affected include at least effects on testicular size, timing of puberty, set-point of the testicular-pituitary feedback interactions, and certain facets of the male pattern of sexual behavior.

TABLE 2

**ACUTE AND LONG-TERM EFFECT OF NEONATAL TREATMENT (BETWEEN 1-15 DAYS OF AGE) WITH A GnRH ANTAGONIST ON DEVELOPMENT AND FUNCTION OF THE PITUITARY-TESTICULAR AXIS (Huhtaniemi *et al.*, 1986; Kolho *et al.*, 1988; Kolho and Huhtaniemi, unpublished results).**

| | Acute effects (age 16 days) | Long-term effects (age 90-150 days) |
|---|---|---|
| Testicular weight (mg) | | |
| control | 40.7 ± 2.6 | 1290 ± 78 |
| Antagonist | 15.7 ± 5.1** | 701 ± 50** |
| Seminal vesicle wt. (mg) | | |
| control | 4.45 ± 0.57 | 599 ± 61 |
| antagonist | 1.89 ± 0.39** | 577 ± 66 |
| Pituitary LH ( g/gland) | | |
| control | 19.1 ± 4.2 | 392 ± 47 |
| antagonist | 10.9 ± 1.2 | 535 ± 83 |
| Pituitary FSH (μg/gland) | | |
| control | 10.2 ± 1.3 | 47.9 ± 6.4 |
| antagonist | 1.70 ± 0.34** | 54.1 ± 11 |
| Serum LH (ng/ml) | | |
| control | 5.12 ± 1.04 | 26.8 ± 3.6 |
| antagonist | 1.92 ± 0.27* | 32.0 ± 8.3 |
| Serum FSH (ng/ml) | | |
| control | 197 ± 28 | 235 ± 32 |
| antagonist | 147 ± 15 | 427 ± 50* |
| Serum testosterone (ng/ml) | | |
| control | 0.81 ± 0.19 | 5.59 ± 1.0 |
| antagonist | 0.14 ± 0.02** | 11.3 ± 1.6* |

**, $p < 0.01$; *, $p < 0.05$ vs. control.

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