# MINIREVIEW

# Fetal Leydig Cells: Cellular Origin, Morphology, Life Span, and Special Functional Features (43493)

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*Abstract.* **The Leydig cells, responsible for testicular androgen production, have two growth phases during the life-span of mammals. The fetal population appears during fetal life and is responsible for the androgen-induced differentiation of the male genitalia. The fetal Leydig cells disappear after birth, and the other population, the adult Leydig cells, appears during puberty and persists for the whole adult life. The fetal Leydig cells, evidently due to the intrauterine endocrine milieu and their special functional requirements in genital differentiation, differ both morphologically and functionally from the adult population. The purpose of this review is to elucidate the special features of the mammalian fetal Leydig cell population, which presents an intriguing experimental model for studies of function and regulation of steroidogenic cells.** [P.S.E.B.M. 1992, Vol 201]

The Leydig cells, responsible for androgen production, appear in the testis during the embryonic period. Their active androgen production starts early and is essential for normal masculine differentiation (Fig. 1). Fetal testicular activity continues until the neonatal period and is followed by hormonal quiescence, which is characteristic of the prepubertal testis. The second phase of testicular endocrine activity starts at puberty and continues throughout life. Two distinct populations, or growth phases, of Leydig cells are responsible for the fetal-neonatal and adult periods of testicular steroidogenesis. The former growth phase is called the fetal and the latter the adult Leydig cell population. They differ in a number of features, such as morphology, hormone production, tropic and paracrine regulation, and physiological functions. Such differences are expected, and physiologically meaningful, considering the dissimilar hormonal milieus where the two cell populations function, i.e., *in utero* and *ex utero*. Moreover, testicular androgens have very different physiological functions in the fetus and the adult. In the fetus, androgens are responsible for morphogenesis, whereas in the adult, they function more in the maintenance of male sexual characteristics.

Characteristic and unique for the fetal Leydig cells, in comparison to those in the adult, are the continuous structural and functional changes from their first appearance toward the end of their presence in the neonatal testis. After birth, the fetal-type Leydig cells continue their function until they are gradually replaced by the adult type of Leydig cells at approximately the onset of puberty (1). The structural features of the fetal and adult Leydig cells at the electron-microscopic level are generally similar, but their functional behavior is considerably different, possibly even more than we know at the present.

Recognition of these special features may help us to better understand the ontogeny of male reproductive functions and the pathogenesis of their disturbances. In this review, we focused primarily on two mammalian species, the human and the rodent (rat or mouse), and have attempted to draw general conclusions on fetal Leydig cells from these two models.

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## Origin, Differentiation, and Morphology of the Fetal Leydig Cells

The gonad as a whole, aside from the germ cells, is a direct local outgrowth from the mesonephros. The Leydig cells, as all other somatic cells of the gonads, are thus subspecialized derivatives of the nephrogenic cord which arises from the paraxial mesoderm. The differentiation of the mammalian interstitial Leydig cells starts after the basic histological differentiation of the testis into its main components (Figs. 2 and 3): the epithelial testicular cords and the interstitium (2–5). From this sequence of events, one can conclude that the Leydig cells develop from undifferentiated interstitial mesenchymal cells, and that they have no role in the formation of the testicular cords, i.e., in the primary sexual differentiation of the gonads. Their main function before puberty is the endocrine regulation (through production of androgenic steroids) of masculine differentiation of the extragonadal genital organs and sex-related functions (Fig. 1).

The human fetal Leydig cell precursors become discernible among the undifferentiated mesenchymal cells during the eighth week of fetal age, i.e., the tenth week of gestation (3). In some of the mesenchymal cells, the nucleus starts to grow in size. The amount of the cytoplasm increases by addition of mitochondria, granular endoplasmic reticulum, lipid droplets, and particularly agranular endoplasmic reticulum, but not of Reinke crystals, which have been observed only in adult cells (Figs. 4 and 5). The cells first become elongated and finally polygonal and reach a diameter of about 20 $\mu$m. The triggering factor for Leydig cell differentiation is not known. The testis determining factor (Fig. 1) is currently thought only to regulate Sertoli cell differentiation and the organization of testicular cords (6, 7).

In the human, the functional maturation of the Leydig cells (see below) proceeds in concert with the morphological differentiation, as indicated by the appearance of the histochemical reaction of the 3$\beta$-hydroxysteroid dehydrogenase enzyme at the age of 8 weeks (8). After initiation of differentiation in Leydig cells, their number correlates with the plasma human chorionic gonadotropin (hCG) concentration (9). At this time, they are also stimulated *in vitro* by hCG (10, 11).

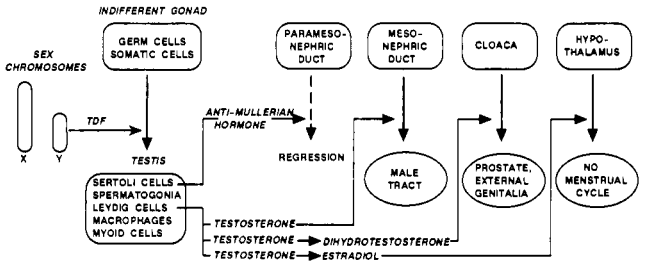


**Figure 1.** Schematic drawing of developmental and regulatory relationships in prenatal sexual differentiation of male mammals. The indifferent stages are in the top row of boxes. Their development is depicted by thick vertical arrows into the cells within the testis box and organs in the ovals, both in the bottom row. The role of Leydig and other cells and factors in the differentiation of the reproductive system is indicated by thin horizontal arrows starting from their origin and pointing at their target processes in each case. TDF, testis determining factor.

A few cells often get attached to each other and form clusters like those in Fig. 3. By the 14th week of gestation in the human, tightly apposed Leydig cells fill up the interstitial space, with only occasional mesenchymal cells dispersed between the dominating Leydig cells (Fig. 4). During the next week, they reach their relative maximum of $48 \times 10^6$ cells/pair of testes and occupy 50% of the cross-sectional area of the testis (3, 8, 12). The Leydig cells are connected by gap junctions (13), which means that they are electrically coupled and molecules of less than 1000 mol wt can pass freely from cell to cell. The functional significance of these connections is not yet understood. Another inexplicable finding is the occurrence of basement membrane segments (Fig. 6) around the Leydig cells in some species (14).

The undifferentiated mesenchymal cells in the testicular interstitium (Fig. 4) also give rise to the peritubular myoid cells, lymphatic and vascular endothelial cells, and macrophages (Fig. 1). The last type of cell differentiates between 14 and 18 weeks of fetal age (16–20 weeks of gestation) (15) and thus probably has no role in the differentiation of Leydig cells. The macrophages react with antibodies to myelomonocytic cells and probably differentiate within the testis (15). Macrophages are sometimes seen in contact with fetal Leydig cells during the maturity and involution phases and may have regulatory interactions. However, they do not seem to actively phagocytose degenerating Leydig cells.

The relative number of Leydig cells starts to decrease around the age of 16 weeks because of the fast growth of the testis, even though their absolute number remains constant until about the 24th week (8, 12). During the remainder of the fetal period, the absolute number of Leydig cells decreases progressively to $18 \times 10^6$ cells/pair of testes just before birth (12). The reduction of the Leydig cell population takes place by degeneration and complete destruction of some cells (Fig. 7) and by a 50% reduction in the volume of the remaining cells (3, 12). This process coincides with and parallels the decrease in plasma hCG concentration (16), but whether these two phenomena have a causal relationship is not known. The Leydig cells present in the human fetal testis can thus be divided into three different phases: the differentiation phase (fetal ages 8–14 weeks), the fetal maturity phase (14–18 weeks), and the involution phase (18–38 weeks) (3). A simple and teleological explanation for the Leydig cell involution is that the physiological demand for androgens is lower during

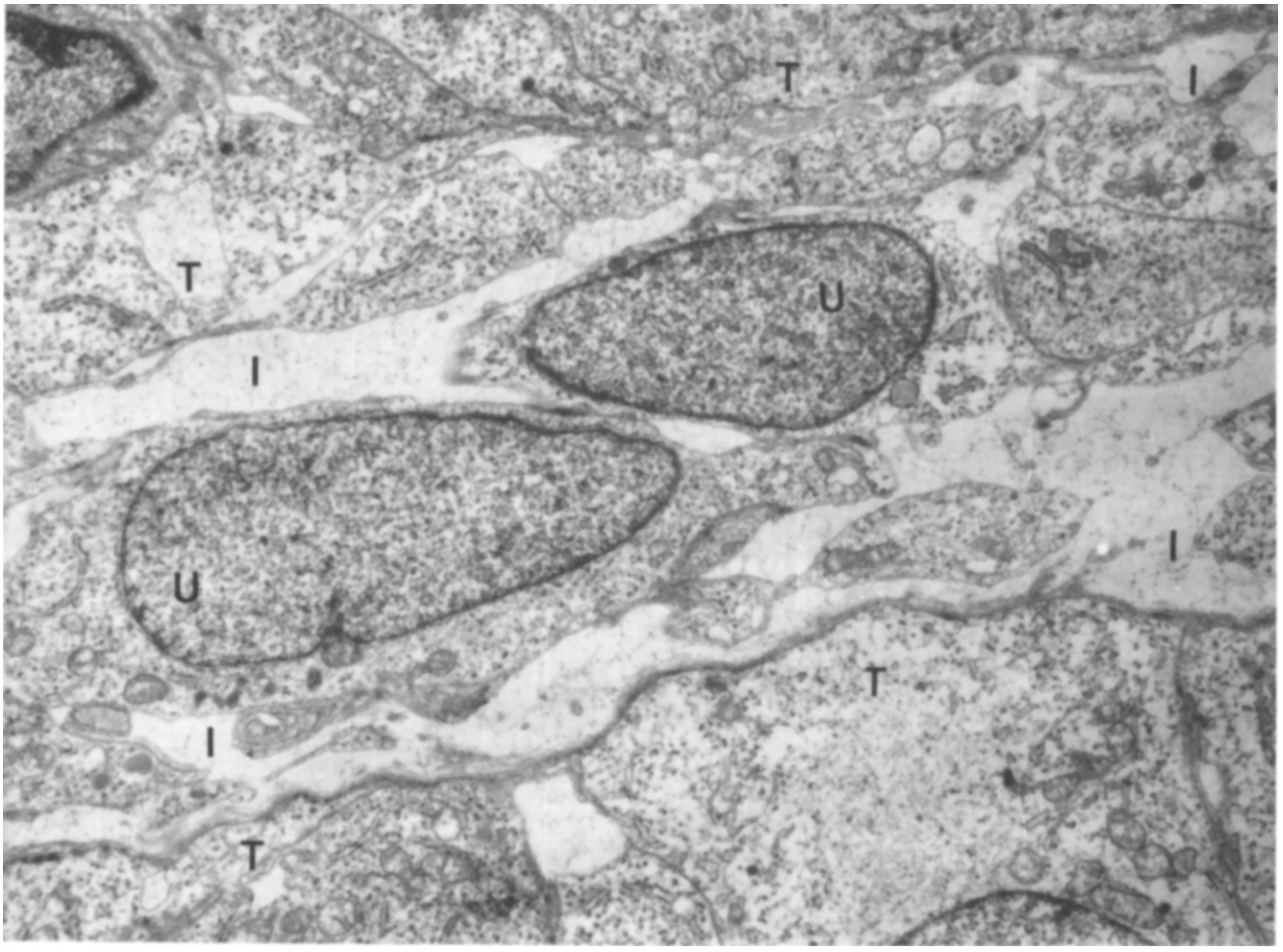


**Figure 2.** Undifferentiated mesenchymal cells in the early interstitium (I) between two organizing testicular cords (T). The cell (U) on the left may have started its differentiation into a Leydig cell because its cytoplasm has slightly enlarged. Electron micrograph of human embryo at the age of 6 weeks (original magnification ×8300).

the second half of pregnancy, after differentiation of the male genital organs. This regulatory behavior is analogous to that observed in adult seasonally breeding animals.

In the rat, the Leydig cells start to develop in the late fetal period (at about three fourths of the way through the prenatal period). In the human, this occurs at about one fifth of the way through the prenatal period. The cytodifferentiation from undifferentiated mesenchymal cells proceeds quickly between embryonic-fetal (F) Days 15 and 17 through similar stages as described before for human Leydig cells (2, 17–20). Functional maturation probably precedes the structural differentiation, as suggested by the presence of 3$\beta$-hydroxysteroid dehydrogenase enzyme activity by the age of Day F 15 (21). Testicular steroidogenesis, as well as gonadotropin responsiveness in the Leydig cell, corresponds with ultrastructural development (see below).

The relative number of Leydig cells in both the fetal rat and human testis changes with respect to time. However, the profile of numerical change is quite different between the species (8, 12, 22). In the rat, the absolute number of Leydig cells ($260 \times 10^3$ per pair of testes) reaches an acme at Day F 19.5. In the human, two peaks are seen. The first peak occurs at 13–16 weeks ($48 \times 10^6$ cells), and a second at the age of 3 months postpartum (8, 12, 22). If only the fetal type of Leydig cell is considered in the rat, there is also a second peak in total number that occurs at about 14 days of postnatal life. This peak is obscured, however, because the decline in number is masked by the concomitant increase in the number of adult-type Leydig cells. These appear in the rat relatively earlier than in the human (1). The absolute numbers reported for Leydig cells are in fairly good agreement and vary in number per pair of testes from $50 \times 10^3$ at the age of Day F 17 (23) through $376 \times 10^3$ (24) on Day F 19 , $260 \times 10^3$ (22) on Day F 19.5, to a final count of 160 and $180 \times 10^3$ (1, 23) on Days F 20 and F 21, respectively.

The fetal Leydig cells in the rat do not form large confluent regions like those in the human. They remain as individual cells for a longer duration, but toward the

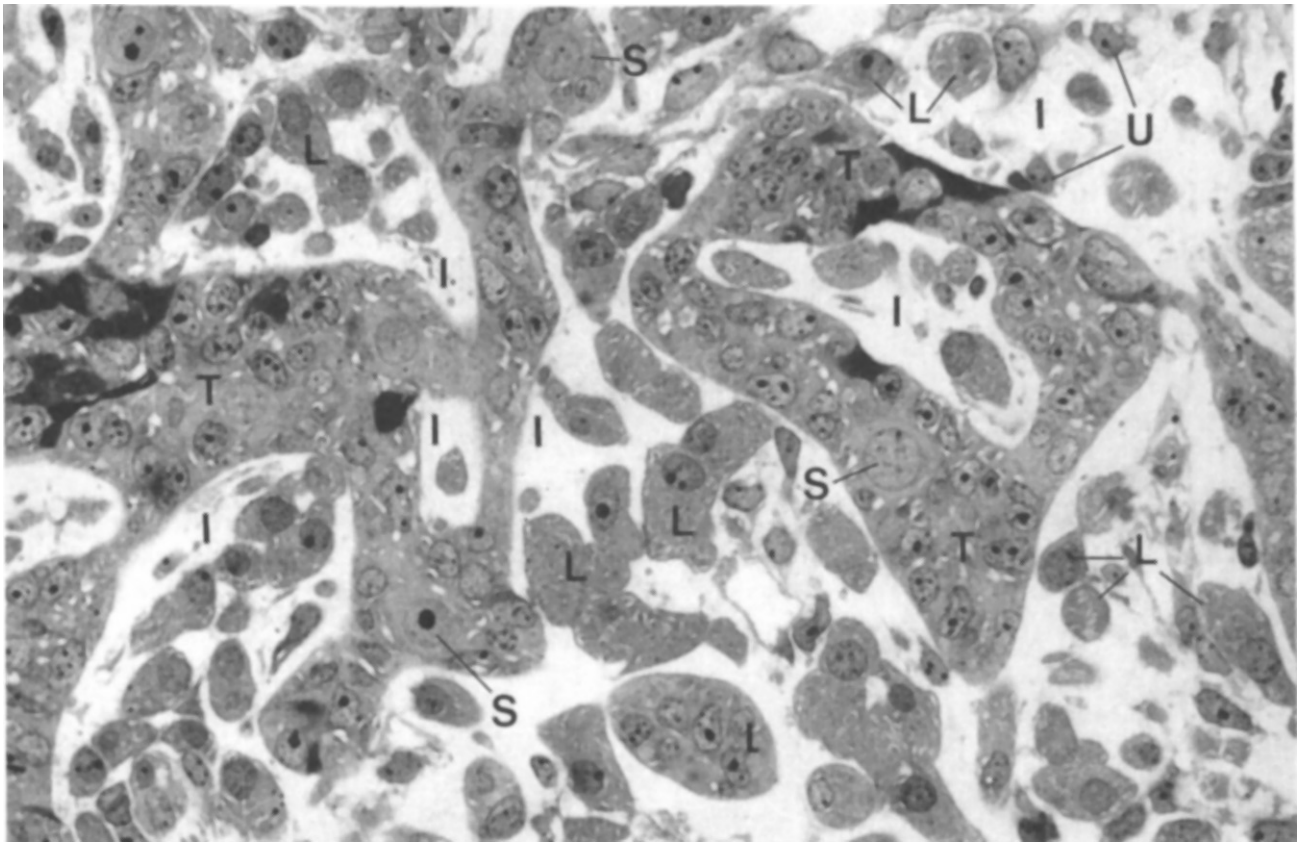


**Figure 3.** Leydig cells (L) in the interstitium (I) between the pleomorphic testicular cords (T), which consist of Sertoli cells and occasional spermatogonia (S). Some of the Leydig cells are organized in small clusters (e.g., (L) bottom, middle of the figure). There are also remaining undifferentiated mesenchymal cells (U). Light micrograph of pig embryo at the age of 30 days (original magnification ×680).

end of pregnancy, the form irregularly outlined groups (14). The differentiating Leydig cells have small patches of ultrastructurally and immunocytochemically identifiable basement membrane (Fig. 6), which increases in area with advancing cell differentiation (14). The addition of L-azetidine 2-carboxylic acid, a proline competitor, into the medium of organ culture of differentiating testes inhibits differentiation of Leydig cells and their steroidogenic function (25). This inhibition was prevented by addition of an excess of proline into the medium. These findings suggest that the extracellular matrix has a role in Leydig cell differentiation, perhaps related to their grouping into epithelial-like glandular islets. This also adds laminin, collagen type IV, and other basement membrane components to the list of Leydig cell products. Other hints to unknown, but probably important, properties of the Leydig cell surface are offered by the observations of specific nonimmunological binding of adult mouse Leydig cells to lymphocytes and macrophages (26, 27). The cells of the latter type appear in the rat testicular interstitium at Day F 19 (28). Macrophages are known to produce several regulatory factors, which may have local regulatory effects on Leydig cells (28).

The regulation of Leydig cell differentiation and steroid biosynthesis is apparently under the control of pituitary gonadotropins throughout life. As an unphysiological experiment, caffeine administration to the mother decreased the number of rat fetal Leydig cells and their testosterone biosynthesis (29), and may offer new insight into the mechanisms regulating the proliferation of these cells. In organ culture, fetal calf serum has been found to prevent the formation of testicular cords, but not the structural and functional differentiation of Leydig cells (30). This suggests that Leydig cell differentiation does not depend on normal histological organization of the Sertoli cells, but may still be related to their function factors, which, like anti-müllerian hormone, may remain intact.

The presently available reports on various other mammals (pig, mouse, guinea pig, hamster, rabbit, and monkey) show that the differentiating fetal Leydig cells are basically similar endocrine cells with steroid-synthesizing ultrastructural characteristics and functional properties. There is, however, great variation in the timing and relative lengths of the different stages of the Leydig cell development until full maturity is reached after puberty.

### Early Stages of Testicular Steroidogenic Activity

Steroidogenesis in fetal Leydig cells starts as soon as these cells have differentiated morphologically. In

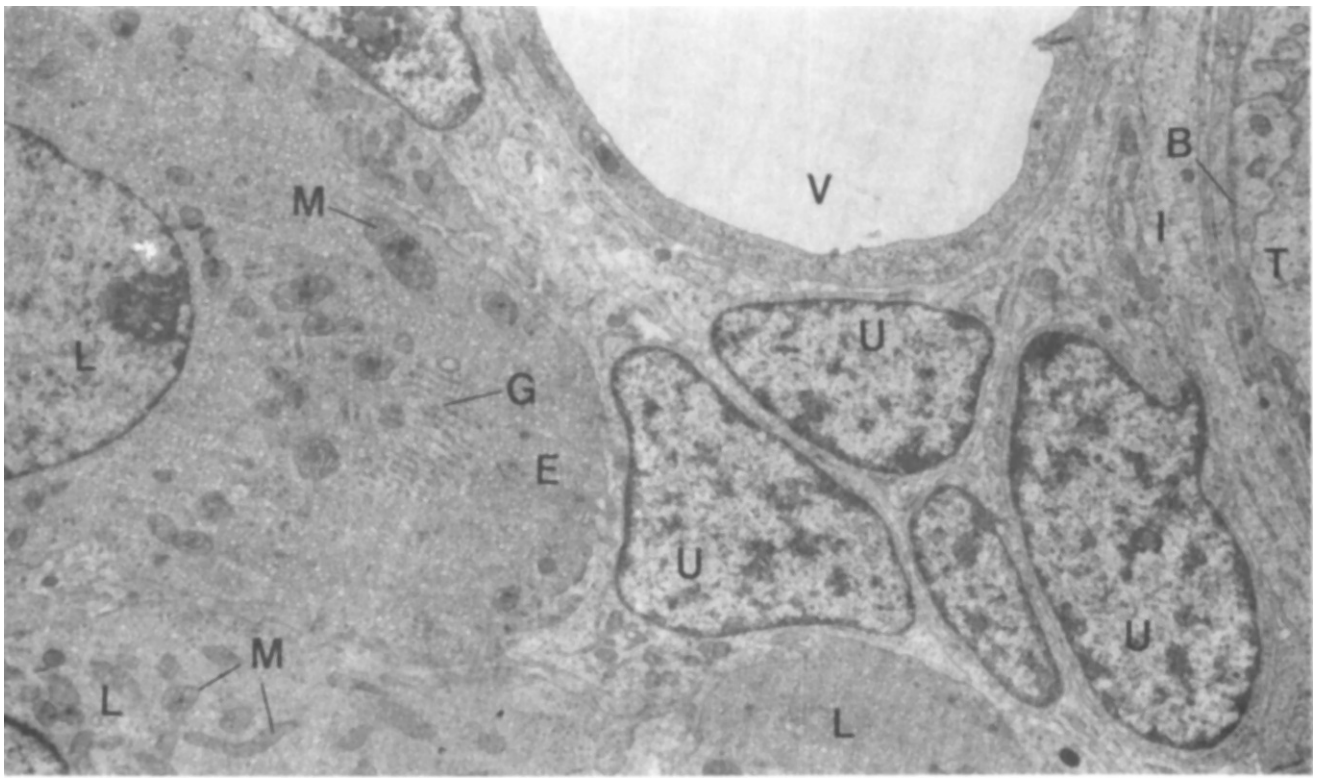


**Figure 4.** The basic structural and functional components of the fetal testicular interstitium at the time of its endocrinologically most active phase. The Leydig cells (L) are large, and the cytoplasm is filled with abundant agranular endoplasmic reticulum (E), which occupies all the space between the mitochondria (M), small stacks of granular endoplasmic reticulum (G), and other organelles. Next to the blood vessel (V), there are few undifferentiated mesenchymal cells (U). On the right, there is a small segment of the testicular cord which consists of Sertoli cells and is sealed toward the interstitium (I) by a continuous basement membrane (B). Electron micrograph of the testis of a human fetus at the age of 15 weeks (original magnification ×7600).

the human, this takes place at the fetal age of 8 weeks (3). Intrauterine androgen production reaches its maximum during Weeks 12–14 (Fig. 8). Thereafter, there is a steep decline in steroidogenesis toward the end of gestation. A short peak of reactivation occurs between Months 1–3 postpartum, until the quiescent prepubertal period of steroidogenesis is attained after about 6 months of age (Fig. 8) (for reviews, see Refs. 16, 31, and 32). Although the physiological function of the fetal testosterone peak in male genital differentiation is clear, the postnatal elevation in circulating testosterone obviously represents an adaptational phenomenon due to the increased capacity of plasma sex-steroid-binding proteins (33, 34). The androgen levels in the fetal testis and circulation parallel the proportion of Leydig cells in the testis tissue (see above). The next phase of testicular steroidogenesis, through activity of the adult population of Leydig cells, starts at puberty and continues for the rest of life.

In the rat, the differentiation of Leydig cells starts at fetal age 15.5–16.5 days (20), and the onset of testosterone synthesis occurs simultaneously (Figs. 8 and 9) (35, 36). It rises sharply during subsequent days and attains its maximum on days F 18.5–19.5 (22, 37). Although testicular testosterone content stays relatively stable for the rest of gestation (and the first postnatal days), its concentration shows a clear decline (22). The decline in testicular steroidogenesis continues after birth until the age of about 15 days, after which it stays low until about 30 days of age (Figs. 8 and 10) (22). Although the Leydig cell concentration of testis tissue increases about 4-fold between 15 and 30 days, no concomitant increase in steroidogenesis is seen. This indicates that the new generation of Leydig cells initially has very low steroidogenic activity. Androgen production in the pubertal rat testis is rapidly reactivated around Day 40 of life, and 5$\alpha$-reduced androgens predominate the steroid profile at this age (38). As the Leydig cells mature, the capacity for 5$\alpha$-reduction diminishes (38). This steroidogenic phase is due to the adult population of Leydig cells, and therefore is beyond the scope of this review.

### Development of Hypothalamic-Pituitary-Testicular Interactions

It is required for the onset of gonadotropin action in the testis that the pituitary gland initiate gonadotropin synthesis and secretion, and the testis must have functional gonadotropin receptors. Furthermore, hypothalamic control of gonadotropin secretion must be

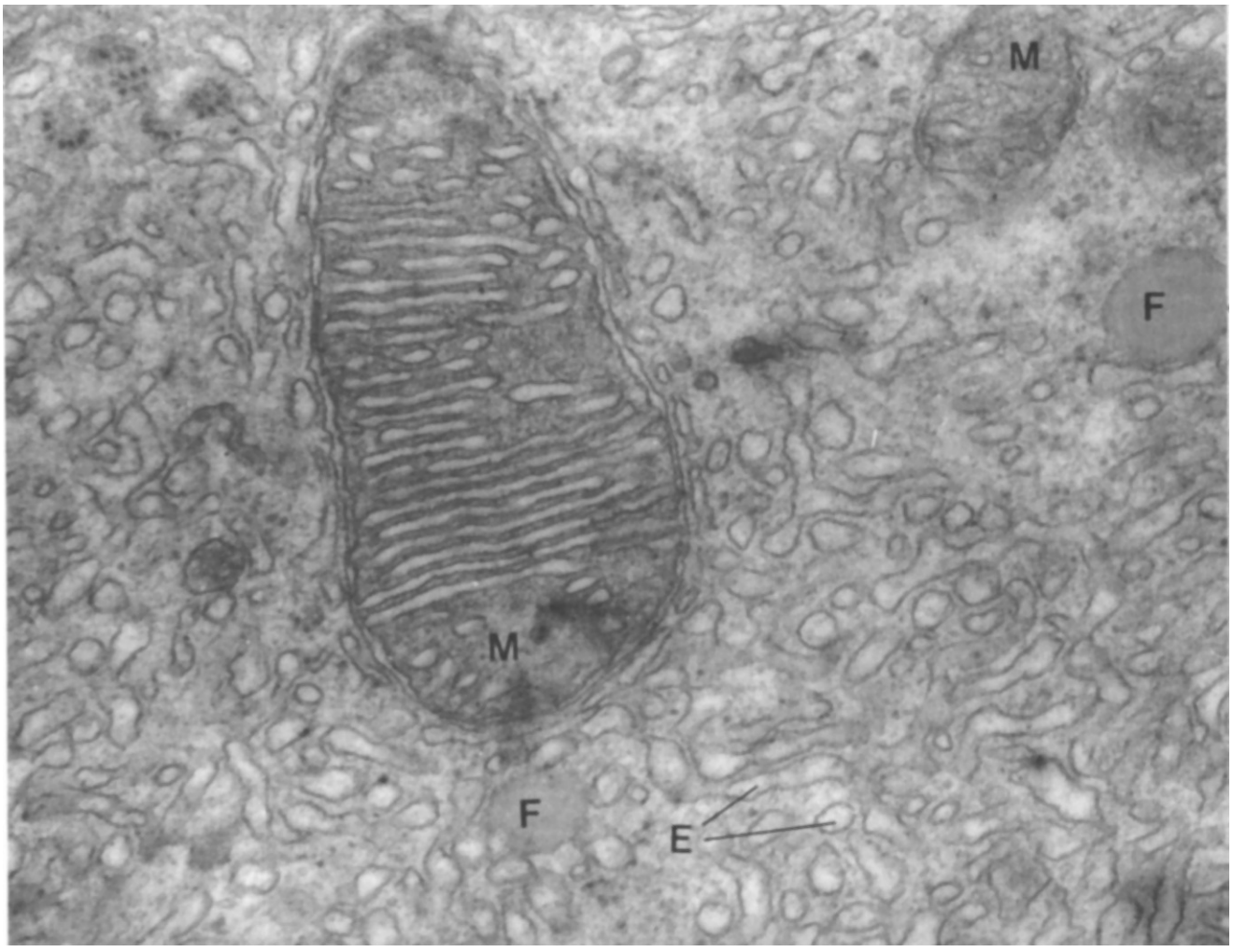


**Figure 5.** High magnification of Leydig cell cytoplasm comprising the working organelles of steroid synthesis: tubularly organized agranular endoplasmic reticulum (E), mitochondria with lamellar cristae (M), and lipid droplets (F). Electron micrograph of the testis of a human fetus at the age of 15 weeks (original magnification ×61,000).

functional. Although gonadal feedback to the hypothalamic-pituitary level is an essential part of this system in the adult, it may be the last link to develop in the fetal period (39, 40). The initial source of gonadotropic stimulus in some species (e.g., the rat) is the fetal pituitary, whereas in others (e.g., the human), it is chorionic gonadotropin.

The gonadotropin-releasing hormone (GnRH)-secreting neurons originate in the medial olfactory placode and enter the forebrain with the nervus terminalis (41–43). This migration is needed before hypothalamic control of gonadotropin secretion through GnRH is possible. It occurs in the mouse between Days F 12 and F 15. In the rat, GnRH is first identifiable at the ventral olfactory bulb on Day F 15 (44), and it may initially reach the pituitary by diffusion, since the mature portal system connecting the GnRH axons in the median eminence of the hypothalamus with the pituitary gland does not develop until Days F 20–F 21 (43). Most studies agree that the fetal rat gonadotropes are differentiated on Day F 16, which is the first day of appearance of luteinizing hormone (LH) in the anterior pituitary (reviewed recently in Ref. 44). However, when assessed by the presence of GnRH receptors, the gonadotropes are detectable as early as Day F 13 (44).

LH, secreted by the fetal pituitary gland, appears in the circulation of the rat on Day F 17 (45). This takes place after the initiation of testicular testosterone synthesis, and appearance of LH receptors on Day F 15.5 (Fig. 9) (35, 37). Thus, the onset of LH secretion precedes the steepest increase of testicular steroidogenesis and LH receptor content on Day F 18 (37). It seems, therefore, that pituitary LH synthesis and testicular steroidogenesis are initiated independently of the reactive tropic stimuli. Once the secretion of LH is established, a major increase occurs in the amount of testicular LH receptors and testosterone production. Gonadal feedback to the hypothalamic-pituitary level

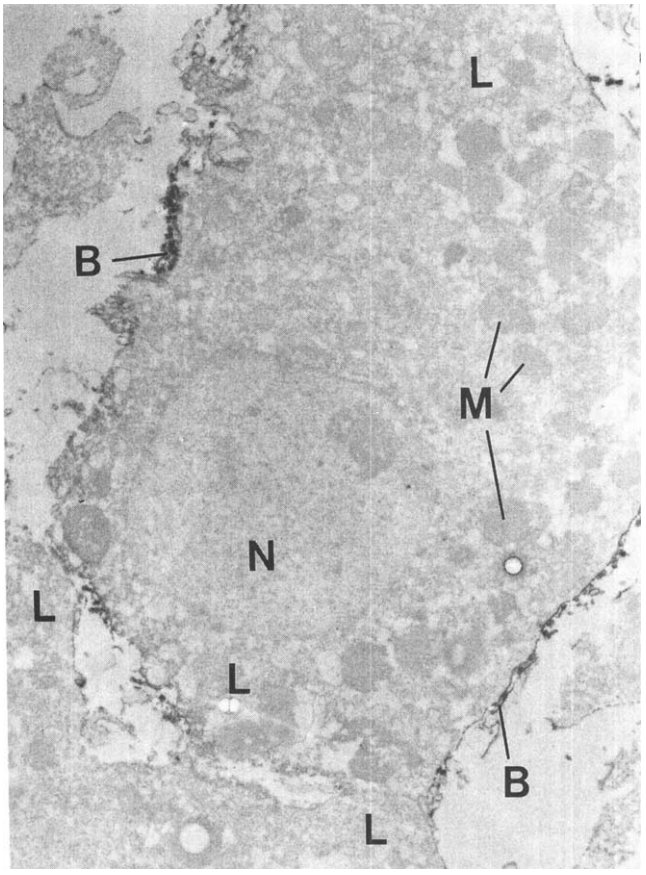


**Figure 6.** Immunocytochemical localization of laminin-containing patches (B) as black precipitation on the surface of a Leydig cell (L). There is no reaction on the nucleus (N), mitochondria (M), or elsewhere in the cytoplasm. The patches (B) also contain collagen type IV and other basement membrane components, and they are seen as lamina densa in conventional electron microscopy. Electron micrograph with mild fixation and no background staining. Rat fetus at the age of 17 days (original magnification ×5800) (courtesy of Dr. Teijo Kuopio).

is apparently the last link to develop in the hypothalamic-pituitary-gonadal circuit (39).

The mechanism that induces LH receptors in the developing gonad is still unknown. Since this appears slightly before LH can be measured in the circulation, Day F 15.5 (Fig. 9) versus Day F 17 (45), LH itself cannot be the initial stimulus, although it maintains its own receptors in the adult gonad (46–48). Furthermore, it is difficult to envision a mechanism of action for LH before its receptors are present. The role of FSH, although clear in the adult (46–48), is also unlikely in the fetal induction of LH receptors, since they seem to appear in the testis several days before pituitary follicle-stimulating hormone (FSH) secretion can be detected (45). The role of prolactin, also an active inductor of LH receptors in the adult gonad (46–48), is unlikely for the same reason. The secretion of this pituitary hormone does not start until the last days (F 19–21) of gestation (45, 49).

When studying the ontogeny of LH receptors and LH responsiveness in the neonatal rat ovary, the expression of truncated versions of LH receptor mRNA was found to precede that of the full-size message by at least 10 days (50). Hence, the LH receptor gene may be constitutively expressed in the mesonephric cells destined to become target cells of LH long before their actual differentiation starts. The LH receptor may then be switched on by a change in the alternative splicing pattern of the cognate mRNA. Whether the same applies to the fetal testis is still unknown. Thus, instead of being a transcription factor, the putative inducer of the LH receptor may have its main action on alternative splicing or translation of the constitutively expressed LH receptor mRNA.

In the human, the development of the anterior pituitary starts between Weeks 4 and 5 of fetal life (for a review, see Ref. 51). As in the rat, the development of the hypothalamic-pituitary connections occurs later. The median eminence is discernible by Week 9 and GnRH is first detectable in human fetal hypothalamus by Weeks 9 and 10 of gestation (16), but the hypothalamohypophysial vascular system is not functional until Week 12. Because of the missing vascular connections, GnRH may initially reach the pituitary by diffusion. LH and FSH appear in the pituitary by Week 10, and their secretion starts by Weeks 11 and 12 (16, 51, 52). Hence, there is a close temporal relationship between the onset on GnRH synthesis and that of LH and FSH also in the human.

In the human fetus, gonadotropins attain the maximum levels by Week 16, when the levels in women are as high as in castrated adults (16). In early studies, human fetal gonadotropin secretion was extensively studied using aborted material (16, 52). Very recently, these earlier findings were confirmed with normal human fetal blood samples obtained during cordocentesis, and measured using the novel ultrasensitive immunometric assay methods (53).

There is an interesting sex difference in human fetal gonadotropin levels. The levels in female fetuses are in the postmenopausal adult range, but in the male, the LH and FSH levels stay considerably lower (51–53). At this time, the testes are actively producing androgens and inhibin/activin peptides (see below), but the ovaries are still inactive. Hence, at this age, the fetal testis, but not the fetal ovary, participates in the negative feedback control of the pituitary. The decline of gonadotropins occurs in both sexes during the latter half of gestation. Since the ovaries still remain endocrinologically inactive, this has been interpreted to mean that the fetal hypothalamic-pituitary level has started responding to the high circulating levels of placental estrogens (and progesterone).

A third gonadotropin, i.e., placental hCG, must be taken into account in human fetal development. hCG attains its peak levels around Week 12 of fetal age, and

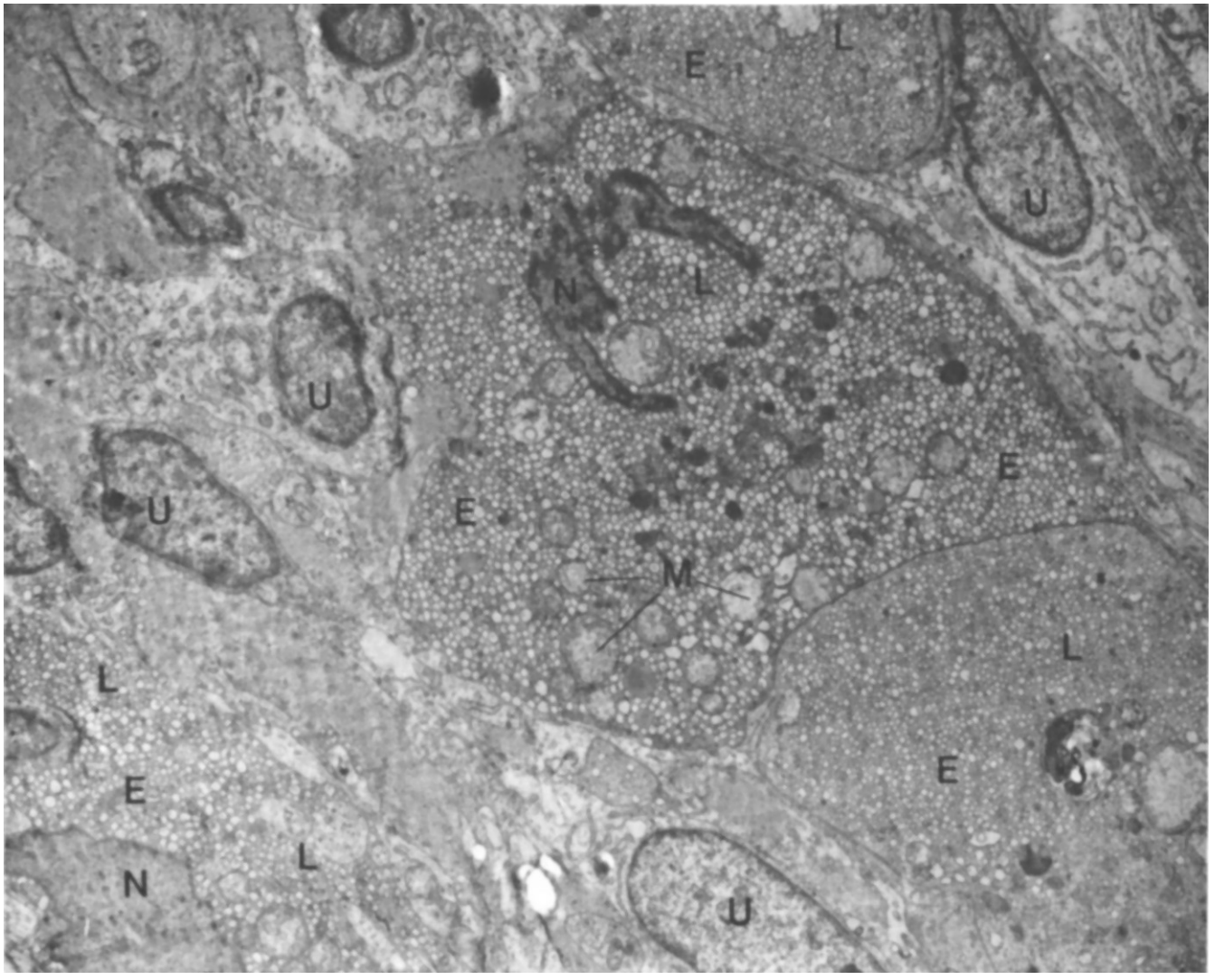


**Figure 7.** Leydig cells (L) in testicular interstitium at various stages of degeneration. The nuclei (N) are condensed into abnormal shapes and are undergoing lysis. The tubular agranular endoplasmic reticulum has transformed into vesicles (E) and the mitochondrial cristae (M) are destroyed. Undifferentiated mesenchymal cells (U) remain present between the Leydig cells. Electron micrograph of the human fetus at the age of 23 weeks (original magnification ×5000).

declines to a nadir at 20 weeks (16). It is a potential stimulator of the fetal testes. In fact, the highest levels of fetal testicular steroidogenesis correlate much more closely with the peak of hCG in fetal circulation than with the levels of fetal pituitary gonadotropins (16, 53).

There is a considerably body of information indicating that hCG is responsible for human fetal testicular steroidogenesis during the intrauterine peak of testicular activity. We, as well as others, have shown that fetal testes contain LH receptors and that hCG is able to stimulate fetal testicular steroidogenesis (11, 54–59). An example of such an experiment is shown in Figure 11. However, Word *et al.* (60) recently were unable to show such hCG stimulation, and concluded that fetal testicular steroidogenesis is autonomous and not responsive to gonadotropins. The reason for the discrepant findings is not readily apparent. Our initial studies (11) were carried out using quarters of fetal testes. One pair of quarters was incubated in the absence, and the three remaining pairs in the presence, of three concentrations of hCG. A clear 2- to 4-fold stimulation of testosterone formation was observed in these incubations using a physiological range of hCG concentrations found in fetal circulation (16). The same finding was made subsequently by perifusing fetal testes with a piece of human placenta (56). The release of hCG and progesterone from the placenta was documented, and there was a clear testicular testosterone response. Similar stimulation by hCG was demonstrated in human fetal testicular cultures (58, 59). It seems feasible that testicular steroidogenesis is initiated without gonadotropic stimulation, as shown by Wilson *et al.* (61) in the rabbit testis. However, the bulk of information available indicates that the peak of the synthesis of testosterone by

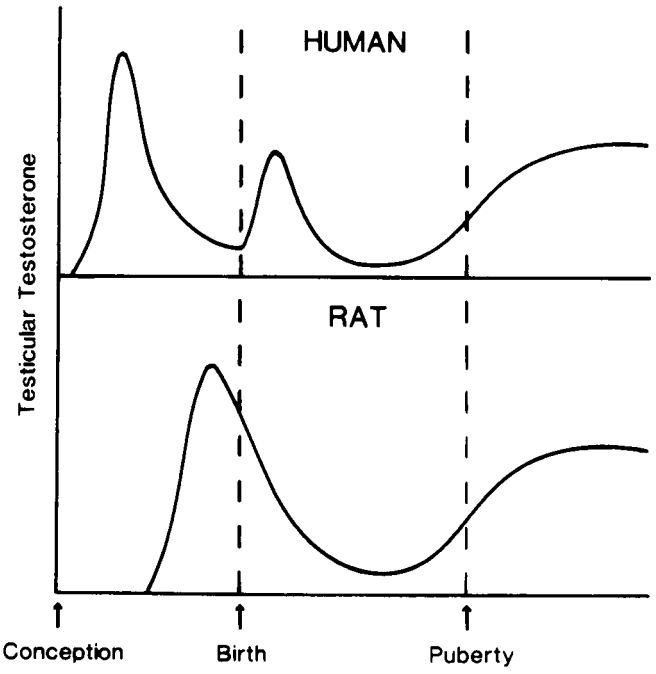


**Figure 8.** Schematic presentation of testicular testosterone levels in the human and rat between conception and adult life.

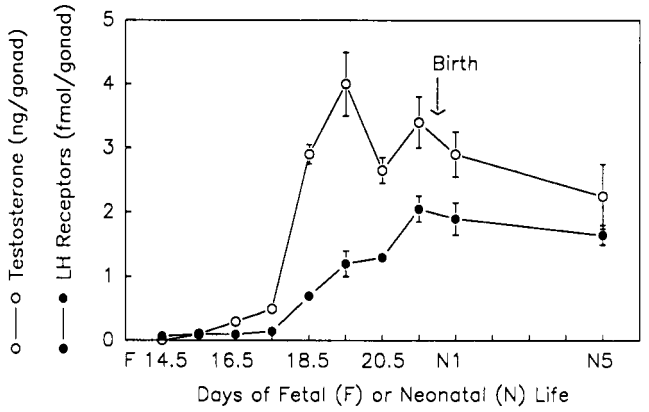


**Figure 9.** Content of LH receptors and testosterone in the rat testis between Day 14.5 of fetal life and Day 5 postpartum. Each point is the mean ± SE of six replicate determinations (37).

the fetal testes is under gonadotropic stimulation (32, 62). In the rat, the tropic stimulus originates from the fetal pituitary, in the human it is hCG.

## Special Characteristics of Fetal Testicular Steroidogenesis

The steroidogenic capacity of the fetal Leydig cells is clearly higher than that of the adult cells. We have shown this both by measuring the amount of endogenous steroids in testis tissue per number of Leydig cells and by assessing the steroidogenic capacity of the Leydig cells *in vitro* (22, 63). The adult Leydig cells are under negative modulation by a number of hormonal or paracrine influences (see Ref. 64). The high steroid

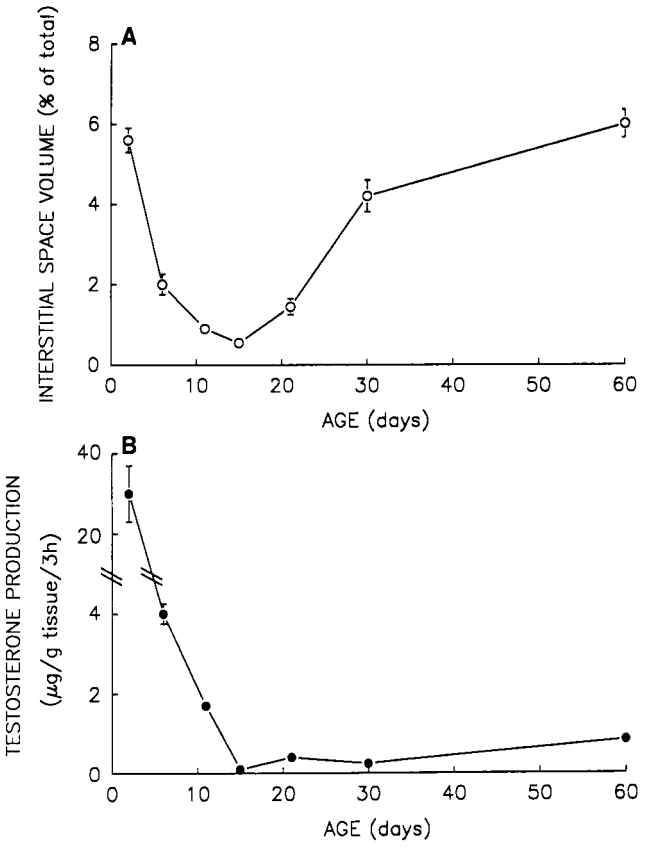


**Figure 10.** (A) Percentage of interstitial tissue in the rat testis (volume density) between 2 and 60 days of age. Each point represents the mean ± SE of measurements from six tissue preparations. (B) hCG-stimulated (3 IU/ml) testosterone production in a 3-hr incubation by decapsulated testes of rats aged 2–60 days. Each bar represents the mean ± SE of data from five testes (63).

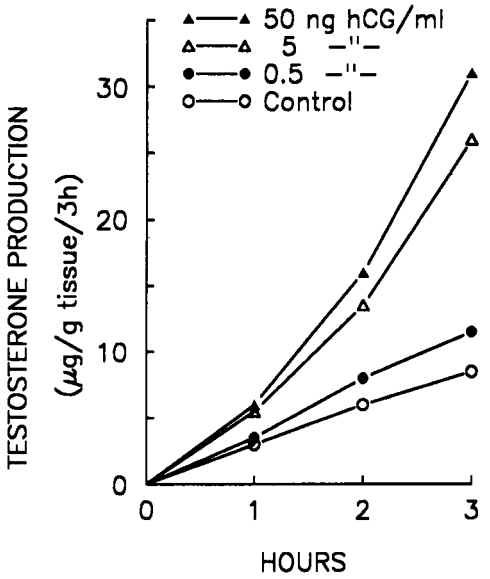


**Figure 11.** hCG-stimulated testosterone production by slices of testis tissue from a human midterm fetus. Each point is the mean of two incubations (11).

production of the fetal cells may be due to the absence of many of these negative influences.

In the fetal rat testis, steroidogenesis per Leydig cell is most abundant on Days F 17 and 18, but it clearly declines toward the end of gestation (22, 63). The reason for the decline is not apparent, and its time range is obviously too narrow to be explained by the appearance of new steroidogenically less active Leydig cells. Decreasing gonadotropic stimulation at the end of gestation is not an explanation either, since, in fact, circulating LH levels increase during the last days of gestation (65, 66). The fetal testis can probably use extratesticular steroids (from placenta, mother, and/or other fetal tissues) as substrates for its androgen production. The pronounced drop in maternal serum progesterone levels during the last days of gestation (67) may, therefore, be the explanation for the apparent decrease of androgen production before birth, since fewer substrates are available for testicular steroid conversions.

The second decline in testicular steroid concentrations during the first 2 weeks of life can be explained by the decrease in total number of Leydig cells, since the steroid concentration per Leydig cells does not change markedly at the same time (22) (Fig. 10). The postnatal nadir is around day 15 postpartum, which also represents the nadir of the proportion of testicular Leydig cells. When steroidogenesis is reactivated after this age, the steroid concentration per Leydig cell stays much lower than *in utero* for the rest of life (Fig. 10). The difference in steroidogenesis in favor of the fetal population of Leydig cells is even greater if the steroidogenic capacity *in vitro*, instead of endogenous content, is compared (63).

Concerning the quality of testicular steroidogenesis, one apparent difference between the fetal and adult Leydig cells is the low aromatase activity of the former (68, 69). The implications of this feature for the special functional characteristics of the fetal testis will be discussed below. Another qualitative difference is the very low 5$\alpha$-reductase activity of the rat fetal testis (35, 70, 71). The physiological significance of this finding, if any, remains open. Both aromatase and 5$\alpha$-reductase are especially active in the immature testis, where the Sertoli cell is the preferential site of these conversions (38, 72).

Human testicular testosterone synthesis is activated between Weeks 8 and 12 by fetal life, and it attains its maximum between Weeks 12 and 14 (73, 74). Since a considerable amount of 5-ene steroids are produced before the period of increased testosterone production, the increase evidently occurs through activation of the 3$\beta$-hydroxysteroid dehydrogenase enzyme. The drop in fetal testicular steroidogenesis between Weeks 16 and 20 is mainly due to a decrease of the 17-hydroxylase/17-20-lyase cytochrome P-450 activity. Fetal testicular testosterone concentration bears a close temporal correlation with the gene expression of the cholesterol side chain cleavage and 17-hydroxylase/17-20-lyase cytochrome P-450 enzymes in this organ (75).

When concentrations of the endogenous steroids are compared between fetal and adult human testes, several differences are evident (74, 76–78). The 3-fold higher concentration of testosterone in fetal testes may be due to the higher volume density of Leydig cells in the fetal testis, but the fetal testis seems to contain clearly higher levels of two other 3-keto-4-ene steroids, progesterone and androstenedione. In contrast, the contents of several steroid sulfate conjugates, especially those with the 3$\beta$-hydroxy-5-ene structure, are higher in the adult testis. A short peak of endogenous estradiol, around Week 12 of gestation, was also found in fetal testes (74), but this hormone may be trapped by the tissue from circulation, since the aromatase activity of the human fetal testis is very low (59). This is in contrast to the important role of testes as a source of estrogens in the adult male (79).

Another pathway of intratesticular testosterone metabolism to 5$\alpha$-dihydrotestosterone seems to be equally active in the fetal and adult human testis (80), which is in contrast to the rat, in which testicular 5$\alpha$-reductase activity is low in the fetus and neonate (see above). 5$\alpha$-Dihydrotestosterone has been proposed to play a role in the regulation of spermatogenesis, but its physiological role in the fetal testis is still open.

### Special Characteristics of Fetal Leydig Cell Responses to Gonadotropins

The rat fetal Leydig cells respond to LH stimulation with increased cAMP production and increased steroidogenesis from Day F 15.5 onward (35, 37, 63, 81). In addition to stimulation, adult rat testes display inhibitory responses to high doses of LH or hCG (46, 82). This is due to a loss of the homologous receptors (i.e., LH receptor down-regulation) and blockade of steroidogenesis either at the cholesterol side chain cleavage step (early lesion) or at the 17-hydroxylase/17-20 lyase step (late lesion). When a down-regulating dose of hCG was given to neonatal rats (83), or when fetal Leydig cells were challenged with a high concentration of LH in culture (81), no signs of either LH receptor down-regulation or the steroidogenesis lesions were seen. Instead, both the Leydig cell LH receptor levels and the steroidogenic capacity increased (Figs. 12 and 13). The pattern of the fetal-type up-regulation of LH receptors and steroidogenesis after LH/hCG treatment gradually shifts to the adult-type inhibition as the fetal Leydig cells are replaced by the adult population in the prepubertal animal (83). The difference between the fetal and adult Leydig cells is seen both at the LH receptor mRNA and receptor protein level (84). Likewise, the negative heterologous regulation of lactogen receptors

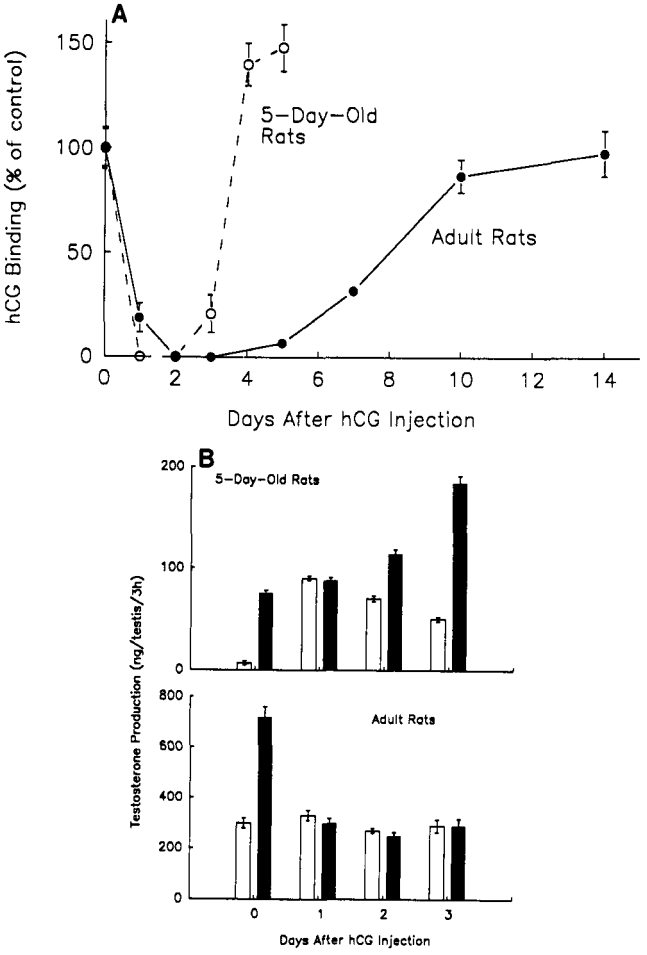


**Figure 12.** (A) Available LH receptors, monitored by [$^{125}$I]iodo-hCG binding, in the testis tissue of neonatal (5-day-old) and adult rats after subcutaneous injection of 600 IU/kg of hCG. (B) *In vitro* testosterone formation by decapsulated testes of rats similarly treated with hCG, before (0) and 1, 2, and 3 days after the injection. The open bars represent basal testosterone production and the closed bars represent production in the presence of 3 IU/ml of hCG. Each group represents measurements from five animals (63).

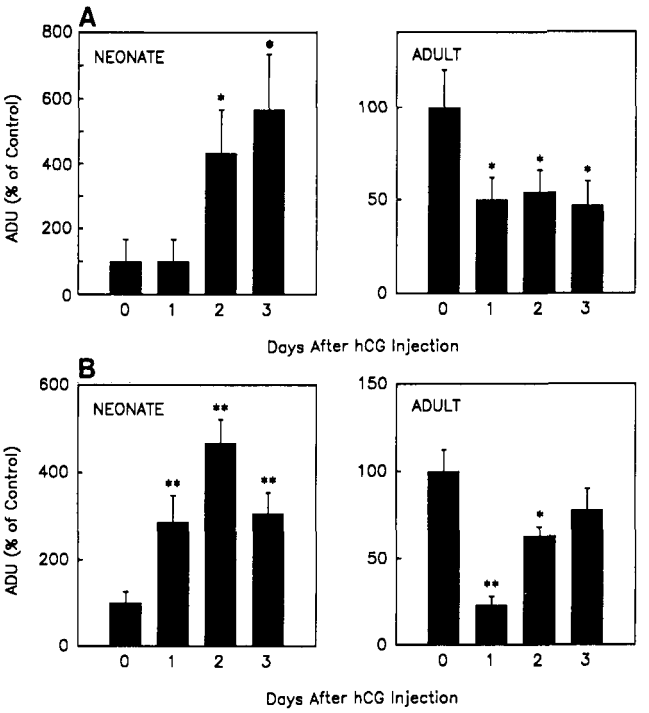


**Figure 13.** Testicular concentrations of mRNA for (A) 17-hydroxylase/17-20 lyase-cytochrome P-450 lyase and the (B) LH receptor before (0) and up to 3 days after a 600 IU/kg injection of hCG in neonatal (5-day-old) and adult rats. ADU, arbitrary densitometric units. Each pair is the mean ± SE of measurements from five to 12 animals. * $P < 0.05$; ** $P < 0.01$ as compared with 0-day level (84).

by LH/hCG, apparent in the adult testis, is missing in the fetal-neonatal testis (85).

Although the molecular mechanisms behind the functional differences are still unknown, several explanations are available. The gonadotropin-induced blockage of androgen formation in the adult Leydig cells appears to be an estrogen-mediated phenomenon (68, 69). The fetal-neonatal population of Leydig cells does not respond to estrogen-induced inhibition of steroidogenesis (83, 84). The circulating fetal estrogen levels may also be neutralized by $\alpha$-fetoprotein, at least in rodents (83). Furthermore, estrogen production, aromatase activity, and estrogen receptors are unmeasurable or very low in the fetal Leydig cells (68, 69, 82, 83). Both develop gradually as the fetal Leydig cells are replaced by the adult population during the postnatal development. In the adult testis, estrogen then triggers, through its receptor, an intratesticular feedback loop that leads to impaired activity, especially of the 17-hydroxylase/17-20-lyase cytochrome P-450 enzyme, and the blockade of androgen production (82). A similar response can be induced in the fetal Leydig cells only after prolonged culture with estrogen (68, 69).

This functional difference between the fetal and adult Leydig cells makes sense physiologically. If the fetal Leydig cells were inhibited by high gonadotropin levels and estrogens as effectively as the respective adult cells, they probably could not produce the high-level androgen synthesis that is vital for male-type sexual differentiation. The fetal circulation has high levels of estrogens, progestins, and hCG of placental origin. These concentrations are so high that similar levels would probably be inhibitory in adult circulation.

The LH-induced down-regulation of LH receptors is partly due to increased internalization and degradation of the receptor and partly due to suppressed expression of the LH receptor gene (87). The absence of this phenomenon in the fetal Leydig cells is at least partly due to the absence of suppression of LH receptor mRNA expression. We showed recently that the suppressed gene expression of the LH receptor mRNA is not observed in the neonatal testis after hCG treatment (84). Whether the internalization of LH/hCG receptor complexes is also different from that of the adult Leydig cells is not known.

Very little is known about the biochemical mechanisms responsible for the high steroidogenic capacity of the fetal Leydig cells and for the apparent resistance to a number of negative regulatory effects encountered in the adult cells. It is possible that there are some endocrine or paracrine factors in fetal circulation or testis that inhibit the mechanisms responsible for the inhibitory effects. It is also possible that the inhibiting mechanisms are not functional in the fetal testis, but are a result of specific activation during the postnatal development. It is tempting to speculate that mechanisms as basic as the various components of the transmembrane signal transduction system appear gradually, and the fetal-type function of the Leydig cells is a result of insufficient maturation of this system. Even the mRNA of the fetal Leydig cell LH receptors may be regulated by different promoter sequences that are not under the regulatory influences involved in receptor down-regulation. The multiple mRNA species of LH receptor and likelihood of multiple translation initiation sites of the gene speak for this possibility (88).

There is some evidence that the signal transduction system involved in suppression of cAMP production is not functional in the fetal Leydig cells (89). Warren (90) recently observed that the inhibitory guanine nucleotide-binding regulatory protein-mediated inhibition of testosterone production is not functional in animals younger than 23 days. We have now extended these observations to younger animals and found that the appearance of the effect of the inhibitory guanine nucleotide-binding regulatory protein on testicular steroidogenesis is absent in the fetal Leydig cells. It appears concomitantly with the transition of the fetal to adult Leydig cell dominance around Days 10–15 postpartum (91). Whether this is related to the absence of LH receptor down-regulation and the steroidogenic lesions is not yet known.

In conclusion, it seems that many mechanisms negatively modulating testosterone synthesis are absent in the fetal Leydig cells. In contrast, the adult cells are protected from excessive gonadotropin stimulation and androgen synthesis by a multitude of inhibitory modulation mechanisms. In the fetus, the importance of active androgen synthesis is known, but we do not know why the adult is so effectively protected from too active androgen production.

**Paracrine Regulation of Fetal Leydig Cells**

As in the adult, it is obvious that gonadotropins do not provide the only exogenous signal regulating the fetal Leydig cells. The paracrine/autocrine regulation of Leydig cell function in the adult has recently received considerable attention (64, 92). Given the special endocrine milieu of the fetal period, it is also obvious that the paracrine modulation of Leydig cells may have its specific fetal-type features. Our current knowledge about these aspects of the fetal Leydig cell function is even sparser than our knowledge of the role of gonadotropins. The role of different growth factors as paracrine modulators of adult testicular function was recently studied extensively, but the developmental aspects of this regulation are still almost totally unknown. However, unlike the gonadotropin regulation, no clear picture is yet available on the special features of the autocrine and paracrine regulation of the fetal Leydig cells.

The recent cloning of anti-müllerian hormone (AMH) has greatly advanced our knowledge of this Sertoli cell-derived fetal hormone. AMH has a paracrine role in inducing müllerian duct regression in the male embryo. In addition, some other findings suggest that it may also play a role within the testis, and also in the Leydig cells. In this regard, the finding that AMH is an aromatase inhibitor in the fetal ovary (93) is of particular interest. Since aromatase activity is very low in fetal Leydig cells (see above), it is possible that AMH, coming from Sertoli cells, has an inhibitory effect on aromatase activity of the fetal Leydig cells. AMH arrests meiosis in adult murine oocytes (94), and an analogous function in the inhibition of spermatogenesis in the fetal testis has been proposed. Furthermore, AMH may synergize with testosterone in supporting testicular descent (95).

Both adult and fetal Leydig cells produce opioid peptides (96). Although no features specific to the fetal Leydig cells are known in the production or action of these peptides, they may have a special role during the early stages of development. Leydig cell $\beta$-endorphin production is stimulated by LH (97), and in the adult, it inhibits a number of Sertoli cell functions (96). In the fetus and neonate, the paracrine effect of $\beta$-endorphin may be to contribute to the quiescent state of the testis before the onset of sexual maturation. Both stimulatory and inhibitory effects of other opioid peptides have been shown in fetal-neonatal Leydig cells (96, 98). Interestingly, $\beta$-endorphin suppresses the FSH-stimulated proliferation of isolated neonatal Sertoli cells (99) and may, therefore, be one of the paracrine factors contributing to the neonatal determination of the Sertoli cell pool size. This effect is distinctive to the fetal-neonatal period, since the proliferation of Sertoli cells ceases at 10–20 days of age (99).

Inhibins are another groups of paracrine factors produced both by fetal and adult testes. Inhibin peptides are produced both by the Leydig and Sertoli cells (100), and hence the Leydig cells can be both the origin and target of the inhibin-mediated paracrine (or autocrine) regulation. The role of inhibin seems to be greatly dependent upon the developmental stage (100). Therefore, we can expect that in Leydig cells, the inhibins have specific functions that are different from those in the adult.

Although the role of inhibin and activin in the function of the fetal Leydig cells has not been addressed, we can make some conclusions from the existing data on the presence and effects of these peptides and their cognate mRNA in the developing rat testis. Only the $\alpha$-subunit is consistently expressed in the interstitial (Leydig) cells from Day F 14 fetuses (101). The $\beta_A$ subunit seems to be transiently expressed in the Leydig cells between birth and Day 30 of life (102–104), and Lee *et al.* (105) have demonstrated activin-like activity in spent media of cultured Leydig cells from 17-day-old rats. The $\beta_B$-subunit message was demonstrated over the interstitial cells (104). Although the roles of inhibin and activin in the development of the embryonic testis are apparent, there is no direct evidence of biologically active activin or inhibin production by the fetal Leydig cells. Only production of $\alpha$-subunit by these cells has been documented. More information is available on the function of the fetal Leydig cells as a target of inhibin and activin action. Activin inhibits (106–108), and inhibin stimulates (106) LH-dependent steroidogenesis of adult Leydig cells and Leydig cell lines in culture. The same has been shown in the neonatal rat testis (106), which suggests that the paracrine modulation is already functional in the fetal Leydig cells, but it is not known whether the fetal cells differ in this respect from the adult ones.

Two studies have addressed the presence of inhibin peptides in the primate fetal gonad (109, 110). It seems that inhibin $\alpha$-subunit is present in the midterm human fetal Leydig and Sertoli cells, whereas both $\beta$-subunits are present only in interstitial (Leydig) cells. With development, as inferred from observation on late gestation rhesus monkey fetuses, $\alpha$-subunit expression was shifted only to the tubules, and only the $\beta_B$-subunit was present in both the tubules and interstitium. Thus, there is a shift in the subunit localization within the testis during embryonic development. These changes also suggest a shift in the function of the inhibin peptides within the testis during development, but additional details remain obscure. We also do not know about the functional correlates of inhibin, activin, and the fetal Leydig cell.

There are several observations suggesting the presence and age-specific effects of several growth factors in the fetal Leydig cells. Transforming growth factor-$\alpha$ is present in the 1-day-old rat Leydig cells, where it may be involved in the autocrine regulation of Leydig cells or may act as a paracrine regulator of growth and differentiation of the seminiferous tubules (111). Since this growth factor is similarly located in the adult testis (111), is not known whether this fetal form of epidermal growth factor has any special functions during the early stages of testicular development. Hansson *et al.* (112) showed recently that all cells of the neonatal rat testis contained insulin-like growth factor-1 immunoreactivity, whereas only the spermatogenic cells of the adult testis were immunopositive. Again, this finding suggests a special autocrine/paracrine action of insulin-like growth factor-1 in the developing testis.

There are several other examples of differential effects of growth factors and paracrine effectors on fetal and adult Leydig cell function. Androgens and glucocorticoids are inhibitory to LH-dependent steroidogenesis of adult Leydig cells, but not of the fetal cells (113). Antidiuretic hormone inhibits the same function in neonatal and adult cells, but not in the fetus (90, 113). The effects of tumor necrosis factor and interleukin 1 are different (114). Since these agents are mitogenic to normal adult cells and inhibitory to rapidly dividing malignant cells, these actions appear to parallel their effects on Leydig cells, i.e., stimulation of adult and inhibition of the rapidly dividing fetal Leydig cells. Both suppress testosterone production by the fetal Leydig cells in culture rather than augment it, as they do for adult testis cells. In contrast, the effects of GnRH (acute stimulation and long-term inhibition) and corticotropin-releasing factor (inhibition) on Leydig cell steroidogenesis seem to be similar in the fetus and adult (115, 116). The neonatal or immature testis is frequently used as a model in studies of Leydig cell function, but such results should not be directly extrapolated to the adult situation. The effects may be closely dependent upon the nature of the cell population studied, whether fetal, immature, or adult.

## Concluding Remarks

The fetal period represents a very active period of testicular endocrine function. A specific fetal-type population of Leydig cells is responsible for this activity. The functions of testosterone in the fetus are important, although very different from those observed in the adult. These activities also occur in an endocrinological environment that is very different from that of the adult. For these reasons, it is expected that the structural and functional features of the fetal Leydig cells have numerous special characteristics in comparison to the adult. Constant morphological and functional changes are typical of these cells during fetal life, a feature that differentiates them from the constant adult population of Leydig cells. The functional activity of the fetal Leydig cells clearly precedes their morphological maturation. Characterizing the special features of Leydig cells, and contrasting them with the respective events in the adult Leydig cells, provides us with an alternative perspective in attempts to unravel the functional features of steroidogenic cells.

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