The functional maturation of the Sertoli cell and Leydig cell in the mammalian testis

G. M. H. Waites, Alison C. Speight and N. Jenkins*

Department of Physiology & Biochemistry, University of Reading, P.O. Box 228, Whiteknights, Reading RG6 2AJ, U.K.

Introduction

The morphological features and associations of the Sertoli and Leydig cells in the adult have clear implications for their functional characteristics and for their development in the maturing testis. For example, adult Sertoli cells form intimate cellular associations with germ cells through both physical contact and gap junctions with significance for metabolic cooperation between the two cell types. Inter-Sertoli cell junctional specializations are influential in the context of the 'blood–testis barrier'. They also establish the polarity of these cells with their basal aspect against a peritubular myoid cell layer and a luminal aspect from which fluid is secreted. Leydig cells are associated with the peritubular myoid cells and with the interstitial blood vessels, being surrounded to varying extents according to species by interstitial spaces and fluid. There is a positive correlation between the amount of testosterone secreted by perfused testes *in vitro* of various species and the volume of smooth endoplasmic reticulum in their Leydig cells (see Ewing & Zirkin, 1983). The transport of the secretory products of the Sertoli and Leydig cells depends upon dynamic exchanges with the various fluids secreted by the testis (see reviews by: Waites, 1977; Steinberger & Steinberger, 1977; Fritz, 1978; Setchell, 1978; Means *et al.*, 1980; Purvis & Hansson, 1981; Parvinen, 1982; Waites & Gladwell, 1982; Ritzén & Syed, 1985; Sharpe, 1984).

Thus, the adult features of the Sertoli and Leydig cells are relevant when considering their functional maturation in the developing testis.

Prenatal development

The precursor cell types of the Sertoli and Leydig cells appear early in fetal life (see Gondos, 1977). The former are believed to arise from the gonadal blastema and the latter from undifferentiated mesenchymal cells in the interstitium (see Pelliniemi & Dym, 1980). Thus, sexual differentiation of the male gonad starts with the formation of the testicular or seminiferous cords at 13 days of gestation in the rat (Jost, Magre, Cressent & Perlman, 1974; Jost, Magre & Ageloupoulou, 1981), 26 days in the pig (Pelliniemi, 1975; Pelliniemi & Lauteala, 1981) and 42 days in the human (Pelliniemi & Dym, 1980).

The testicular cords are believed to be directed by the presence of the H–Y antigen and its binding to receptors on the somatic gonadal blastema cells (Wachtel, Ohno, Koo & Boyse, 1975; Ohno, 1976; Wachtel & Koo, 1981; Silvers, Gasser & Eicher, 1982; Gore-Langton, Tung & Fritz, 1983; see Ritzén & Syed, 1985). In the pig fetus at 27 days of age testicular cords surround interstitial spaces which contain small, irregularly shaped, undifferentiated cells. Primordial germ cells, while capable of free migration in the open channels of the interstitium, nevertheless associate themselves with the precursor Sertoli cells. The latter have one or two large nucleoli and are the first stage at which anti-Müllerian hormone (AMH) activity appears in the pig (Tran, Meusy-Dessolle & Josso, 1977). By

* Present address: Biological Laboratory, University of Kent, Canterbury CT2 7NJ, U.K.

35 days onwards, pig Sertoli cells have a well developed Golgi apparatus and morphologically appear to be well equipped for protein synthesis (van Vorstenbosch, Spek, Colenbrander & Wensing, 1984).

Steroidogenesis begins in the fetal pig testis at 30–35 days (Raeside & Sigman, 1975) at which time the interstitial cells have differentiated and contain abundant smooth endoplasmic reticulum (Pelliniemi & Lauteala, 1981). A similar steroidogenic capacity becomes apparent in the human fetal gonad at 8 weeks (Huhtaniemi, Ikonen & Vihko, 1970). The proportion of interstitial tissue then increases to a peak at around 12 weeks fetal age and is followed by an involutionary phase (Niemi, Ikonen & Hervonen, 1967; Pelliniemi & Niemi, 1969). The stimulation of fetal androgen production is associated with the peak in serum hCG at 12 weeks rather than with LH or FSH secretion which is not detected in human fetal plasma until later (Kaplan, Grumbach & Aubert, 1976).

Fetal Sertoli cells actively intervene in the organization of the male gonad through the secretion of the glycoprotein AMH early in fetal life, i.e. at 14.5 days in the rat (Picon, 1970; Josso, Picard & Tran, 1977, 1980). AMH has been localized immunocytochemically in the rough endoplasmic reticulum of the developing bovine Sertoli cell where it is first detectable at 42–43 days of fetal life and reaches peak concentrations at 50–80 days corresponding with the period of Müllerian duct regression (Vigier, Picard & Josso, 1982; Vigier, Tran, du Mesnil du Buisson, Heyman & Josso, 1983; Josso & Picard, 1985). Recent evidence indicates that AMH dephosphorylates proteins on the plasma membrane of Müllerian duct cells and thus antagonizes the effects of epidermal growth factor (Donahoe, Hutson, Fallat, Kamagata & Budzik, 1984).

Receptors for LH are first detected in the rat fetal testis at 15.5 days and achieve maximal concentration at birth (Warren, Huhtaniemi, Tapanainen, Dufau & Catt, 1984). The binding of FSH in the fetal rat testis increased from about 17.5 days until birth which could be attributed to an increase in the number of FSH receptors per Sertoli cell, an increase in the number of Sertoli cells or to both. Mitosis of Sertoli cells occurs during the growth of the testicular cords (Pelliniemi & Dym, 1980), during the late fetal period in rats under the influence of FSH (Orth, 1984) and is well-established at birth and then declines in early postnatal life (rat: Steinberger & Steinberger, 1971; Nagy, 1972; lamb: Courot, 1971; Hochereau-de Reviers & Courot, 1978).

Birth intervenes in the developmental process at different stages in testicular maturation in different species and this should be borne in mind when considering postnatal events.

Postnatal development

Sertoli cell

Practically all of the functional characteristics of the Sertoli cell undergo marked changes between birth and puberty. For example, androgen binding protein (ABP) is a well-characterized protein that binds testosterone and 5 α -dihydrotestosterone with high affinity and is considered to be a postnatal 'marker' for protein secretion by the Sertoli cell and to be under the direct control of FSH (see Means *et al.*, 1980; DePhilip, Feldman, Spruill, French & Kierszenbaum, 1982). ABP is detectable in fetal rat serum from Day 16 of gestation in both sexes and therefore its prime source at this stage may be the liver (Carreau, Musto, Bercu, Bardin & Gunsalus, 1985), although a local testicular content of Sertoli cell origin is not ruled out. After birth ABP reappears in the circulation of only male rats and rises sharply to peak values at around 3 weeks. With the formation of the blood–testis barrier and the start of secretion of tubular fluid by the Sertoli cells, ABP is diverted to the epididymis and less appears in the circulation.

Since the early 1970s most of our knowledge concerning the functional maturation of Sertoli cells derives from primary cell culture studies. When cells from rats of about 3 weeks of age are cultured in a defined medium, they secrete several polypeptides, ranging in molecular weight from approximately 16 000 to 140 000 (Wilson & Griswold, 1979; Kissinger, Skinner & Griswold, 1982).

Text-fig. 1. Proportion (%) of total [³H]leucine incorporation into Sertoli cell-secreted proteins. Sertoli cell cultures were prepared from the testes of lambs of 2, 6, 8 or 12 weeks of age. [³H]Leucine was added at Day 6 of culture. After 24 h its incorporation into cellular and secreted protein was separately determined. Values are mean $\pm$ s.e.m. *** $P < 0.001$ compared to value at 2 weeks. Approximate time of appearance of primary spermatocytes (PS) in the lamb testis.

Amongst these are ABP (Fritz, Kopec, Lam & Vernon, 1974), plasminogen activator, a tissue protease (Lacroix, Smith & Fritz, 1977), transferrin and ceruloplasmin, iron and copper transport proteins respectively (Skinner & Griswold, 1980; Kissinger *et al.*, 1982) and galactosyl transferase, an enzyme involved in the glycosylation of proteins (D. W. Hamilton, personal communication). The patterns of proteins secreted from Sertoli cells obtained from rats at 20 and 60 days of age were similar to each other but differed from those of Sertoli cells from 10-day-old rats (Kissinger *et al.*, 1982). Sertoli cells cultured from lambs at different maturational stages demonstrate a significant age-dependent increase in the proportion of newly synthesized protein for secretion as measured by the incorporation of [³H]leucine; protein secretion is increasing at the time when spermatogenesis is starting (Text-fig. 1; Speight, Clifford & Waites, 1985). Some of the secreted proteins in the rat were shown to arise from peritubular myoid cells. Such cells in co-culture can influence the efficiency of ABP secretion by Sertoli cells (Tung & Fritz, 1980; Hutson & Stocco, 1981) and act co-operatively with Sertoli cells to synthesize the various components of the extracellular matrix of the basal lamina (Fritz, 1985).

In most mammalian species, Sertoli cells spend long periods associated with only one germ cell type, the pre-spermatogonium, e.g. lamb (3–4 months; Courot, 1971), pig (3–4 months; Peyrat, Meusy-Desolle & Garnier, 1981), human (10–12 years; Camatini, Franchi, de-Curtis, Anelli & Masera, 1982), whereas the Sertoli cell of the rat becomes associated with proliferating germ cells within hours of birth. An age-dependent secretion of lactate and pyruvate by rat Sertoli cells has been shown (Jutte, Jansen, Grootegoed, Rommerts & van der Molen, 1983): lactate production was stimulated by added FSH and testosterone, and the effect was most pronounced at 4 weeks of age (Text-fig. 2a). Additional stimulation was also elicited by insulin (Oonk, Grootegoed, Reuvers & van der Molen, 1985). Spent medium from cultured Sertoli cells from 4-week-old rats stimulated leucine incorporation into isolated germ cells, an effect which could be accounted for by the amounts of pyruvate and lactate present (Text-fig. 2b; Jutte *et al.*, 1982, 1983). These products of

Text-fig. 2. The potential support of germ cell function by Sertoli cell products of glycolysis (data from Jutte *et al.*, 1983). **(a)** Sertoli cells from prenatally irradiated rats incubated for 24 h in medium without hormones (○) or with added FSH (7.5 NIH-FSH-S1 U/ml) and testosterone (200 ng/ml) (●). * $P < 0.05$ compared to value at 3 weeks; ** $P < 0.05$ compared to value at 4 weeks. Approximate time of appearance of primary spermatocytes (PS), early and late spermatids (ES, LS) in the rat testis. **(b)** Stimulatory effect of leucine incorporation into germ cells of spent medium from FSH + testosterone stimulated Sertoli cells from 4-week-old rats (B) compared to fresh medium (A). * $P < 0.01$ compared to value for A.

glycolysis have also been shown to increase ATP levels in isolated round spermatids (Mita & Hall, 1982). It is suggested, therefore, that germ cells may depend upon Sertoli cells for their metabolic support.

Several of the activities of the Sertoli cell are stimulated by FSH especially in the immature animal. The number of FSH receptors increases slowly until approximately 80 days of age in the lamb (Barenton, Hochereau-de Reviers, Perreau & Saumande, 1983), in association with increasing numbers of Sertoli cells during this period (Courot, 1962). Thereafter sharp increases in the number of both LH and FSH receptors coincide with the onset of spermatogenesis.

Leydig cell

In most mammalian species, the high plasma testosterone concentrations achieved shortly after birth decline and remain low until just before puberty (Ewing & Zirkin, 1983). The concentration of LH receptors, expressed as moles per mg testicular protein declines postnatally in the lamb and pig (Barenton *et al.*, 1983; Sundby, Torjesen & Hansson, 1983). This change may be due in part to the differential growth of the tubular and interstitial elements within the testis (Waites, Wenstrom, Crabo & Hamilton, 1983), although atrophy of a proportion of the Leydig cell population has been observed during this period in rats (Lee & Burger, 1983). The concentration of LH receptors remains constant during the first 5 days of life in rats yet the testosterone concentrations within the testis decline rapidly (Warren *et al.*, 1984).

Several steroidogenic enzymes display age-dependent activities which do not parallel the numbers of Leydig cells in the testis (Preslock, 1980; Payne, Chase & O'Shaughnessy, 1982). The activity of 5 α -reductase in rats reaches a peak at 15–20 days of age and results in an abundance of

Text-fig. 3. The augmentation by porcine FSH (50 ng/ml) of binding of ¹²⁵I-labelled hCG and of hCG-provoked testosterone secretion by the Leydig cells of 3-week-old pigs when co-cultured with Sertoli cells (adapted from the data of Reventos *et al.*, 1983). nd = undetectable.

5 α -reduced androgens in plasma and a paucity of testosterone. Another example of altered steroidogenesis in early postnatal life is the change in the ratio of androstenedione to testosterone which occurs during the prepubertal period in the bull (Lindner, 1969).

The increasing plasma concentrations of LH during puberty result in an increase in the number of active Leydig cells with parallel elevations in the testicular content of the steroidogenic enzymes 17 α -hydroxylase, C17–20 lyase and Δ_5 -3 β -hydroxysteroid dehydrogenase–isomerase (Payne *et al.*, 1982), suggesting that the activities of these enzymes within each Leydig cell do not change during development. Controversy surrounds the question of whether there are one or two different functional populations of Leydig cells (Payne *et al.*, 1982), an issue which may relate to the method used to separate the cells (Aquilano & Dufau, 1984).

The activity of aromatase (a multi-enzyme complex which converts androgens to oestrogens) in rat Sertoli cells declines during puberty while at the same time it increases in the Leydig cells (Rommerts, de Jong, Brinkmann & van der Molen, 1982; see Ritzén & Syed, 1985). Despite this, the total production of oestrogen remains at < 0.1% of androgen output in most mammalian species, with the exception of the stallion and boar (Amann & Schanbacher, 1983). Implants of oestradiol depress plasma androgen concentrations in lambs (Jenkins & Waites, 1983), and conversely immunization against oestradiol stimulates androgen production in rats (Nishihara & Takahashi, 1983) and lambs (N. Jenkins, P. G. Knight, C. M. Howles, B. A. Morris & G. M. H. Waites, unpublished observations). Studies *in vitro*, however, suggest that although the Leydig cells of neonatal animals have oestrogen receptors (Brinkmann, Mulder, Lamerstahlhofen, Mechielsen & van der Molen, 1972; Moger, 1980; Benahmed, Bernier, Ducharme & Saez, 1982), the concentrations of oestradiol required to impair the steroidogenic function of Leydig cells directly are probably never achieved *in vivo*, and therefore a suppression of LH secretion is a more likely explanation of the effects of oestradiol on androgen production.

Inter-cellular communication

The co-culture of pig Leydig with Sertoli cells has shown that FSH, acting via the Sertoli cell, stimulates the secretion of testosterone by the Leydig cells (Text-fig. 3; Reventos, Benahmed,

Text-fig. 4. Age-dependent changes in plasma FSH and LH concentrations and some associated functions of Sertoli and Leydig cells in the rat. **(a)** Testicular FSH receptors, expressed as FSH bound (c.p.m.) per testis for fetal values (data from Warren *et al.*, 1984) and as pmol per testis for postnatal values (data from Ketelslegers, Hetzel, Sherins & Catt, 1978) are compared with postnatal plasma FSH concentrations (data from Ketelslegers *et al.*, 1978). Bioassayable testicular AMH was detectable at Day 14.5 of fetal life and remained at a similar concentration until Day 4 after birth and subsequently declined (data from Picon, 1970). Serum ABP concentrations declined during fetal life, reflecting decreased synthesis in the liver, and increased after birth due to increased synthesis by the Sertoli cell; the subsequent decline corresponds to the establishment of the blood–testis barrier and the excretion of ABP towards the epididymis (data from Carreau *et al.*, 1985). **(b)** Testicular LH receptors expressed as fmol per testis for fetal values (data from Warren *et al.*, 1984) and as pmol per testis for postnatal values (data from Ketelslegers *et al.*, 1978) are compared with postnatal plasma LH concentrations (data from Ketelslegers *et al.*, 1978). Testicular testosterone was detectable from Day 15.5 of fetal life and increased to a maximum concentration by Day 18.5 and remained at a similar concentration through birth but decreased by Day 5 after birth (data from Warren *et al.*, 1984). Plasma testosterone concentration was elevated 2–8 days after birth and then declined to remain low until Days 35–55 when a rapid increase was observed, starting about 15 days after the onset of the increase in LH receptors (data from Ketelslegers *et al.*, 1978).

Tabone & Saez, 1983). The chemical nature of the messengers which may mediate Sertoli–Leydig cell communication remains uncertain, although evidence confined to rats has identified a gonadotrophin-releasing hormone-like peptide in Sertoli cell secretions which binds to receptors on the Leydig cells (Sharpe, 1982, 1984). Prolactin has been shown to induce Leydig cell receptors for LH, but its role in testicular maturation remains ill-defined (Payne *et al.*, 1982).

The compensatory changes that follow hemicastration in the prepubertal period may provide insights into the mechanisms of normal testicular development. Most studies indicate that FSH secretion is increased by neonatal hemicastration and, in lambs, is secondarily suppressed back to control levels at 8–10 weeks of age, i.e. before the onset of spermatogenesis (Walton, Evins, Hillard & Waites, 1980; Waites *et al.*, 1983). It was postulated that this FSH modulation might be a manifestation of ‘inhibin’ secretion by the FSH-stimulated prepubertal Sertoli cell of the lamb as previously suggested for the rat (Steinberger, 1979). Another feature of the response to hemicastration in rats, lambs, and bulls is the maintenance of normal plasma androgen concentrations despite the loss of steroidogenic tissue and any compensation which might occur through an increase in Leydig cell numbers (Cunningham, Tindall, Huckins & Means, 1978; Barnes, Longnecker, Reisen & Woody, 1980; Jenkins, Speight & Waites, 1985). The altered androgen production may be stimulated indirectly by the FSH acting on Sertoli cells since an increase in LH secretion in response to hemicastration is less evident. The sensitivity of Leydig cells to LH is increased after hemicastration in bulls (Boockfor, Barnes & Dickey, 1983) but not in lambs (Jenkins & Waites, 1983).

Conclusion

There is clear evidence, therefore, that the Sertoli cell and the Leydig cell exhibit specific functional activities at different stages in their maturation (Text-fig. 4). It is less clear how the age-dependent development of the two cell types is interrelated and co-ordinated. The ‘signals’ that pass between these cells are likely to be steroid from the Leydig cells and predominantly peptide from the Sertoli cells, but how these signals are initiated or modulated remains unknown. Co-culture experiments are beginning to yield some information, but ultimately, an intimate understanding of testicular maturation in terms of co-ordinated cell–cell interactions will need elucidation in the intact animal.

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