Sertoli Cell Development of Pig Testis in the Fetal and Neonatal Period

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ABSTRACT

The Sertoli cells of pig fetuses from 35 days postcoitum until 1 mo after birth have been investigated by light and electron microscopy in decapitated animals and their control littermates, as well as in untreated animals. Until 52 days postcoitum, Sertoli cells change in form during the formation of sex cords but from then onwards they are rather uniform. They primarily display an elongated nonindented nucleus with one or more prominent nucleoli, a well-developed Golgi apparatus, and in the basal compartment below or beside the nucleus, a large lipid droplet. There are large quantities of rough endoplasmic reticulum (RER) from 52 days postcoitum onwards, often with complex whorl forms and a parallel arrangement of profiles with relatively few ribosomes. After birth their numbers seem to be somewhat less, and by 1 mo after birth the RER profiles are often shorter and almost free of ribosomes. Clustered ribosomes are found in large quantities throughout the period under investigation. Especially in the early fetal period, the endoplasmic reticulum (ER) profiles show prominently filled cisternae. Mitochondria are mostly long and slender, or small and ovoid. Most have lamellar cristae, but mixed lamellar-tubular cristae can also be seen. Between decapitated, control and untreated animals no obvious ultrastructural differences could be observed. The peritubular cell sheath surrounding the sex cords did not show signs of differentiation into a layer of myoid cells.

INTRODUCTION

In the pig the gonad becomes ultrastructurally recognizable as a testis at approximately 26 days postcoitum (p.c.) (Pelliniemi, 1975a). Leydig cells cannot be identified before 30 days p.c. (Pelliniemi et al., 1979), although the testis is already capable of producing testosterone (Stewart and Raeside, 1976). Testosterone concentration peaks at approximately 37 days p.c. and declines afterwards (Ford et al., 1980). From 35 days p.c. onwards the changes in morphology of Leydig cells, important in steroidogenesis, match the biochemical data: a developmental wave lasting until approximately 60 days p.c. which is pituitary independent, and a second wave which is pituitary dependent. This latter period begins at approximately 70 days p.c. and lasts until approximately 1 mo after birth (term is 114 days p.c.) (van Vorstenbosch et al., 1982, 1984).

Morphological data on the developing pig Sertoli cell are scarce. The early sex cords consist mainly of precursor Sertoli cells (sustentacular cells) and a relatively small number of primordial gonocytes (Pelliniemi, 1975a,b). The development of a follicle-stimulating hormone (FSH) dependency of these cells has hardly been studied at all.

The fetal (precursor) Sertoli cells produce at least one protein, anti-Müllerian hormone (AMH), which is important in male sexual development because it causes regression of the Müllerian duct. In the pig AMH is produced from 27 days p.c., a high concentration is reached by 33 days p.c. and high levels are present at birth, but decline afterwards. Data on the intermediate period are not available, with the exception of 90 days p.c., at which time the level is also high (Tran et al., 1977, 1981). Mature Sertoli cells synthesize proteins such as androgen-binding protein (ABP) and inhibin. In vitro they are capable of converting testosterone into estrogens (Dorrington et al., 1978). However, little is known about at what age Sertoli cells acquire these functions (Colenbrander et al., 1982).

In the pig, fetal hypophysectomy by decapitation does not seem to effect Sertoli cell de-
development, at least not at the light microscopic level, during the later fetal period when the pituitary activity is high. This is in sharp contrast to Leydig cell development which is pituitary dependent (Colenbrander et al., 1979; van Vorstenbosch et al., 1982, 1984). Quantitative studies of sex cord development, however, have shown a significant increase in diameter and a decrease in cord length (Colenbrander et al., 1979). The latter is also observed in hypophysectomized rhesus monkeys (Gulyas et al., 1977).

The present study was set up to provide quantitative light microscopic and qualitative electron microscopic data on pig Sertoli cell development. Fetal decapitation experiments were carried out to determine a possible lutetinizing hormone (LH) and FSH dependency of this development. The period chosen (30 days p.c. until 1 mo postpartum) covers the first and second developmental waves of Leydig cell development, which includes the period of testicular descent. Attention has been focused on organelles involved in protein and steroid synthesis.

MATERIALS AND METHODS

Testes of fetuses of Yorkshire and Dutch Landrace crossbred pigs were studied. At 42 days p.c. a number of fetuses was decapitated according to the method of Stryker and Dziuk (1975); littermates served as controls. Moreover, a number of fetuses of normal sows (untreated animals) were used to check the reliability of the control fetuses, since the handling of the uterus for the decapitation procedure might have affected the normal development of these controls. The number of animals used, sample data and the number of litters are given in Table 1. The period investigated ranged from 35 days p.c. to 30 days postpartum (p.p.).

Materials were handled and processed as reported elsewhere (van Vorstenbosch et al., 1982). In brief: a double fixation in 2.5% glutaraldehyde followed by 1% OsO4 according to Sabatini et al. (1963) was succeeded by block staining in 2% uranyl acetate prior to dehydration in graded series of acetone. Materials were cleared in propyleneoxide and embedded in either DER 1-mixture (Lockwood, 1964) or in Durcupan ACM (Fluka Chemical Co., Hauppauge, NY). Semithin sections were stained with either paragon or toluidine blue. Thin sections were stained with lead citrate (Venable and Coggshall, 1965). A Philips 201G microscope was used at 60 kV with a 30-µm objective aperture.

On semithin sections of true cross-sectioned seminiferous tubules, diameters were measured and the number of nuclei of both germ cells and Sertoli cells were counted in normal, control and decapitated animals at the ages shown in Table 1. Results were analyzed with the one-way analysis of variance (ANOVA F1) at a level of significance: P<0.05. The correlation coefficient was calculated between the number of nuclei and the surface of the tubular cross sections. For the calculation of the surfaces it was assumed that the true cross sections were circles.

Reference standards of negative-stained crystals of catalase showing the 8.12 nm periodicity (Polysciences, Warrington, PA) were used to estimate the diameters of cytoplasmic filaments and to classify the microfilaments if their diameter was less than 8 nm and as intermediate filaments if their diameter was between 8 and 12 nm.

RESULTS

Light Microscopy

At 35 days postcoitum the sex cords formed an anastomosing network consisting of cell plates and cord-like structures of 2-3 cell layers thickness. The primitive sex cords were surrounded by numerous large Leydig cells, but in the peripheral interstitium these cells were

TABLE 1. Experimental design: the number of animals observed in each group.

<table>
<thead>
<tr>
<th>Age in days (±) 1 daya</th>
<th>Postcoitum (p.c.)</th>
<th>After birthb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35    52  62  75  80  90  100</td>
<td>Term 10  20  30</td>
</tr>
<tr>
<td>Decapitated animalsc</td>
<td>5     5     5     3     3     6     7</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>10    10    5     6     6     4     12</td>
<td></td>
</tr>
<tr>
<td>Normal (untreated) animals</td>
<td>9     5     5     2     3     4     5</td>
<td></td>
</tr>
<tr>
<td>Litter</td>
<td>2     6     4     6     3     4     7</td>
<td></td>
</tr>
</tbody>
</table>

a± One day variation in the sampling data; the age of the animals was precisely known.
bTerm at 113 days p.c.
cDecapitation at 42 days p.c.
dLitter origin of the neonatal piglets unknown.
FIG. 1. Thirty-five days postcoitum (p.c.)-untreated animal. The early sex cords formed an anastomosing plate-like network (asterisk). X 500.

FIG. 2. Fifty-two days p.c-control animal. Sex cords were clearly formed but still anastomosed at the periphery of the testis. Numerous germ cells could be observed. X 200.

FIG. 3. Fifty-two days p.c-control animal. Sex cords varied in diameter. Germ cells (single, pairs and clusters) could be observed. X 500.
scarce. In the sex cords the majority of the cells were precursor-Sertoli cells (sustentacular cells) and only a few gonocytes could be observed (Fig. 1).

At 52 days p.c. the organization of the sex cords was changed. Centrally, the anastomosing cords and plates were transformed into single straight solid cords but towards the testis periphery anastomoses still existed (Fig. 2). A number of gonocytes were in contact with the basal membrane of the cords and these cells were now, by definition, spermatogonia. Pairs, and sometimes small groups of gonocytes could be observed (Fig. 3). The number of Leydig cells decreased. Sex cord organization in the decapitated animals did not differ from control animals.

From 62 days p.c. onwards the organization of the sex cords was stable until the end of the observation period. However, there was a shift in the number of Sertoli cells and gonocytes. The diameter of the sex cords of both normal and control animals increased rapidly between 35 and 52 days p.c.; thereafter there was hardly any increase until the end of the observation period. The sex cord diameter in decapitated animals at 62 days p.c. differed significantly from those in controls and normal animals. At birth the difference in diameter between controls and decapitated animals was (59.7 μm

FIG. 4. Term-control animal. Except for its diameter the sex cord at term did not differ from that at 52 days p.c. X 500.

FIG. 5. Term-decapitated animal. The sex cord diameter was larger, the same as in controls. Leydig cells had almost disappeared and the interstitium was intruded by erythrocytes. X 500.
**SEROTLI CELL DEVELOPMENT IN THE PIG**

Electron Microscopy

At 35 days p.c. the precursor Sertoli cells (hereafter termed Sertoli cells) rested upon a well-developed basal lamina which sometimes seemed to consist of several layers. The Sertoli cells had a polygonal or conical form. Their longitudinal axis was radially directed. The intercellular space had a variable width. Cell-to-cell contacts were mostly loose, and only superficial interdigitations could be observed. Coated pits and other signs of pinocytosis were scarce.

Between Sertoli cells, and also between Sertoli cells and gonocytes, gap junction-like cell connections were scarce and of variable dimension. Bundles of tightly packed microfilaments could be observed parallel and adjacent to the basal cell membrane within most Sertoli cells. However, they did not appear in every Sertoli cell. They were seen throughout the observation period. The very tight packing made reliable measurements impossible. They appeared to be smaller than intermediate filaments and seemed to originate from electron-dense plaques on the cell membrane (Figs. 10 and 11). In the basal part of the cells a crisscross arrangement of intermediate filaments of approximately 10 nm diameter could be observed. Some Sertoli cells showed large quantities of these intermediate filaments (Fig. 12). Sometimes they showed a close spatial relationship with the nuclear membrane or with the mitochondrial outer membranes.

The nucleus showed mostly a slightly irregular form but seldom displayed deep indentations. Most cells had one or two large nucleoli (Fig. 13). The Golgi apparatus was well

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**FIG. 6.** Changes in sex cord diameters. The average values with standard deviations are given: control animals (open circles), normal, untreated animals (solid circles), and decapitated animals (asterisks). The same symbols are used in Figs. 7 and 8.

± 4.9 and 73.9 μm ± 7.5, respectively) which is approximately a 15% increase (Figs. 4, 5 and 6).

Only a few gonocytes per cross section could be observed at 35 days p.c. Their number increased until 52 days p.c. and from then onwards it was rather constant until birth in normal as well as in controls. Figure 7 shows that the number of germ cells were identical for normal-control and decapitated animals until 80 days p.c. After birth there was a sudden drop to a level half that from before birth in normal animals. In decapitated animals the number of gonocytes per cross section was significantly higher than in controls from 80 days p.c. to birth (Fig. 7).

The number of Sertoli cells per cross section also increased substantially between 35 and 52 days p.c.; thereafter their number increased linearly with time, but apparently at a slower pace. After birth the number of Sertoli cells per cross section dropped slightly. The number of Sertoli cells per cross section in decapitated animals at birth differed significantly from those in controls and in the normal animal (Fig. 8).

Between the mean of the numbers of gonocytes and Sertoli cells of normal, control and decapitated animals at the various ages on the one hand, and the surface of the sex cord cross sections on the other, there was a correlative coefficient of 0.96 (Fig. 9).

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**FIG. 7.** Changes in the number of gonocytes per cross-sectioned tubule.
number of sertoli cells per cross-sectioned tubule

FIG. 8. Changes in the number of Sertoli cells per cross-sectioned cord in normal, control and decapitated animals.

developed and consisted of several stacks of dictyosomes. Coated and uncoated vesicles were present in varying quantities (Fig. 14). In the Golgi area a centriole was seen during the whole observation period. In rare cases a 9+0 cillum in a narrow tunnel could be observed. In most cases single cilia were surrounded by a much wider space.

The mitochondria very often displayed an elongated-ovoid form. Their cristae were mostly of the lamellar form; the tubular-lamellar form was rare. The endoplasmic reticulum (ER) and the mitochondria frequently showed a close spatial relationship and very often a peculiar arrangement of ribosomes on the membranes could be seen: the juxta-mitochondrial membrane was usually occupied with ribosomes, while the opposite membrane was smooth. The cisternae were wide and filled with finely dispersed flocculent material (Figs. 15 and 16). If there were only a few profiles of ER present, then very frequently each showed an intimate relationship with a mitochondrion. Sometimes the filled cisternae became very large, showing "pouch"-like invaginations, and each of such invaginations contained a mitochondrion (Fig. 17). These complexes may have originated from a fusion process of the single ER-mitochondrial complexes. ER free of ribosomes was not observed. Sometimes the ER formed stacks of parallel-arranged profiles.

Besides a few lysosome-like bodies, some strongly electron-dense, ovoid membrane-bound bodies could be observed, most likely belonging to the lysosomal family. One or a few large lipid droplets per cell were present in its basal compartment.

At 52 days p.c. Sertoli cells differed from Sertoli cells at 35 days p.c. The changes were not as profound as one would expect from the rearrangements of the sex cords. The cords were surrounded by a single basal lamina. The Sertoli cells seemed to be more elongated and showed deep pouches in which the gonocytes were embedded. Frequently, the Sertoli cell separated gonocytes by very thin cell projections (Fig. 18). The intercellular spaces between Sertoli cells, and between Sertoli cells and gonocytes, were mostly uniform. Interdigitations were poorly developed. Coated pits were a common finding both on the basal cell membranes of the Sertoli cells and between Sertoli cells and gonocytes; however, between joining Sertoli cells they were infrequent. Pinocytotic vesicles were very rare. The nuclei appeared more elongated, but otherwise displayed no clear differences to those at 35 days p.c. Large nucleoli were still a regular observation and the Golgi apparatus was well developed. The quantity of coated and uncoated vesicles was considerable but varied from cell to cell. Mitochondria frequently showed long and slender forms, however ovoid mitochondria were also common. The cristae at this age mainly displayed a lamel-
FIG. 10. Fifty-two days p.c.-control animal. Sertoli cells adjacent to one another showed a uniform narrow intercellular space (arrowheads). Bundles of microfilaments (small arrows) seemed to originate from electron-dense plaques on the cell membrane. A large lipid droplet (L) can be seen. X21,000.

FIG. 11. Fifty-two days p.c.-decapitated animal. A section parallel to the basal cell membrane showing a plate-like layer of microfilaments. X21,000.

FIG. 12. Fifty-two days p.c.-control animal. A crisscross arrangement of intermediate filaments could be observed in the basal cell compartment of the Sertoli cell. X35,000.
FIG. 13. Thirty-five days p.c.-untreated animal. Highly complex organized nucleoli were common in early pig Sertoli cells. X21,000.

FIG. 14. Fifty-two days p.c.-control animal. The Golgi apparatus was well developed. Numerous coated and uncoated vesicles could be observed. A cilium in a narrow tunnel (cell invagination?) could be seen in the Golgi area (arrow). X21,000.
FIG. 15. Thirty-five days p.c.-untreated animal. A peculiar arrangement of ribosomes on the ER-juxta-mitochondrial membranes could be observed in the Sertoli cells. X 36,000.

FIG. 16. Thirty-five days p.c.-untreated animal. Higher magnification of a similar complex. The juxta-mitochondrial-ER membrane was occupied with ribosomes, while the opposite membrane was smooth. X 55,000.

FIG. 17. Thirty-five days p.c.-untreated animal. Mitochondrial - ER complex. The cisternae fused, creating a large cistern within its membrane pocket-like pouches in which the mitochondria are located. Note that the juxta-mitochondrial ER membrane contained ribosomes. X 25,000.
FIG. 18. Fifty-two p.c.-decapitated animal. Very thin cell processes of the Sertoli cells enveloped the gonocytes (G). X 25,000.

FIG. 19. Fifty-two days p.c.-control animal. The ER was organized in a loose concentric whirl. The membranes were smooth over large areas but still contained patches of ribosomes. At the bottom left is a gonocyte (G). X 21,000.

FIG. 20. Fifty-two days p.c.-decapitated animal. the ER was more parallel arranged. Also one could observe transitional ER. X 25,000.
lar form. The ER was arranged either in parallel arrays of profiles or in concentric whirls; both are a regular finding (Fig. 19, 20). Their quantity varied but extremely well-developed systems were seen mostly. The relationship between mitochondria and ER was not so evident at 52 days p.c. The ER was irregularly covered with patches of ribosomes but areas completely devoid of ribosomes could also be observed. The cisternae were filled with finely dispersed flocular material. The number of free ribosomes and polysomes seemed rather constant. SER free of ribosomes was not observed.

In all Sertoli cells a few strongly electron-dense, membrane-bound bodies could be observed. Lysosomes (identified on morphological criteria) were also present. In most Sertoli cells a very large lipid droplet could be seen in the basal part of the cell. From 52 days onwards mesenchymal cells formed a loose incomplete sheath around the sex cords.

At 62 days p.c. the ultrastructure of Sertoli cells was very similar to that at 52 days p.c. From 62 days until the end of the observation period, the sex cords hardly changed. The nuclei showed mostly a smooth appearance and indentations typical of the adult Sertoli cells were very scarce. The nucleoli were prominent, and the Golgi apparatus remained well developed. Until about 90 days p.c. the ER was predominantly composed of concentrically arranged whirls, or in the form of parallel stacks. Cisternae were mostly filled. Free ribosomes and polysomes were abundant.

At the end of this period no signs were observed of a differentiation of the peritubular mesenchymal cells into a myoid cell layer surrounding the sex cords. Between untreated animals, controls and decapitated animals no obvious morphological differences were apparent throughout the whole observation period.

**DISCUSSION**

In the pig the undifferentiated gonad and the very early testis have been investigated ultrastructurally until 27 days p.c. (Pelliniemi, 1975a,b, 1976). During that period no smooth endoplasmic reticulum (SER) can be observed in Sertoli cells, with the exception of a very few small concentric profiles which are continuous with rough endoplasmic reticulum (RER). The early pig testis can produce testosterone even before Leydig cells can be identified morphologically (Raeside and Sigman, 1975). In that period, 3β-hydroxysteroid dehydrogenase (HSD) has been demonstrated histochemically in the sex cords (Moon and Raeside, 1972). 3β-HSD, however, is known to be localized in the SER (Christensen and Gillim, 1969). In the period of testicular differentiation dealt with in this study, SER was not observed in the Sertoli cells. Neither this study nor those of Pelliniemi (1975a,b, 1976) showed evidence of mitochondria with pure tubular cristae. The spatial relationship between mitochondria and endoplasmic reticulum (ER) we observed in the early fetal period wanes from 52 days p.c. onwards. A similar change in relationship can be observed in Leydig cells during this period. The ER described seems to be a transitional form between SER and RER. This is also observed for Sertoli cells in the fetal guinea pig (Black and Christensen, 1969) and in the fetal mouse (Almond and Singh, 1980). Transitional ER can produce steroids (Dallner et al., 1966). Possibly the complex mitochondrion-ER is involved in steroid synthesis in the early fetal period. Until the end of the observation period we did not find as much SER as was observed for 6-wk-old piglets (Chevalier, 1978).

The early Sertoli cell produces relatively high concentrations of anti-Müllerian hormone (AMH), a glycoprotein important in male sexual differentiation (Tran et al., 1977). This protein is also produced at high concentrations at 90 days p.c. Shortly after birth the concentration drops sharply and then rises again (Tran et al., 1981). In the bovine Sertoli cells AMH is localized in the cisternae of the RER (Tran and Josso, 1982). The Golgi apparatus seems not to be involved in the secretion process. Secretion granules, frequently observed in protein synthesizing cells, are absent (Tran and Josso, 1982). Our ultrastructural observations are fully compatible with those of Tran et al. (1981). Therefore it seems quite possible that AMH synthesis in the pig occurs during the entire fetal period.

Intermediate filaments were found but in much lower quantities as in Leydig cells of the same developmental period (van Vorstenbosch et al., 1982, 1984). In the Sertoli cells they show the same spatial relationship with the nuclear and mitochondrial outer membranes as in Leydig cells (Aguas, 1981; van Vorstenbosch et al., 1982). Whirls, characteristic for Leydig cells in this period, were never observed. The bundles of microfilaments adjacent to the cell membrane have been described for
Sertoli cells in the rat (Ramos and Dym, 1979). In Sertoli cells of adult pigs small bundles of microfilaments have been observed (Toyama, 1975). Toyama et al. (1979) claim an actin-like nature for these filaments. Actin is known to exist in the immature pig Sertoli cells and, in vitro, its degree of organization is controlled by testosterone and FSH. Testosterone controls the organization and FSH the disorganization of these actin-like filaments (Chevalier and Dufaure, 1981), and in this way cell morphology. It is also claimed that actin plays a role in steroid synthesis (Hall et al., 1980) and in its secretion (Welsh et al., 1980).

Decapitation causes a deprivation of gonadotropins and as a consequence the disappearance of the Leydig cells, resulting in extremely low testosterone concentrations. Although the fetal pituitary produces LH at 45 days p.c. and FSH at 60 days p.c. (Danchin and Dubois, 1982), it seems unlikely that this organ releases gonadotropins in a "physiological dose" before 70 days p.c. (Colenbrander et al., 1982). Therefore it is not surprising that leydig cells show no ultrastructural effect as a result of decapitation before 70 days p.c. (van Vorstenbosch et al., 1982, 1984). It could be argued that the placenta might produce gonadotropin-like substances at a "physiological dose level." However, for the pig this possibility seems unlikely (Amoroso, 1970; Saunders and Porter, 1980). The undisturbed development of Sertoli cells in the decapitated fetal pig strongly suggests that this development is not controlled by gonadotropic hormones.

In the hypophysectomized fetal rhesus monkey (Gulyas et al., 1977) and in the fetal decapitated pig (Colenbrander et al., 1979) an increase in tubular (cord) diameter and a reduction in length of the tubules (cords) can be observed. The close similarity in cellular organization of Sertoli cells in normal, control and decapitated animals does not give a clue for the obvious difference in sex cord diameter and the number of cells per cross section. The positive correlation between the number of cells and cord surface per cross section indicates an increase in cells but not in cell dimension, since there is a constant ratio of the number of cells per surface-unit in normal, control and decapitated animals. An increase in the total number of cells is unlikely, since the number of germ cells is about equal in both control and decapitated animals (Colenbrander et al., 1979) and no indications were found of an increase in the total number of Sertoli cells.

Because decapitated animals lack LH and FSH as well as testosterone sources, the differences in diameter could be explained in terms of a rearrangement of the cytoskeleton according to the schema of Chevalier and Dufaure (1981). This rearrangement might have caused a rounding of the Sertoli cells, possibly resulting in a reshaping of the form of the cords. Another possible explanation for the reshaping of the cords might be found in a change of the intra-testicular pressure resulting from the absence of the Leydig cell component of the testis.

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