

POSTNATAL DEVELOPMENT OF INTERSTITIAL
ENDOCRINE (LEYDIG) CELLS IN PIG'S TESTES –
ELECTRON MICROSCOPY STUDY

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Summary

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The normal ultrastructure of interstitial endocrinocytes in testes of pigs aged from 1 day to 8 months was determined by electron microscopy. It was found out that these cells had similar ultrastructural traits during the entire experimental period – a very well developed agranular reticulum of tubulovesicular type and numerous mitochondria with tubulous cristae, evidence for active steroidogenesis.

Key words: interstitial endocrinocytes, pig, testis, ultrastructure

INTRODUCTION

Interstitial endocrinocytes or Leydig cells have always being a subject of scientific interest in testis research due to their essential role. Their primary function is to secrete testosterone needed for spermatogenesis and reproduction (Herrera *et al.*, 1983), but they produce substantial amounts of other steroids as well (Raeside *et al.*, 2006; Davidoff *et al.*, 2009).

Three stages in Leydig cells development in boars are reported: foetal and perinatal, that are transient, and a final third stage – from the puberty onward (Lunstra *et al.*, 1986).

The individual Leydig cell volume exhibits significant variations during the postnatal development of porcine testes. During the first five months, Leydig cell volume increase correlated to plasma testosterone concentrations (Franca *et al.*, 2000).

The total Leydig cell volume in pig's testes is extremely high and represents

one third from the total testicular volume (Wagner & Claus, 2004).

Varieties in the rearrangement of agranular endoplasmic reticulum (AER) in Leydig cells are reported by Aguas (1983). He has observed vesicle-like dilatations along with the tubular array containing residual bodies and electron-lucent inclusions similar to cholesterol or cholesterol esters crystals.

During the postnatal development, the ultrastructure of interstitial endocrinocytes in boars exhibits both progressive and regressive trends (Dierichs *et al.*, 1974; Herrera *et al.*, 1983).

In boars fed rations deficient in zinc, the ultrastructure of Leydig cells was altered, exhibiting reduction and disorganization of endoplasmic reticulum and appearance of whorls of cytoplasmic filaments (Hesketh, 1982).

The purpose of the present study was to investigate the normal ultrastructure of

Leydig cells in boar's testes during their postnatal development, with the aim to provide a background for comparison in the interpretation of pathological changes and abnormalities occurring in these cells.

MATERIALS AND METHODS

After castration of pigs at the age of 1 day, 1, 2, 3, 4, 5, 6, 7 and 8 months, 1 mm³ pieces were obtained from each testis for electron microscopy. The samples were processed as followed:

- prefixation in buffered 5% glutaraldehyde for 2 hours at 4 °C;
- washing in cacodylate buffer for 5 min;
- fixation in 2% osmium tetroxide in cacodylate buffer for 1 h at 4 °C.
- washing in cacodylate buffer for 5 min;
- dehydration in ascending ethanol series;
- clearing in propylene oxide – twice for 20 and 10 min;
- infiltration with propylene oxide and Durcupan (1:1) for 30 min;
- embedding in Durcupan (Fluka, Switzerland). Embedded material is left in a thermostat at 56 °C for 48 h;
- cutting on an ultramicrotome (Reichert-Jung, Austria). The obtained ultrathin sections (70 nm) are mounted on copper grids;
- contrasting with uranyl acetate and lead citrate (Reynolds, 1963);
- observation and documentation on electron microscope Opton-10 C (Zeiss, Oberkochen, Germany).

RESULTS

At one day of age, Leydig cells at a various degree of differentiation prevailed in the interstitium of porcine testes. The

similarity in their ultrastructure was marked in all age groups. Their shape was usually polygonal or ovoid.

The nuclei were relatively big, with spherical or ovoid shape, located at one of cell's ends and rarely exhibited irregular borders of the nuclear envelope. In some Leydig cells, a well-defined perinuclear space and a large number of pores on the nuclear membrane were observed (Fig. 1). The major part of the chromatin was scattered among the cytoplasm, but part of it was found concentrated under the nucleolemma. Most nuclei contained one, rarely multiple well-developed nucleoli, appearing as network of matrix fibrils and a condensed heterochromatin.

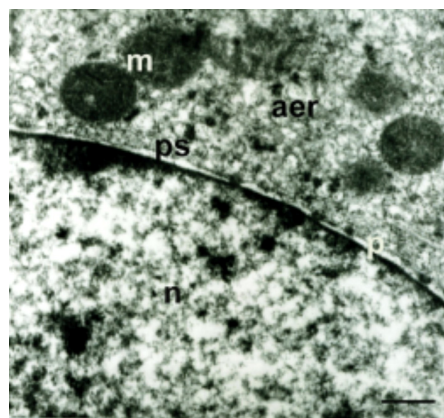


Fig. 1. Leydig cell in the testis of 1-day-old pig: n – nucleus; aer – agranular endoplasmic reticulum, m – mitochondria, ps – perinuclear space. Bar = 0.5 µm.

Among all organelles, agranular endoplasmic reticulum was the most abundant and was found almost everywhere in the cytoplasm, usually concentrated in compact lamellar masses appearing as anastomizing tubulous and vesicular formations.

Mitochondria were the other prevalent cell organelles. They were usually spheri-

cal in shape and exhibited a preferential pericentral pattern of accumulation. In some Leydig cells, the mitochondrial matrix showed an unusually high electron density (Fig. 2) while in others the electron density was moderate with irregularly oriented tubulous cristae within (Fig. 3).

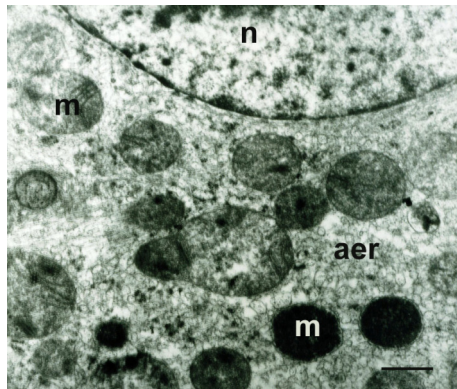


Fig. 2. Leydig cell in the testis of 1-day-old pig: n – nucleus; aer – agranular endoplasmic reticulum, m – mitochondria. Bar=1 μ m.

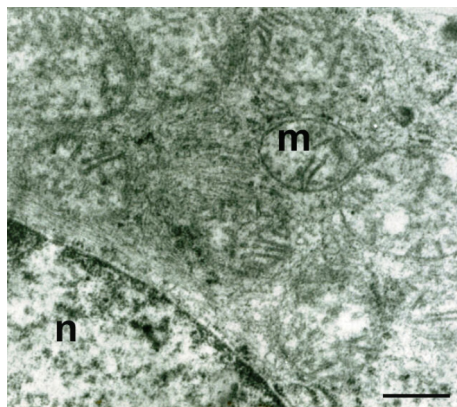


Fig. 3. Leydig cell in the testis of 1-day-old pig: n – nucleus; m – mitochondria. Bar=1 μ m.

The Golgi complex, the cistern of granular endoplasmic reticulum and lysosomes were less often and less distinctly

seen by electron microscopy.

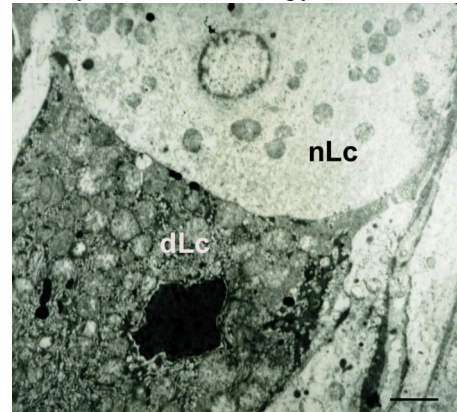


Fig. 4. Testis of a 2-month-old pig: nLc – normal Leydig cell; dLc – degenerating Leydig cell. Bar=3.5 μ m.

By the age of one month, the traits of Leydig cells did not differ substantially compared to the previous studied age.

In 2-month-old pigs, apart Leydig cells described at earlier ages, single cells undergoing degeneration, with pyknotic nucleus, dense chromatin and darker vacuolated cytoplasm were observed in testicular interstitium (Fig. 4).

Leydig cells still prevailed as an element in the interstitium of testes in pigs at the age of 3 months, and did not show any structural changes as compared to preceding ages. At the age of 4 months, most Leydig cells were completely differentiated. They contained a large amount of agranular endoplasmic reticulum of a tubulovesicular type and multiple round mitochondria with moderately electron dense matrix and tubulous cristae (Fig. 5).

Leydig cells in the testicular interstitium of 5-month-old boars were highly differentiated. They were with a large spherical, eccentric nucleus; their cytoplasm was filled with enlarged tubules and vesicles from the agranular endoplasmic reticulum and numerous round

mitochondria, with tubulous cristae (Fig. 6).

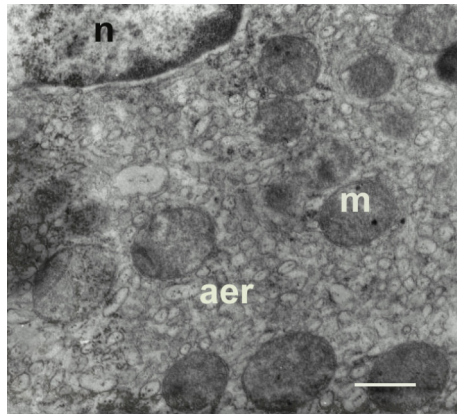


Fig. 5. Leydig cell in the testis of 4-month-old pig: n – nucleus; aer – agranular endoplasmic reticulum; m – mitochondria. Bar = 0.8 μ m.

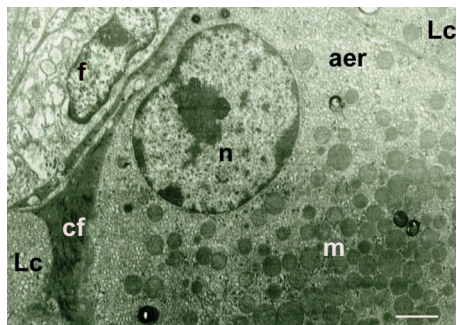


Fig. 6. Testis of a 5-month-old pig: n – nucleus of the Leydig cell; aer – agranular endoplasmic reticulum, m – mitochondria, Lc – Leydig cell, f – fibroblast, cf – collagen fibres. Bar = 1.5 μ m.

At 6 months of age, Leydig cells in the interstitium were differentiated, with very well developed agranular endoplasmic reticulum consisting of enlarged tubules and vesicles and numerous round mitochondria with tubulous cristae. The appearance of described organelles suggested an active steroidogenesis (Fig. 7).

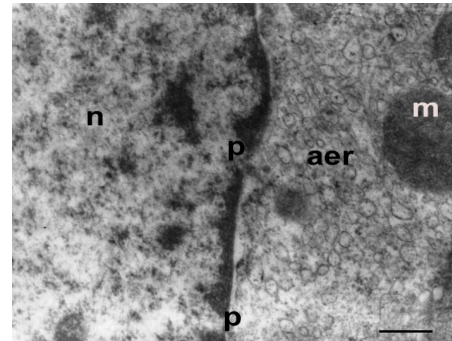


Fig. 7. Leydig cell in the testis of 6-month-old pig: n – nucleus; p – pores, aer – agranular endoplasmic reticulum; m – mitochondria. Bar = 0.5 μ m.

In boars aged 7 and 8 months, the ultrastructure of Leydig cells were similar to that described at 6 months of age. Electron microscopy provided evidence that they were completely differentiated and corresponding to sexually mature animals.

DISCUSSION

In this electron microscopic study, the ultrastructural traits of Leydig cells in pigs' testes of 1 day to 8 months of age are described. Their structure was similar over the entire period of the study.

The commonest cell organelle in Leydig cells was the agranular endoplasmic reticulum representing a complex system of anastomizing tubular and vesicular cisterns. Other abundant cell organelles were mitochondria, part of them were unusually electron-dense, and others with lighter matrix, penetrated by irregularly oriented tubulous cristae.

Our results were very similar to Leydig cell structure in testes of some pig breeds reported by other researchers (Dierichs *et al.*, 1974; Aguas, 1983; Lunstra *et al.*, 1986; Zibrin *et al.*, 2000).

The very good development of these

two organelles provides evidence for the active steroidogenesis that takes place in Leydig cells. The membranes of the agranular endoplasmic reticulum contain most enzymes involved in steroid biosynthesis. Mitochondria participate in the first stages of steroid hormones production (conversion of cholesterol in pregnenolone). Apart testosterone, interstitial endocrine cells in boars produce also a large amount of estrogens (Wrobel & Bergmann, 2006).

Unlike other animal species, porcine Leydig cells normally do not contain lipid droplets, but in some pathological states they could appear due to disorganization of the agranular endoplasmic reticulum and impaired steroid synthesis (Hesketh, 1982; Zibrin *et al.*, 2000; Pinart *et al.*, 2001).

The single dark cells observed at the age of 2 months, with pyknotic nuclei and vacuolated cytoplasm, determined as degenerating Leydig cells, are described in bulls (Wrobel, 1990), rabbits (Crabo, 1963) and humans (Nistal *et al.*, 1986). Despite the smaller size of other normal Leydig cells at that age, their ultrastructural traits were not changed.

At all studied ages, no Reinke crystalloids or paracrystalline inclusions were detected, the fusion of which produces the crystalloids themselves, as described in humans by Kerr (1991).

In conclusion, the steroid-producing apparatus of Leydig cells in boars is very well developed and composed of agranular endoplasmic reticulum and mitochondria. They contained neither cytoplasmic lipid drops nor crystalloids.

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