Foetal Leydig Cells and the Neuroendocrine System

Duška Šklebar¹, Kristina Šemanjki², Marina Kos³, Ivan Šklebar¹ and Davor Ježek²

¹ Departments of Neurology and Anaesthesiology, General Hospital «Bjelovar», Bjelovar, Croatia
² Department of Histology and Embryology, School of Medicine, University of Zagreb, Zagreb, Croatia
³ Department of Clinical Pathology «Ljudevit Jurak», University Hospital «Sestre milosrdnice», Zagreb, Croatia

Abstract

It has been shown that adult human Leydig cells express a number of neuroendocrine markers, and, therefore, could be considered as a part of the neuroendocrine system in the adult. A limited number of studies have dealt with the dynamics of development of human foetal Leydig cells, whereas studies on their neuroendocrine nature are still extremely rare. Therefore, the aim of our study was to investigate the development of human foetal Leydig cells in different weeks of gestation (wg) and to check if these cells express certain markers characteristic of the diffuse neuroendocrine system (DNS). Qualitative, quantitative histological studies and immunohistochemical analyses of human foetal testicular tissue have been performed. According to our data, Leydig cells formed a dynamic population of cells within the interstitium of testes in the period between 15 and 36 wg. The total number of Leydig cells of human foetal testes changed through different stages of gestation by means of 'pulsatile' dynamics (most likely, by following the variable level of gonadotropins). At early stages of development (15–17 wg) immunohistochemical reactions for the expression of neuron specific enolase (NSE) were positive within sex cords and between them, in the interstitium. Pro-spermatogonia in the sex cords were positive, as well as elongated spindle-shaped cells of the interstitium (very likely, precursors of Leydig cells). During the later stages of development (28–36 wg), excluding the pro-spermatogonia, the interstitial Leydig cells were also positive. The results of the immunohistochemical analyses (the expression of NSE) confirmed the hypothesis that human foetal Leydig cells were of neuroendocrine nature.

Key words: Leydig cells, human, foetus, development, neuroendocrine system

Introduction

Leydig cells are situated within the interstitial compartment of testes, between the seminiferous tubules. In the adult, these cells produce testosterone, a male sex hormone that influences a wide range of organs, including the brain, bones, bone marrow, skin, liver, kidney as well as seminiferous tubules of the testis. Observed at ultrastructural level, these cells have round or oval nucleus with a lot of euchromatin, abundant cisternae of smooth endoplasmic reticulum and mitochondria with cristae, glycogen granules and a moderate number of lipid droplets. A special feature of adult Leydig cells are Reinke’s crystals found in humans, primates and New Zealand rabbit. By producing foetal testosterone, Leydig cells play a key role during the human development in the differentiation of sex cords and Wolffian ducts (from which ductus deferens and epididymis arise). Another form of testosterone, dihydrotestosterone, stimulates masculinisation of external genitalia (penis, scrotum) and the prostate.

It seems that testicular macrophages largely influence the function of Leydig cells, especially testosterone production. Morphological studies on the rat testis have revealed a specific ultrastructural coupling of macrophages and Leydig cells. Namely, Leydig cells project slender cytoplasmic processes towards deep coated channels of macrophages. The channels are limited by an electron dense part of the macrophage membrane. It can be supposed that these channels could be sites of an intensive exchange of molecules/signals between the two cell

Received for publication June 1, 2007.
populations\(^6,8\). Leydig cells are the major source of testosterone\(^9\), but are capable of producing many non-steroidal (»neuroendocrine«) factors like \(\beta\) endorphin\(^10\) and prodymorphin\(^11\) which are known to act as paracrine factors affecting macrophage function. In the human testis with an impairment of spermatogenesis, the structure of Leydig cells, macrophages and the production of testosterone are altered when compared to controls\(^12,15\).

Surprisingly, a number of studies have shown that adult human Leydig cells, apart from their steroidogenic characteristics, express a number of molecules typical for neurons and glial cells. Thus, the presence of neuronal markers, synaptic and storage vesicle proteins (synaptophysin and chromogranin), neurofilament cytoskeletal proteins, enzymes involved in the synthesis of catecholamines, neurohormones and their receptors, neuropeptides and their receptors (substance \(P\), neuropeptide \(Y\), \(\beta\)-endorphin, methionine-enkephalin, neuropeptide tyrosine etc.) as well as components of NO/cGMP system have been demonstrated in these cells. Moreover, a significant number of glial cells markers such as galactocerebroside, 2′-3′cyclic nucleotide 3-phosphodiesterase, glial fibrillary acidic protein and A2B5 protein are expressed in the adult human Leydig cells (in addition, some of these antigens were discovered in the mouse, rat and hamster Leydig cells)\(^16\). Although the significance of the above-mentioned neuronal and glial markers is not fully understood, it has been shown that some of these molecules could influence the level of testosterone production (for example, substance \(P\) and NO)\(^17,18\).

The human foetal Leydig cells do not appear earlier than the 8 \(wg\) and are fully differentiated by the 10 \(wg\). During the 15–16 \(wg\), these cells increase in number, reaching approximately 24 million cells per testis\(^5,10\). The Leydig cell number remains stable until the 26 \(wg\), but afterwards the number progressively decreases to approximately 9 million cells per testis just before birth\(^19\). This reduction coincides with low hCG plasma concentrations\(^20\). Leydig cell regression is thought to take place by degeneration and destruction of these cells\(^19\).

Since there is a lack of data on the neuroendocrine characteristics of foetal human Leydig cells, in the current study we wanted to investigate the expression of neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP), synaptophysin, protein S-100 and chromogranin in foetal human testicles. In addition, the developmental dynamics of foetal Leydig cells has been assessed by a qualitative and quantitative histological analysis.

### Materials and Methods

A total of 39 human foetal testicles from 15 to 36 \(wg\) were used in the study. The tissue samples were obtained during the routine paedopathological section of miscarried children during 1994 (approved by the Ethical Committee of Clinic for Gynaecology and Obstetrics, Clinical Hospital Centre »Zagreb«). Testicles have been weighed at the 8 \(wg\), 15 \(wg\), and 20 \(wg\) and are fully differentiated by the 10 \(wg\). During the 15–16 \(wg\), these cells increase in number, reaching approximately 24 million cells per testis\(^5,10\). The Leydig cell number remains stable until the 26 \(wg\), but afterwards the number progressively decreases to approximately 9 million cells per testis just before birth\(^19\).

A pilot stereological measurement has been made in order to determine the number of microscopic fields \((n)\) needed for a reliable data assessment. This measurement has been carried out on 20 microscopic fields. After the preliminary measurement, de Hoff’s formula\(^25\) has been applied:

\[
N = \frac{Na}{Ni} \times Vt
\]

where:
- \(N\) – absolute number of Leydig cells
- \(Na\) – numerical density of Leydig cells
- \(Ni\) – numerical density of the cells in the interstitial compartment determined by optical dissector\(^24\)
- \(Vt\) – testis volume

Statistical analysis has been done using a »SAS« biostatistical package (University of Zagreb, School of Public Health »Andrija Štampar«)\(^26\). Individual data were processed by a personal computer. In addition to general statistics, parts of the programme such as Pearson’s coefficient of correlation and regression analysis were applied.

### Results

Qualitative histological analysis on H+E slides demonstrated that human foetal Leydig cells appeared in 2 forms: one cell form was oval, with a centrally or eccentrically positioned nucleus (and sometimes well-visible nucleolus), whereas another was elongated with a round or oval nucleus situated mainly in the middle of the cell.
The population of foetal Leydig cells changed with time/gestation weeks. Thus, in the period from 15–24 wg, as well as 27–36 wg, the cells could be easily recognized by their shape (Figure 1). In the period from 25–27 wg, foetal Leydig cells resembled fibroblasts i.e. their mesenchymal precursors (Figure 2). The described cells occupied the interstitial space between sex cords. Sex cords had 40–50 \( \mu \)m and were composed of pro-spermatogonia and foetal Sertoli cells. The future lamina propria consisted of several gentle layers of peritubular cells (Figures 1 and 2). In general, sex cords and associated blood vessels in the interstitium grew progressively from 15–36 wg having a rather tortuous structure (Figures 1 and 2).

IHC analysis pointed out a positive expression of NSE in the foetal testis interstitium as well as within sex cords. In the early stages of foetal development (15–17 wg) elongated, spindle-shaped cells were positive (Figure 3). In the period of 18–36 wg, Leydig cells were positive for NSE (+++) (Figure 4). Moreover, in both of the above-mentioned periods, a strong expression of NSE (+++) was discovered in pro-spermatogonia within sex cords, whereas foetal Sertoli and peritubular cells were negative (Figures 3 and 4). No positive results were obtained applying antibodies to S-100, GFAP, synaptophysin and chromogranin.

Stereological analyses demonstrated a pulsatile (oscillatory) development of foetal Leydig cells during the observed foetal period. However, N values steadily increa-

Fig. 1. Human foetal testis in 19 wg. Within the interstitial tissue, Leydig cells (arrowheads) could be identified. These cells have an oval or elongated form. Sex cords are limited by a gentle layer of peritubular cells and are lined by pro-spermatogonia (arrow) and foetal Sertoli cells. Blood vessels (stars). H&E staining. 400x, bar = 100 \( \mu \)m.

Fig. 2. Foetal testis in the 25 wg. The majority of Leydig cells have elongated form (arrowheads) or fibroblast-like architecture. Pro-spermatogonia (arrows). Blood vessels (stars). H&E staining. 400x, bar = 100 \( \mu \)m.

Fig. 3. Immunohistochemical reactions for NSE. Human foetal testis in week 17 of development. Within the testis interstitium, elongated cells (arrowheads) are positive (putative Leydig cells precursors). Within the sex cords, pro-spermatogonia (arrows) strongly express NSE. Blood vessel (star). Diaminobenzidine (DAB) staining. 400x, bar = 100 \( \mu \)m.

Fig. 4. Foetal testis in 32nd wg. Leydig cells (arrowheads) are positive for NSE, as well as pro-spermatogonia (arrows). Diaminobenzidine (DAB) staining. 400x, bar = 100 \( \mu \)m.
Discussion

In general, Leydig cells are considered to be of mesodermal (mesenchyme) origin. The mesenchyme of the genital ridge is thought to give rise to the interstitium of the testis, including steroid producing Leydig cells. The two described forms of foetal Leydig cells observed on H+E slides indicate the possible dual nature of these cells: the oval much resembles the epithelial-derived population of cells, whereas the elongated or spindle-shaped form could be connected to the mesenchymal/fibroblast cell line. Epithelial-like Leydig cells could originate from other sources, not only from the mesoderm of the genital ridge. Thus, a possible source of these cells could be the neural crest/tube, since it is well known that it gives rise to dorsal root ganglions, medulla of suprarenal gland and enteric ganglia. A significant number of cells from neural tube undergo a rather complex migration to reach their «destination» in the suprarenal glands or the gut. It can be presumed that some of them, while populating the suprarenal medulla, migrate to the genital ridge, which is in the close vicinity. Our IHC results support this assumption, since the expression of NSE was shown in developing Leydig cells and their precursors. However, expression of GFAP, synaptophysin, S-100 and chromogranin was negative. This finding is in contrast to IHC results on the adult human Leydig cells (i.e. positive expression of GFAP, synaptophysin and chromogranin). One can assume that, due to the extremely low level of the above-mentioned antigens in foetal Leydig cells and application of commercially available antibodies (that are optimized for the detection of »high« concentrations of neuronal or glial antigens in central nervous system or tumours) IHC results were negative. Recent studies clearly indicated a presence of neuronal and glial markers in both the developing and adult human testis27–29.

Stereological analyses pointed out a pulsatile developmental dynamics of foetal Leydig cells. This could be due to the activity of foetal hypophysis. It is known that gonadotropins are released from adenohypophysis in an oscillatory manner, which could strongly influence the development of the foetal testis. However, N showed a steady and stable increase in the observed gestational weeks. This could be the effect of human chorionic gonadotropin secreted by the placenta. This hormone has relatively stable levels during the foetal period (in addition to foetal LH and FSH), and could stimulate the growth of Leydig cells30.

In conclusion, we propose that foetal Leydig cells have a dual nature: they are steroidogenic cells that produce significant levels of testosterone during the foetal development; however, since they express NSE (and other neuronal and glial markers found in other studies), they could be considered as a part of the neuroendocrine system or paraneurons.

Acknowledgements

The study has been supported by Ministry of Science, Education and Sport, grant No. 108-1080399-0383.
REFERENCES


D. Ježek

Dept. of Histology and Embryology, School of Medicine, University of Zagreb, Šalata 3, HR-10000 Zagreb, Croatia
e-mail: davorjezek@yahoo.com

LJUDSKE FETALNE LEYDIGOVE STANICE – DA LI SU DIO FETALNOG NEUROENDOKRINOG SUSTAVA?

SAŽETAK