Neuroendocrine properties of human foetal Leydig cells

Ježek D.¹, Šklebar D.², Kos M.³, Grahovac G.¹, Šemanjski K.¹ and Šklebar I.²

¹Dept. Histology and Embryology, School of Medicine, University of Zagreb; ²General Hospital Bjelovar; ³Dept. for Clinical Pathology „Ljudevit Jurak“, Clinical Hospital „Sisters of Mercy“, Zagreb, Croatia.
E-mail: davorjezek@yahoo.com

Introduction. Leydig cells are situated within the interstitial compartment of testis, between seminiferous tubules. In the adult, these cells produce testosterone, a male sex hormone that influences a wide range of organs, including brain, bones, bone marrow, skin, liver, kidney as well as seminiferous tubules of the testis. Ultrastructurally, 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 [1,2]. A special feature of adult Leydig cells are Reinke's crystals found in humans, primates and New Zealand rabbit [3]. By producing foetal testosterone, Leydig cells play a key role during the human development in 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 prostate [4,5].

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, neurokinin, β-endorphin, methionine-enkephalin, neuropeptide tyrosine ect.) 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 (some of these antigens were discovered in the mouse, rat and hamster Leydig cells) [6]. 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, NO) [7,8].
**Aim of the study.** 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 stereology.

**Materials & methods.** A total of 39 human foetal testicles form 15\textsuperscript{th} to 36\textsuperscript{th} week of gestation were used in the study. The tissue samples were obtained during the routine paedopathological section of miscarried children during year 1994 (approved by the ethical committee). After the section, testicles have been weighed by means of a precise scale to obtain their mass/volume for stereological analysis. The samples were fixed in Bouin’s fluid, dehydrated and embedded in paraffin. Paraffin blocks were cut extensively in order to provide sections for hematoxylin & eosin (H+E) and immunohistochemical staining (IHC). Monoclonal antibodies to NSE (1:100), GFAP (1:200), synaptophysin (1:25), chromogranin (1:100) and S-100 (1:600) in combination with an appropriate EnVision kit (Dako, Glostrup, Denmark) were applied. Stereological analysis of foetal human Leydig cells has been performed on H+E stained slides to assess the developmental dynamics of these cells (total number of Leydig cells per testis \( /N_L_c/ \) has been determined).

**Results.** Qualitative histological analysis on H+E slides demonstrated that human foetal Leydig cells appeared in 2 forms: one cell form was oval, with centrally or eccentrically positioned nucleus (and sometimes well-visible nucleolus), whereas another was elongated with 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 form 15\textsuperscript{th} to 24\textsuperscript{th} as well as 27\textsuperscript{th} to 36\textsuperscript{th} week, the cells could be easily recognized by their shape. In the period from 25\textsuperscript{th} to 27\textsuperscript{th} week, foetal Leydig cells much resembled fibroblasts i.e. their mesenchymal precursors. The described cells occupied interstitial space between sex cords. Sex cords had 40-50 µm and were composed of pro-spermatogonia and foetal Sertoli cells. The future lamina propria consisted of several gentle layers of peritubular cells. In general, sex cords and associated blood vessels in the interstitium grew progressively from 15\textsuperscript{th} to 36\textsuperscript{th} week of gestation achieving rather tortuous structures.

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\textsuperscript{th}-17\textsuperscript{th} week of gestation) elongated, spindle-shaped cells (Leydig cells precursors?) were positive. In the period of 18\textsuperscript{th} to 36\textsuperscript{th} week, Leydig cells were positive to NSE (++). Moreover, in the above-mentioned
period a strong expression of NSE (+++) was discovered in pro-
spermatogonia within sex cords, whereas foetal Sertoli and peritubular cells
were negative. No positive results were achieved applying antibodies to S-
100, GFAP, synaptophysin and chromogranin.

Stereological analysis demonstrated a pulsatile (oscillatory)
development of foetal Leydig cells during the observed foetal period.
However, NLc values steadily increased with gestational weeks. A strong
positive correlation between NLc and weeks of gestation has been found
(r=0.9321; P<0.001).

**Discussion.** In general, Leydig cells are considered to be of
mesodermal (mesenchyme) origin. 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:
oval form much resembles epithelial-derived population of cells, whereas
elongated or spindle-shaped form could be connected to mesencymal
/fibroblast cell line. Epithelial-like Leydig cells could originate from other
sources, not only mesoderm of the genital ridge. Thus, a possible source of
these cells could be neural crest/tube, since it is well known that it gives
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
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 developing and adult human testis [9,10,11].

Stereological analysis 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, NLc showed a steady and
stable increase in the observed gestational weeks. This could be the effect
of human chorionic gonadotropin secreted by placenta. This hormone that has relatively stable levels during the foetal period (in addition to foetal LH and FSH) could stimulate the growth of Leydig cells.

In conclusion, we propose that foetal Leydig cells have 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 a neuroendocrine system or paraneurons. Some of these cells, therefore, could be derivatives of neural crest/tube.

References:

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