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Neuroendocrine properties of human foetal Leydig cells

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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 Zeland 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 form 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 (synaptophisin and chromogranin), neurofilament cytoskeletal proteins, enzymes involved in the synthesis of catecholamines, neurohormones and their receptors, neuropeptides and their receptors (substance P, neurokinin, β-endrophin, methoinine-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 galactocerebrozide, 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 15th to 36th 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 /NLc/ 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 15th to 24th as well as 27th to 36th week, the cells could be easily recognized by their shape. In the period from 25th to 27th 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 15th to 36th 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 (15th-17th week of gestation) elongated, spindle-shaped cells (Leydig cells precursors?) were positive. In the period of 18th to 36th 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 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 the 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 stabile increase in the observed gestational weeks. This could be the effect

of human chorionic gonadotropin secreted by placenta. This hormone that has relatively stabile 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.

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