FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 48, No. 4, 2010
pp. 491-506

100th anniversary of the discovery of the human adrenal fetal zone by Stella Starkel and Lesław Węgrzynowski: how far have we come?

Ludwik K. Malendowicz

Department of Histology and Embryology, Poznań University of Medical Sciences, Poznań, Poland

Abstract: Year 2010 marks a centennial anniversary of the description by Stella Starkel and Lesław Węgrzynowski, Polish students of the Faculty of Medicine, University of Lwów, the fetal zone of the human fetal adrenal gland. In 1911 both, Starkel and Węgrzynowski were graduated from the Faculty of Medicine of Lwow University. The paper appeared in the German Arch. Anat. Physiol. and its original title was "Beitrag zur Histologie der Nebeniere bei Feten und Kindern“ ("Contribution to histology of adrenals of fetuses and children"). The studies were performed on 100 adrenal glands obtained from fetuses (from 6th month of gestation) and up to 5-year-old children. They described the fetal zone as a "medullary zone”, also as "immature cortex", which undergoes involution in first years of life. To commemorate this discovery, this review aimed to present the most important achievements of studies on the development and involution of the human adrenal fetal zone.

Key words: human adrenal fetal zone, development, structure, function, growth regulation, Stella Starkel, Lesław Węgrzynowski

Introduction

The year of 2010 marks a centennial anniversary of the description by Stella Starkel and Lesław Węgrzynowski, Polish students of the Faculty of Medicine, University of Lwów, of the fetal zone in the human fetal adrenal gland [1]. Their investigations were performed in the Institute of Pathological Anatomy. In 1911, both Starkel and Węgrzynowski were graduated from the Faculty of Medicine of Lwow University. In the entire scientific world the priority of their discovery is unquestionable and their publication is still cited in international literature. What did the study concern? The paper appeared in the German Arch. Anat. Physiol. and its original title was "Beitrag zur Histologie der Nebeniere bei Feten und Kindern" ("Contribution to histology of adrenals of fetuses and children"). Studies were performed on 100 adrenal glands obtained from fetuses (from 6th month of gestation) and up to 5-year-old children. They described the fetal zone as a "medullary zone", also as "immature cortex" which undergoes involution in first years of life. Their original drawning is shown (Fig. 1). They also noted that in neonates the weight of human fetal adrenal gland undergoes a rapid and notable decrease. Prof. Heinrich Wilhelm Waldeyer, the editor of Arch. Anat. Physiol. in his commentary stressed the importance and originality of the paper. Starkel and Węgrzynowski had also published their findings in Polish, in the Warsaw medical journal, entitled "Medycyna and Kronika Lekarska" (Medicine and Medical Chronicle) [2].

In 1961 H. Kuński recalled this great discovery in the Pol. Med. Sci. Hist. Bull. [3]. The centennial anniversary of the first description of the human adrenal fetal zone also deserves a special recall and gives an opportunity to present the most important findings on the development of the human adrenal gland, in particular its fetal zone.

An outline of the human adrenal development

Results of extensive studies on human adrenal gland development allowed Sucheston and Cannon [4] to divide this process into five phases: (i) condensation of the celomic epithelium (3-4 weeks of gestation); (ii) proliferation and migration of celomic epithelial cells (4-6 weeks of gestation); (iii) morphological differen-

Correspondence: Ludwik K. Malendowicz, Dept. Histology and Embryology, Poznań University of Medical Sciences, 6 Święcicki Str., 60-781 Poznań, Poland; tel.: (+4861) 8546444, fax.: (+4861) 8546440, e-mail: lkm@amp.edu.pl

Folia Histochem Cytobiol. 2010:48(4): 491 (491-506)
10.2478/v10042-010-0062-7

Fig. 1. Original drawing of the human fetal adrenal gland from Starkel and Węgrzynowski (1910) [1]. Adrenal gland of "6-month-old embryo. t – capsule; a – mature cortical cells; b – immature cortical cells; sg – groups of sympathogonia".

tiation of fetal adrenal cortical cells into two distinct zones (8-10 weeks of gestation); (iv) decline and disappearance of the fetal zone (first 3 postnatal months); and (v) establishment and stabilization of the adult zonal pattern (10-20 yr of age) (Fig. 2).

Adrenal cortex morphogenesis starts at weeks 3-4 of intrauterine life with appearance of local thickening of celomic epithelium, called also the adrenal anlage. Subsequently, cells of the anlage migrate to the cranial end of the mesonephros (weeks 4-6 of intrauterine development) forming adrenal placode (adrenal blastema) [5]. Also cells originating from the urogenital ridge (developing from the intermediate mesoderm) migrate to adrenal placode, thus forming the adreno-gonadal ridge. At this stage of development, expression of *SF1* (steroidogenic factor 1 gene) takes place in cells destined to become the steroidogenic cells of the adrenals and gonads. Adrenal cortex originates from the cephalic part of the adreno-gonadal ridge, while steroidogenic cells of the gonads originate from its caudal portion.

Around the developing adrenals, specialized mesenchymal cells migrating from the area of Bowman's capsule form adrenal capsule (weeks 8-9 of intrauterine development). At this stage of development neural crest-derived cells migrate into the gland and they will differentiate into medullary chromaffin cells. Numerous islands of small, neural-crest derived cells, called also as sympathogonias, are dispersed among the steroidogenic cells, especially within the fetal zone. At this stage of development steroidogenic cells are organized into two distinct components. Centrally localized cells, with abundant, eosinophilic cytoplasm, form adrenal fetal zone, while small steroidogenic cells with basophilic cytoplasm, form a very narrow definitive cortex only in the vicinity of adrenal capsule. Subsequently, the transitory (or intermediate) zone differentiates from the definitive cortex. This zone, localized in between definitive and fetal zones, is composed of cells similar to that of the definitive zone. Both definitive and transitory zones are also called the neocortex (Figs. 3, 4).

Origin of fetal and definitive zone cells of human adrenal gland is controversial [6]. It had been suggested that they may originate from two distinct waves of migrating celomic cells, or from the local differentiation of a common progenitor cell. Some authors also suggest that fetal adrenocortical cells originate from two distinct types of progenitor cells – one specific for the fetal zone and another for the neocortex.

As mentioned above, growth of fetal adrenals depends mainly on enlargement of the fetal zone, which may occupy 70-85% of the gland volume [7-12]. Rich vascular supply of the fetal zone probably is connected with the potent secretory activity of its cells. At the end of gestation, a developing adrenal gland is organized into distinct zones: glomerulosa, transitory (appears between weeks 22-24, the future zona fasciculata) and dominant fetal zone. Immediately after birth, the fetal zone undergoes rapid involution, but its elements may persist up to the end of the second year of life [13,14]. In human adrenal gland zona reticularis starts to develop at the age of 4-6 or even 8 years, while structure typical to adults stabilizes at the age of 10-20 years [4,11,15,16].

Adrenal weight during human ontogenesis

Intrauterine growth of the human adrenal glands is rapid and depends primarily on enlargement of the fetal zone. From weeks 8-10 up to midterm their weight doubles weekly. Subsequently, from week 20 to the term, adrenals weight increases from ca 0.5 g to 8-10 g. Thus, this phase of adrenal growth is also prominent, however its dynamics is lower than in the first half of intrauterine development [7,11,13,17-22]). At midgestation the size of adrenals is almost equal to that of fetal kidneys and at term they are similar in weight to adult adrenals (weight of both glands averages at 8-10 g). Immediately after birth adrenals undergo a physiologic involution (drop of

Folia Histochem Cytobiol. 2010:48(4): 492 (491-506)
10.2478/v10042-010-0062-7

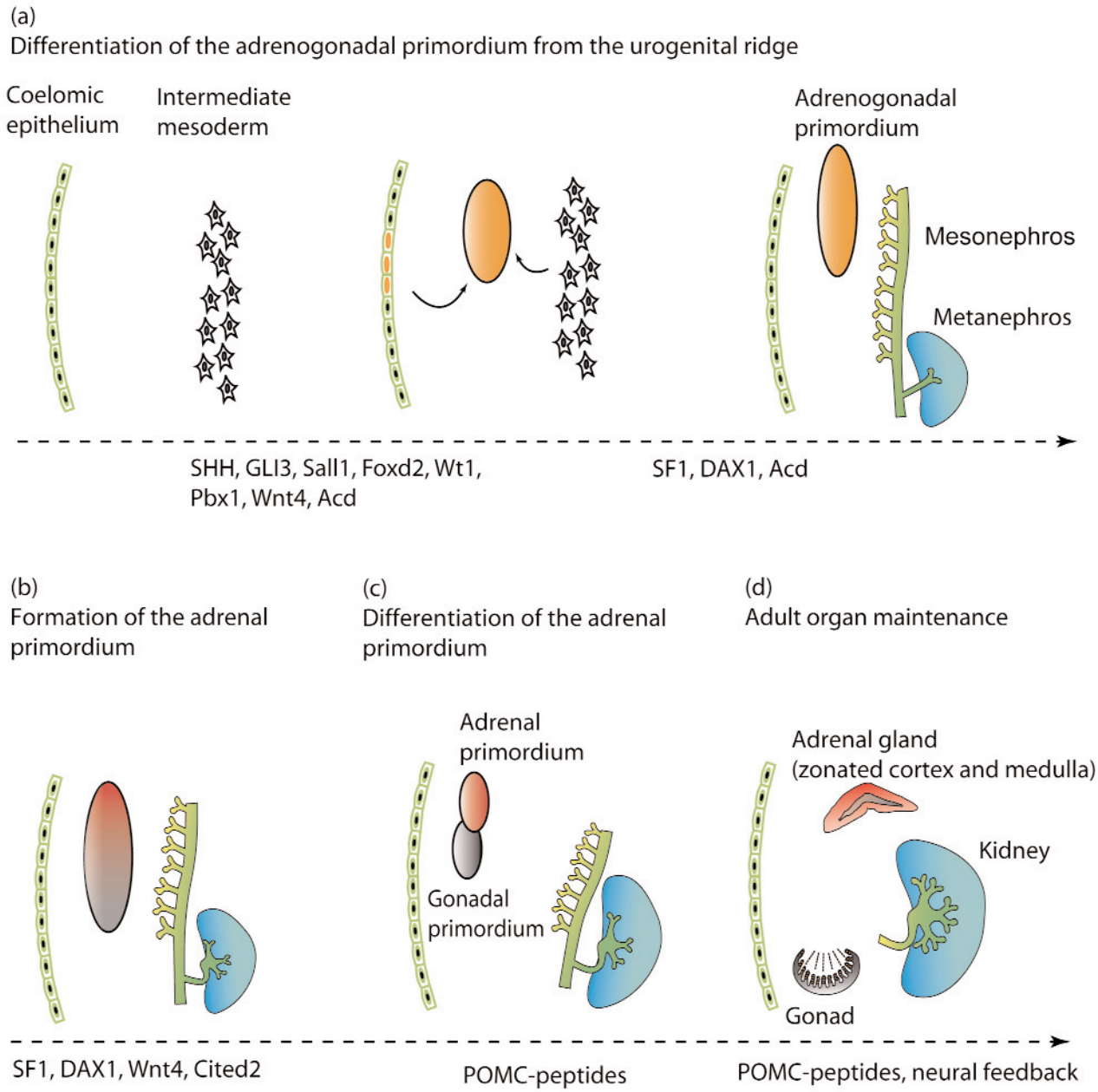

Fig. 2. Outline of the development of the adrenal cortex. Factors implicated in regulating particular stages of adrenocortical development are shown [data from analyses of human genetic diseases (capital letters) or mouse models (first letter capitalized)]. After: Else, T., Hammer, G.D.: Genetic analysis of adrenal absence: agenesis and aplasia. Trends Endocrinol. Metab., 16: 458-468 (2005) [6] modified.

weight to around 2 g), and only at the end of puberty they again attain the size similar to that seen in the newborns. At term, the relative adrenal weight is 10-20- fold higher than that of the adult adrenals (Fig. 5).

In anencephalic fetuses, which do not secrete ACTH, adrenal gland weight is notably lower than in normal ones, and this difference starts to occur before week 20 of intrauterine life [4,23,24].

Recently, a size of the fetal adrenal gland may be evaluated ultrasonographically. Results of such studies also prove a dynamic increase in adrenal gland weight and their sudden involution in neonates [25-29].

Morphological aspects of human adrenal gland development

Histologic aspects. Since the first description of the human adrenal fetal zone by Starkel and Węgrzynowski [1,2], numerous studies were performed on its development, structure and function. A comprehensive

Folia Histochem Cytobiol. 2010:48(4): 493 (491-506)
10.2478/v10042-010-0062-7

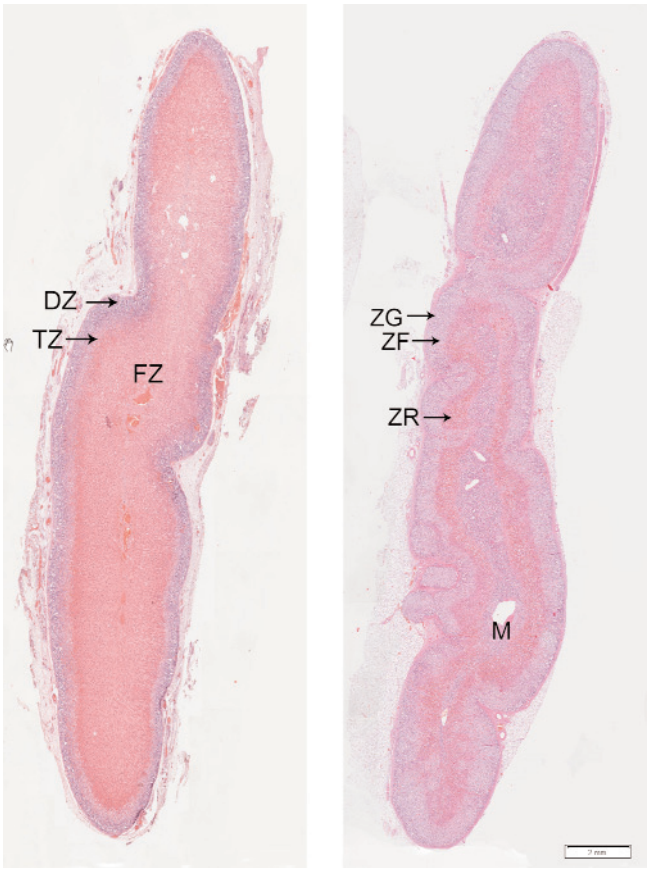

Fig. 3. General structure of the fetal adrenal gland at week 30 of intrauterine life (left) and of adult man (right). DZ – definitive zone; ZG – zona glomerulosa; TZ – transitional zone; ZF – zona fasciculata; FZ – fetal zone; M – medulla. H+E staining. Magnifications shown by bars. Courtesy of dr. Maria Chmielnicka-Kopaczyk, Department of Biochemistry and Pathomorphology, Poznań University of Medical Science.

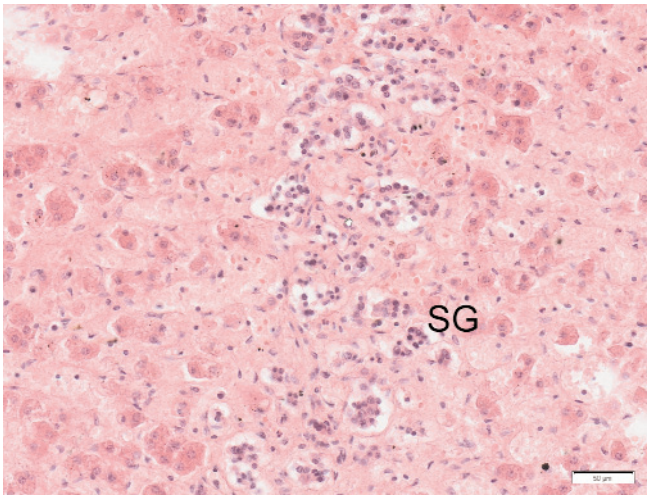

Fig. 4. Human adrenal fetal zone at week 30 of intrauterine life. In fetal zone numerous differentiating chromaffin cells (SG – sympathogonia) are seen. H+E staining. Magnifications shown by bars. Courtesy of dr. Maria Chmielnicka-Kopaczyk, Department of Biochemistry and Pathomorphology, Poznań University of Medical Science.

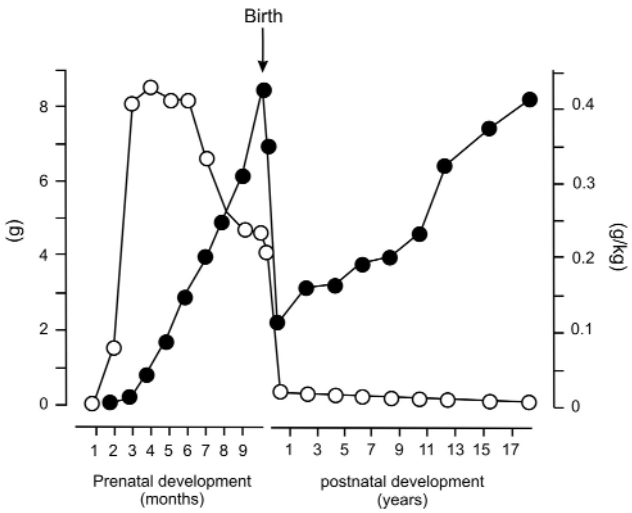

Fig. 5. Absolute and relative human adrenal gland weights during intrauterine and postnatal life's. Absolute (circles) and relative (black circles) adrenal weights. After Neville, A.M., O'Hare, M.J.: The Human Adrenal Cortex. Springer-Verlag, Berlin, 1982 [174], modified.

review of the earliest reports is presented in the excellent monograph of Bachmann [13]. The original observations of the Polish students were confirmed in 1911 by Elliot and Armour [30]. Out of the earlier reports on the subject, publications of Keene and Hewer [31], Tobeck [32], Uotila [33], Swinyard [17], Velican [34], [35], Lantan [36,37] and Wołynska [38] should be mentioned. All of them provided detailed cytological descriptions of developing adrenal gland. Human adrenal fetal zone cells are large cells, with eosinophilic cytoplasm and usually round nucleus. They differ significantly from cells of the definitive zone. Cells of the zone are small, with basophilic cytoplasm with only few lipid droplets, thus they are of the proliferative type.

Electron microscopy. In human adrenal fetal zone electron microscopy revealed ultrastructural features characteristic of all steroid-secreting cells. Results of these studies were reviewed by Nussdorfer [39] and all of them consistently described ultrastructural features of the cells as well as their appearance in the course of development. As early as from week 8 of intrauterine life, fetal zone cells possesses an abundant smooth endoplasmic reticulum, numerous mitochondria with tubular cristae, well developed Golgi apparatus and lipid droplets [40-48]. In contrast to fetal zone cells, cytoplasm of cells of the definitive and transitory zones contains numerous free ribosomes and small mitochondria with membranous cristae.

Of interest is that in cells of adrenal fetal zone of anencephalic fetuses large number of lipid droplets,

Folia Histochem Cytobiol. 2010:48(4): 494 (491-506)
10.2478/v10042-010-0062-7

rough endoplasmic reticulum, tubular or vesicular profiles of smooth endoplasmic reticulum and mitochondria with poorly developed cristae are seen [49].

Morphometry, stereology. Quantitative morphological studies revealed notable changes in cellular and zonal composition of the developing human adrenal gland. The volume of adrenocortical cells showed correlation with gestational age only in the definitive zone of the developing adrenal, while in fetal zone cell volume was rather stable, not related to pregnancy duration [50]. Other studies revealed that from the beginning of development, fasciculata cell volume (transitory zone) was markedly higher than that of definitive zone cells and lower than in fetal zone cells [8]. The average cell volume of the definitive and transitory zones remained constant during the fetal period, while the average cell volume of fetal zone cells increased gradually from the 9th to the 20th fetal week and afterwards remained unchanged. Also zonation of the fetal adrenals changes notably during the intrauterine development. Gray and Abramovich [24] reported that at midgestation in normal fetuses 76.5% of the gland volume was occupied by the fetal zone. In other reports, at the end of fetal period volume of fetal zone attained over 8200 mm^3 and occupied 69-70% of the total gland volume, while respective values for definitive and transitory zones and medulla were 1665 mm^3 (14%), 833 mm^3 (7%) and 1071 mm^3 (9%) [8,9,51,52]). The above mentioned changes are accompanied by notable changes in the number of adrenocortical cells. In normal fetuses, at the end of gestation, the definitive zone (zona glomerulosa) contains ca 3.5×10^9 cells, the transitory one (presumptive zona fasciculata) 0.7×10^9 and the fetal zone approximately 3×10^9 cells.

In anencephalic fetuses, due to the lack of fetal ACTH, development of fetal adrenal is profoundly disturbed. At midgestation, the volume of the adrenal gland occupied by the fetal zone in the anencephalic fetuses was 57.5%, as compared with 76.5% in normal fetuses [24]. Systemic description of the development of adrenal gland of anencephalic fetuses, in comparison with the normally developing gland, revealed that at the end of gestation volume of the fetal zone is approximately 8-fold lower than in the normal fetus, comprising only 25% of the total gland volume [9,51-53]. For the remaining zones the values were: definitive zone – 1501 mm^3 (around 90% of the normal value and 39% of the total gland volume); transitory zone – 770 mm^3 (around 92% and 20% respectively), and medulla – 539 mm^3 (around 50% and 14%, respectively). Thus, in anencephalic fetuses the growth of adrenal medulla is also retarded while the growth of the definitive zone remains rather unaffected. These changes depend on the profound changes in the size and number of parenchymal cells in developing human adrenal glands.

Histochemistry, immunohistochemistry, immunofluorescence, in situ hybridization. Numerous publications on human adrenal fetal zone contain data on localization of different cellular components in the gland. Initially they were identified by means of classic histological and histochemical methods, later on by immunohistochemistry and finally by *in situ* hybridization. In early period of those studies developing adrenal glands were characterized in a rather general manner, afterwards the studies were more selective and directed preferentially toward the most important steroidogenic enzymes (for example 3β-hydroxysteroid dehydrogenase – 3βHSD), growth factors and their receptors as well as toward expression of genes regulating development of the gland and adrenocortical steroidogenesis.

Aming the general studies important observations of Jirasek [5] should be mentioned. He described localization of numerous hydrolases, peptidases and dehydrogenases in the human adrenal blastema. Similar studies were performed by Lichnovský and Lojda [54] on adrenals of fetuses from 8-20 weeks of gestation.

As far as elements of intracellular signaling pathways are concerned, during the second trimester of gestation expression of αs subunit of the heterotrimeric G_s protein was detected by immunofluorescence in all adrenal cell types [55]. On the other hand, αi1-2 protein was restricted to the definitive zone, whereas αi3 labeling was mainly expressed in the fetal zone. Chromaffin cells of developing glands expressed αs, αq, and αo1 subunits. Furthermore, the neocortex of the fetal adrenal glands displayed only minimal StAR immunostaining, whereas moderate to intense staining was found in the transitory and fetal zones [56,57]).

Studies performed on 31 human fetal adrenal specimens from weeks 14 – 40 of gestation revealed that immunoreactivity for SF1 (Ad4BP) was present in the nucleus of adrenocortical parenchymal cells in almost all cells of the definitive, transitional and fetal zones in all cases examined [57].

Series of publications present general characteristics of steroidogenic enzymes in the developing human adrenal gland [58-62]. Results of these studies suggest that adrenals acquire the ability to synthesize and secrete steroid hormones between weeks 6-8 of intrauterine development. In developing adrenals localization of such specific steroidogenic enzymes as 21-hydroxylase [62], 11β-hydroxylase [63], 11β-hydroxysteroid dehydrogenase type 2 [64], 17α-hydroxylase [65], and aldosterone synthase [66] has also been described. Generally, activity of steroidogenic enzymes and regulatory proteins is higher in the fetal zone than in neocortex.

Of special importance are studies on localization in the developing human adrenal gland of the key enzymes in particular steroidogenic pathways: 3β-hydroxysteroid dehydrogenase/4-5 izomerase

Folia Histochem Cytobiol. 2010:48(4): 495 (491-506)
10.2478/v10042-010-0062-7

(3βHSD) and 17α-hydroxylase/17,20 lyase. Both enzymes are required for cortisol synthesis. When only 3βHSD is expressed, cell possesses the ability to synthesize mineralocorticoids, while expression of 17α-hydroxylase/17,20 lyase only indicates synthesis of C19 steroids, the androgens. In developing human adrenal glands the main androgen involves dehydroepiandrosterone or its sulfate (DHEA, DHEAS).

In a developing human adrenal gland localization of 3βHSD changes in relation to the stage of development [11,57,67-74]). Analyses performed by Messiano and Jaffe [11]indicate that human adrenal fetal zone, both on mRNA and protein levels, does not express 3βHSD. In that zone, however, 17α-hydroxylase/17,20 lyase complex is present [65]. In the transitory zone of the gland (the future zona fasciculata), expression of both enzymes is found in weeks 25-30 of intrauterine life, while in the definitive zone (zona glomerulosa) expression of 3βHSD appears between weeks 22 and 24. Since functional data suggest fetal adrenal cortisol secretion starting at weeks 6-8 of development [75], such a late expression of 3βHSD is difficult to understand. One of explanations may be, that in the human fetal adrenal gland cortisol is not synthesized *de novo* from cholesterol but from progesterone.

Another topic of studies on human fetal adrenal gland focuses on the search for specific markers of cells in particular zones of the gland. Identification of such markers could help to obtain zone-specific fractions of adrenocortical cells [76,77]. Such specific markers of adrenocortical cells have not been found yet. In definitive zone cells a high levels of metallopanstimulin-1, novH (human nephroblastoma overexpressed (*novH*) gene) and p-glycoprotein mRNA are present, while in fetal zone the low density lipoprotein (LDL) receptor is predominantly expressed. As expected, chromogranin A is a typical marker of developing chromaffin cells.

Quantitative aspects of steroidogenic pathways in neocortex and fetal zone of the human adrenal gland

Introduction of modern techniques expanded our knowledge of steroidogenic pathways in developing adrenals and on their regulation. In this regard application of cell cultures of definitive and fetal adrenal zones revealed significant functional differences between them. This section presents some of such data, obtained using quantitative methods.

Considering steroidogenic pathways, the adrenal fetal zone carries a higher number of LDL-binding sites than definitive zone does and in both zones ACTH stimulates LDL-binding [78,79]. Similar differences are observed in *de novo* cholesterol synthesis and, again, ACTH stimulates this synthesis.

Out of corticosteroidogenesis regulating intracellular pathways, basal adenylate cyclase activity is 2- to 3-fold higher in definitive zone than in fetal zone membrane fractions [80]. In adrenals of anencephalics, the basal adenylate cyclase activity is reduced to 3-5% of that observed in normal adrenals [81]. Likewise, the specific activity of protein kinase-C and the amount of kinase-C protein are significantly higher in fetal zone than in definitive zone, while they are very low in adrenals of anencephalic fetuses [82].

Cholesterol desmolase (CYP11A1, the enzyme responsible for transformation of cholesterol into pregnenolone) activity, is 3-4-fold higher in the fetal zone than in the definitive one. Activity of this enzyme is also present in adrenals of anencephalic fetuses [83]. As mentioned earlier, special attention in these studies has been focused on 3βHSD activity in developing human adrenal. In this regard, in cultures of fetal zone and definitive zone cells of human fetal adrenal, 3βHSD expression cannot be detected until ACTH is added. ACTH induces the 3βHSD type II activity and mRNA expression. A higher level of 3βHSD mRNA is present in definitive zone compared with fetal zone cells [72].

As far as mineralocorticoid synthesis is concerned, using the RNase protection assay, aldosterone synthase (CYP11B2) mRNA is observed in the RNA from the neocortex, while RT-PCR reveals the presence of CYP11B2 mRNA in both neocortex and fetal zone [63,66]. Also notable differences between neocortex and fetal zone are seen in sulfotransferase activity. The highest rate of sulfurylation of pregnenolone and DHEA is found in the cytosol of the fetal zone (5 nmol min^{-1} mg $protein^{-1}$). The corresponding activities in fetal neocortex, anencephalic, and adult adrenocortical tissues amount to one tenth of that in the fetal zone [83].

Steroidogenesis in the developing human adrenal gland

Human fetal adrenal is a highly steroidogenic organ. For most of gestation, fetal zone secretes large quantities of DHEA and DHEAS, attaining the production rate of up to 100-200 mg/day. The rate of steroid secretion by the fetal adrenals may be 5-fold higher than that by the adrenals of adults at rest [78]. Functional data indicate cortisol and DHEAS secretion as early as at weeks 6-8 of gestation, while aldosterone secretion starts after weeks 20-24 [11,75].

The earliest studies on steroidogenic capacity of developing human adrenal gland were based on identification and quantitation of steroids in extracts of the gland, in cord blood and amniotic fluid. Subsequently, adrenal slice or homogenate assays demonstrated in fetal adrenals the presence of enzyme systems required

Folia Histochem Cytobiol. 2010:48(4): 496 (491-506)
10.2478/v10042-010-0062-7

to convert acetate to steroids [for review see 19,84,85]. Initial studies also revealed responsiveness of the human fetal adrenal cortex to ACTH [86-89]. Furthermore, early studies documented a lowered steroid production by adrenals of anencephalics [90-92].

Introduction of cell cultures further expanded our knowledge on steroid secretion by definitive and fetal zone cells. The studies demonstrated cortisol secretion by neocortex and DHEA/DHEAS by fetal zone as well as stimulating effects of ACTH on their secretion [93-101].

A general agreement has been reached that in cord blood from human fetuses between 10 and 20 weeks of gestation, concentrations of cortisol, aldosterone and DHEA/DHEAS are higher in the umbilical artery than in the umbilical vein, indicating that these steroids are produced by the fetus [11,102-106]. As expected, lowered corticosteroid levels are present in fetal blood of anencephalics [106,107].

Cortisol and DHEA/DHEAS secretion by fetal adrenal is well recognised and the findings leave no doubts. However, more questions concern aldosterone synthesis. Aldosterone has been isolated from human fetal adrenal glands as early as in 1956 [108]. Subsequent perfusion experiments revealed that the gland is capable of converting progesterone to aldosterone [87,109]). Moreover, the aldosterone concentration in fetal plasma is 2-12 times higher than that in the corresponding mothers [110,111]. Early in gestation mineralocorticoid production by the human fetal adrenal cortex is very low but it increases during the third trimester. Its concentration in human amniotic fluid also increases markedly from the weeks 14-16 of gestation till term [112,113]. Human fetal adrenal aldosterone biosynthesis was confirmed by means of tissue culture studies [114]. Later it was found that subcapsular region of the fetal adrenal is the main source of this hormone [97]. Aldosterone synthesis by definitive zone of human fetal adrenal was confirmed in subsequent publications [11,115].

Proliferative activity of fetal adrenocortical cells and apoptosis

It is well known that mutual interrelationship between cell proliferation and their elimination, primarily via apoptosis, governs growth and patterning of developing adrenal gland.

In adult adrenal gland proliferating cells are observed primarily in the subcapsular region of adrenal cortex. In this regard, in 1883 Gottschau [116] introduced a hypothesis on centripetal migration of adrenocortical cells from the subcapsular region into the center of the gland (zona reticularis), where parenchymal cells undergo elimination [13]. This hypothesis is confirmed by the recently applied molecular biology techniques, the results of which suggest clonal centripetal migration of adrenocortical cells [117].

Centripetal migration of cells from the definitive to the fetal zone was also observed in the developing human adrenal gland [5,7,31]. The earliest reports have based primarily on the observation of dividing cells in the glands. Introduction of immunohistochemistry extended these observation. In the earliest periods studied (weeks 6-8 of intrauterine development) proliferating-cell nuclear antigen (PCNA) positive cells were observed in the central part of developing fetal zone while the peripheral part was immunonegative. At week 8 of development, both definitive and fetal zones exhibited similar proliferative activity. At this stage of development the middle proliferative center of the fetal zone disappeared, and all cells of this zone had similar PCNA reactivity [118]. In other studies PCNA-immunopositive cells were observed only in the narrow surface zone of the primitive adrenal cortex [119,120]. Proliferation of fetal adrenal cells from 12 to 25 weeks of development was also assessed by PCNA and Ki-67 method [62]. PCNA and Ki-67 immunopositive cells were visible only in cells of the definitive zone and indexes of proliferation from 40% and 25% respectively, decreased gradually and were lower than 1% at the 25th week of intrauterine life. Similar studies (from 10 to 25 week of development) revealed that between weeks 10-14, PCNA indexes were similar in definitive and fetal zones of the gland and increased significantly between weeks 15-20 of gestation [121]. After 14 weeks, the mitotic index of the definitive zone was higher than that of fetal zone (approximately 59% vs. 39%). Despite the variable results, these studies suggest that adrenocortical cells proliferate in the definitive zone and migrate centripetally to form the fetal zone [11].

There are several descriptions of apoptosis in developing human adrenal gland. At all stages of gestation apoptotic nuclei were not observed in the definitive zone of developing adrenal. Only scattered apoptotic nuclei were seen in central part of the fetal zone and their number increases with the length of gestation [121,122]. In postnatal period, apoptotic index of fetal zone cells was highest during the second week of life, and subsequently declined (from ca 30% to 20%) [14,16,123].

Involution of the fetal zone

As mentioned, during two weeks after birth, the fetal zone of human adrenal cortex practically disappears, however fragments of the zone may persist in the adrenals till the end of the 2 year of life [13,14,124]. It is worth to emphasize marked individual variations in the rate of involution of that zone.

Folia Histochem Cytobiol. 2010:48(4): 497 (491-506)
10.2478/v10042-010-0062-7

The sudden postnatal involution of the fetal zone is connected with notable hemorrhagic lesions and the entire process may be divided into 2 distinct phases, the rapid one, lasting from the birth till the end of the second week of life, followed by a slower one [14,123]. As revealed by stereology, this decrease is highly correlated with the decrease of both the volume of fetal zone and quantity of its cells. During the first two weeks of postnatal development, volume of the fetal zone decreases from ca 70% to 3% of total adrenal volume and the quantity of its parenchymal cells from around 40% to 5% of all parenchymal cells. Furthermore, the volume of fetal zone stroma (connective tissue and blood vessels) expands notably during the first day of postnatal life and decreases from day 5th onward.

Factors regulating human fetal adrenal growth and steroidogenesis

It is well known that ACTH secreted by the fetal pituitary gland is a major factor regulating growth and steroidogenic capacity of the human adrenal fetal zone [11,125]. In congenital malformations connected with the absence of pituitary gland, for example anencephaly, development of the fetal zone is severely impaired. In anencephalics fetal zone development is comparable to that seen at 15-16th week of gestation [4,9,23,24,51-53,126].

Rapid postnatal involution of the fetal zone indicates that ACTH is not a sole factor regulating this zone and maintaining its structure and function. Moreover, not gestational age but parturition itself is the basis for fetal adrenal involution [29]. This implicates that placental factors are also involved in maintaining the fetal adrenal. And in fact, numerous factors have been identified that affect growth and function of the fetal zone during intrauterine life. These factors may act independently from, or in concert with, ATCH. Some of those factors are mentioned below.

Placental factors

hCG (human chorionic gonadotropin) extracted from pregnancy urine, but not highly purified one, stimulates thymidine incorporation into cultured fetal zone cells and does not affect DHEA/DHEAS output [97,127,128]. These findings suggest that the extracted hCG contained an unidentified compound stimulating cultured cells.

CRH (corticotrophin releasing hormone) is produced also by the placenta. In cultured fetal adrenocortical cells CRH stimulates all elements of DHEAS synthetic pathway [129-133].

ACTH is also produced by human placenta [134-137]. However, ACTH is not a growth factor *per se* and *in vitro* inhibits proliferation of adrenocortical cells. On the contrary, *in vivo* ACTH stimulates initially hypertrophy and later on mitotic activity of adrenocortical cells and possibly this effect may be mediated by growth factor(s) [11,138].

Growth factors and neuropeptides

IGF-I and IGF-II (insulin-like growth factor) stimulate proliferation of fetal adrenal cortical cells in a dose-dependent fashion [11,59,60,139,140]. Authors suggest that IGF-II may act as a mediator, in concert with bFGF and possibly EGF, of the tropic action of ACTH in induction of the rapid growth of the human fetal adrenal cortex during midgestation. It is believed that IGF system is the most important controller of fetal zone growth.

EGF (epidermal growth factor) stimulates thymidine incorporation into cultured fetal zone cells and has no effect on DHEA secretion [128].

bFGF (basic fibroblast growth factor) stimulates proliferation of both fetal and definitive zone cells [141-143].

Midkine (heparin-binding growth factor) selectively stimulates proliferation of definitive zone cells of the human fetal adrenal gland [144].

TNF (tumor necrosis factor) inhibits the ACTH-induced production of cortisol in cultures of human fetal adrenals [71,114]. These results show that TNF suppresses the synthesis of cortisol and shifts the steroid secretory pattern towards androgen production.

Activin A inhibits proliferation of cultured human fetal adrenal cells [145,146].

TGF-beta (transforming growth factor beta) dose-dependently inhibits the growth of fetal neocortical cells, an effect partially prevented by ACTH [146-152]. Of interest is that in the fetal zone, ACTH and TGF-beta have additive inhibitory effects on cell proliferation.

GH (growth hormone) and beta-lipotropin inhibits DHEAS secretion by cultured fetal zone cells [97].

ANP (atrial natriuretic peptide) inhibits basal and ACTH-stimulated steroid secretion by fetal zone cells [153]. The inhibitory effect of ANP on fetal steroidogenesis depends on inhibition of cholesterol side chain cleavage or transfer of cholesterol to the mitochondria.

PACAP (pituitary adenylate-cyclase activating peptide) significantly increases cortisol and DHEAS secretion by cultured fetal adrenal cells [154-156].

Nuclear receptor/transcription factors

A growing body of literature demonstrates the role of transcription factors in regulating development of the adrenal gland. However, experimental data are only available for animals [6,157,158].

Folia Histochem Cytobiol. 2010:48(4): 498 (491-506)
10.2478/v10042-010-0062-7

Analyses of inherited disorders suggest that some transcription factors are also involved in the development of human fetal adrenals (Fig. 2). One of such factors is GLI3 (GLI family zinc finger 3). Mutations in this transcription factor are causing Pallister-Hall syndrome and in some patients with this syndrome absence or hypoplasia of the adrenal glands were reported [159,160].

Also mutations of DAX1 [dosage-sensitive sex reversal congenital adrenal hypoplasia (AHC), a critical region on the X chromosome] were originally found in patients with the cytomegalic form of AHC. AHC combines two distinct histological patterns in the adrenal cortices, the miniature adult and cytomegalic forms. In the miniature adult form of AHC, the small amount of residual adrenal cortex is composed primarily of permanent adult cortex with normal structural organization. In the cytomegalic form of AHC, the residual adrenal cortex is structurally disorganized with scattered irregular nodular formations of eosinophilic cells, with the adult permanent zone absent or nearly absent [158,161]). This inherited disorder is commonly manifested as an early-onset adrenal insufficiency syndrome [162-164].

Recently Achermann *et al*. [165] suggested that mutations in SF1 (steroidogenic factor-1 or Ad4BP) result in adrenal failure in humans.

Data from global gene profiling

Modern global gene profiling study revealed numerous novel factors associated with the development or differentiated function of the adrenal cortex. By DNA microarrays, Rainey *et al*. [166] compared expression levels of several thousand transcripts (from 7075 to 9182 cDNA elements) between the human fetal and adult adrenal gland. Out of them, 69 transcripts were found to have a greater than 2.5-fold difference in expression between fetal and adult adrenals. The most pronounced differences were found for IGF-II (25-fold higher expression in fetal adrenals) and 3βHSD (24-fold higher expression in adults). Generally, expression of genes connected with growth, development and steroid biosynthesis was higher in fetal adrenals. In adult adrenals, on the other hand, the higher expression of genes connected to cellular immunity and signal transduction pathways was found. It remains, however, important that the vast majority of the 69 transcripts have not been studied with regard to adrenal function. Some of these data were detailed in the subsequent study [167]. DHEA sulfotransferase transcript (SULT2A1) was present at 13-fold higher levels in the fetal than the adult adrenals. 3β-Hydroxysteroid dehydrogenase type II (HSD3B2) mRNA was 127-fold higher in the adult adrenal gland while StAR, 21-hydroxylase, 11β hydroxylase, and aldosterone synthase mRNA abundance did not differ significantly.

The fetoplacental unit

As mentioned earlier, during most of gestation the fetal adrenal gland secretes large amount of DHEAS, which is used by the placenta to produce estrogens. These observations led Diczfalusy [168-172] to introduce the idea of a single endocrine steroidogenic unit of those two organs, the fetoplacental unit. Early studies revealed distinct variations in the pattern of steroidogenic enzyme distribution in fetal adrenal and placenta. In some instances, enzymes absent from one tissue are present in another, so that fetus and placenta together can synthesize steroids which they could not synthesize in separation. Some of the steroid hormones, partly metabolized in the placenta, are passed to the fetus for modification; others originate in the fetal adrenal gland and act as precursors for hormones that can be synthetized by the placenta. The human placenta does not produce estrogens *de novo* from cholesterol and cannot convert pregnenolone or progesterone into C19 steroids because it lacks the enzyme of 17α-hydroxylase/17,20 lyase (CYP17). Therefore, placenta produces estrogens by aromatization of the C19 precursors, namely DHEA and the 16α-hydroxy-DHEA (16-OH-DHEA), which are synthesized predominantly in the fetal adrenal and liver, respectively. In placenta they are aromatized to estradiol, estrone and estriol [173]. It is of interest that a similar fetoplacental steroidogenic unit is not present in most other species [174].

Conclusions

The year of 2010 marks the centenary of the first description of the human adrenal fetal zone. This transitory zone still intrigues world scientists. Despite extensive investigations, the origin, development, growth and steroidogenic function of the human adrenal fetal zone is not fully recognized. Notable progress has been made in understanding zonal and cellular composition of the gland. Also mechanisms of steroidogenesis, including the feto-placental unit, and their regulation are rather well known. With advance of the modern techniques of molecular biology and molecular engineering, future studies will likely be directed into the regulatory mechanism of expression of genes governing the development of the human fetal adrenal gland. In this regard, global gene profiling by Rainey *et al*. [166] has opened a new field of investigations. Identification of numerous genes not previously known to be associated with the development or specialized function of the human adrenal cortex, with great probability will rapidly expand our understanding of these processes.

Folia Histochem Cytobiol. 2010:48(4): 499 (491-506)
10.2478/v10042-010-0062-7

Fig. 6. Stella Starkel. From: Węgrzynowska [175].

Fig. 7. Lesław Wegrzynowski. From: Domosławski [177].

Addendum

Stella Maria Starkel (married name Węgrzynowska) (1885 – 1969)

Stella Maria Starkel was born on August 10, 1885 in Lwów. Her grandfather, Józef Cyryl Starkel (1807-1876) was an esteemed physician, deeply involved in life of the city of Tarnów, Eastern Poland. Her father, Romuald Starkel (1850-1888), was a teacher and journalist. Starkels' mother, Władysława Boczkowska, was married to widower Romuald Starkel, with 12-year-old daughter Maria. This marriage gave two daughters – Stella and Janina and son Władysław. Despite poverty caused by premature death of Romuald, all children completed University education [175].

Since at that time there were no schools for girls, Stella Starkel home schooled. In 1904 she started medical studies at Faculty of Medicine, Lwów University (Fig. 6). It is worth to mention that at that time only 2 girls were students of the Faculty of Medicine. During studies, together with Lesław Węgrzynowski, her future husband, she worked in the Institute of Pathological Anatomy, where as first in the world they described human adrenal fetal zone. In 1911 both received diplomas of "doctor medicinae universae".

During the World War I she became engaged in dentistry [176]. She also worked, together with her husband, Lesław Węgrzynowski, in military hospital and field laboratories in Moravian Brno. After World War I she worked in the laboratory of the SPROVAC, serum and vaccine factory owned by her husband.

After World War II Stella Węgrzynowska worked in laboratories of the Antituberculosis Sanatorium in Bukowiec, near Kowary and Oborniki Śląskie.

Stella Maria Węgrzynowska died on April 7, 1969 in Oborniki Śląskie, near Wrocław, Poland.

Lesław Węgrzynowski (1885-1956)

Lesław Węgrzynowski was born on September 17, 1885, in Rohatyn (Western Ukraine, not far from Lwów) to the family with long patriotic and medical traditions. His father, Władysław, was a physician while mother – Zofia Gluzińska – also grow up in famous physicians' family.

In 1904 he received the general certificate of education and started medical studies at the Faculty of Medicine, Lwów University. During medical school together with Stella Starkel, his future wife, he worked in the Institute of Pathological Anatomy, where as first in the world they described human adrenal fetal zone. In 1911 both received diplomas of "doctor medicinae universae".

After graduation he became interested in pneumophthisiatric diseases, especially in lung tuberculosis (TB). For 2 years he studied bacteriological and serological as well as clinical aspects of TB in the world-known clinics of prof. F. Krause and Robert Koch Institute (Berlin), dr. L. Brauer (Hamburg) and dr. Turban (Davos, Switzerland). After return to Lwów, Węgrzynowski worked in the Institute of Pharmacology, at that time chaired by prof. Lech Popielski.

In 1914 he became a chief of sanitary service of the Polish Eastern Legion (Polish armed forces created in August 1914 in Galicia, the southern district of Poland) [176,177]. The Legion was disbanded on 21 September 1914 and Węgrzynowski was taken to Austrian army. He was a chief of military hospital and field laboratories in Moravian Brno. At that time he had worked together with his wife, Stella from Starkels'. Since 1918 he served in the Polish Army. During the Polish – Ukrainian war Węgrzynowski headed the sanitary service of the Main Command of the Lwów

Defence forces. After the war he was even the chief of the board conferring Lwów Defence Cross.

After the World War I Węgrzynowski was employee of the Lwów University and developed his interest in pneumo-phthisiatric diseases. Up to 1939 he was a chief of the Outpatient TB Department in Lwów and of Antituberculosis Sanatorium in Hołosko Wielkie by Lwów. At the beginning of the World War II he served in the sanitary command of the Lwów, later on (up to 1941) he was nominated by Soviets as chairman of the Lwów Phthisiatric Clinic of the Kijow Institute of Tuberculosis. Arrested by Soviets, he avoided death only, because somebody informed them that in 1914, when Lenin was in Poronin and Cracow (Poland) Węgrzynowski treated him (two available sources cited this fact). After entering of German forces to Lwów, Węgrzynowski and his family moved to Warszawa. In Warszawa he worked as a physician and participated in conspiracy activities. He was a consultant to Ujazdowski Hospital and chief of the Antituberculosis Sanatorium in Świder (near Warszawa).

In conspiracy he was functioning in the Governmental Delegation for Poland (of the Polish Government in Exile, London) saving the life of numerous Jews. He had hided them in his clinics as a patients while they waited further placement. Followed by Gestapo, he went underground and took the pseudonym of Bartosz. During the Warsaw Uprising Bartosz served as the head of sanitary service of the I District Warszawa Śródmieście of the Polish Home Army [see the www page 178].

After World War II he restarted a function of the Polish Red Cross Sanatorium in Zakopane. Between years 1946 – 1947 he was director of the Antituberculosis Sanatorium in Bukowiec, near Kowary and a cofounder of the Polish Society of Studies on Tuberculosis.

On August 9, 1949 Węgrzynowski became employed as a deputy professor in the Department of Phthisiatry, Faculty of Medicine, Wrocław University (later on Medical Academy) [176,177]) (Fig. 7). As a victim of the Secrete Police action (code name "Medicine") [179] he was dismissed in 1951. Until the end of his life he worked in the Municipal Antituberculosis Outpatient Clinic in Wrocław. He died in there on June 10, 1956.

References

[1] Starkel S, Wegrzynowski L. Beitrag zur Histologie der Nebenniere bei Feten und Kindern. *Arch Anat Physiol*. 1910;8:214-235.

[2] Starklówna S, Węgrzynowski L. O nadnerczach u dzieci na podstawie własnych badań (Część I) [Polish] (Adrenal in children – results of original investigations). *Medycyna i Kronika Lekarska*. 1910;30-34:667,692,709,736,753.

[3] Kuński H. Priority of Polish investigators in the discovery of the fetal zone in human adrenal. *Pol Med Sci Hist Bull*. 1961;4:166.

[4] Sucheston ME, Cannon MS. Development of zonular patterns in the human adrenal gland. *J Morphol*. 1968;126:477-491.

[5] Jirasek, J.: Human Fetal Endocrines.1980; Martinus Nijhoff, 4. London, pp 69-82.

[6] Else T, Hammer GD. Genetic analysis of adrenal absence: agenesis and aplasia. *Trends Endocrinol Metab*. 2005;16:458-468.

[7] Crowder RE. The development of the adrenal gland in man, with special reference to origin and ultimate location of cell types and evidence in favor of the "cell migration" theory. *Contemp Embryol*. 1957;251:195-209.

[8] Bocian-Sobkowska, J., Malendowicz, L.K., Woźniak, W. Cytological aspects of the human adrenal cortex development in the course of intra-uterine life. *Histol Histopathol*. 1993;8:725-730.

[9] Bocian-Sobkowska J, Wozniak W, Malendowicz LK, Ginda W. Stereology of human fetal adrenal medulla. *Histol Histopathol*. 1996;11:214-216.

[10] Serón-Ferré M, Jaffe RB. The fetal adrenal gland. *Ann Rev Physiol*. 1981;43:141-162.

[11] Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev*. 1997;18:378-401.

[12] Ehrhart-Bornstein M, Breidert M, Guadanucci P, Wozniak W, Bocian-Sobkowska J, Malendowicz LK, Bornstein SR. 17 alpha-Hydroxylase and chromogranin A in 6th week human fetal adrenals. *Horm Metab Res*. 1997;29:30-32.

[13] Bachmann R. Die Nebenniere. W: Handbuch der mikroskopischen Anatomie des Menschen. Tom VI, part 5. Springer Verlag, Berlin, Gottingen, Heidelberg. 1954; pp. 952.

[14] Bocian-Sobkowska J, Woźniak W, Malendowicz LK. Postnatal involution of the human adrenal fetal zone: stereologic description and apoptosis. *Endocr Res*. 1998;24:969-973.

[15] Dhom G. The prepuberal and puberal growth of the adrenal (adrenarche). *Beitr Pathol*. 1973;150:357-377.

[16] Hui XG, Akahira JI, Suzuki T, Nio M, Nakamura Y, Suzuki H, Rainey WE, Sasano H. Development of the human adrenal zona reticularis: morphometric and immunohistochemical studies from birth to adolescence. *J Endocrinol*. 2009;203:241-252.

[17] Swinyard CA. Growth of the human suprarenal glands. *Anat Rec*. 1943;87:141-150.

[18] Chester-Jones I. The adrenal cortex. Cambridge University Press, London (1957).

[19] Deane HW. The anatomy, chemistry and physiology of adrenocortical tissue. In: Deane, H.W., (ed) *Handbuch der experimentellen Pharmakologie*. 1962; Vol 14, Part 1. Springer, Berlin Göttingen Heidelberg, pp 1-185.

[20] Carr BR, Casey ML. Growth of the adrenal gland of the normal human fetus during early gestation. *Early human devel*. 1982;6:121-124.

[21] Vinson GP, Whitehouse B, Hinson J. *The Adrenal Cortex*. Prentice Hall. 1992.

[22] Folligan K, Bouvier R, Targe F, Morel Y, Trouillas J. [Development of the human adrenal glands]. *Ann Endocrinol*. (Paris). 2005;66:325-332.

[23] Sucheston ME, Cannon MS. Microscopic comparison of the normal and anencephalic human adrenal gland with emphasis on the transient-zone. *Obstet Gynecol*. 1970;35:544-553.

[24] Gray ES, Abramovich DR. Morphologic features of the anencephalic adrenal gland in early pregnancy. *Am J Obstet Gynecol*. 1980;137:491-495.

[25] Rosenberg ER, Bowie JD, Andreotti RF, Fields SI. Sonographic evaluation of fetal adrenal glands. *Am J Roentgenol*. 1982;139:1145-1147.

[26] Hata K, Hata T, Kitao M. Ultrasonographic identification and measurement of the human fetal adrenal gland in utero. *Int J Gynecol Obstet*. 1985;23:355-359.

Folia Histochem Cytobiol. 2010:48(4): 501 (491-506)
10.2478/v10042-010-0062-7

[27] Hata K, Hata T, Kitao M. Ultrasonographic identification and measurement of the human fetal adrenal gland in utero: Clinical Application. *Gynecol Obstet Invest.* 1988;25:16-22.
[28] Hauffa BP, Menzel D, Stolecke H. Age-related changes in adrenal size during the first year of life in normal newborns, infants and patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency: comparison of ultrasound and hormonal parameters. *Eur J Pediatr.* 1988;148:43-49.
[29] Ben-David S, Zuckerman-Levin N, Epelman M, Shen-Orr Z, Levin M, Sujov P, Hochberg Z. Parturition itself is the basis for fetal adrenal involution. *J Clin Endocrinol Metab.* 2007;92:93-97.
[30] Elliot TR, Armour RG. The development of the cortex in the human suprarenal glands and its condition in hemicephaly. *J Pathol*. 1911;15:481-488.
[31] Keene MFL, Hewer EE. Observations on the development of the human suprarenal gland. *J Anat.* 1927;61:302-324.
[32] Tobeck A. Ueber die Lipoid- und Eisenablagerungen in Nebennieren und Hoden im Säuglingsalter. *Virchows Arch*. 1928;267:690-715.
[33] Uotila UU. The early embryological development of the fetal and permanent adrenal cortex in man. *Anat Rec.* 1940;76:183-203.
[34] Velican C. La zone transitoire de la cortico-surrenale humaine. *Arch Anat Microsc*. 1948;37:73-81.
[35] Rotter W. Die Strukturen der fötalen und kindlichen Nebennierenrinde. *Verh Dtsch Ges Path.* (Dortmund). 1950;170-175, 276-277.
[36] Lanman JT. Fetal zone of the adrenal gland; its developmental course, comparative anatomy, and possible physiological functions. *Medicine*. 1953;32:389-430.
[37] Lanman JT. The adrenal fetal zone: its occurrence in primates and a possible relationship to chorionic gonadotropin. *Endocrinology*. 1957;61:684-691.
[38] Wołyńska M. The foetal development of the human adrenal gland [Polish]. *Folia Morph*. (Warsz).1956;7:35-51.
[39] Nussdorfer GG. Cytophysiology of the adrenal cortex. *Int Rev Cytol.* 1986;98:1-395.
[40] Ross MH, Pappas GD, Lanman JT, Lind J. Electron microscope observations on the endoplasmic reticulum in the human fetal adrenal. *Biophys Biochem Cytol.* 1958;4:659-666.
[41] Ross M H. Electron microscopy of the human fetal adrenal cortex. The human adrenal cortex (eds. A. R. Currie, T. Symington and J. K. Grant) 1962; p.558-569. Baltimore: Williams & Wilkins
[42] Hatakeyama S. Submicroscopic study on the developmental morphokinetics of the human embryonal and new born adrenal cortex. *Acta path jap.* 1966;16:253-286.
[43] Johannisson E. The foetal adrenal cortex in the human. Its ultrastructure at different stages of development and in different functional states. *Acta Endocrinol.* 1968; (Copenh) 130[Suppl]:1-107.
[44] Johannisson E. Aspects of the ultrastructure and function of the human fetal adrenal cortex. Contrib. *Gynecol. Obstet*. 1979;5:109-130.
[45] Idelman S. Ultrastructure of the mammalian adrenal cortex. *Int Rev Cytol*. 1970;25:181- 281.
[46] McNutt NS, Jones AL. Observations of the ultrastructure of cytodifferentiation in the human fetal adrenal cortex. *Lab Invest.* 1970;11:513.
[47] Mäusle E. Elektronenmikroskopische Befunde an der menschlichen Neugeborenen-Nebenniere. *Beitr Pathol Anat.* 1972;146:221-247.
[48] Fujita H, Ihara T. Electron-microscopic observations on the cytodifferentiation of adrenocortical cells of the human embryo. *Anatom Embryol*. 1973;142: 267-281.
[49] Murakoshi M, Osamura Y, Watanabe K, Kuroshima Y. Enzyme histochemical and ultrastructural studies in human adrenal glands of normal and anencephalic fetuses. *Tokai J Exp Clin Med.* 1984;9:119-137.
[50] Pajor L, Németh A, Illés T. Functional morphology of the adrenal cortex in newborns. I. Morphometric study. *Acta Morphol Hung.* 1986;34:31-37.
[51] Bocian-Sobkowska J, Malendowicz LK, Woźniak W. Comparative stereological study on zonation and cellular composition of adrenal glands of normal and anencephalic human fetuses. I. Zonation of the gland. *Histol Histopathol*. 1997;12:311-317.
[52] Bocian-Sobkowska J, Malendowicz LK, Woźniak W. Comparative stereological studies on zonation and cellular composition of adrenal glands of normal and anencephalic human fetuses. II. Cellular composition of the gland. *Histol. Histopathol.* 1997;12:391-399.
[53] Bocian-Sobkowska J, Malendowicz LK, Woźniak W, Kopaczyk M. Adrenal glands in anencephaly. *Folia Morphol.* (Warsz). 1996;55:214-216.
[54] Lichnovský V, Lojda Z. Enzyme histochemistry in the developing suprarenal gland of human embryos. *Acta Univ Palacki Olomuc Fac Med.* 1989;122:91-100.
[55] Breault L, Chamoux E, Lehoux JG, Gallo-Payet N. Localization of G protein alpha-subunits in the human fetal adrenal gland. *Endocrinology*. 2000;141:4334-4341.
[56] Pollack SE, Furth EE, Kallen CB, Arakane F, Kiriakidou M, Kozarsky KF, Strauss JF 3rd. Localization of the steroidogenic acute regulatory protein in human tissues. *J Clin Endocrinol Metab.* 1997;82:4243-4251.
[57] Narasaka T, Suzuki T, Moriya T, Sasano H. Temporal and spatial distribution of corticosteroidogenic enzymes immunoreactivity in developing human adrenal. *Mol Cell Endocrinol.* 2001;174:111-120.
[58] Di Blasio AM, Voutilainen R, Jaffe RB, Miller WL. Hormonal regulation of messenger ribonucleic acids for P450scc (cholesterol side-chain cleavage enzyme) and P450c17 (17 alpha-hydroxylase/17,20-lyase) in cultured human fetal adrenal cells. *J Clin Endocrinol Metab*. 1987;65:170-175.
[59] Mesiano S, Mellon SH, Jaffe RB. Mitogenic action, regulation and localization of insulin-like growth factors in the human fetal adrenal gland. *J Clin Endocrinol Metab*. 1992;76:968-976.
[60] Mesiano S, Coulter CL, Jaffe RB. Localization of cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17 alpha-hydroxylase/17, 20-lyase, and 3 beta-hydroxysteroid dehydrogenase isomerase steroidogenic enzymes in human and rhesus monkey fetal adrenal glands: reappraisal of functional zonation. *J Clin Endocrinol Metab*. 1993;77:1184-1189.
[61] Goto, M., Brickwood, S., Wilson, D.I., Wood, P.J., Mason, J.I., Hanley, N.A.: Steroidogenic enzyme expression within the adrenal cortex during early human gestation. *Endocr Res.* 2002;28:641-645.
[62] Folligan K, Bouvier R, Targe F, Morel Y, Trouillas J. Histological and molecular study of fetal human adrenal cortex (12-36 wk)] *Ann Endocrinol* .(Paris) 2005;66:519-526.
[63] Freije WA, Pezzi V, Arici A, Carr BR, Rainey WE. Expression of 11 beta-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) in the human fetal adrenal. *J Soc Gynecol Investig*. 1997;4:305-309.
[64] Coulter CL, Smith RE, Stowasser M, Sasano H, Krozowski ZS, Gordon RD. Expression of 11beta-hydroxysteroid dehydrogenase type 2 (11betaHSD-2) in the developing human adrenal gland and human adrenal cortical carcinoma and adenoma. *Mol Cell Endocrinol*. 1999;154:71-77.
[65] Hanley NA, Rainey WE, Wilson DI, Ball SG, Parker KL. Expression profiles of SF-1, DAX1, and CYP17 in the human

fetal adrenal gland: potential interactions in gene regulation. *Mol Endocrinol*. 2001;15:57-68.
[66] Coulter CL, Jaffe RB. Functional Maturation of the Primate Fetal Adrenal *in Vivo:* 3. Specific Zonal Localization and Developmental Regulation of CYP21A2 (P450c21) and CYP11B1/CYP11B2 (P450c11/Aldosterone Synthase) Lead to Integrated Concept of Zonal and Temporal Steroid Biosynthesis. *Endocrinology*. 1998;139: 5144-5150.
[67] Goldman AS, Yakovac WC, Bongiovanni AM. Development of activity of 3β-hydroxysteroid dehydrogenase in human fetal tissues and in two anencephalic newborns. *Metab*. 1966;26:14-22.
[68] Simonian MH. ACTH and thyroid hormone regulation of 3 beta-hydroxysteroid dehydrogenase activity in human fetal adrenocortical cells. *J Steroid Biochem*. 1986;25:1001-1006.
[69] Doody KM, Carr BR, Rainey WE, Byrd W, Murry BA, Strickler RC, Thomas JL, Mason JI. 3β-Hydroxysteroid dehydrogenase/isomerase in the fetal zone and neocortex of the human fetal adrenal gland. *Endocrinology*. 1990;126:2487-2492.
[70] Dupont E, Luu-The V, Labrie F, Pelletier G. Ontogeny of 3β-hydroxysteroid/ 5- 4 isomerase (3β-HSD) in human adrenal gland performed by immunocytochemistry. *Mol Cell Endocrinol.* 1990;75:R7-R10.
[71] Voutilainen R, Ilveskmaki V, Miettinen P. Low expression of 3β-hydroxy-5-ene steroid dehydrogenase gene in human fetal adrenals in vivo; adrenocorticotropin and protein kinase C-dependent regulation in adrenocortical cultures. *J Clin Endocrinol Metab*. 1991;72:761-767.
[72] Mason JI, Ushijima K, Doody KM *et al*. Regulation of expression of the 3 beta-hydroxysteroid dehydrogenases of human placenta and fetal adrenal. *J Steroid Biochem Mol Biol*. 1993;47: 151-159.
[73] Parker Jr CR, Faye-Petersen O, Stankovic AK, Mason JI, Grizzle WE. Immunohistochemical evaluation of the cellular localization and ontogeny of 3β-hydroxysteroid dehydrogenase/delta 5-4 isomerase in the human fetal adrenal gland. *Endocr Res*. 1995;21:69-80.
[74] Coulter CL, Goldsmith PC, Mesiano S, Voytek CC, Martin MC, Mason JI, Jaffe RB. Functional maturation of the primate fetal adrenal *in vivo*: 2. Ontogeny of corticosteroid synthesis is dependent upon specific zonal expression of 3β-hydroxysteroid dehydrogenase/isomerase (3βHSD). *Endocrinology.* 1996;137:4953-4959.
[75] Lanman JT. The adrenal gland in the human fetus: An interpretation of its physiology and unusual developmental pattern. *Pediatrics*. 1961;27:140-158.
[76] Muench MO, Ratcliffe JV, Nakanishi M, Ishimoto H, Jaffe RB. Isolation of definitive zone and chromaffin cells based upon expression of CD56 (neural cell adhesion molecule) in the human fetal adrenal gland. *J Clin Endocrinol Metab*. 2003;88:3921-3930.
[77] Ratcliffe J, Nakanishi M, Jaffe RB. Identification of definitive and fetal zone markers in the human fetal adrenal gland reveals putative developmental genes. *J Clin Endocrinol Metab*. 2003;88:3272 – 3277.
[78] Carr BR, Simpson ER. Lipoprotein utilization and cholesterol synthesis by the human fetal adrenal gland. *Endocr Rev.* 1981;2:306-326.
[79] Carr BR, Ohashi M, Simpson ER. Low density lipoprotein binding and de novo synthesis of cholesterol in the neocortex and fetal zones of the human fetal adrenal gland. *Endocrinology.* 1982;110:1994-1998.
[80] Carr BR, Milburn J Jr, Wright EE, Simpson ER. Adenylate cyclase activity in neocortex and fetal zone membrane fractions of the human fetal adrenal gland. *J Clin Endocrinol Metab*. 1985;60:718-722.
[81] Carr BR. Adenylate cyclase activity in membrane fractions of adrenal tissue of human anencephalic fetuses. *J Clin Endocrinol Metab*. 1986;63:51-55.
[82] Rainey WE, Mason JI, Cochet C, Carr BR. Protein kinase-C in the human fetal adrenal gland. *J Clin Endocrinol Metab*. 1988;67:908-914.
[83] Korte K, Hemsell PG, Mason JI. Sterol sulfate metabolism in the adrenals of the human fetus, anencephalic newborn, and adult. *J Clin Endocrinol Metab.* 1982;55:671-675.
[84] Bloch E, Benirschke K. Synthesis *in vitro* of steroids by human fetal adrenal slices. *J Biol Chem*. 1959; 234:1085-1089.
[85] Shibusawa H, Sano Y, Okinaga S, Arai K. Studies on 11 beta-hydroxylase of the human fetal adrenal gland. *J Steroid Biochem*. 1980;13:881-887.
[86] Villee DB, Engel LL, Loring JM, Villee CA. Steroid hydroxylation in human fetal adrenals: Formation of 16 alpha-hydroxyprogesterone, 17-hydroxyprogesterone and deoxycorticosterone. *Endocrinology*. 1961;69:354-372.
[87] Dufau ML, Villee DB. Aldosterone biosynthesis by human fetal adrenal *in vitro. Biochim Biophys Acta*. 1969;176:637-641.
[88] Serón-Ferré M, Lawrence CC, Jaffe RB. Role of hCG in regulation of the fetal zone of the human fetal adrenal gland. *J Clin Endocrinol Metab*. 1978;46:834-837.
[89] Serón-Ferré M, Lawrence CC, Siiteri PK, Jaffe RB. Steroid production by definitive and fetal zones of the human fetal adrenal gland. *J Clin Endocrinol Metab*. 1978;47:603-609.
[90] Fransden VA, Stakemann G. The site of production of oestrogenic hormones in human pregnancy. Hormone excretion in pregnancy with anencephalic foetus. *Acta Endocrinol.* (Copenh) 1961;38:383-391.
[91] Honnebier WJ, Jobis AC, Swaab DF. The effect of hypophyseal hormones and human chorionic gonadotropin (HCG) on the anencephalic fetal adrenal cortex and parturition in the human. *J Obstet Gynaecol Br Common*. 1974;81:423-438.
[92] Carr BR, Parker CR Jr, Porter JC, MacDonald PC, Simpson ER. Regulation of steroid secretion by adrenal tissue of a human anencephalic fetus. *J Clin Endocrinol Metab*. 1980;50:870-873.
[93] Kahri AI, Huhtaniemi I, Salmenperä M. Steroid formation and differentiation of cortical cells in tissue culture of human fetal adrenals in the presence and absence of ACTH. *Endocrinology*. 1976;98:33-41.
[94] Branchaud CL, Goodyer CG, Hall CG, Arato JS, Silman RE, Giroud CJP. Steroidogenic activity of hACTH and related peptides on the human neocortex and fetal adrenal cortex in organ culture. *Steroids* 1978;31:557-571.
[95] Branchaud CL, Goodyer CG, Shore P, Lipowski LS, Lefebvre Y. Functional zonation of the midgestation human fetal adrenal cortex: fetal versus definitive zone use of progesterone for cortisol synthesis. *Am J Obstet Gynecol*. 1985;151:271-277.
[96] Mason JI, Rainey WE. Steroidogenesis in the human fetal adrenal: a role for cholesterol synthesized de novo. *J Clin Endocrinol Metab*. 1987;64:140-147.
[97] Fujieda K, Faiman C, Reyes FI, Thliveris J, Winter JS. The control of steroidogenesis by human fetal adrenal cells in tissue culture. II. Comparison of morphology and steroid production in cells of the fetal and definitive zones. *J Clin Endocrinol Metab.* 1981;53:401-405.
[98] Fujieda K, Faiman C, Reyes FI, Winter JS. The control of steroidogenesis by human fetal adrenal cells in tissue culture. III. The effects of various hormonal peptides. *J Clin Endocrinol Metab*. 1981;53:690-693.
[99] Simonian MH, Gill GN. Regulation of the fetal human adrenal cortex: effects of adrenocorticotropin on growth and function of monolayer cultures of fetal and definitive zone cells. *Endocrinology*. 1981;108:1769-1779.

Folia Histochem Cytobiol. 2010:48(4): 503 (491-506)
10.2478/v10042-010-0062-7

[100] Mason JI, Hemsell PG, Korte K. Steroidogenesis in dispersed cells of human fetal adrenal. *J Clin Endocrinol Metab*. 1983;56:1057-1062.
[101] Simonian MH, Capp MW. Characterization of steroidogenesis in cell cultures of the human fetal adrenal cortex: comparison of definitive zone and fetal zone cells. *J Clin Endocrinol Metab*. 1984;59:643-651.
[102] Lytt I, Gardner LI, Walton RE. Plasma 17-Ketosteroids of Full-Term and Premature Infants. *J Clin Invest*. 1954;33:1642-1645.
[103] Migeon CJ, Keller AR, Holmstrom EG. Dehydroepiandrosterone, androsterone and 17-hydroxycorticosteroid levels in maternal and cord plasma in cases of vaginal delivery. *Bull Johns Hopkins Hosp.* 1955;97:415-421.
[104] Murphy BE. Steroid arteriovenous differences in umbilical cord plasma: evidence of cortisol production by the human fetus in early gestation. *J Clin Endocrinol Metab.* 1973; 36:1037-1038.
[105] Murphy BE, Clark SJ, Donald IR, Pinsky M, Vedady D. Conversion of maternal cortisol to cortisone during placental transfer to the human fetus. *Am J Obstet Gynecol.* 1974;118:538-541.
[106] Easterling WK Jr, Simmer HH, Dignam WJ, Frankland MV, Naftolin F. Neutral C19-steroids and steroid sulfates in human pregnancy II. Dehydroepiandrosterone sulfate, 16α-hydroxydehydroepi-androsterone, and 16α-hydroxydehydroepiandrosterone sulfate in maternal and fetal blood of pregnancies with anenphalic and normal fetuses. *Steroids*. 1966;8:157-178.
[107] Nichols J, Lescure OL, Migeon CJ. Levels of 17-hyrdroxycorticosteroids and 17-ketosteroids in maternal and cord plasma in term anencephaly. *J Clin Endocrinol Metab*. 1958;18:444-452.
[108] Bloch E, Benirschke K, Rosemberg E. C19 steroids, 17alpha-hydroxycorticosterone and a sodium retaining factor in human fetal adrenal glands. *Endocrinology*. 1956;58:626-633.
[109] Pasqualini JR, Wiqvist N, Diczfalusy E. Biosynthesis of aldosterone by human foetuses perfused with corticosterone at mid-term. *Biochim Biophys Acta*. 1966;121:430-431.
[110] Bayard F, Ances IG, Tapper AJ, Weldon VV, Kowarski A, Migeon CJ. Transplacental passage and fetal secretion of aldosterone. *J Clin Invest*. 1970;49:1389-1393.
[111] Beitins IZ, Bayard F, Levitsky L, Ances IG, Kowarski A, Migeon CJ. Plasma aldosterone concentration at delivery and during the newborn period. *J Clin Invest*. 1972;51:386-394.
[112] Blankstein J, Fujieda K, Reyes FI, Faiman C, Winter JS. Aldosterone and corticosterone in amniotic fluid during various stages of pregnancy. *Steroids*. 1980;36:161-165.
[113] Sippell WG, Müller-Holve W, Dörr HG, Bidlingmaier F, Knors D. Concentrations of Aldosterone, Corticosterone, 11-Deoxycorticosterone, Progesterone, 17-Hydroxyprogesterone, 11-Deoxycortisol, Cortisol, and Cortisone Determined Simultaneously in Human Amniotic Fluid throughout Gestation. *J Clin Endocrionol Metab*. 1981;52:385-392.
[114] Voutilainen R. Steroidogenesis in tissue culture of fetal and adult human adrenals with special reference to cortisol/corticosterone ratio and aldosterone secretion. *J Steroid Biochem*. 1979;10: 115-120.
[115] Nelson HP, Kuhn RW, Deyman ME, Jaffe RB. Human fetal adrenal definitive and fetal zone metabolism of pregnenolone and corticosterone: alternate biosynthetic pathways and absence of detectable aldosterone synthesis. *J Clin Endocrinol Metab*. 1990;70:693-698.
[116] Gottschau M.: Struktur und embryonale Entwicklung der Nebennieren bei Saugetieren. *Archiv Anat Phys.* 1883;412-458.
[117] Morley SD, Viard I, Chung BC, Ikeda Y, Parker KL, Mullins JJ. Variegated expression of a mouse steroid 21-hydroxylase/beta- galactosidase transgene suggests centripetal migration of adrenocortical cells. *Molec Endocrinol*. 1996;10:585-598.
[118] Bocian-Sobkowska J, Woźniak W, Zabel M, Surdyk-Zasada J. Proliferating-cell nuclear antigen (PCNA) in the human adrenal glands at the end of the embryonic period. *Acta Histochem*. 1998;100: 279-286.
[119] Tauber Z, Lichnovský V, Kolár Z. Proliferating cell nuclear antigen in tissues and organs of human embryos. *Acta Univ Palacki Olomuc Fac Med*. 1995;139:29-32.
[120] Tauber Z, Lichnovský V, Bocek M, Kolár Z. [PCNA (proliferating cell nuclear antigen) in the tissues and organs of the human fetus. II] *Acta Univ Palacki Olomuc Fac Med.* 1996; 140:29-32.
[121] Spencer SJ, Mesiano S, Lee JY, Jaffe R.B. Proliferation and apoptosis in the human adrenal cortex during the fetal and perinatal periods: implications for growth and remodeling. *J Clin Endocrinol Metab*. 1999;84:1110-1115.
[122] Chamoux E, Breault L, Lehoux JG, Gallo-Payet N. Involvement of the angiotensin II type 2 receptor in apoptosis during human fetal adrenal gland development. *J Clin Endocrinol Metab.* 1999;84:4722-4730.
[123] Bocian-Sobkowska J. Morphometric study of the human suprarenal gland in the first postnatal year. *Folia Morphol (Warsz)*. 2000;58:275-284.
[124] Benner, M.C: Studies on the involution of the fetal cortex of the adrenal glands. *Am J Pathol.* 1940;16:787-798.
[125] Pepe GJ, Albrecht ED. Regulation of the primate fetal adrenal cortex. *Endocr Rev*. 1990;11:151-176.
[126] Benirschke K, Bloch E, Hertig AT. Concerning the function of the fetal zone of the human adrenal gland. *Endocrinology*. 1956;58:598-625.
[127] Voutilainen R, Kahri AI, Salmenpera M. The effects of progesterone, pregnenolone, estriol, ACTH and hCG on steroid secretion of cultured human fetal adrenals. *J Steroid Biochem*. 1979;10:695-700.
[128] Abu-Hakima M, Branchaud CL, Goodyer CG, Murphy BE. The effects of human chorionic gonadotropin on growth and steroidogenesis of the human fetal adrenal gland *in vitro*. *Am J Obstet Gynecol*. 1987;156:681-687.
[129] Chakravorty A, Mesiano S, Jaffe RB. Corticotropin-releasing hormone stimulates P450 17α-hydroxylase/17,20-lyase in human fetal adrenal cells via protein kinase C. *J Clin Endocrinol Metab*. 1999;84:3732-3738.
[130] Parker CR Jr, Stankovic AM, Goland RS. Corticotropin-releasing hormone stimulates steroidogenesis in cultured human adrenal cells. *Mol Cell Endocrinol*. 1999;155:19-25.
[131] Sirianni R, Mayhew BA, Carr BR, Parker CR Jr, Rainey WE. Corticotropin-releasing hormone (CRH) and urocortin act through type 1 CRH receptors to stimulate dehydroepiandrosterone sulfate production in human fetal adrenal cells. *J Clin Endocrinol Metab*. 2005;90:5393-5400.
[132] Sirianni R, Rehman KS, Carr BR, Parker CR Jr, Rainey WE. Corticotropin-releasing hormone directly stimulates cortisol and the cortisol biosynthetic pathway in human fetal adrenal cells. *J Clin Endocrinol Metab*. 2005;90:279-285.
[133] Rehman KS, Sirianni R, Parker CR Jr, Rainey WE, Carr BR. The regulation of adrenocorticotrophic hormone receptor by corticotropin-releasing hormone in human fetal adrenal definitive/transitional zone cells. *Reprod Sci*. 2007;14:578-587.
[134] Liotta A, Osathanondh R, Ryan KJ, Krieger DT.: Presence of corticotropin in human placenta: demonstration of *in vitro* synthesis. *Endocrinology*. 1977;101:1522-1528.
[135] Liotta AS, Krieger DT.: In vitro biosynthesis and comparative posttranslational processing of immunoreactive precur-

Folia Histochem Cytobiol. 2010:48(4): 504 (491-506)
10.2478/v10042-010-0062-7

sor corticotropin/beta-endorphin by human placental and pituitary cells. *Endocrinology*. 1980;106:1504-1511.
[136] Goland RS, Wardlaw SL, Stark RI, Brown Jr LS, Frantz AG.: High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. *J Clin Endocrinol Metab*. 1986;63:1199-1203.
[137] Petraglia F, Sawchenko PE, Rivier J, Vale W. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature*. 1987;328:717-719.
[138] Dallman MF. Control of adrenocortical growth in vivo. *Endocr Res*. 1984-5;10:213-242.
[139] Shigematsu K, Niwa M, Kurihara M, Yamashita K, Kawai K, Tsuchiyama H. Receptor autoradiographic localization of insulin-like growth factor-I (IGF-I) binding sites in human fetal and adult adrenal glands. *Life Sci*. 1989;45:383-389.
[140] Mesiano S, Katz SL, Lee JY, Jaffe RB. Insulin-like growth factors augment steroid production and expression of steroidogenic enzymes in human fetal adrenal cortical cells: implications for adrenal androgen regulation. *J Clin Endocrinol Metab.* 1997;82:1390-1396.
[141] Crickard K, Ill CR, Jaffe RB. Control of proliferation of human fetal adrenal cells in vitro. *J Clin Endocrinol Metab.* 1981;53:790-796.
[142] Hornsby PJ, Sturek M, Harris SE, Simonian MH. Serum and growth factor requirements for proliferation of human adrenocortical cells in culture: comparison with bovine adrenocortical cells. *In Vitro*. 1983;19:863-869.
[143] Mesiano S, Mellon SH, Gospodarowicz D, Di Blasio AM, Jaffe RB. Basic fibroblast growth factor expression is regulated by corticotropin in the human fetal adrenal: A model for adrenal growth regulation. *Proc Natl Acad Sci USA*. 1991;88:5428-5432.
[144] Ishimoto H, Muench MO, Higuchi T, Minegishi K, Tanaka M, Yoshimura Y, Jaffe RB. Midkine, a heparin-binding growth factor, selectively stimulates proliferation of definitive zone cells of the human fetal adrenal gland. *J Clin Endocrinol Metab*. 2006;91:4050-4056.
[145] Spencer SJ, Rabinovici J, Jaffe RB. Human recombinant activin-A inhibits proliferation of human fetal adrenal cells in vitro. *J Clin Endocrinol Metab.* 1990;71:1678-1680.
[146] Spencer SJ, Rabinovici J, Mesiano S, Goldsmith PC, Jaffe RB. Activin and inhibin in the human adrenal gland. Regulation and differential effects in fetal and adult cells. *J Clin Invest.* 1992;90:142-149.
[147] Riopel L, Branchaud CL, Goodyer CG, Zweig M, Lipowski L, Adkar V, Lefebvre Y. Effect of placental factors on growth and function of the human fetal adrenal in vitro. *Biol Reprod.* 1989;41:779-789.
[148] Parker CR Jr, Stankovic AK, Harlin C, Carden L. Adrenocorticotropin interferes with transforming growth factor-beta-induced growth inhibition of neocortical cells from the human fetal adrenal gland. *J Clin Endocrinol Metab*. 1992;75:1519-1521.
[149] Stankovic AK, Dion LD, Parker CR Jr. Effects of transforming growth factor-beta on human fetal adrenal steroid production. *Mol Cell Endocrinol.* 1994;99:145-151.
[150] Stankovic AK, Grizzle WE, Stockard CR, Parker CR Jr. Interactions between TGF-beta and adrenocorticotropin in growth regulation of human adrenal fetal zone cells. *Am J Physiol*. 1994;266:E495-E500.
[151] Lebrethon MC, Jaillard C, Naville D *et al*. Regulation of corticotropin and steroidogenic enzyme mRNAs in human fetal adrenal cells by corticotropin, angiotensin-II and transforming growth factor-β1. *Mol Cell Endocrinol*. 1994;106:137-143.
[152] Stankovic, A.K., Parker, C.R. Jr.: Receptor binding of transforming growth factor-beta by human fetal adrenal cells. *Mol. Cell. Endocrinol.* 1995;109:159-165.
[153] Carr BR, Mason JI. The effects of alpha-human atrial natriuretic polypeptide on steroidogenesis by fetal zone cells of the human fetal adrenal gland. *Am J Obstet Gynecol.* 1988;159:1361-1365.
[154] Breault L, Yon L, Montéro M, Contesse V, Delarue C, Fournier A, Lehoux JG, Vaudry H, Gallo-Payet N. Presence of PACAP and PACAP receptors in the human adrenal gland: possible role in fetal development. *Endocr Res*. 1998;24:961-962.
[155] Breault L, Yon L, Montéro M, Chouinard L, Contesse V, Delarue C, Fournier A, Lehoux JG, Vaudry H, Gallo-Payet N. Occurrence and effect of PACAP in the human fetal adrenal gland. *Ann N Y Acad Sci.* 2000;921:429-433.
[156] Yon L, Breault L, Contesse V, Bellancourt G, Delarue C, Fournier A, Lehoux JG, Vaudry H, Gallo-Payet N. Localization, characterization, and second messenger coupling of pituitary adenylate cyclase-activating polypeptide receptors in the fetal human adrenal gland during the second trimester of gestation. *J Clin Endocrinol Metab.* 1998;83:1299-1305.
[157] Coulter CL. Functional biology of the primate fetal adrenal gland: advances in technology provide new insight. *Clin Exp Pharmacol Physiol.* 2004;31:475-484.
[158] Kempná P, Flück CE. Adrenal gland development and defects. Best Pract. *Res Clin Endocrinol Metab*. 2008;22:77-93.
[159] Hall JG, Pallister PD, Clarren SK Beckwith JB, Wiglesworth FW, Fraser FC, Cho S, Benke PJ, Reed SD.: Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus and postaxial polydactyly-a new syndrome? Part I: Clinical, causal, and pathogenetic considerations. *Am J Med Genet.* 1980;7:47-74.
[160] Kang S, Graham Jr. JM, Olney AH *et al*. GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. *Nature Genetics.* 1997;15:266-268.
[161] Phelan JK, McCabe ER. Mutations in NR0B1 (DAX1) and NR5A1 (SF1) responsible for adrenal hypoplasia congenita. *Hum Mutat.* 2001;18:472-487.
[162] Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER *et al*. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature*. 1994;372:635-641.
[163] Guo W, Burris TP and McCabe, E.R.B. Expression of DAX-1, the gene responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism, in the hypothalamicpituitary-adrenal/gonadal axis. *Biochem Mol Med*. 1995;56:8-13.
[164] McCabe ERB. DAX1: Increasing Complexity in the Roles of this Novel Nuclear Receptor. *Mol Cell Endocrinol.* 2007;265-266:179-182.
[165] Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet*. 1999;22:125-126.
[166] Rainey WE, Carr BR, Wang ZN, Parker CR Jr. Gene profiling of human fetal and adult adrenals. *J Endocrinol.* 2001;171:209-215.
[167] Rehman KS, Carr BR, Rainey WE. Profiling the steroidogenic pathway in human fetal and adult adrenals. *J Soc Gynecol Invest.* 2003;10:372-380.
[168] Diczfalusy E. Endocrine functions of the human fetoplacental unit. *Fed Proc* . 1964;23:791-798.
[169] Diczfalusy E. Steroid metabolism in the feto-placental unit. In: Pencile A, Finzi C (eds): The Feto-Placental Unit. 1969;p 65. Amsterdam, Excerpta Medica.
[170] Diczfalusy E. Fetoplacental hormones and human gestation. *Basic Life Sci*. 1974;4(PT. B):385-402.

Folia Histochem Cytobiol. 2010:48(4): 505 (491-506)
10.2478/v10042-010-0062-7

[171] Pasqualini JR, Lowy J, Wiqvist N, Diczfalusy E. Biosynthesis of cortisol from 3β, 17 ,21-trihydroxypregn-5-en-20-one by the intact human foetus at midpregnancy. *Biochim Biophy Acta*. 1968;152:648-650.
[172] Telegdy G, Weeks J W, Wiqvist N, Diczfalusy E. Acetate and cholesterol metabolism in the human foeto-placental unit at midgestation. Steroids synthesized and secreted by the placenta. *Obstetr Gynecol Surv.* 1970;25:652-654.
[173] Rainey WE, Rehman KS, Carr BR. The human fetal adrenal: making adrenal androgens for placental estrogens. *Semin Reprod Med.* 2004;22:327-336.
[174] Neville AM, O'Hare MJ. *The human adrenal cortex: pathology and biology, an integrated approach.* Springer-Verlag. 1982; pp. 354.
[175] Węgrzynowska K. *Śladami Ojca, lekarza i żołnierza*. Wrocław 1992.
[176] Bulzacki K. *Zawsze wierni Tobie Polsko.* Jelenia Góra 1990.
[177] Domosławski Z. *Miesięcznik informacyjny AM we Wrocławiu,* August 2004;10 (90).
[178] Słynni Lwowianie: http://www.lwow.home.pl/lwowianie4.html; (as for 2010.05.31)
[179] Kamiński Ł. *Służba Bezpieczeństwa wobec nauki polskiej. Zarys problematyki.* W: Seminare a studie Vyzkumneho Centra pro Dejiny Vedy z let 2002-2003. Praha, 2003; pp. 445-465.

Submitted: 20 July, 2010
Accepted after reviews: 11 August, 2010