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# Hydroxysteroid Dehydrogenases – Biological Role and Clinical Importance – Review

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<http://dx.doi.org/10.5772/54149>

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## 1. Introduction

Hydroxysteroid dehydrogenases (HSDs) belong to the NADPH/NAD<sup>+</sup>-dependent oxidoreductases, which interconvert ketones and the corresponding secondary alcohols. As their names imply, they catalyze the oxidoreduction in different positions of steroidal substrates (3 $\alpha$ -, 3 $\beta$ -, 11 $\beta$ -, 17 $\beta$ -, 20 $\alpha$ - and 20 $\beta$ -position). The steroid-converting HSDs play central roles in the biosynthesis and inactivation of steroid hormones, but some of them are also involved in the metabolism of diverse non-steroidal compounds [1]. The HSDs are integral parts of systemic (endocrine) and local (intracrine) mechanisms. In target tissues they convert inactive steroid hormones to their corresponding active forms and viceversa, thus modulating the transactivation of steroid hormone receptors or other elements of the non-genomic signal transduction pathways. Therefore, HSDs act as molecular switches allowing pre-receptor modulation of steroid hormone action [2].

It is also well recognized that human and certain other primates are unique among animal species in having adrenals that secrete large amounts of inactive steroid precursors including dehydroepiandrosterone (DHEA). These steroids do not bind to the androgen receptor but exert either estrogenic or androgenic action after their conversion into active estrogens and/or androgens in target tissues [3]. Imbalanced action of sex steroid hormones, i.e. androgens and estrogens, is involved in the pathogenesis of various severe diseases in human. Hormone-dependent cancers are commonly lethal both in women and in men, with breast cancer being the most prevalent cancer in women and prostate cancer in men in several Western countries [4]. In addition, there are various other common hormone-dependent diseases, such as polycystic ovary syndrome (PCOS) and endometriosis, having poorly understood aetiology and lacking efficient pharmacological treatment [5, 6]. However, changes in circulating hormone concentrations do not explain all pathophysiological processes occurred in hormone-dependent tissues. A more inclusive explanation is provided by paracrine and intracrine action of sex steroids, namely the

regulation of intratissue hormone concentrations by expression of steroidogenic enzymes. The modulation of local sex steroid production using pharmaceutical compounds is also a valuable treatment option for developing of novel therapies against hormonal diseases [7]. In the view of successful practice of inhibiting of non-HSD enzymes (aromatase and  $5\alpha$ -reductase) [8, 9], recent attempt are made for development of HSD inhibitors as therapeutic strategy. Several of HSD enzymes are also considered as promising drug targets and inhibitors, for example most of the isoforms of  $17\beta$ -HSD enzyme [10].

In this review, we summarise the data from the literature and our own data on the main HSDs ( $11\beta$ -HSD,  $3\beta$ -HSD  $17\beta$ -HSD) focusing our attention on the localization/tissue distribution and regulation of the enzyme isoforms and their role in normal and pathological processes as revealed by experimental models and clinical observations. The review would provide better understanding on multifunctionality of HSDs and their relevance to the clinic and that would be helpful for scientists and clinicians, working in a new challenging area of development of HSD-inhibitors as new drugs for hormone-related diseases.

## **2. Steroid hormones and role of hydroxysteroid dehydrogenases in steroidogenesis: steroidogenic pathways and general regulatory mechanisms**

Steroid hormones are produced by the gonads, adrenal gland and placenta and they play vital role in physiological and reproductive processes. Structurally, steroids have a basic or common nucleus called the cyclopentanoperhydrophenanthrene, consisting of three, six-membered fully hydrogenated (perhydro) phenanthrene rings designated A, B and C, and one five-membered cyclopentane ring designated D (Fig 1, right top). In 1967, the International Union of Pure and Applied Chemistry (IUPAC) established rules for the number of carbons in a steroid and thus its biological action can be predicted. For instance, 21-carbon steroids have progestogenic or corticoid activity, 19-carbon steroids have androgenic activity and 18-carbon steroids have estrogenic activity. Cholesterol is a 27-carbon steroid that gives rise pregnenolone (21-carbon) after cleavage of its side chain. Pregnenolone is subsequently converted to progesterone, which in turn give rise androgens or corticoids. Androgens are subjected to aromatization of ring A thus giving rise estrogens [11]. The pathways of steroidogenesis differ between species, but the pathways of human steroidogenesis are shown in the Figure 1. [12]. Cholesterol is the precursor of the steroid hormones, providing backbone of the steroid molecule. The enzymes involved in the synthesis of steroid hormones can be divided into two major classes of proteins: the cytochrome P450 heme-containing proteins (CYP) and the hydroxysteroid dehydrogenases (HSD) [13, 14]. These enzymes are primarily expressed in the gonads, adrenal and placenta. Interestingly, some of these enzyme activities have been demonstrated in non-endocrine tissues, where they may be involved in important paracrine and autocrine actions. This is particularly the case in the human fetus where steroid precursors circulates at high levels and could be metabolized within tissues to produce active steroid hormones. The first class of steroidogenic enzymes, CYP proteins called hydroxylases catalyze reaction of

hydroxylation (introduction of hydroxyl group –OH into organic compound) and cleavage of the steroid substrate utilizing molecular oxygen and nicotinamide adenine dinucleotide phosphate (NADPH, reduced) as the source of reductive potential. Several enzymes are included: cytochrome P450 cholesterol side-chain cleavage enzyme (P450<sub>scc</sub>, *CYP11A1*), cytochrome P450 17 $\alpha$ -hydroxylase (P450<sub>c17</sub>, 17 $\alpha$ -hydroxylase, 17-20 lyase, *CYP17A1*), P450 aromatase (aromatase, *CYP19A1*), 21 $\alpha$ -hydroxylase (*CYP21A*), 11 $\beta$ -hydroxylase (*CYP11B1*) and aldosterone synthase (*CYP11B2*). The second class of steroidogenic enzymes, HSD enzymes called alcohol oxydoreductases catalyze the dehydrogenation of hydroxysteroids. Acting as oxydoreductases, HSD enzymes require nicotinamide adenine dinucleotide (NAD, oxidized) and/or NADPH as electron acceptor/donor. HSD enzymes include: 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD), 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) and 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD). While each P450 enzyme is the product of a single gene, the HSD enzymes have several isoforms that are products of distinct genes [15]. There are four types, classified by the number of the carbon acted upon.

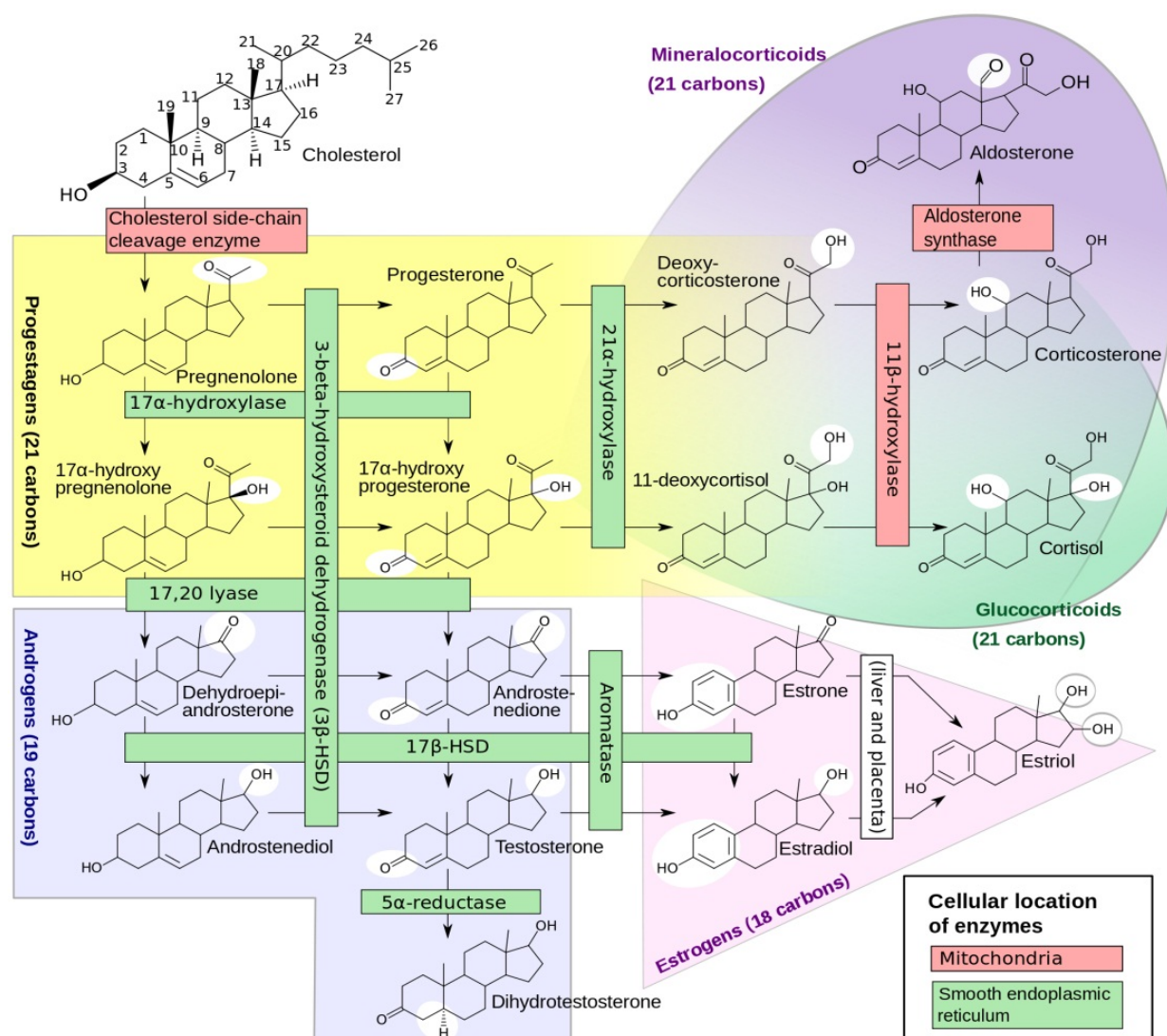
In all species, the first and rate-limiting step in steroidogenesis, in particular androgen biosynthesis, is conversion of the C27 cholesterol to the C21 steroid, pregnenolone (Figure 1). This reaction is catalyzed by cytochrome P450<sub>scc</sub> enzyme located in the inner mitochondrial membrane. Pregnenolone diffuses across the mitochondrial membrane and it is further metabolized by enzymes associated with the smooth endoplasmic reticulum. These enzymes are: 1) cytochrome P450<sub>c17</sub>, which catalyzes the conversion of the C21 steroids pregnenolone or progesterone to the C19 steroids dehydroepiandrosterone or androstenedione, respectively; 2) 3 $\beta$ -HSD ( $\Delta^5$ - $\Delta^4$  isomerase), which catalyzes the conversion of the  $\Delta^5$  hydroxysteroids - pregnenolone or dehydroepiandrosterone to the  $\Delta^4$  ketosteroids - progesterone or androstenedione, respectively; 3) 17 $\beta$ -HSD (17-ketosteroid reductase), which catalyzes the final step in the biosynthesis of testosterone [16].

Corticosteroids (mineralocorticoids and glucocorticoids, C-21 carbons) derive from progestagens (progesterone and 17 $\alpha$ -OH progesterone) after hydroxylation of carbon-21 by the enzyme 21 $\alpha$ -hydroxylase. So, aldosterone and corticosterone share the first part of their biosynthetic pathway. The last part is mediated either by aldosterone synthase (for aldosterone) or by 11 $\beta$ -hydroxylase (for corticosterone). These enzymes are nearly identical (they share 11 $\beta$ -hydroxylation and 18-hydroxylation functions). Aldosterone synthase is also able to perform 18-oxidation. 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) catalyzes the conversion of active cortisol to inert 11 keto-products (cortisone), or vice versa, thus regulating the access of glucocorticoids to the steroid receptors.

The steroidogenic pathways/steroid output are controlled by complex regulatory mechanisms that involved wide range of factors like pituitary trophic hormones, growth factors, cytokines and steroids. The major factors, expressed since early fetal life, are steroidogenic acute regulatory protein (StAR) and Steroidogenic Factor-1 (SF-1). StAR actively transports cholesterol from the outer to the inner mitochondrial membrane and allows *CYP11A* (located in the inner membrane) access to cholesterol [17]. Cell specific expression of StAR and P450 enzymes are regulated by Steroidogenic Factor-1 (SF-1), which binds to promoter region of StAR gene and of all CYP genes, activating their expression [18, 19]. The most compelling

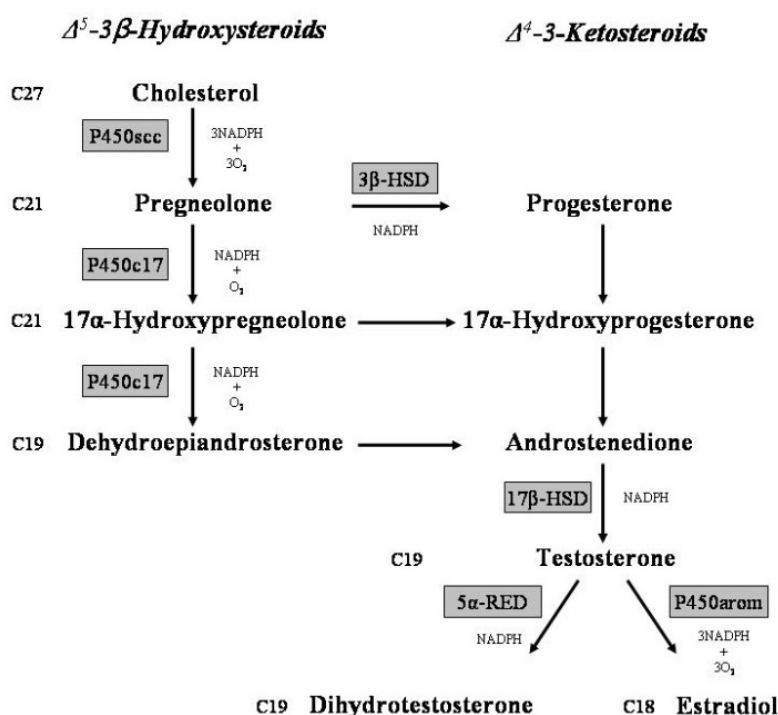
evidence for the essential requirement for StAR in steroidogenesis is provided by StAR-specific knockout mice and human mutations that caused the potentially lethal condition known as congenital lipoid adrenal hyperplasia. It is not surprising that 46XY individuals with mutated SF1 have XY sex reversal, indicative of disrupted fetal testosterone biosynthesis and masculinization. In mice with Leydig cell-specific knockout of SF-1 gene there is lack of *CYP11A* and StAR expression resulting in adrenal and gonadal agenesis [20-23]. The activity of P450<sub>scc</sub> enzyme is regulated by mitochondrial environment [24] and the vital role of this enzyme is demonstrated by homozygous mutation of *CYP11A* gene that is lethal due to inability of placenta to produce progesterone [25]. Consequently, 46XY genetic males with partial inactivation of *CYP11A* exhibit major deficiencies in masculinization [26, 27].

The combined enzymatic actions of 3 $\beta$ -HSD and P450<sub>c17</sub> catalyze the overall conversion of pregnenolone to androstenedione, the precursor of testosterone. This conversion can occur via one of two main pathways, either via  $\Delta 4$  or  $\Delta 5$  pathway and the preferred route is both species- and age-dependent. [14] (Figure 2.).



**Figure 1.** Pathways of human steroidogenesis [12].





**Figure 2.** Steroid biosynthetic pathways as adapted to Payne, 2007 [16]

The  $\Delta^4$  pathway (pregnenolone, progesterone, androstenedione, testosterone) was the first indentified route in rat testis and subsequently shown to be preferred one. In the human and higher primates, as well as in pig and rabbit the  $\Delta^5$  pathway predominates in the adult and fetal testis because human P450c17 enzyme readily converts 17 $\alpha$ -hydroxypregnenolone to dehydroepiandrosterone (DHEA), but has little enzyme activity when 17 $\alpha$ -hydroxyprogesterone is the substrate. In the rat, P450c17 readily cleaves both the  $\Delta^4$  and  $\Delta^5$  C21 steroids, but in contrast to the human, it has a preference for the  $\Delta^4$  pathway. In the mouse the  $\Delta^4$  pathway dominates before puberty but in adult animals the  $\Delta^5$  pathway may also contribute to overall testosterone production. Therefore, differences in preferred pathways between species are likely to depend upon relative substrate affinity of P450c17 enzyme [6, 14].

The clinical importance of P450c17 enzyme is demonstrated by numerous reports on CYP17A gene mutations [28, 29, 30]. Both male and female patients are hypertensive because overproduction of mineralocorticoids as well as impaired production of cortisol. Affected females exhibit abnormal sexual development resulting in primary amenorrhea. Male patients are phenotypic females due to the deficiency of testosterone production.

### 3. 3 $\beta$ -HSD gene family – function, tissues distribution, regulation and clinical importance

The 3 $\beta$ -HSD was described in 1951 and later characterized as bifunctional dimeric enzyme required for the biosynthesis of all classes of steroid hormones (glucocorticoids, mineralocorticoids, progestagens, androgens, and estrogens). Therefore the 3 $\beta$ -HSD controls

the critical steroidogenic reactions in the adrenal cortex, gonads, placenta, and peripheral target tissues [31]. The  $3\beta$ -HSD isoforms catalyze the conversion of the  $\Delta^5$ - $3\beta$ -hydroxysteroids - pregnenolone,  $17\alpha$ -hydroxypregnenolone, and DHEA, to the  $\Delta^4$ -3-ketosteroids - progesterone,  $17\alpha$ -hydroxyprogesterone, and androstenedione, respectively. Two sequential reactions are involved in the conversion of the  $\Delta^5$ - $3\beta$ -hydroxysteroid to a  $\Delta^4$ -3 ketosteroid. The first reaction is the dehydrogenation of the  $3\beta$ -hydroxysteroid, requiring the coenzyme  $\text{NAD}^+$ , yielding the  $\Delta^5$ -3-keto intermediate, and reduced NADH. The reduced NADH, activates the isomerization of the  $\Delta^5$ -3- keto steroid to yield the  $\Delta^4$ -3-ketosteroid (Figure 2.). Stopped-flow spectroscopy studies show that NADH activates the isomerase activity by inducing a time-dependant conformational change in the enzyme [15, 32]. Using histochemical and imunohistochemical techniques  $3\beta$ -HSD activity was detected to the smooth endoplasmic reticulum and mitochondrial cristae and later in the microsomal fraction suggesting that  $3\beta$ -HSD is a membrane-associated enzyme [16]. Submitochondrial fractionation studies showed that  $3\beta$ -HSD is in a functional steroidogenic complex with P450scc located in the inner mitochondrial membrane [33, 34], that provides the enzyme with immediate substrate metabolized from cholesterol. However,  $3\beta$ -HSD activity could be preferentially distributed to the mitochondria under certain physiological conditions [35, 36].

**Isoforms:** Structural studies of  $3\beta$ -HSD family characterized several isoforms, products of distinct genes. The number of isozymes varies in different species. The isoenzymes differ in tissue distribution, catalytic activity (whether they function predominantly as dehydrogenases or reductases), in substrate and cofactor specificity, and in subcellular distribution [6]. So far, two isoforms were reported in human (h)  $3\beta$ -HSD, six in mouse, four in rat and three in hamster. Multiple  $3\beta$ -HSD isoenzymes have been cloned from several other species, further illustrating that the  $3\beta$ -HSD gene family is conserved in vertebrate species. The human type I  $3\beta$ -HSD gene (*HSD3B1*) encodes an enzyme of 372 amino acids predominantly expressed in the placenta and peripheral tissues (skin, mammary gland, prostate, and several other normal and tumor tissues) [37, 38]. In comparison, the type II gene (*HSD3B2*), which encodes a protein of 371 amino acids, shares 93.5% identity with the type I and it is almost exclusively expressed in the adrenals ovaries and testes. It is most homologous to the type I gene expressed in mice, rats and other species [39, 40]. The structure of *hHSD3B1* and *hHSD3B2* genes consists of four exons which are included within a DNA fragment of 7.8 kb and genes are assigned to chromosome 1p13.1 [41].

The rat type I and II  $3\beta$ -HSD proteins are expressed in the adrenals, gonads, kidney, placenta, adipose tissue, and uterus and share 93.8% identity. The type III protein shares 80% identity with the type I and II proteins but, in contrast to other types, it is a specific 3-ketosteroid reductase (KSR) [42, 43]. The type III gene is exclusively expressed in male liver, and there is marked sexual dimorphic expression, which results in pituitary hormone-induced gene repression in the female rat liver [44]. The rat type IV protein shares 90.9%, 87.9%, and 78.8% identity with types I, II, and III proteins, respectively. Furthermore, types I and IV possess a  $17\beta$ -HSD activity specific to  $5\alpha$ -androstane- $17\beta$ -ol steroids, thus suggesting a key role in controlling the bioavailability of the active androgen dihydrotestosterone DHT

[45, 46, 47]. Concerning to an enzyme having dual activity, such secondary activity could be explained by binding of the steroid in the inverted substrate orientation, in this case C-17 rather than C-3 position. [47].

To date, six distinct cDNAs encoding murine members of the  $3\beta$ -HSD family have been cloned and all of them are highly homologous and encode a protein of 372 amino acids. Functionally, the different forms fall into two distinct classes of enzymes -  $3\beta$ -HSD types I, II and III function as dehydrogenase/isomerases, and are essential for the biosynthesis of active steroid hormones whereas  $3\beta$ -HSD type IV and type V (analogous to rat type III) function as 3-KSRs and they are involved in the inactivation of active steroid hormones [48, 49]. In the adult mouse  $3\beta$  HSD I is expressed in gonads and adrenal gland, whereas  $3\beta$ -HSD II and III are expressed in liver and kidney. The type V isoenzyme is expressed only in the liver of the male mouse and the expression starts in late puberty. The type VI isoenzyme is the earliest isoform expressed during the first half of pregnancy in cells of embryonic origin and in uterine tissue suggesting that this isoenzyme may be involved in the local production of progesterone, required for the successful implantation and/or maintenance of pregnancy [50]. In the adult mouse,  $3\beta$ -HSD type VI appears to be the only isoenzyme expressed in skin. The aminoacid sequences among the different isoforms and between mouse and human isoforms show a high degree of identity. Mouse  $3\beta$ -HSD I has 84% identity to mouse VI, and 71% identity to human II [31, 50].

**Tissue distribution:** As  $3\beta$ -HSD gene family is widely expressed within the steroidogenic organs (adrenal, ovary and testis) as well as in peripheral tissues, the distribution and local regulation will be described separately for each organ.

*Adrenal:* The onset of  $3\beta$ -HSD expression in the fetal primate adrenal cortex correlates with the ability of the definitive zone to synthesize aldosterone and also allows cortisol production by transitional zone cells. Although  $3\beta$ -HSD is not expressed to a high degree in the fetal cortex, P450c17 is expressed, thereby directing the steroidogenic pathway toward  $\Delta 5$ -hydroxysteroid (*i.e.*, DHEA) production. There is zone-specific steroid secretion pattern dependent on the relative expression levels of  $3\beta$ -HSD, P450c17 and P450 21 $\alpha$ -hydroxylase (P450c21) that serve as molecular markers of the adrenocortical developmental state [51, 52]. After birth, the coexpression of  $3\beta$ -HSD and P450c21 leads to aldosterone production, whereas the coexpression of  $3\beta$ -HSD and P450c17 results in production of cortisol. The expression of P450c17 along with low levels of  $3\beta$ -HSD expression leads to synthesis of DHEA. The differential expression of the enzymes required for zonal-specific steroid production in the adrenal is under the control of multiple factors as Adrenocorticotrophic hormone (ACTH), Epidermal Growth Factor (EGF), Fibroblast Growth Factors (FGFs), Insulin-like Growth Factors (IGFs), thyroid hormone (T3), Transforming Growth Factor- $\beta$  (TGF $\beta$ ) [31, 53, 54]. Therefore, there appears to be a complex interplay of factors controlling adrenal development, and combinations of these factors could be involved in the regulation of  $3\beta$ -HSD and other steroidogenic enzymes *in vivo*.

*Ovary:* Ontological studies for  $3\beta$ -HSD have shown that fetal human ovaries are steroidogenically quiescent except for a window late in gestation [55], so most of the



estrogens seen by the primate fetus are of placental origin [56].  $3\beta$ -HSD is not expressed in mouse and rat ovary until first week after birth. This is in contrast to testicular expression because androgen production by the male embryo is critical for male sexual development [57]. PCOS is an ovarian disorder associated with hyperthecosis of the ovary and elevated serum LH, insulin, and androgen levels. Several studies provide evidence of aberrant  $3\beta$ -HSD regulation in polycystic thecal cells although the mechanisms are unclear [58].

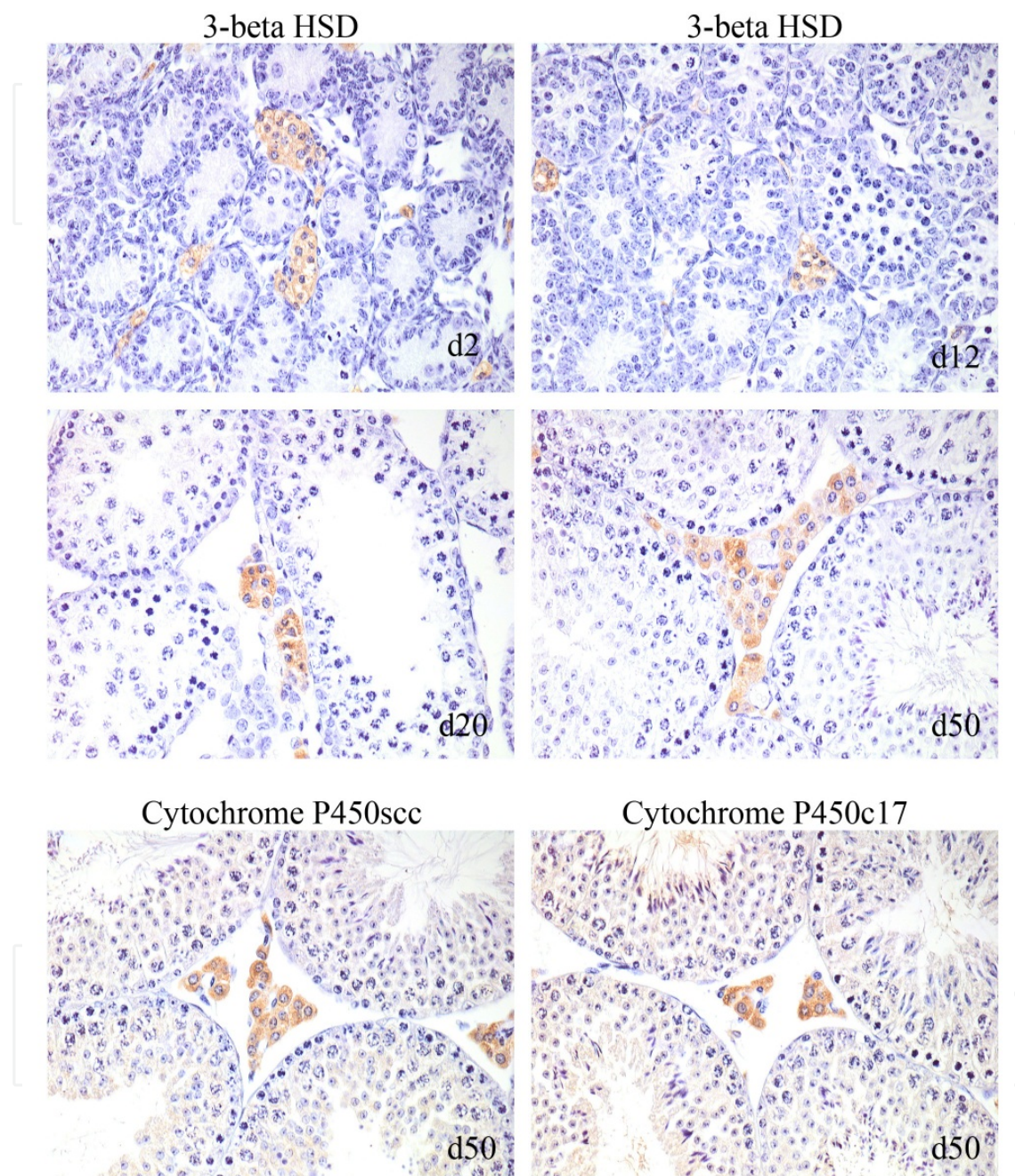
Preantral/antral *follicular* expression studies show  $3\beta$ -HSD mRNA and protein expression in the human ovary initially in the theca and then in the granulosa layer as folliculogenesis continues [59]. In nonprimate species,  $3\beta$ -HSD has been shown to have different expression patterns. In the rat, preantral, antral, and preovulatory rat follicles showed  $3\beta$ -HSD expression in the theca, but no expression was seen in the granulosa layer [60]. In contrast to rodents, pigs, and primates,  $3\beta$ -HSD expression in the cow was seen in all the stages of the preovulatory follicle in both theca and granulosa layers [61]. Pituitary hormones are the primary means of the regulation of the steroidogenesis in the ovary. The gonadotropins, FSH and LH cause an increase in  $3\beta$ -HSD expression concomitantly with other steroidogenic enzymes. The role of prolactin (PRL) on primate  $3\beta$ -HSD is unclear, although PRL was shown to be inhibitory. Interestingly in postmenopausal women 30% of circulating  $\Delta^4$ -DIONE is of ovarian origin [62]. These studies suggest that ovarian steroid production in postmenopausal women continues, but the decline in pituitary control dramatically changes the steroid profile. After ovulation, *Corpus Luteum* (CL) is developed to secrete large amount of progesterone that is controlled in part by the amount of  $3\beta$ -HSD. The enzyme is considered as a marker for progesterone production of the CL [63]. In primates, LH/hCG action through LH receptor provides a primary mean of luteotropic support [64, 65]. In addition, FSH increased  $3\beta$ -HSD protein and mRNA levels in human granulosa-luteal cells, and this effect could be enhanced by insulin [66]. Although the direct control of  $3\beta$ -HSD by PRL in humans has yet to be demonstrated, PRL has been shown to up-regulate  $3\beta$ -HSD transcriptional activity *in vitro* [67]. During regression of CL (luteolysis) the expression of  $3\beta$ -HSD dramatically decreased and there is evidence that LH is mainly involved in induction of luteolysis [68].

*Testis*: Testis is the major place for production of androgens, mainly testosterone although local conversion/reduction of testosterone to dihydrotestosterone (DHT) by  $5\alpha$ -Reductase ( $5\alpha$ -Red) occurred in the following part of reproductive system (epididymis and prostate). Within the testis, the Leydig cells (LC) are primary place for steroidogenesis as they are only cell type in the male that expressed all of the enzymes essential for the conversion of cholesterol to testosterone [16]. During development two distinct population of LCs arise sequentially, namely fetal and adult LC population, being differentially regulated [20]. Immunohistochemical studies have revealed that human Leydig cells express  $3\beta$ -HSD as early as 18 wk of gestation. During gestation in human,  $3\beta$ -HSD expression is an indicator of testicular androgen production. Adult Leydig cells arise postnatally and encompass three developmental stages: progenitor, immature and adult Leydig cells [69]. Rat testes of postnatal day 15 showed  $3\beta$ -HSD localization to the smooth ER in precursor Leydig cells and that points the beginning of differentiation of adult LC population. At this time point

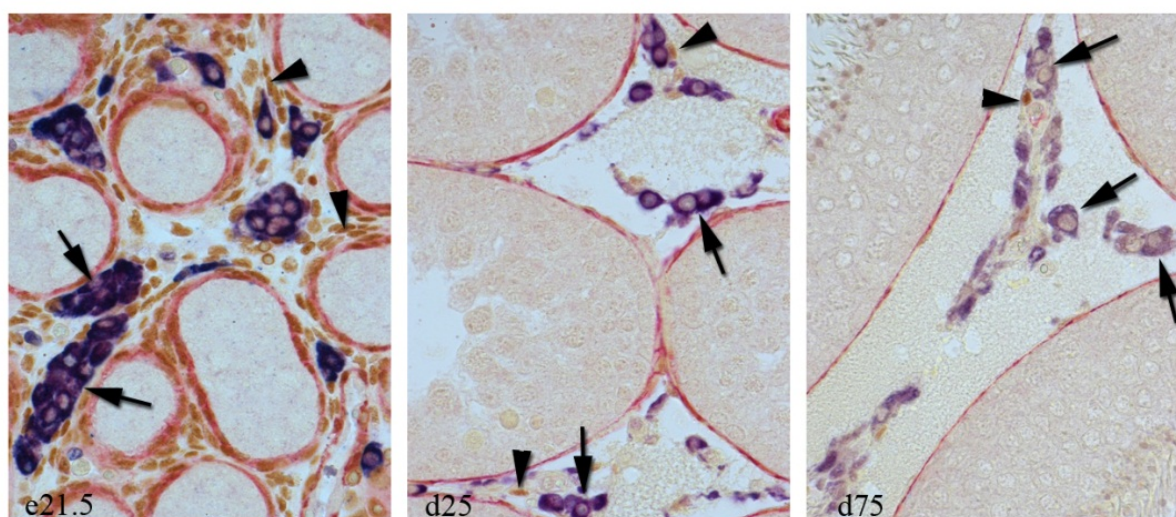
LC expressed P450scc and P450c17, as well. Therefore an antibody against  $3\beta$ -HSD is highly applicable as a marker for visualization both, fetal and adult LC. The expression of  $3\beta$ -HSD protein overlapped with expression of other steroidogenic enzymes, P450scc and P450c17, clearly demonstrated on Figure 3 and that was confirmed by other authors [70]. Development of triple co-localization immunohistochemical technique allows distinguishing of presumptive progenitors cells from adult or fetal LC that is very helpful to study kinetic and differentiation pattern of LCs (Figure 4) [71]. Application of IHC for  $3\beta$ -HSD is widely used by many authors in quantification studies of LC under normal and experimental/pathological conditions, especially those of hormonal manipulations [72].  $3\beta$ -HSD immunohistochemistry is also useful tool for validation of EDS (ethane dimethanesulfonate) model for selective ablation of adult LC and thus testosterone withdrawal. The major regulator of postnatal testicular expression of  $3\beta$ -HSD in rodents and human is the LH, acting via LH receptor located in LCs. That is in contrast to the fetal testis where an independent mechanism is suggested [73]. Steroids and growth factors (EGF, TGF $\beta$ , FGFs, Activin A) are also suggested to control the expression of  $3\beta$ -HSD [31].

*Peripheral tissues;* Expression of  $3\beta$ -HSD in peripheral tissues such breast, prostate, placenta, liver, blain and skin will be briefly described in relation to clinical importance. Sex steroids are well recognized to play a predominant role in the regulation of cell growth and differentiation of normal mammary gland as well as in hormone-sensitive breast carcinomas. Estrogens stimulate cell growth of hormonesensitive breast cancer cells, whereas androgens exert an antiproliferative action in breast cancer cells [74]. Stage II/III infiltrating ductal primary breast tumors demonstrated  $3\beta$ -HSD activity [75], and  $3\beta$ -HSD protein was seen in 36% of breast carcinoma samples tested [76, 77]. The  $3\beta$ -HSD expressed in human placenta is the peripheral isoform, type I  $3\beta$ -HSD, and it is under differential regulatory control than the adrenal/gonadal isoform, type II  $3\beta$ -HSD [78, 79]. In the prostate epithelium  $3\beta$ -HSD expression was colocalized with  $17\beta$ -HSD type V in normal conditions.  $3\beta$ -HSD was found in human hyperplastic prostates suggesting the capacity of the human prostate for local androgen production, that increase the hypertrophic potential of the organ [80, 81]. Hepatic  $3\beta$ -HSD expression is presumed to be important in the metabolism and inactivation of steroids.  $3\beta$ -HSD activity in human liver microsomes was shown to be three times higher for the reduction of DHT to  $3\Delta$ -DIOL than the reverse reaction [82]. The circulating levels of steroids might affect regulation of  $3\beta$ -HSD activity in the liver, principally through altering Growth Hotmone (GH) and PRL levels, and thereby resulting in feedback on steroid degradation [83]. In skin  $3\beta$ -HSD was confined to keratinocytes, co-expressed with  $17\beta$ -HSD. Aberrant expression of these enzymes results in increased scalp DHT levels and possibly acceleration of the balding process in genetically predisposed men and women [84, 85].  $3\beta$ -HSD expression was reported in the central nervous system (CNS) and peripheral neurons demonstrating the importance of steroid hormones for growth maturation and differentiation of nerve tissue. For instance,  $3\beta$ -HSD together with P450scc are expressed in the hippocampus, dentate gyrus, cerebellum, olfactory bulb, and Purkinje cells of the rat brain with highest levels in cerebellum [86] as well as in cultured neuronal cells [87]





**Figure 3.** Immunoexpression of steroideogenic enzymes ( $3\beta$ -HSD, cytochrome P450<sub>scc</sub> and cytochrome P450<sub>c17</sub> in the Leydig cells (DAB-brown) of postnatal mouse testis after birth to sexual maturity (d2-neonatal, d12-prepubertal, d20-pubertal, d50-adult) x400.



**Figure 4.** Triple immunostaining for  $3\beta$ -HSD (blue),  $\alpha$ -smooth muscle actin (red) and COUP TFII (brown) in fetal (embryonal day 21.5) and postnatal (pubertal-d25 and adult-d75) rat testes. Fetal and adult LCs (arrows) are clearly distinguishable from presumptive progenitor cells (arrowheads) x400.

**Regulation:** The regulation of  $3\beta$ -HSD gene family is quite complex process involving multiple signal transduction pathways that are activated by growth factors, steroids and cytokines and they are differentially dependent on ontogeny and tissue distribution. Initial studies investigating the transcriptional regulation of the human *HSD3B2* gene are primarily focused on the trophic hormones, including ACTH in the adrenal cortex, LH/human chorionic gonadotropin (hCG) in theca cells and corpus luteum, as well as LH in testicular Leydig cells. cAMP is well known intracellular mediator of trophic hormone stimulation of  $3\beta$ -HSD expression but mechanisms by which cAMP stimulate transcription of the *HSD3B2* gene are not clear yet. [31].

Gonadal expression of human  $3\beta$ -HSD II and mouse  $3\beta$ -HSD I is dependent on SF-1 as described for the gonadal-specific expression of the P450 steroidogenic enzymes [88]. Studies on mouse *Hsd3b1* promoter identified three potential SF-1 consensus binding sites [89]. The regulation of *HSD3B2* human gene expression involved the transcription factors of Stat family (signal transducers and activators of transcription) [90]. Interestingly, the Stat5 knockout mice displays luteal failure [91]. DAX-1 (dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on X chromosome gene-1) was originally isolated by positional cloning from patients with DAX-mutation exhibiting adrenal congenita hypoplasia associated with hypogonadotropic hypogonadism. The studies examining the effects of DAX-1 overexpression on adrenal cell showed suppression of steroidogenesis associated with inhibition of the expression of StAR, P450<sub>scc</sub>, and  $3\beta$ -HSD [92]. The exact mechanisms by which DAX-1 overexpression affects  $3\beta$ -HSD expression remain unclear. Interestingly, transcription factors belonging to the GATA family are emerging as novel regulators of steroidogenesis. In fetal and adult adrenals and gonads several target genes for GATA protein were identified such as StAR, *CYP11A*, *CYP17A*, *CYP19A*, *HSD17B1*, human *HSD3B1* and *HSD3B2* [93]. Moreover, deregulation of GATA expression and/or activity might be relevant to pathological processes associated with aberrant *HSD3B2*



expression such as adrenal insufficiency, male pseudohermaphroditism and polycystic ovary syndrome (PCOS) [31]. Immune cell populations in the ovary undergo changes during the reproductive cycle and cytokines from these immune cells (Interleukin-4, IL-4) have been shown to affect steroidogenesis, mediated by Stat [94]. Some growth factors like members of the TGF $\beta$  family and nerves growth factor have been shown to regulate *HSD3B2* gene expression [95-97]. There is growing evidence in the literature that steroid hormones modulate type II 3 $\beta$ -HSD expression. For example, glucocorticoids stimulate the expression of 3 $\beta$ -HSD in adrenal cells [98], whereas androgens inhibit 3 $\beta$ -HSD expression in the adrenal cortical cells and in testicular Leydig cells [99, 100]. There are number of questions concerning the mechanisms of steroids and the action of their receptors. In relation to structure-function aspects the question is what is the influence of known steroid agonists and antagonists on the efficacy of activation? What is the effect of other nonsteroid factors, which are known to activate other intracellular signaling pathways on steroid-regulated transcription?

#### *Clinical importance of 3 $\beta$ -HSD genetic deficiency:*

Homozygous mutations in *HSD3B1* are lethal in human due to interruption of pregnancy before the end of the first trimester because 3 $\beta$ -HSD I protein is required for progesterone synthesis in the placenta (as described above for *CYP11A*). Many mutations in the *HSD3B2* gene have been identified and are summarized in a review by Simard et al. 2005 [31]. The classical 3 $\beta$ -HSD deficiency results from mutations in the *HSD3B2* gene (the *HSD3B1* gene in these patients is normal) and it can be divided, depending upon the severity of the salt-wasting (salt-wasting or non-salt-wasting forms). The classical 3 $\beta$ -HSD deficiency is a rare form of congenital adrenal hyperplasia (CAH) accounting for about 1–10% of cases of CAH. The salt-losing forms of CAH are a group of life-threatening diseases that require prompt recognition and treatment. Indeed, the autosomal recessive mutations in the *CYP21*, *CYP17*, *CYP11B1*, and *HSD3B2* genes encoding steroidogenic enzymes can cause CAH, each resulting in different biochemical consequences and clinical features. In these cases the cortisol secretion is impaired resulting in compensatory hypersecretion of ACTH and consequent hyperplasia of the adrenal cortex. However, only deficiencies in 21-hydroxylase (*CYP21*) and 11 $\beta$ -hydroxylase (*CYP11B1*) predominantly result in virilizing disorders. Indeed, in patients with the classical form of these two defects, the most noticeable abnormality in the sexual phenotype is the masculinization of the female fetus due to oversynthesis of adrenal DHEA. Male individuals suffering from classical 3 $\beta$ -HSD deficiency present hypospadias. On the other hand, the complete or partial inhibition of 3 $\beta$ -HSD activity in the adrenals and ovaries was not accompanied by a noticeable alteration in the differentiation of the external genitalia of female patients. The reason for this striking difference in phenotype between the male and female individuals is that the deficiency of 3 $\beta$ -HSD in the fetal testis results in lowering of the T levels below the levels required for the normal development of male external genitalia.

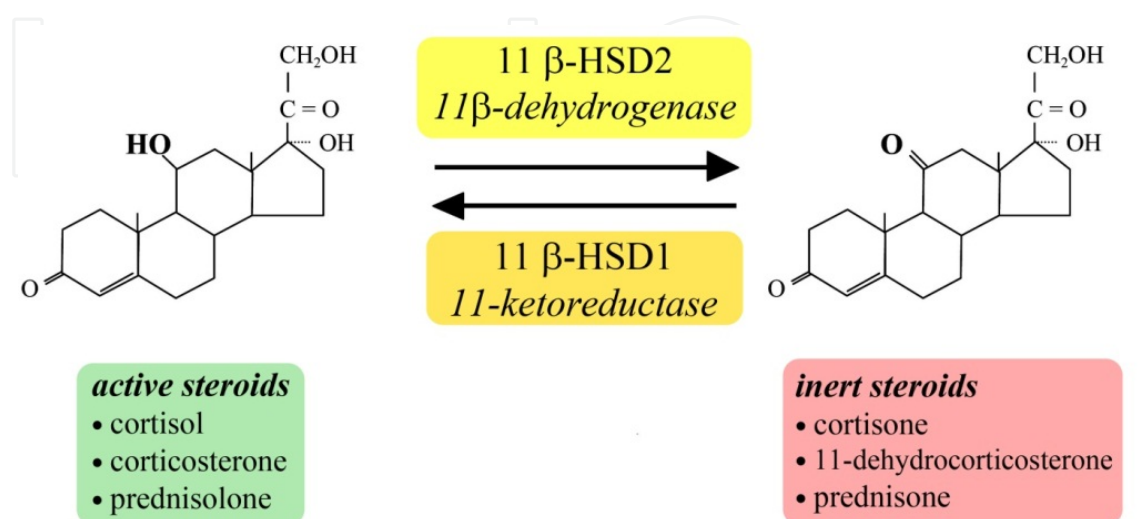
The basal plasma levels of  $\Delta$ 5-3 $\beta$ -hydroxy steroids such as pregnenolone (PREG), 17OH-PREG, and DHEA are elevated in affected individuals. An elevated ratio of  $\Delta$ 5/ $\Delta$ 4-steroids is



considered to be the best biological parameter for the diagnosis of  $3\beta$ -HSD deficiency. The best criteria for the correct diagnosis of this disorder now appears to be a plasma level of 17OH-PREG but 17OH Progesterone (17OH-PROG) also should be measured for correct diagnosis of  $3\beta$ -HSD deficiency. It is well recognized that plasma levels of 17OH-PROG and  $\Delta^4$ -DIONE and other  $\Delta^4$ -steroids are frequently elevated in  $3\beta$ -HSD-deficient patients. Such observations are consistent with a functional type I  $3\beta$ -HSD enzyme that is expressed in peripheral tissues. Moreover, the peripheral type I  $3\beta$ -HSD activity could explain why certain patients were initially misdiagnosed as suffering from 21-hydroxylase deficiency, in view of elevated levels of 17OH-PROG and mild virilization seen in girls at birth. Therefore, measurement of the levels of 17OH-PREG should be performed when an elevated level of 17OH-PROG has been observed in a female neonate without ambiguity of external genitalia or if the patient is a male pseudohermaphrodite [31].

#### 4. $11\beta$ -hydroxysteroid dehydrogenase – biological role in the regulation of glucocorticoid metabolisms and cortisol levels

The glucocorticosteroids exert diverse actions throughout the body and many of them have important implications in the reproduction and metabolite syndrome. It was recognized that within potential target cells, the actions of glucocorticoids are modulated by  $11\beta$ -hydroxysteroid dehydrogenases ( $11\beta$ -HSD) which catalyse the reversible inactivation of cortisol and corticosterone to their inert 11-ketosteroid metabolites, cortisone and 11-dehydrocorticosterone, respectively [101]. The actions of physiological glucocorticoids (cortisol and corticosterone) are modulated by isoforms of the enzyme  $11\beta$ -HSD (Figure 5, [108]). To date, two isoforms of  $11\beta$ -HSD have been identified: 1)  $11\beta$ -HSD1 acts predominantly as an NADP(H)-dependent reductase that converts inactive circulating 11-ketosteroids, into active glucocorticoids generating active cortisol or corticosterone; 2)  $11\beta$ -HSD2 is a high affinity NAD<sup>+</sup>-dependent enzyme that catalyses the inactivation of glucocorticoids [102-107]. Although the biochemistry of  $11\beta$ -HSD is well established, the physiological significance of glucocorticoid metabolism by these enzymes is still not fully



**Figure 5.**  $11\beta$ -hydroxysteroid dehydrogenase ( $11\beta$ -HSD) (Adopted by Seckl et al., 2004 [108])

understood. The enzymatic inactivation of cortisol and corticosterone by  $11\beta$ -HSD enzymes appears to be of central importance for protection of gonadal steroidogenesis, prevention of intra-uterine growth retardation and metabolite syndrome.

This review focuses on the importance of  $11\beta$ -HSD isoenzymes in the developing and aging testis, ovary, adrenal gland, placenta and adipose tissue. The current work aims to provide recent understanding of the biological roles played by  $11\beta$ -HSD in different processes and diseases including reproduction, adrenal gland function, cystic ovarian disease, and the metabolite syndrome. In addition, this review summarizes recent knowledge based on human data and genetic models on the clinical importance of  $11\beta$ -HSD in relation to metabolite syndrome.

## 5. $11\beta$ -hydroxysteroid dehydrogenase in developing testis- marker for differentiation of the Leydig cells

The enzyme  $11\beta$ -hydroxysteroid dehydrogenase ( $11\beta$ -HSD) is hypothesized to modulate LCs steroidogenesis by controlling the intracellular concentration of glucocorticoids. By doing so,  $11\beta$ -HSD can protect the LCs against the suppressive effect of glucocorticoids [109-112]. Glucocorticoids have been found to directly inhibit the transcription of genes encoding the key enzymes of testosterone biosynthesis [113,114]. Excessive glucocorticoid exposure suppress androgen synthesis and thus decrease serum testosterone (T) levels by inducing LC apoptosis and reducing the number of LCs per testis [115,116]. The effects of glucocorticoids on LCs are not only associated with the classic glucocorticoid receptor-mediated mechanism but possibly through the plasma membrane receptor or prereceptor-mediated action by the glucocorticoid metabolizing enzyme  $11\beta$ -HSD1 [117]. Both isoforms of  $11\beta$ -HSD are localized in testicular LCs [118-121]. Recent studies showed that reductase activity predominates in both human and rat type 1  $11\beta$ -HSD [109]. In contrast, the other  $11\beta$ -HSD isoform, type 2, has been found to be exclusively oxidative [118,110,131]. Predominance of oxidative activity results in glucocorticoid inactivation, whereas the reductive activity of the enzyme has an opposite effect [109]. Hu et al. [122] postulated that inhibition of  $11\beta$ -HSD1 in rats *in vivo*, increases intracellular active glucocorticoid concentration and thereby affects serum T concentration and steroidogenic enzyme expression in the LCs. The above mentioned data suggest an important role of  $11\beta$ -HSD1 in modulating intracellular corticosterone concentrations and, in turn, for a direct effect of glucocorticoids on LCs. On the other hand,  $11\beta$ -HSD type 1 mRNA and its activity was decreased corticosterone deficiency, and it seems that LCs need to maintain their intracellular concentration of corticosterone for normal function [123].

Several authors have demonstrated that  $11\beta$ -HSD in LCs is predominantly an oxidase [109-111] and the enzyme has been suggested as a marker for the functional maturity of rat adult LCs [111,112,124,125]. The appearance of  $11\beta$ -HSD correlates with the postnatal increase in testicular weight, LCs number, total surface area of the intracellular membranes and T production by LCs [112]. Neumann *et al.* [126] reported a temporal coincidence of the first appearance of elongated spermatids in the seminiferous epithelium and the first

histochemical demonstration of 11 $\beta$ -HSD in the rat LCs on 35 pnd. The developmental pathway of ALCs population is accompanied with an increase in the 11 $\beta$ -HSD activity and thus the enzyme can be used as a marker for steroidogenic differentiation of LCs [112,124,126,127]. Examination of 11 $\beta$ -HSD in the LCs revealed that both oxidative and reductive activities were barely detectable in the progenitors (PLCs), intermediate in immature type (ILCs), and highest in ALCs. The ratio of the two activities favored reduction in PLCs and ILCs and oxidation in ALCs [109]. Clear recognizable oxidative activity of 11 $\beta$ -HSD is present from 31 pnd onward, first in single ALCs and later in majority of these cells [127]. ALCs population expresses high levels of 11 $\beta$ -HSD oxidative activity [109,125] and enzymatic behavior of 11 $\beta$ -HSD in LCs is not consistent with the presence of type 1 alone [127,128]. Developmental analysis of 11 $\beta$ -HSD in rat LCs revealed that 11 $\beta$ -HSD reductive activity predominated in LCs precursors, whereas in adult LCs, the enzyme was primarily oxidative [118]. This switch, observed in the predominant direction of catalysis of 11 $\beta$ -HSD from reduction to oxidation in adult LCs, may protect this cell type from glucocorticoid-mediated inhibition of steroidogenesis. It was demonstrated that the adult LCs expressed not only 11 $\beta$ -HSD type 1, an oxidoreductase, but also type 2, an unidirectional oxidase [129, 130]. Due to its high affinity for glucocorticoid substrates and exclusively oxidative activity, 11 $\beta$ -HSD type 2 may also play a protective role in blunting the suppressive effects of glucocorticoids on LCs steroidogenesis. The inhibition of 11 $\beta$ -HSD1 predominantly lowered reductase activity whereas by inhibition of 11 $\beta$ -HSD2 alone, the oxidase activity was more prominently suppressed [131]. Recently, it has been reported that products such 7 $\alpha$ -hydroxytestosterone significantly switched 11 $\beta$ -HSD1 oxidoreductase activities toward reductase in developing rat testis and thus regulates the direction of 11 $\beta$ -HSD1 activity in LCs [132]. It seems that the switch of 11 $\beta$ -HSD activity from reduction to oxidation during the transition from PLCs to ALCs [109] can be associated with the presence of 11 $\beta$ -HSD2.

As mentioned above the main function of glucocorticoids in adult LCs is inhibition of T biosynthesis [111]. Glucocorticoids directly regulate T production in LCs through glucocorticoid receptor (GR)-mediated repression of the genes that encode T biosynthetic enzymes [143,109]. The response of LCs to glucocorticoids depends not only on the number of GR and the circulating concentration of glucocorticoids, but also on the ratio of 11 $\beta$ -HSD oxidative and reductive activities [144]. When oxidation predominates over reduction, 11 $\beta$ -HSD decreases the intracellular availability to active glucocorticoid, attenuating GR-mediated responses [118]. In this way, T production is maintained in the presence of normal serum concentrations of corticosterone and it is inhibited only if 11 $\beta$ -HSD oxidative capacity in LCs is reduced.

By using experimental model for treatment with ethane-dimethnesulphonate (EDS) of mature rats our studies provided new data about expression pattern of 11 $\beta$ -HSD during renewal of LCs population [133]. The quantitative immunohistochemical analysis of 11 $\beta$ -HSD2 pattern after EDS treatment revealed progressive increases in the reaction intensity during postnatal development (on d 21 after EDS) and reached a maximum on d35 and that is a turning point in the development from immature to mature LCs [133]. These changes in 11 $\beta$ -HSD2 expression are consistent with previous data about structural and functional

maturation of the new population of LCs after EDS [134,135]. Therefore, 11 $\beta$ -HSD2 can be a useful marker for ALCs differentiation and the reaction intensity might be associated with increased 11 $\beta$ -HSD oxidative activity that occurred during the transition from PLCs to ALCs in postnatal rat testis [109,127]. Moreover, the gene profiling of rat PLCs, immature LCs and ALCs showed increased expression of 11 $\beta$ -HSD2 gene that is in parallel with enhanced 11 $\beta$ -HSD2 enzyme activity during postnatal development [136]. Together with previous studies [126] the data from EDS model suggest the relationship between 11 $\beta$ -HSD and kinetics of spermatid differentiation and restoration of T production by new LCs population.

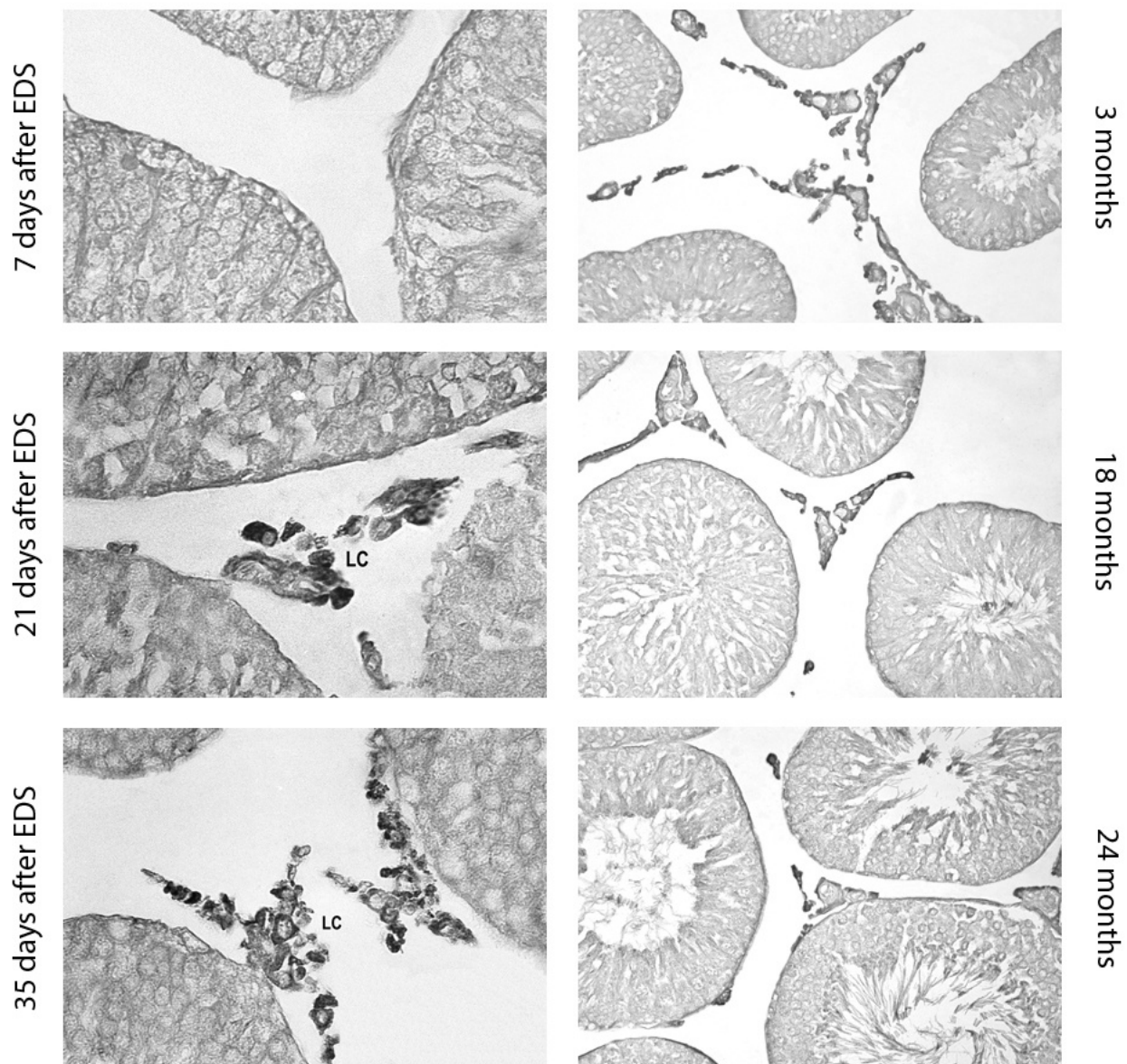
## **6. 11 $\beta$ -hydroxysteroid dehydrogenase in aging testis- role in the response of Leydig cells to the glucocorticoids**

It has been established that circulating levels of testosterone decrease with age in both male rodents and men [137]. It was demonstrated by analyzing cohorts of healthy men and rodents that the decline in androgen levels result from specific age-related changes in the male reproductive system and not secondarily from increased disease frequency associated with the aging process, [138,139]. Data indicated that the hypothalamic-pituitary axis in the aging individuals is still intact [140]. Indeed, it is unlikely that the deficiency in the hypothalamic-pituitary axis are primarily responsible for age-related changes in steroidogenesis. The reduced ability of aging LCs to produce T might be caused by events occurring outside these cells that impinge upon them or by events that occur within LCs themselves [141]. It seems that functional changes in LCs themselves rather than their loss cause reduced steroidogenesis during aging [142].

Our data demonstrated that aging affects T production not only through the direct suppression of 3 $\beta$ -HSD, a key marker for LCs steroidogenic activity but also through the inhibition of 11 $\beta$ -HSD type 2 and insulin-like 3 (INSL3) factor that are involved in functional maturation of the adult LCs [146]. These data suggest that increasing functional hypogonadism in aging male rats is likely caused by dedifferentiation of the LCs themselves. Our findings for reduced 11 $\beta$ -HSD type 2 expression in aging LCs provide new evidence for the functional properties of this enzyme in rat testis and bring an additional elucidation of the intracellular mechanisms underlying the decrease in T production accompanying aging. Significant diminished expression of 11 $\beta$ -HSD type 2 in LCs with aging implies suppression in 11 $\beta$ -HSD oxidative capacity resulting in elevated inhibitory potency of corticosterone on T production [136]. The reduced expression of 11 $\beta$ -HSD type 2 in aging rat LCs is also suggestive for decline in LCs protection ability as opposed to adverse effect of glucocorticoids on T production [146]. Inhibition of 11 $\beta$ -HSD 2 oxidative activity by treatment with 11 $\beta$ -HSD 2 antisense oligomer results in excess of glucocorticoids due to lowering the rate of their inactivation [136]. On the other hand, the elevated levels of corticosterone caused decline in oxidative activity of 11 $\beta$ -HSD leading to impaired LCs steroidogenesis [147]. Therefore, the reduction of 11 $\beta$ -HSD type 2 oxidase occurred during LC aging [146] appears to be a key event that leads to down-stream deficits in the response of LCs to prevent glucocorticoid-mediated suppression of steroidogenesis. (Figure.6)



### 11 beta - HSD type 2 in developing and aging Leydig cells



**Figure 6.** 11 $\beta$ -HSD type 2 in developing Leydig cells (LC)- 7, 21 and 35 days after EDS; and aging Leydig cells- 3, 18 and 24-months of age. x 400.

### 7. 11 $\beta$ -hydroxysteroid dehydrogenase in the adrenal gland - expression profile under conditions of testosterone withdrawal

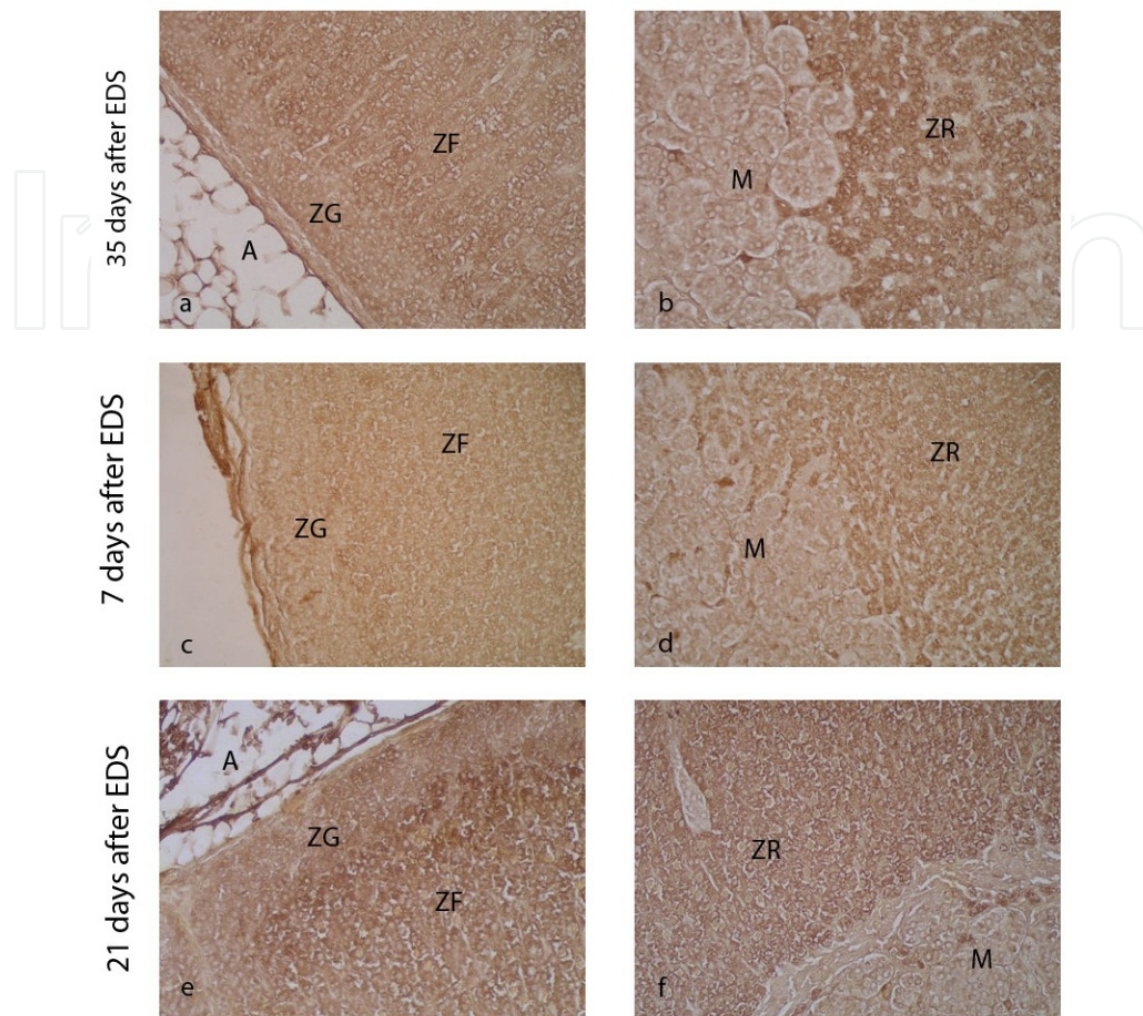
As mentioned above, the enzyme 11 $\beta$ -HSD catalyzes the interconversion of glucocorticoids to inert metabolites in man and rodents and plays a crucial role in regulating the action of corticosteroids. Inhibition of 11 $\beta$ -HSD allows access of cortisol or corticosterone to the mineralocorticoid receptors where they act as mineralocorticoids [148]. Northern blot analyses revealed expression of mRNAs encoding both 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 in the whole rat



adrenal gland. *In situ* hybridization of rat adrenal cortex and medulla demonstrated specific localization of 11 $\beta$  HSD1 mRNA predominantly to the cells at the corticomedullary junction, within the inner cortex, suggesting that the oxoreductase enzyme may serve to maintain high medullary glucocorticoid concentrations required for catecholamine biosynthesis. In contrast, 11 $\beta$ -HSD2 mRNA was more uniformly distributed in the cortex and was low/absent in the medulla [149, 150]. The expression of 11 $\beta$ -HSD2 has been demonstrated in rat adrenal gland by immunohistochemical and molecular analyses and the 11 $\beta$ -HSD2 antigen was confined to the zona fasciculata and zona reticularis, but not in the zona glomerulosa or medulla [149-151]. The ubiquitous presence of 11 $\beta$ -HSD2 in sodium-transporting epithelia revealed that mineralcorticosteroid action is facilitated by this enzyme which metabolizes glucocorticoids and allows aldosterone to bind to the nonselective mineralcorticoid receptor [151].

Using EDS experimental model in adult rats [152] we found that the dynamic of 11 $\beta$ -HSD2 expression correlated with the changes of serum T levels following the exposure after EDS [153]. The lowest 11 $\beta$ -HSD2 staining intensity was found 7 days after EDS followed by progressive increase in the immunoreactivity on day 14 and 21 after EDS [152]. Moreover, the restoration of 11 $\beta$ -HSD2 activity on day 14 after EDS corresponded with unchanged glandular and serum corticosterone levels in treated rats on day 15 reported by Plecas et al. [154]. Enzymatic assays on tissue homogenates showed extensive conversion of corticosterone to its 11 $\beta$ -dehydro product in an NAD<sup>+</sup>-dependent manner in adrenal gland [151]. Using enzyme histochemistry a strong reduction was found in the activity of NADH<sub>2</sub>-cytochrome-C-reductase that is involved in NAD<sup>+</sup>-synthesis as a cofactor in the adrenal gland after EDS treatment of adult rats [155]. Immunohistochemical analysis revealed that the 11 $\beta$ -HSD2 expression pattern in adrenal gland of EDS treated rats [152] is very similar to the enzyme histochemical profile of NADH<sub>2</sub>- cytochrome-C-reductase [155], supporting the view that 11 $\beta$ -HSD2 acts as high-affinity NAD<sup>+</sup>-dependent dehydrogenase in the rat adrenal gland [151]. On the other hand, the increase in the expression of 11 $\beta$ -HSD2 in rat adrenal gland on day 14 after EDS treatment [152] coincided with the appearance of the repopulation of testosterone-producing Leydig cells in the testis [135]. These data suggested a possible role of the gonadal steroids, especially of testosterone, as modulators of the adrenal gland functional activity and they are consistent with previously reported results related to the direct impact of testosterone on the key steps in the adrenal gland steroidogenesis [156]. The above mentioned findings characterized 11 $\beta$ -HSD2 (high-affinity NAD<sup>+</sup>- dependent unidirectional dehydrogenase) as a potential target of testosterone action in rat adrenal cortex. Our data from EDS experimental model provided new evidence for expression of 11 $\beta$ -HSD2 in the adrenal gland under conditions of testosterone withdrawal. The EDS results bring additional elucidation on the functional significance of 11 $\beta$ -HSD system in rat adrenal gland and the regulatory role of testosterone in its activity [152]. Together with our previous studies [135,153], these data suggested the relationship between 11 $\beta$ -HSD2 expression in adrenal gland and kinetics of restoration of testosterone production during renewal of testicular adult LCs population after EDS treatment. (Figure 7)

## 11 beta HSD type 2 immunoreactivity in rat adrenal gland zones



**Figure 7.** 11 $\beta$ -HSD2 immunoreactivity in rat adrenal gland zones. 35 days after EDS (a, b); 7 days after EDS (c, d); 21 days after EDS (e, f). 11 $\beta$ -HSD2- immunoreactivity in the zona fasciculata (ZF) and zona reticularis (ZR), and the adipocytes of adrenal capsula adipose (A). Less sensitive were the adrenocorticoocytes of zona glomerulosa (ZG). No positive signals in the medulla (M). x 200.

## 8. 11 $\beta$ –hydroxysteroid dehydrogenase in the ovary – cellular localization/distribution and relation to Polycystic Ovaries Syndrome and obesity in women

Glucocorticoids exert their effects in all parts of the body and they are involved in a number of physiological processes, including female reproduction. The ovary is also affected by the glucocorticoids and it is well known that the reproductive function may be impaired in cases of adrenal hyperactivity. The ovaries express glucocorticoid receptors and one of the prominent glucocorticoids affecting ovarian function is the cortisol [157]. Ovaries lack the necessary enzymes for cortisol synthesis and cortisol is not produced *de novo* [158] but it was delivered by the circulation. The 11 $\beta$ -HSD enzymes play a crucial role in controlling the

tissue concentration of cortisol. The two types of 11 $\beta$ -HSD (1 and 2) with opposite action modifies cortisol exposure by interconversion between active and inactive glucocorticoids [159,160].

In the human ovary expression of 11 $\beta$ -HSD types 1 and 2 is well documented. 11 $\beta$ -HSD type 2 expression is most prominent during the luteal phase in the corpus luteum and in non-luteinized granulosa cells from follicles before the mid-cycle surge of gonadotrophins. In contrast 11 $\beta$ -HSD type 1 is only seen in granulosa cells from preovulatory follicles [161]). As a result, developmentally regulated pattern of 11 $\beta$ -HSD types 1 and 2 promotes high levels of cortisol during the mid-cycle surge of gonadotrophins, immediately prior to ovulation, whereas reduced levels are maintained throughout the rest of the menstrual cycle [162,163]. Therefore the high levels of local free cortisol are suggested to act as anti-inflammatory agent that limited the tissue damage occurring in connection with follicular rupture [163,164]. This considerations suggest that the regulation of concentration of biologically active cortisol in the ovary may be an important physiological mechanism by which glucocorticoids affect female reproductive organs.

The polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder among premenopausal women. The symptoms include the consequences of excessive androgen production (hyperandrogenemia), anovulation and infertility. The hallmark of PCOS is follicular maturation arrest and hyperandrogenemia that is believed to be a critical component of the syndrome [165, 66]. Studies regarding the pathophysiology of PCOS focus attention to primary defects in the hypothalamic–pituitary axis, ovarian function, insulin secretion and action but none of these hypotheses can fully elucidate the multiple clinical phenotypes of PCOS [167-169]. Insulin resistance and the associated compensatory hyperinsulinemia and centripetal obesity, perhaps reflect an association and linkage of the insulin gene with PCOS [170]. PCOS is of unknown etiology, but several lines of evidence suggest that there is an underlying genetic cause for PCOS. Ovarian androgen production occurs primarily in the theca cells and examination of the metabolism of radiolabeled steroid hormone precursors and steady-state levels of mRNAs, encoding steroidogenic enzymes, revealed that there are multiple alterations in the steroidogenic machinery of PCOS theca cells [171-173]. These observations are consistent with the notion that dysregulation of androgen biosynthesis is intrinsic property of PCOS theca cells and that PCOS may develop as a consequence of a primary genetic abnormality in ovarian androgen production [174]. Elevated adrenal androgen levels are common in PCOS, but the underlying pathogenetic mechanisms are poorly understood. One proposed contributing mechanism is altered cortisol metabolism. Moreover, PCOS and obesity are independently associated with increased expression of 11 $\beta$ -HSD1 mRNA in subcutaneous abdominal tissue from lean and obese women with and without PCOS. Decreased peripheral insulin sensitivity and central obesity were associated with increased expression of 11 $\beta$ -HSD1 but not of 11 $\beta$ -HSD2 mRNA expression [175]. Previous studies have described an increased 5 $\alpha$ -reduction of cortisol and impaired regeneration of cortisol from cortisone by 11 $\beta$ -HSD1 in PCOS, supporting the concept of an altered cortisol metabolism in PCOS [176].



In the rare syndrome of cortisone reductase deficiency, impaired ability of 11 $\beta$ -HSD1 to convert cortisone to cortisol, results in compensatory activation of ACTH secretion and adrenal hyperandrogenism [177,178]. This syndrome has been associated with the polymorphisms in the *HSD11B1* gene, which encodes 11 $\beta$ -HSD1, and female patients affected by cortisone reductase deficiency exhibited hyperandrogenism and a phenotype resembling PCOS [179,180]. Lower ratios of cortisol/cortisone metabolites in urine in patients with PCOS were found compared to controls, suggesting a reduced 11 $\beta$ -HSD1 activity [179]. Gambineri et al., [180] reported that polymorphism, predicting lower peripheral regeneration of cortisol by 11 $\beta$ -HSD1, is related to PCOS status and it is associated with increased adrenal hyperandrogenism in lean PCOS. These data strongly support a role for the *HSD11B1* gene in the pathogenesis of PCOS. According to Gambineri et al. [180], the association of the *HSD11B1* genotype with PCOS was mainly attributable to lean rather than obese PCOS patients, suggesting that in obese PCOS women adrenal hyperandrogenism must have a different pathogenetic mechanism as hyperinsulinemia [181] or increased cortisol clearance [182]. The above mentioned findings differ from studies by San Milla'n et al. [183] and White [184] where no association between *HSD11B1* genotype and PCOS was found. This fact suggests that *HSD11B1* polymorphisms may be relevant only in some subgroups of patients and that the pathogenesis of PCOS is different among the different phenotypes of the syndrome [180]. Recently, the functional consequences in these polymorphisms in *HSD11B1* gene were examined and the results confirm previous reports that the variant in *HSD11B1* confer increased 11 $\beta$ -HSD1 expression and activity, that are associated with the metabolic syndrome [183, 185] but are not associated with the prevalence of PCOS [186]. These findings are confirmed by study by Mlinar et al. [187], reporting that PCOS is not associated with increased *HSD11B1* expression. The elevated expression of this gene correlates with markers of adiposity and predicts insulin resistance and an unfavorable metabolic profile, independently of PCOS.

## 9. 11 $\beta$ –hydroxysteroid dehydrogenase in adipose tissue – relation to obesity and metabolic syndrome

The metabolic syndrome describes a cluster of risk factors like insulin resistance, type 2 diabetes, dyslipidemia, hypertension [188] and co-occurrence of visceral (abdominal, central) obesity. There are strong morphological and metabolic similarities between the Cushing's syndrome of endogenous or exogenous glucocorticoid excess and the metabolic syndrome [189]. Glucocorticoid excess exerts opposing effects on adipose tissue, with an increase in central fat deposition through stimulation of preadipocyte differentiation, gluconeogenesis and triglyceride synthesis, while peripheral fat is reduced as a result from increased lipolysis and lipoprotein lipase downregulation [108]. Glucocorticoid-induced obesity has been investigated in animal models and in humans. It has been shown that cortisol levels are modestly elevated in patients with the metabolic syndrome and tend to be normal or even reduced in simple obesity [189].

The preponderance of data suggest that the intracellular glucocorticoid reactivation was elevated in adipose tissue of obese rodent models and humans [108, 190]. The enzyme that

mediates this activation, locally within tissues, is 11 $\beta$ -HSD1 that converts inactive metabolite cortisone to active cortisol, thereby amplifying local glucocorticoid action [104]. 11 $\beta$ -HSD1 expression in adipose tissue was first reported by Monder and White [144] and it is thought to be a dehydrogenase. Studies in leptin-resistant obese rats revealed that obesity was associated with an increase in 11 $\beta$ -HSD1 in abdominal adipose tissue [191]. In human subcutaneous abdominal adipose tissue, 11 $\beta$ -HSD1 activity is increased both *in vivo* and *in vitro* and the enhanced 11 $\beta$ -HSD1 activity in biopsies is accompanied by elevated 11 $\beta$ -HSD1 mRNA levels [108]). It is interesting to note, that increased subcutaneous adipose 11 $\beta$ -HSD1 is associated with insulin resistance in obesity, but it is not linked specifically with visceral fat accumulation or hypertension [192]. The mechanisms underlying the increase in adipose 11 $\beta$ -HSD1 activity in obesity and metabolic syndrome are still not fully understood. 11 $\beta$ -HSD1 transcription is regulated by many factors like cytokines, sex steroids, growth hormone, insulin and induced weight loss [193-195].

The key question is whether increased 11 $\beta$ -HSD1 in adipose tissue is a cause or a consequence of obesity and it is associated with metabolic syndrome. In order to determine this, mice over-expressing 11 $\beta$ -HSD1 selectively in adipose tissue have been generated, using the adipocyte fatty acid binding protein (aP2) promoter [196, 197]. The adipose-selective 11 $\beta$ -HSD1 transgenic mice exhibited elevated intra-adipose, but not systemic corticosterone levels, as well as the major features of the metabolic syndrome-abdominal obesity, hyperglycaemia, insulin resistance, dyslipidaemia and hypertension. Conversely, transgenic mice with overexpression of 11 $\beta$ -HSD1 in liver showed an attenuated metabolic syndrome with modest insulin resistance and hypertriglyceridemia, hypertension and fatty liver, but with normal body weight [198]. 11 $\beta$ -HSD1-knock-out mice fed on a high-fat diet are protected from obesity and metabolic complications [199-201]. Recently, polymorphisms in *HSD11B1*, the gene encoding 11 $\beta$ -HSD1, have been associated with components of the metabolic syndrome [186, 202-205]. Moreover, subjects with single nucleotide polymorphisms (SNPs) in *HSD11B1* gene exhibit increased adipose 11 $\beta$ -HSD1 expression and increased whole-body 11 $\beta$ -HSD1 activity, associated with increased prevalence of the metabolic syndrome. These findings strengthen the view that variations in 11 $\beta$ -HSD1 activity influence the metabolic profile and provide a new evidence that *HSD11B1* gene influence enzyme activity *in vivo* [186].

## 10. 11 $\beta$ -HSD and metabolite syndrome - clinical importance

Based on human data and genetic models, 11 $\beta$ -HSD1 seems to be cause and promising pharmaceutical target for the treatment of metabolic disease. In mice, the increased enzyme activity in adipose tissue enhances local glucocorticoid levels and produces a metabolic syndrome [196], whereas the decreased enzyme activity protects against obesity and the metabolic syndrome [200, 201]. In human, 11 $\beta$ -HSD1 expression is elevated in adipose tissue in obesity [206], whereas inhibition of 11 $\beta$ -HSD1 enhances insulin sensitivity and provides a new approach to treat type 2 diabetes [207-209]. Polymorphisms in the *HSD11B1* gene that encodes 11 $\beta$ -HSD1 have been associated with type 2 diabetes [203] and hypertension [204, 205]. On the other hand, a polymorphism that predicts 11 $\beta$ -HSD1 deficiency may protect



against obesity and its metabolic consequences because of impaired regeneration of cortisol in adipose tissue [180].  $11\beta$ -HSD1 inhibition is a tempting target for treatment of the metabolic syndrome and its complications. Selective  $11\beta$ -HSD1 inhibitors in rodents cause weight loss, improve insulin sensitivity and delay progression of cardiovascular disease [210-212]. Pharmacological inhibition of  $11\beta$ -HSD1 with the anti-ulcer drug carbenoxolone has provided evidence that cortisol regeneration influences insulin sensitivity, particularly glycogen turnover in healthy human subjects and in patients with type 2 diabetes [207, 208]. This corroborated the notion that the enzyme may be an attractive option to treat the metabolic disease [108, 190, 202, 212, 213]. Moreover,  $11\beta$ -HSD1 gene knock-out ( $11\beta$ -HSD1<sup>-/-</sup>) mice exhibited cardioprotective phenotype with improved glucose tolerance and lipid profile, reduced weight and visceral fat accumulation in condition of chronic high-fat feeding [190, 200, 201, 214]. These data support the beneficial effects of  $11\beta$ -HSD1 inhibitors to lower intracellular glucocorticoid levels and to treat both obesity and its metabolic complications.

### **11. $11\beta$ –hydroxysteroid dehydrogenase and pregnancy – role of $11\beta$ -HSD type 2 as a protective barrier for fetus to overexposure to glucocorticoids; implication in intrauterine growth retardation**

In mammals, glucocorticoids are important for fetal growth, tissue development and maturation of various organs (surfactant production by the fetal lung, gut enzymes activation and development of the brain and liver). However, supraphysiological levels of glucocorticoids have been shown to cause fetal growth retardation in mammalian models and in human. A number of studies in animal models have examined the effects of prenatal exposure to synthetic glucocorticoids on the fetal development and offspring biology. Maternal glucocorticosteroid treatment reduces birth weight of the offspring and adults exhibit hypertension, hyperinsulinemia, increased hypothalamic–pituitary–adrenal (HPA) axis activity and altered affective behavior [215, 216]). Moreover, human intrauterine growth retardation is associated with high maternal and fetal concentrations of glucocorticoids [217]. Normally, fetal physiological glucocorticoid levels are much lower than maternal levels [218]. The physiological fetoplacental barrier to glucocorticoid exposure is placental  $11\beta$ -HSD2 that catalyses the rapid conversion of active cortisol and corticosterone to physiologically inert cortisone and corticosterone [219].  $11\beta$ -HSD2 acts as a protective barrier to glucocorticoids but a small proportion of maternal glucocorticoid passes through the placenta [220] thus, maternal stress elevates fetal glucocorticoid levels [221]. Different factors are involved in the regulation of placental  $11\beta$ -HSD2 expression - progesterone, estrogen, hypoxia, infection and proinflammatory cytokines reduce placental  $11\beta$ -HSD2 activity. Conversely, placental  $11\beta$ -HSD2 activity is stimulated by glucocorticoids, retinoids and leptin [221]. Studies in rats and human indicate that the deficiency in placental  $11\beta$ -HSD2 activity results in high fetal exposure to maternal glucocorticoids, with subsequent effects on fetal development and birth weight and offspring biology - high plasma cortisol levels, permanent hypertension, hyperglycemia and increased HPA axis activity was present through the adult life [222-224]. Moreover, individuals homozygous for deleterious mutations

of *HSD11B2* gene encoding 11 $\beta$ -HSD have low birth weight. Intrauterine growth retardation in human is associated with increased fetal cortisol levels and reduced placental 11 $\beta$ -HSD2 activity [217]. Studies on prenatal exposure to 11 $\beta$ -HSD inhibitors such as glycyrrhetic acid and carbenoxolone have indicated that these agents cause fetal growth retardation and adult offspring changes that are very similar to those that are caused by prenatal exposure to glucocorticoids such as dexamethasone (readily crosses the placenta) [221]). Mice that are homozygous for disrupted alleles of *HSD11B2* (i.e. 11 $\beta$ -HSD2 $^{-/-}$  mice) also have lower birth weight and the offspring display anxiety-related behaviors in adulthood. It seems that the conditions of increased fetal glucocorticoid levels, in response to different maternal restrictions, sometimes have persistent effects in the offspring - so-called concept of developmental physiological programming and that placental 11 $\beta$ -HSD2 is a key player in fetal programming [215, 216, 221].

## 12. 17 $\beta$ -HSD dehydrogenase and multifunctional isoforms: localization, function and relevance to clinical therapeutic strategies

17 $\beta$ Hydroxysteroid dehydrogenases (17 $\beta$ -HSDs, 17HSD/KSRs) are NAD(H)- and/or NADP(H)-dependent enzymes that catalyze the oxidation and reduction of active 17 $\beta$ -hydroxy- and low active/inactive 17-ketosteroids, respectively. In the presence of substantial excess of a suitable cofactor and/or in the absence of a preferred cofactor, 17HSD/ KSRs can be compelled to catalyze both oxidative and reductive reactions. Depending on their reductive or oxidative activities, they modulate the intracellular concentration of inactive and active steroids. Acting as oxidoreductases at the 17-position of the steroid, they play a key role in estrogen/androgen steroid metabolism by catalyzing the final steps of steroid biosynthesis. Both estrogens and androgens have the highest affinity for their receptors in the 17 $\beta$ -hydroxy form and hence, 17HSD/KSR enzymes regulate the biological activity of the sex hormones. 17KSR activities are essential for estradiol and testosterone biosynthesis in the gonads, but they are also present in certain extragonadal tissues and can convert low-activity precursors to their more potent forms in peripheral tissues. Instead, 17HSD activities tend to decrease the potency of estrogens and androgens and consequently may protect tissues from excessive hormone action [10, 225].

Up to now, 14 different subtypes have been identified in mammals and they differ in tissue distribution, sub-cellular localization, function and catalytic preference (oxidation or reduction using the cofactor NAD(H) and NADP(H), respectively) (Table 1). In fact, 17 $\beta$ -HSDs have diverse substrate specificities in vivo as they also catalyze the conversions of other substrates than steroids as for example lipids or retinoids. Until recently, besides 17 $\beta$ -HSD3 and 17 $\beta$ -HSD14, 17 $\beta$ -HSD1 and 2 were thought to be exclusively converting sex steroids. However, the participation of the two latter enzymes (17 $\beta$ -HSD1 and 2) in retinoic acid metabolism recently was suggested. Other 17 $\beta$ -HSD types were already known to be multifunctional and some of them play important roles in different metabolic pathways.

17 $\beta$ -HSD7 is mainly involved in cholesterol synthesis, 17 $\beta$ -HSD4 is implicated in  $\beta$ -oxidation of fatty acids, 17 $\beta$ -HSD5 participates in both prostaglandin and steroid

metabolism, and 17 $\beta$ -HSD12 is required in fatty acid elongation. 17 $\beta$ -HSD10 catalyzes the oxidation of short chain fatty acids. 17 $\beta$ -HSD6 and 9 play a role in retinoid conversion. For some 17 $\beta$ -HSDs, the physiological function is not yet clear. For several types of 17 $\beta$ -HSDs participation in the pathophysiology of human diseases has been postulated [225]. The specificity of each 17 $\beta$ -HSD subtype for a preferred substrate together with distinct tissue localization, suggests that these proteins are promising therapeutic targets for diseases like breast cancer, endometriosis, osteoporosis, and prostate cancer. For some of them, their

Type	Gene	Function	Disease-associations	References
1	HSD17B1	Steroid (estrogen) synthesis	Breast and prostate cancer, endometriosis	[226, 227]
2	HSD17B2	Steroid (estrogen, androgen, progestin) inactivation	Breast and prostate cancer, endometriosis Abnormal eye development	[10,226, 227]
3	HSD17B3	Steroid (androgen) synthesis	Pseudohermaphroditism in males associated with obesity, prostate cancer	[10,228]
4	HSD17B4	Fatty acid $\beta$ -oxidation, steroid (estrogen, androgen) inactivation	D-specific bifunctional protein-deficiency, prostate cancer	[229]
5	HSD17B5	Steroid (androgen, estrogen, prostaglandin) synthesis	Breast and prostate cancer	[230,231]
6	HSD17B6	Retinoid metabolism, 3 $\alpha$ -3 $\beta$ -epimerase, steroid (androgen) inactivation?		[232]
7	HSD17B7	Cholesterol biosynthesis, steroid(estrogen) synthesis	Breast cancer	[233, 234]
8	HSD17B8	Fatty acid elongation, steroid inactivation, estrogens, androgens	Polycystic kidney disease	[235, 236]
9	HSD17B9	Retinoid metabolism		[237]
10	HSD17B10	Isoleucine, fatty acid, bile acid metabolism, steroid (estrogen, androgen) inactivation	X-linked mental retardation MHB deficiency Alzheimer's disease	[238] [239]
11	HSD17B11	Steroid (estrogen, androgen) inactivation, lipid metabolism?		[240]
12	HSD17B12	Fatty acid elongation, steroid(estrogen) synthesis		[241, 242]
13	HSD17B13	Not demonstrated		[243]
14	HSD17B14	Steroid (estrogen, androgen?) inactivation, fatty acid metabolism	Breast cancer, prognostic marker	[244, 245]

**Table 1.** Human 17 $\beta$ -Hydroxysteroid dehydrogenases

expression level can be used as prognostic marker in breast or prostate cancer. The selective inhibition of the concerned enzymes might provide an effective treatment and a good alternative for treatment of steroid dependent diseases [246]. Having in mind multifunctionality 17 $\beta$ -HSD enzymes, the biological and clinical aspects of each isoform will be described separately.

**17 $\beta$ -HSD type1:** 17 $\beta$ -HSD1 catalyzes the activation of estrone (E1) to the most potent estrogen estradiol (E2), predominantly considered as an enzyme of estradiol biosynthesis. It is abundantly expressed in granulosa cells of developing follicles and variable amounts of the enzyme are also expressed in human breast epithelial cells. The enzyme is known to have a crucial role in the development of estrogen-dependent diseases. Based on the in vitro studies, human (h) 17 $\beta$ -HSD1 has been considered as highly estrogen specific, with markedly lower catalytic efficacy towards androgenic substrates. There is a clear difference in the substrate specificity between human and rodent 17 $\beta$ -HSD1 enzymes; the catalytic efficacy of rodent enzyme in vitro is similar for both androgens and estrogens. According a recent review by Saloniemi et al. [10], the h17 $\beta$ -HSD1 is not fully estrogen-specific but it possesses significant androgenic activity. The enzyme catalyses both oxidative (17-hydroxy to 17-keto) and reductive (17-keto to 17-hydroxy) 17 $\beta$ -HSD activity with a proper cofactor added in vitro. However, in cultured cells, the h17 $\beta$ -HSD1 has been shown to catalyse predominantly the reductive reaction [247]. Although h17 $\beta$ -HSD1 expression in various peripheral tissues is low, its catalytic efficacy is markedly higher than those measured for 17 $\beta$ -HSD7 and 17 $\beta$ -HSD12 [248, 242], suggesting an important role for 17 $\beta$ -HSD1 in peripheral E2 formation. Data from animal models further demonstrated the ability of h17 $\beta$ -HSD1 to enhance estrogen action in target tissues and its decrease after treating the mice with 17 $\beta$ -HSD1 inhibitors [10]. These data suggest that 17 $\beta$ -HSD1 plays a major role in determining the gradient between the E2 concentrations in serum and peripheral tissues. An increased E2/E1 ratio by the 17 $\beta$ -HSD1 point out the pivotal role of 17 $\beta$ -HSD1 in breast cancer, ovarian tumor, endometriosis, endometrial hyperplasia and uterine leiomyoma [249, 250]. Consequently, inhibition of 17 $\beta$ -HSD1 is considered as a valuable therapeutic approach for treatment of these diseases. In vivo evaluation of 17 $\beta$ -HSD1 inhibitors is complicated by the fact that the rodent enzymes only show moderate homology/identity to the human one. Due to these species differences, there is a high probability that inhibitors optimized for activity toward rodent 17 $\beta$ -HSD1 do not inhibit the human enzyme. In addition, rodents and humans vary considerably in enzyme distribution in the different tissues. Attempts to overcome these problems include xenograft models using nude mice.

Recently generated mouse genetic model for overexpression of 17 $\beta$ -HSD1 (HSD17B1-TG mice) by Saloniemi et al [10] provided valuable data about common female reproductive disorders like Polycystic Ovarian Syndrome (PCOS), ovarian carcinogenesis and endometriosis. Overexpression of hHSD17B1 leads to increased androgen exposure during embryonic development that caused androgen-dependent phenotypic alterations in female, such as increased anogenital distance, lack of vaginal opening and combination of vagina with urethra. These alterations observed in the HSD17B1-TG females were effectively rescued by prenatal anti-androgen (flutamide) treatment, further confirming the

dependence of these phenotypes on androgens. Interestingly, the androgen exposure during pregnancy in the HSD17B1-TG mice resulted in benign ovarian serous cystadenomas in adulthood. As ovarian serous borderline tumours are positively associated with a history of PCOS, thus with a history of (foetal) hyperandrogenism, 17 $\beta$ -HSD1 may promote ovarian carcinogenesis via increased estrogen concentration, but also via enhanced androgen production. Endometrial hyperplasia in HSD17B1-TG mice closely resembled human disease and it was efficiently reversed by 17 $\beta$ -HSD1 inhibitor treatment. The data concerning the expression of 17 $\beta$ -HSD1 in normal and diseased human endometrium are not fully conclusive. However, in most of the studies, the 17 $\beta$ -HSD1 expression is detected in normal endometrium, endometriosis specimens and endometriotic cancer. Other 17 $\beta$ -HSD enzymes including 17 $\beta$ -HSD2, 17 $\beta$ -HSD5, 17 $\beta$ -HSD7 and 17 $\beta$ -HSD12 have also been detected in the endometrium under different pathological conditions like endometriosis and PCOS [10]. Collectively, the data suggest that 17 $\beta$ -HSD1 inhibition is one of the several possible approaches to reduce estrogen production both in eutopic and in ectopic endometrial tissue.

**17 $\beta$ -HSD type-2:** 17-HSD/KSR2 converts 17 $\beta$ -hydroxy forms of estrogens and androgens (estradiol, testosterone and 5 $\alpha$ -dihydrotestosterone) to their less active 17-keto forms (estrone, androstenedione and 5 $\alpha$ -androstenedione). The enzyme also possesses 20 $\alpha$ -HSD activity, thereby activating 20 $\alpha$ -hydroxyprogesterone to progesterone. The 17 $\beta$ -HSD2 enzyme is widely and abundantly expressed in both adult and fetal tissues such as placenta, uterus, liver, the gastrointestinal and urinary tracts. Due to its expression pattern and enzymatic characteristics, it has been suggested that the 17 $\beta$ -HSD2 enzyme protects tissues from excessive steroid action [251]. 17 $\beta$ -HSD2 is localised in the endoplasmic reticulum, and it is widely expressed in various estrogen and androgen target tissues both in human and in rodents including breast endometrium, placenta and prostate. Furthermore, the 17 $\beta$ -HSD2 expression in the placenta and in foetal liver and intestine, together with the observed oxidative 17 $\beta$ -HSD2 activity, are the basis for the hypothesis, suggesting a role for the enzyme in lowering the sex steroid exposure of the foetus.

Phylogenetic analyses have indicated that 17 $\beta$ -HSD2 is a close homologue of retinoid-converting enzymes and has a high sequence similarity to retinol dehydrogenase type 1. In addition, studies have shown that retinoic acid (RA) induces expression of 17 $\beta$ -HSD2 in a dose- and time-dependent manner in human endometrial epithelial and placental cells [10]. Recent data from transgenic mice (HADS17B2-TG) provide evidence for importance of 17 $\beta$ -HSD2 for prenatal eye morphogenesis and eye development [10]. These TG mice overexpressing human 17 $\beta$ -HSD2 showed growth retardation, disrupted spermatogenesis, female masculinization, delayed eye opening, squint appearance of the eyes and some of these defects closely resembled those identified in retinoid receptor mutant mice. The most notable changes in the HSD17B1TG mice are well explained by alterations in sex steroid action, whereas in the HSD17B2-TG mice the connection to sex steroids is weaker. The opposite mouse model of deficiency of 17 $\beta$ -HSD2 provide evidence for the essential role of 17 $\beta$ -HSD2. Embryonic death in the HSD17B-KO mice is reported, related to lack of action of 17 $\beta$ -HSD2 enzyme in placenta. Furthermore, the treatment of pregnant female mice with an



anti-estrogen or with progesterone did not prevent the foetal loss of the HSD17B2-KO mice, thus indicating that embryonic deaths is likely not due to the lack of progesterone or due to an increased action of estrogens.

Osteoporosis is well known to occurs in elderly people when the level of active sex steroids decreases. Estrogen replacement therapy is beneficial for the treatment of osteoporosis but it is no longer recommended because of adverse effects (breast, endometrial and ovarian cancers, stroke, thromboembolism). Since  $17\beta$ -HSD2 oxidizes E2 into E1, decreasing the amount of E2 in bone cells, inhibition of this enzyme is a promising approach for the treatment of this disease [225]. Ovariectomized cynomolgus monkeys were used as an osteoporosis model to evaluate the efficacy of  $17\beta$ -HSD2 inhibitors. Decrease in bone resorption and maintenance of bone formation was achieved in this experimental model.

**$17\beta$ -HSD type-3:**  $17\beta$ -HSD/KSR3  $17\beta$ -HSD3 converts  $\Delta 4$ -androstenedione into testosterone and it is essential for testosterone biosynthesis. The enzyme is present exclusively in the testis and the deficiency of the active enzyme results in male pseudohermaphroditism [252]. In addition to the conversion of androstenedione to testosterone, the enzyme is capable of catalyzing conversion of  $5\alpha$ -androstenedione to  $5\alpha$ - dihydrotestosterone as well as estrone to estradiol [108]. Messenger RNA for  $17\beta$ -HSD3 are over-expressed in prostate cancer tissues. As T is known to be responsible for cell proliferation in androgen dependent diseases,  $17\beta$ -HSD3 inhibitors (exerting effects equivalent of chemical castration) could be therapeutics for the treatment of such diseases [225]. Day et al. [253] developed the first xenograft model in castrated mice to evaluate  $17\beta$ -HSD3 inhibitors and strong suppression of tumor growth by 81% was found, suggesting that  $17\beta$ -HSD3 inhibition might be an efficient strategy for the treatment of hormone dependent prostate cancer.

There are only few observations in human male deficient in  $17\beta$ -HSD as rare mutation associated with 46XY disorder of sexual development [254]. Patients with  $17\beta$ -HSD deficiency are usually classified as female at birth (although abdominal testes) but developed secondary male features at puberty with diminished virilization [255].

**$17\beta$ -HSD type-4:** Among  $17\beta$ -HSD/KSRs, type 4 is an unique multifunctional enzyme consisting of  $17\beta$ -HSD/KSR-, hydratase- and sterol carrier 2-like domains.  $17\beta$ -HSD4 is ubiquitously expressed, but in some tissues it shows cell-specific expression. In the brain it is present only in Purkinje cells, in the lung only in bronchial epithelium and in the uterus in luminal and glandular epithelium. The deficiency of  $17\beta$ -HSD4 leads to disease known as Zellweger syndrome [251].

**$17\beta$ -HSD type-5:**  $17\beta$ -HSD/KSR5 is also known as type 2  $3\alpha$ -HSD, and differently from other  $17\beta$ -HSD/KSRs it belongs to the AKR (aldo-keto reductase) family. With other members of the AKR family (type 1  $3\alpha$ -HSD, type 3  $3\alpha$ -HSD and  $20\alpha$ -HSD),  $17\beta$ -HSD5 shares 84%, 86% and 88% identity, respectively. Both human and mouse  $17\beta$ -HSD5 catalyze the conversion of androstenedione to testosterone, and additionally possess  $3\alpha$ -HSD activity. Human  $17\beta$ -HSD5 has been previously identified predominantly as  $3\alpha$ -HSD. Human, but not mouse,  $17\beta$ -HSD5 also converts progesterone to  $20\alpha$ -dihydroprogesterone effectively.  $17\beta$ -HSD5 appears to be involved in the formation of androgens in the testis and several peripheral

tissues. Using specific probes and antibodies, human 17 $\beta$ -HSD5 has been localized in liver, adrenal, testis, basal cells of the prostate, and in prostatic carcinoma cell lines [251]. Recently, up-regulation of 17 $\beta$ -HSD5 was found in breast and prostate cancer [256].

17 $\beta$ -HSD type-6: 17-HSD/KSR6 is part of the catabolic cascade of 5 $\alpha$ -dihydrotestosterone (DHT). The 17 $\beta$ -HSD6 shows low dehydrogenase activity with DHT, testosterone and estradiol and possesses a weak oxidative 3 $\alpha$ -HSD activity. The 17 $\beta$ -HSD6 enzyme shares 65% sequence identity with retinol dehydrogenase type 1 and it is most abundantly expressed in liver and prostate, at least in rodent tissues [251].

17 $\beta$ -HSD type-7: 17 $\beta$ -HSD7 is expressed in the developing follicles and in luteinized cells, being the enzyme of ovarian estradiol biosynthesis. Both rodent and human 17 $\beta$ -HSD7 catalyze exclusively the conversion of estrone to estradiol. The 17 $\beta$ -HSD7 is abundantly expressed in corpus luteum during pregnancy and the enzyme is considered to be important in E2 production, especially during pregnancy. In addition, 17 $\beta$ -HSD7 mRNA has been detected in placental, mammary gland and kidney samples [251]. The 17 $\beta$ -HSD7 enzyme was first characterised as a prolactin receptor-associated protein in the rat corpus luteum, although its role in prolactin signalling has remained unknown.

A role for mouse 17 $\beta$ -HSD7 in cholesterol biosynthesis was also suggested by the studies, showing a similar expression pattern of 17 $\beta$ -HSD7 and cholesterologenic enzymes during mouse embryonic development. Data from HSD17B7-KO mouse embryos evidently showed the essential role of 17 $\beta$ -HSD7 for cholesterol biosynthesis *in vivo*. The lack of 17 $\beta$ -HSD7 resulted in a marked blockage in foetal *de novo* cholesterol synthesis. Histological analysis revealed that the 17 $\beta$ -HSD7 deficiency results in defects in the development of nerve system, vasculature, heart, associated with defect in cholesterol synthesis. HSD17B-KO deficient mice exhibit embryonic lethal phenotypes. These data suggest a possible role of 17 $\beta$ -HSD7 in cholesterol biosynthesis in mice, while its role in E2 production *in vivo* needs further clarification [10].

17 $\beta$ -HSD type-8: The *Ke 6* gene product has been characterized as a protein whose abnormal regulation is linked to the development of recessive polycystic kidney disease in mice and later it was discovered to be a 17 $\beta$ HSD8. In *in vitro* conditions, 17 $\beta$ -HSD8 converts most efficiently estradiol to estrone and, to some extent, it also catalyses oxidative reactions of androgens and the reduction from estrone to estradiol. The 17 $\beta$ -HSD8 is abundant in kidney, liver and gonads. Interestingly, in the ovary, 17 $\beta$ -HSD8 is present in cumulus cells and not in granulosa or luteal cells like 17 $\beta$ HSD1 and 7, respectively [251].

17 $\beta$ -HSD type-10: The 17 $\beta$ -HSD10 has a very broad substrate profile. Interestingly, it has been proposed that this enzyme plays an important role in the pathological processes of Alzheimer's disease (AD), mainly because 17 $\beta$ -HSD10 binds to amyloid- $\beta$  peptide and appears to be up-regulated in patients suffering from this disease [225]. The mechanism by which 17 $\beta$ -HSD10 contributes to the pathology of AD is still not completely understood. The protein-protein interaction of 17 $\beta$ -HSD10 with amyloid- $\beta$  appears to inhibit the enzymatic activity of 17 $\beta$ -HSD10. *In vitro* studies with a potent 17 $\beta$ -HSD10 inhibitor [257] have shown that inhibition of this enzyme can prevent its interaction with the amyloid- $\beta$  peptide,

suggesting  $17\beta$  HSD10 as a potential target for the treatment of AD. Transgenic mice over-expressing human  $17\beta$ -HSD10 suggesting that inhibition of  $17\beta$ -HSD10 could protect from cerebral infarction and ischemia [258].

**$17\beta$ -HSD type-12:** The mammalian  $17\beta$ -HSD12 was initially characterised as a 3-ketoacyl-CoA reductase, involved in the long-chain fatty acid synthesis, particularly essential for brain arachidonic acid synthesis. Both the human and the mouse  $17\beta$ -HSD12 share 40% sequence similarity with  $17\beta$ -HSD3, and the data indicate that  $17\beta$ -HSD12 is an ancestor of  $17\beta$ -HSD3. In human and rodents,  $17\beta$ -HSD12 is expressed universally and the highest expression of  $17\beta$ -HSD12 is detected in tissues involved in the lipid metabolism, including the liver, kidney, heart, and skeletal muscle. In mice, the expression has also been detected in brown and white adipose tissue.  $17\beta$ -HSD12 expression is also regulated by sterol regulatory element binding proteins, identically to that shown to be involved in fatty acid and cholesterol biosynthesis. Interestingly, a reduced expression of  $17\beta$ -HSD12 in cultured breast cancer cells results in significant inhibition of cell proliferation that is fully recovered by supplementation of arachidonic acid. In addition to its putative role in fatty acid synthesis, human  $17\beta$ -HSD12 has been shown to catalyse the conversion of E1 to E2 in cultured cells, and the enzyme was suggested to be a major enzyme converting E1 to E2 in postmenopausal women [10]. Analysis of the HSD17B12-KO embryos indicated that the embryos initiated gastrulation but further organogenesis was severely disrupted. The mutant embryos exhibited severe defects in the neuronal development (ectoderm-derived), they failed to grow several mesoderm-derived structures. Therefore, the embryos at the age of E8.5–E9.5 were devoid of all normal embryonic structures that caused their death.

### 13. Conclusion

HSD enzymes are broadly expressed in all steroidogenic organs as different isoforms with differential localization and function. HSD are key enzymes involved in growth and reproduction and they are considered as suitable targets to modulate the concentration of the potent steroids in case of steroid-dependent diseases. As they could act selectively in an intracrine manner, inhibitors of these enzymes might be superior to the existing endocrine therapies regarding the off-target effects. Although common mechanisms operate in regulation of steroidogenesis, there are some differences/specificities between rodent and human, in particular the susceptibility of fetal testicular steroidogenesis to environmental chemicals with estrogenic/antiandrogenic activity. As the latter appeared to be devoid of effect on fetal human testis, this should be taken into account when dealing with risk assessment of endocrine disruptors for human reproductive health. Species specific differences in steroidogenesis cause real obstacles in investigation of HSD inhibitors. Some of the most active and selective inhibitors were investigated *in vivo* in animal disease-oriented models. They showed efficacy, but none of them reached the clinical trial stage. One reason for this might be the difficulty to identify an appropriate species to conduct the functional assays, as very potent inhibitors of the human enzyme show little activity toward HSD of other species (rodents). In this respect, experiments by using xenograft approach (human tissue xenografting in immunocompromised nude mice) would enable us to develop our

studies for better understanding of regulatory mechanisms of the expression of HSD enzymes. Elucidation of molecular events involved in transcription control of HSD is of great importance for molecular design of new HSD inhibitors and development of new strategies for appropriate treatment of steroid-dependent diseases without use of invasive techniques.

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## Acknowledgement

The authors thank to Professor Richard Sharpe for providing samples from experimental models for hormonal manipulations, Chris McKinnel for technical expertise in immunohistochemistry. We are also grateful to Professor Michail Davidoff and Assoc. Professor Mariana Bakalska for studies on EDS experimental model. Authors' work was supported in part by Grant DEER # 212844 funded by FP7-ENV-CP and Grant # DO 02/113 funded by NF "Scientific Research" of Ministry of Education Youth and Science in Bulgaria.

## 14. References

- [1] Hoffmann F, Maser E. Carbonyl reductases and pluripotent hydroxysteroid dehydrogenases of the short-chain dehydrogenase/reductase superfamily, *Drug Metabolism Reviews* 2007; 39: 87–144.
- [2] Duax WL, Ghosh D, Pletnev V. Steroid dehydrogenase structures, mechanism of action, and disease. *Vitamines & Hormones* 2000; 58: 121–148.
- [3] Labrie F, Luu-The V, Labrie C, Simard J. DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. *Frontiers in Neuroendocrinol* 2001; 22: 185–212.
- [4] Cancer Research: <http://info.cancerresearchuk.org/cancerstats/>
- [5] Giudice L. Clinical practice. Endometriosis. *New England Journal of Medicine* 2010; 362: 2389–2398.
- [6] Goodarzi MO, Dumesic DA, Chazenbalk G & Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nature Reviews Endocrinology* 2010; 7: 219–331.
- [7] Labrie F. Drug insight: breast cancer prevention and tissue-targeted hormone replacement therapy. *Nature Clinical Practice in Endocrinology & Metabolism* 2007; 3: 584–593.



- [8] Schuster D, Laggner C, Steindl TM, Paluszczak A, Hartmann RW, Langer T. Pharmacophore modeling and in silico screening for new P450 19 (aromatase) inhibitors, *Journal of Chemical Information & Modeling* 2006; 46: 1301–1311.
- [9] Aggarwal S, Thareja S, Verma A, Bhardwaj TR, Kumar K, An overview on 5 $\alpha$ -reductase inhibitors. *Steroids* 2010; 75: 109–153.
- [10] Saloniemi T, Jokela H, Strauss L, Pakarinen P and Poutanen M. The diversity of sex steroid action: novel functions of hydroxysteroid (17 $\beta$ ) dehydrogenases as revealed by genetically modified mouse models (Thematic Review). *Journal of Endocrinology* 2012; 212, 27–40.
- [11] Hafez ESE. Hormones, Growth Factors, and Reproduction. In: Hafez ESE.(ed.) *Reproduction in Farm Animals*. Philadelphia: Lea & Febiger; 1993. p59-93.
- [12] Barrett E. Section VIII The Endocrine System. In: Boron WF, Boulpaep EL (eds) *Medical Physiology. A Cellular And Molecular Approach*. Philadelphia, PA: Elsevier/Saunders; 2003 (1<sup>st</sup> edition) p1009-1110.
- [13] Pezzi V, Mathis JM, Rainey WE, Carr BG. Profiling transcript levels for steroidogenic enzymes in fetal tissues. *Journal of Steroid Biochemistry and Molecular Biology* 2003; 87: 181-189.
- [14] Scott HM, Mason JI, Sharpe RM. Steroidogenesis in the fetal Testis and its Susceptibility to Disruption by Exogenous Compounds. *Endocrine Review* 2009; 30: 883-925.
- [15] Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocrine Review* 2004; 25: 947-970.
- [16] Payne AH. Steroidogenic Enzymes in Leydig Cells. In: Payne AH. & Hardy MP. (eds) *The Leydig Cell in Health and Disease*. Totowa, NJ: Human Press Inc; 2007, p157-171.
- [17] Arakane F, Kallen CB, Watari H, Foster JA, Sepuri NB, Pain D, Staybrook SE, Lewis M, Gerton GL, Strauss 3rd JF. The mechanism of action of steroidogenic acute regulatory protein (StAR). StAR acts on the outside of mitochondria to stimulate steroidogenesis. *J Biological Chemistry* 1998; 273: 16339–16345.
- [18] Dube' C, Bergeron F, Vaillant MJ, Robert NM, Brousseau C, Tremblay JJ. The nuclear receptors SF1 and LRH1 are expressed in endometrial cancer cells and regulate steroidogenic gene transcription by cooperating with AP-1 factors. *Cancer Letters* 2009; 275: 127–138.
- [19] Parker KL, Schimmer BP. Transcriptional regulation of the genes encoding the cytochrome P-450 steroid hydroxylases. *Vitamins and Hormones* 1995; 51: p339–370.
- [20] O'Shaughnessy PJ, Johnston H, Baker PJ. Development of Leydig Cell Steroidogenesis. In: Payne AH. & Hardy MP. (eds) *The Leydig Cell in Health and Disease*. Totowa, NJ: Human Press Inc; 2007, p173-179.
- [21] Achermann JC, Ozisik G, Ito M, Orun UA, Harmanci K, Gurakan B, Jameson JL. Gonadal determination and adrenal development are regulated by the orphan nuclear receptor steroidogenic factor-1, in a dose-dependent manner. *Journal of Clinical Endocrinology and Metabolism* 2002; 87: 1829–1833.
- [22] Jeyasuria P, Ikeda Y, Jamin SP, Zhao L, De Rooij DG, Themmen AP, Behringer RR, Parker KL. Cell-specific knockout of steroidogenic factor 1 reveals its essential roles in gonadal function. *Molecular Endocrinology* 2004;1: 1610–1619.

- [23] Stocco DM. The Role of StAR in Leydig Cell Steroidogenesis. In: Payne AH. & Hardy MP. (eds) *The Leydig Cell in Health and Disease*. Totowa, NJ: Human Press Inc; 2007, p149-155
- [24] Black SM, Harikrishna JA, Szklarz GD, Miller WL. The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450<sub>scc</sub>. *Proceedings of the National Academy of Science USA* 1994; 91: 7247–7251.
- [25] Miller WL. Why nobody has P450<sub>scc</sub>(20,22 desmolase deficiency). *Journal of Clinical Endocrinology and Metabolism* 1998;83: 1399–1400.
- [26] Kim CJ, Lin L, Huang N, Quigley CA, AvRuskin TW, Achermann JC, Miller WL. Severe combined adrenal and gonadal deficiency caused by novel mutations in the cholesterol side chain cleavage enzyme, P450<sub>scc</sub>. *Journal of Clinical Endocrinology and Metabolism* 2008; 93: 696-702.
- [27] Pang S, Yang X, Wang M, Tissot R, Nino M, Manaligod J, Bullock LP, Mason JI. Inherited congenital adrenal hyperplasia in the rabbit: absent cholesterol side-chain cleavage cytochrome P450 gene expression. *Endocrinology* 1992;131, 181–186.
- [28] Auchus RJ. The genetics, pathophysiology, and management of human deficiencies of P450<sub>c17</sub>. *Endocrinology, Metabolism Clinics of North America* 2001; 30, 101–119.
- [29] Van Den Akker EL, Koper JW, Boehmer AL, Themmen AP, Verhoef-Post M, Timmerman MA, Otten BJ, Drop SL, De Jong FH. Differential inhibition of 17  $\alpha$ -hydroxylase and 17,20-lyase activities by three novel missense CYP17 mutations identified in patients with P450<sub>c17</sub> deficiency. *Journal of Clinical Endocrinology & Metabolism* 2002; 87: 5714–5721.
- [30] Martin RM, Lin CJ, Costa EM, de Oliveira ML, Carrilho A, Villar H, Longui CA, Mendonca BB. P450<sub>c17</sub> deficiency in Brazilian patients: biochemical diagnosis through progesterone levels confirmed by CYP17 genotyping. *Journal of Clinical Endocrinology & Metabolism* 2003; 88: 5739–5746.
- [31] Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the 3 $\beta$ -hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. *Endocrine Review* 2005; 26: 525-582.
- [32] mas JL, Duax WL, Addlagatta A, Brandt S, Fuller RR, Norris W. Structure/function relationships responsible for coenzyme specificity and the isomerase activity of human type-1 3 $\beta$ -hydroxysteroid dehydrogenase/isomerase. *Journal of Biological Chemistry* 2003; 278: 483–490.
- [33] Cherradi N, Defaye G, Chambaz EM. Dual subcellular localization of the 3 $\beta$ -hydroxysteroid dehydrogenase isomerase: characterization of the mitochondrial enzyme in the bovine adrenal cortex. *Journal Steroid Biochemistry & Molecular Biology* 1993; 46, 773–779.
- [34] Cherradi N, Chambaz EM, Defaye G. Organization of 3 $\beta$ -hydroxysteroid dehydrogenase/isomerase and cytochrome P450<sub>scc</sub> into a catalytically active molecular complex in bovine adrenocortical mitochondria. *Journal Steroid Biochemistry & Molecular Biology* 1995; 55: 507–514.
- [35] Pelletier G, Li S, Luu-The V, Tremblay Y, Belanger A, Labrie F. Immunoelectron microscopic localization of three key steroidogenic enzymes (cytochrome P450<sub>scc</sub>), 3 $\beta$ -

- hydroxysteroid dehydrogenase and cytochrome P450(c17)) in rat adrenal cortex and gonads. *Journal of Endocrinology* 200; 171: 373–383.
- [36] Chapman JC, Waterhouse TB, Michael SD. Changes in mitochondrial and microsomal 3 $\beta$ -hydroxysteroid dehydrogenase activity in mouse ovary over the course of the estrous cycle. *Biology of Reproduction* 1992; 4: 992–997.
- [37] Luu-The V, Lachance Y, Labrie C, Leblanc G, Thomas JL, Strickler RC, Labrie F. Full length cDNA structure and deduced amino acid sequence of human 3 $\beta$ -hydroxy-5-ene steroid dehydrogenase. *Molecular Endocrinology* 1989; 3: 1310–1312.
- [38] Rheume E, Lachance Y, Zhao HF, Breton N, Dumont M, de Launoit Y, Trudel C, Luu-The V, Simard J, Labrie F. Structure and expression of a new complementary DNA encoding the almost exclusive 3 $\beta$ -hydroxysteroid dehydrogenase/  $\Delta$ 5- $\Delta$ 4-isomerase in human adrenals and gonads. *Molecular Endocrinology* 1991; 5: 1147–1157.
- [39] Dumont M, Luu-The V, Dupont E, Pelletier G, Labrie F. Characterization, expression, and immunohistochemical localization of 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase in human skin. *Journal of Investigative Dermatology* 1992; 99: 415–421.
- [40] Lachance Y, Luu-The V, Labrie C, Simard J, Dumont M, de Launoit Y, Guerin S, Leblanc G, Labrie F. Characterization of human 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase gene and its expression in mammalian cells. *Journal of Biological Chemistry* 1992; 267: 3551.
- [41] Morissette J, Rheume E, Leblanc JF, Luu-The V, Labrie F, Simard J 1995 Genetic linkage mapping of HSD3B1 and HSD3B2 encoding human types I and II 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase close to D1S514 and the centromeric D1Z5 locus. *Cytogenetics & Cell Genetics* 1995; 69: 59–62.
- [42] Zhao HF, Labrie C, Simard J, de Launoit Y, Trudel C, Martel C, Rheume E, Dupont E, Luu-The V, Pelletier G. Characterization of rat 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase cDNAs and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. *Journal of Biological Chemistry* 1991; 266: 583–593.
- [43] Simard J, Couet J, Durocher F, Labrie Y, Sanchez R, Breton N, Turgeon C, Labrie F. Structure and tissue-specific expression of a novel member of the rat 3 $\beta$ -hydroxysteroid dehydrogenase/  $\Delta$ 5- $\Delta$ 4 isomerase (3 $\beta$ -HSD) family. The exclusive 3 $\beta$ -HSD gene expression in the skin. *Journal of Biological Chemistry* 1993; 268: 19659–19668.
- [44] Couet J, Simard J, Martel C, Trudel C, Labrie Y, Labrie F. Regulation of 3-ketosteroid reductase messenger ribonucleic acid levels and 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase activity in rat liver by sex steroids and pituitary hormones. *Endocrinology* 1992; 131: 3034–3044.
- [45] Sanchez R, de Launoit Y, Durocher F, Belanger A, Labrie F, Simard J. Formation and degradation of dihydrotestosterone by recombinant members of the rat 3 $\beta$ -hydroxysteroid dehydrogenase/  $\Delta$ 5- $\Delta$ 4 isomerase family. *Molecular & Cellular Endocrinology* 1994; 103: 29–38.
- [46] de Launoit Y, Simard J, Durocher F, Labrie F. Androgenic 17 $\beta$ -hydroxysteroid dehydrogenase activity of expressed rat type I 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase. *Endocrinology* 1992; 130: 553–555.

- [47] Mason JL, Howe BE, Howie AF, Morley SD, Nicol MR, Payne AH. Promiscuous  $3\beta$ -hydroxysteroid dehydrogenases: testosterone  $17\beta$ -hydroxysteroid dehydrogenase activities of mouse type I and VI  $3\beta$ -hydroxysteroid dehydrogenases. *Endocrine Research* 2004; 30: 709–714.
- [48] Payne AH, Clarke TR, Bain PA. The murine  $3\beta$ -hydroxysteroid dehydrogenase multigene family: structure, function and tissue-specific expression. *Journal Steroid Biochemistry & Molecular Biology* 1995; 53: 111–118.
- [49] Payne AH, Abbaszade IG, Clarke TR, Bain PA, Park CH. The multiple murine  $3\beta$  hydroxysteroid dehydrogenase isoforms: structure, function, and tissue- and developmentally specific expression. *Steroids* 1997; 62: 169–175.
- [50] Abbaszade IG, Arensburg J, Park CH, Kasa-Vubu JZ, Orly J, Payne AH 1997 Isolation of a new mouse  $3\beta$ -hydroxysteroid dehydrogenase isoform,  $3\beta$ -HSD VI, expressed during early pregnancy. *Endocrinology* 1998; 138: 1392–1399.
- [51] Belanger B, Belanger A, Labrie F, Dupont A, Cusan L, Monfette G. 1989 Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. *Journal of Steroid Biochemistry* 1989; 32: 695–698.
- [52] Dupont E, Luu-The V, Labrie F, Pelletier G. Ontogeny of  $3\beta$ -hydroxysteroid dehydrogenase/ $\Delta^5$ - $\Delta^4$  isomerase ( $3\beta$ -HSD) in human adrenal gland performed by immunocytochemistry. *Molecular & Cellular Endocrinology* 1990; 74: R7–R10.
- [53] Simonian MH. ACTH and thyroid hormone regulation of  $3\beta$ -hydroxysteroid dehydrogenase activity in human fetal adrenocortical cells. *Journal Steroid Biochem* 1986; 25: 1001–1006.
- [54] Lo MJ, Kau MM, Chen YH, Tsai SC, Chiao YC, Chen JJ, Liaw C, Lu CC, Lee BP, Chen SC, Fang VS, Ho LT, Wang PS. Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats. *American Journal of Physiology* 1998; 274: E238–E245.
- [55] Dupont E, Labrie F, Luu-The V, Pelletier G. Immunocytochemical localization of  $3\beta$ -hydroxysteroid dehydrogenase/ $\Delta^5$ - $\Delta^4$  isomerase in human ovary. *Journal of Clinical Endocrinology & Metabolism* 1992; 74: 994–998.
- [56] Kaplan S, Grumach M. Pituitary and placental gonadotropin and sex steroids in the human and sub-human primate fetus. *Journal of Clinical Endocrinology & Metabolism* 1978; 7: 487–511.
- [57] Grumbach M, Conte F. Disorders of sex differentiation. In: Wilson JD & Foster EW (eds) *Williams textbook of endocrinology*. Philadelphia: W. B. Saunders; 1999: 1303–1425
- [58] Nelson VL, Legro RS, Strauss 3rd JF, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Molecular Endocrinology* 1999; 13: 946–957.
- [59] Doody KJ, Lorence MC, Mason JL, Simpson ER. Expression of messenger ribonucleic acid species encoding steroidogenic enzymes in human follicles and corpora lutea throughout the menstrual cycle. *Journal of Clinical Endocrinology & Metabolism* 1990; 70: 1041–1045.



- [60] Teerds KJ, Dorrington JH. Immunohistochemical localization of  $3\beta$ -hydroxysteroid dehydrogenase in the rat ovary during follicular development and atresia. *Biology of Reprod* 1993; 49: 989–996.
- [61] Voss AK, Fortune JE. Levels of messenger ribonucleic acid for cholesterol side-chain cleavage cytochrome P-450 and  $3\beta$ -hydroxysteroid dehydrogenase in bovine preovulatory follicles decrease after the luteinizing hormone surge. *Endocrinology* 1993; 132: 888–894.
- [62] Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *Journal of Clinical Endocrinology & Metabolism* 1997; 82: 2396–2402.
- [63] Sasano H, Suzuki T. Localization of steroidogenesis and steroid receptors in human corpus luteum. Classification of human corpus luteum (CL) into estrogen- producing degenerating CL, and nonsteroid-producing degenerating CL. *Seminars of Reproductive Endocrinology* 1997; 15: 345–351.
- [64] Duncan WC, Cowen GM, Illingworth PJ. Steroidogenic enzyme expression in human corpora lutea in the presence and absence of exogenous human chorionic gonadotrophin (HCG). *Molecular & Human Reproduction* 1999; 5: 291–298.
- [65] Benyo DF, Little-Ihrig L, Zeleznik AJ. Noncoordinated expression of luteal cell messenger ribonucleic acids during human chorionic gonadotropin stimulation of the primate corpus luteum. *Endocrinology* 1993; 133: 699–704.
- [66] McGee E, Sawetawan C, Bird I, Rainey WE, Carr BR. The effects of insulin on  $3\beta$ -hydroxysteroid dehydrogenase expression in human luteinized granulosa cells. *Journal of the Society for Gynecologic Investigations* 1995; 2: 535–541.
- [67] Feltus FA, Groner B, Melner MH. Stat5-mediated regulation of the human type II  $3\beta$ -hydroxysteroid dehydrogenase/ $\Delta 5$ - $\Delta 4$  isomerase gene: activation by prolactin. *Molecular Endocrinology* 1999; 13: 1084–1093.
- [68] Stocco CO, Deis RP. Participation of intraluteal progesterone and prostaglandin F $2\alpha$  in LH-induced luteolysis in pregnant rat. *J Endocrinol* 1998; 156: 253–259.
- [69] Mendis-Handagama SMLC, Ariyaratne HBS. Differentiation of adult Leydig cell proliferation in the postnatal testis. *Biology of Reproduction* 2001; 65: 660–671.
- [70] Davidoff MS, Middendorff R, Enikolopov G, Riethmacher D, Holstein AF, Muller D. Progenitor cells of the testosterone-producing Leydig cells revealed. *Journal of Cell Biology* 2005; 167: 935–944.
- [71] Kilcoyne K, Sharpe RM, McKinnell C, van den Driesche S, Smith LB, Atanossova N. Putative adult Leydig progenitor cells in the rat are reduced in number following DBP-induced suppression of fetal intratesticular testosterone. *Proceedings of 17<sup>th</sup> European Testis Workshop on Molecular and Cellular Endocrinology of the Testis*. April 20–24 2012, Stockholm, Sweden.
- [72] Atanassova N. Morpho-functional aspect of androgen-estrogen regulation of mammalian testis and male reproductive tract. DSci Thesis, Bulgarian Academy of Sciences, Sofia, 2007.
- [73] Habert R, Lejeune H, Saez JM. Origin, differentiation and regulation of fetal and adult Leydig cells. *Molecular and Cellular Endocrinology* 2001; 179: 47–74.

- [74] Bernstein L, Ross RK 1993 Endogenous hormones and breast cancer risk. *Epidemiology Review* 1993; 15 :48–65.
- [75] Gunasegaram R, Peh KL, Loganath A, Ratnam SS. Expression of  $3\beta$ -hydroxysteroid dehydrogenase-5,4-ene isomerase activity by infiltrating ductal human breast carcinoma in vitro. *Breast Cancer Research & Treatment* 1998; 50: 117–123.
- [76] Reed MJ, Purohit A. Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis. *Endocrine Review* 1997; 18: 701–715.
- [77] Turgeon C, Gingras S, Carriere MC, Blais Y, Labrie F, Simard J. Regulation of sex steroid formation by interleukin-4 and interleukin-6 in breast cancer cells. *Journal of Steroid Biochemistry & Molecular Biology* 1998; 65: 151–162,
- [78] Riley SC, Dupont E, Walton JC, Luu-The V, Labrie F, Pelletier G, Challis JR. Immunohistochemical localization of  $3\beta$ -hydroxy- 5-ene-steroid dehydrogenase/ $\Delta 5$ - $\Delta 4$  isomerase in human placenta and fetal membranes throughout gestation. *Journal of Clinical Endocrinology & Metabolism* 1992; 75: 956–961.
- [79] Morrish DW, Linetsky E, Bhardwaj D, Li H, Dakour J, Marsh RG, Paterson MC, Godbout R. Identification by subtractive hybridization of a spectrum of novel and unexpected genes associated with in vitro differentiation of human cytotrophoblast cells. *Placenta* 1996; 17: 431–441
- [80] Amet Y, Simon B, Quemener E, Mangin P, Floch HH, Abalain JH. Partial purification of  $3\alpha$ - and  $3\beta$ -hydroxysteroid dehydrogenases from human hyperplastic prostate. Comparison between the two enzymes. *Journal of Steroid Biochemistry & Molecular Biology* 1992; 41: 689–692.
- [81] Labrie F, Belanger A, Cusan L, Labrie C, Simard J. History of LHRH agonist and combination therapy in prostate cancer. *Endocrine Related Cancer* 1996; 3, 243–278
- [82] Pirog EC, Collins DC 1999 Metabolism of dihydrotestosterone in human liver: importance of  $3\alpha$ - and  $3\beta$ -hydroxysteroid dehydrogenase. *Journal of Clinical Endocrinology & Metabolism* 1999; 84: 3217–3221.
- [83] Keeney DS, Murry BA, Bartke A, Wagner TE, Mason JI 1993 Growth hormone transgenes regulate the expression of sex-specific isoforms of  $3\beta$ -hydroxysteroid dehydrogenase/ $\Delta 5$ - $\Delta 4$  isomerase in mouse liver and gonads. *Endocrinology* 1993; 133: 1131–1138.
- [84] Anderson D. Steroidogenic enzymes in skin. *Journal of Dermatology* 2001; 11, 293–295.
- [85] Asada H, Linton J, Katz SI 1997 Cytokine gene expression during the elicitation phase of contact sensitivity: regulation by endogenous IL-4. *Journal of Investigative Dermatology* 1997; 108: 406–411.
- [86] Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, Vaudry H 1999 Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. *Pharmacology Review* 1999; 51: 63–81.
- [87] Zwain IH, Yen SS 1999 Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology* 1999; 140: 3843–3852.
- [88] Baker PJ, Johnston H, Abel M, HM C, O'Shaughnessy PJ. Differentiation of adult-type Leydig cells occurs in gonadotropin-deficient mice. *Reproductive Biology & Endocrinology* 2003; 1: 1–9.

- [89] Martin LJ, Taniguchi H, Robert NM, Simard J, Tremblay JJ, Viger RS. GATA Factors and the Nuclear Receptors, Steroidogenic Factor 1/Liver Receptor Homolog 1, Are Key Mutual Partners in the Regulation of the Human 3 $\beta$ -Hydroxysteroid Dehydrogenase Type 2 Promoter. *Molecular Endocrinology* 2005; 19: 2358–2370.
- [90] Darnell Jr JE. STATs and gene regulation. *Science* 1997; 277: 1630–1635.
- [91] und S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 1998; 93: 841–850.
- [92] Lalli E, Bardoni B, Zazopoulos E, Wurtz JM, Strom TM, Moras D, Sassone-Corsi P. A transcriptional silencing domain in DAX-1 whose mutation causes adrenal hypoplasia congenita. *Molecular Endocrinology* 1997; 11: 1950–1960.
- [93] Tremblay JJ, Viger RS. Novel roles for GATA transcription factors in the regulation of steroidogenesis. *Journal of Steroid Biochemistry & Molecular Biology* 2003; 85: 291–298.
- [94] Cote S, Feltus AF, Gingras S, Freeman M, Melner MH, Simard J 2000 IL-4 stimulation of ovarian 3 $\beta$ -hydroxysteroid dehydrogenase/  $\Delta$ 5- $\Delta$ 4-isomerase type II gene expression: mechanisms of activation. *Proceedings of the 82nd Annual Meeting of The Endocrine Society*, 2000, Toronto, Ontario, p 313 (Abstract 1295).
- [95] Rainey WE, Naville D, Mason JI. Regulation of 3 $\beta$ -hydroxysteroid dehydrogenase in adrenocortical cells: effects of angiotensin- II and transforming growth factor  $\beta$ . *Endocrine Research* 1991; 17: 281–296
- [96] Havelock JC, Smith AL, Seely JB, Dooley CA, Rodgers RJ, Rainey WE, Carr BR. The NGFI-B family of transcription factors regulates expression of 3 $\beta$ -hydroxysteroid dehydrogenase type 2 in the human ovary. *Molecular & Human Reproduction* 2005; 11: 79–85
- [97] Martin LJ, Tremblay J. The human 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase type 2 promoter is a novel target for the immediate early orphan nuclear receptor Nur77 in steroidogenic cells. *Endocrinology* 2005; 146: 861–869
- [98] Feltus FA, Cote S, Simard J, Gingras S, Kovacs WJ, Nicholson WE, Clark BJ, Melner MH. Glucocorticoids enhance activation of the human type II 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase gene. *Journal of Steroid Biochemistry & Molecular Biology* 2002; 82: 55–63.
- [99] Perry JE, Stalvey JR. Gonadal steroids modulate adrenal fasciculata 3 $\beta$ -hydroxysteroid dehydrogenase isomerase activity in mice. *Biology of Reproduction* 1992; 46: 73–82.
- [100] Stalvey JR, Clavey SM. Evidence that testosterone regulates Leydig cell 3 $\beta$ -hydroxysteroid dehydrogenase-isomerase activity by a trans-acting factor distal to the androgen receptor. *Journal of Andrology* 1992; 13: 93–99.
- [101] Bush IE, Hunter SA and Meigs RA. Metabolism of 11-oxygenated steroids *Biochemical Journal* 1968; 107: 239–258.
- [102] Lakshmi V and Monder C. Purification and characterization of the corticosteroid 11 $\beta$ -dehydrogenase component of the rat liver 11 $\beta$ -hydroxysteroid dehydrogenase complex *Endocrinology* 1988; 123: 2390–2398.
- [103] Jamieson PM, Chapman KE, Walker BR, Seckl JR. 11 $\beta$ -hydroxysteroid dehydrogenase type 1 is a predominant 11-reductase in the intact perfused rat liver. *Journal of Endocrinol* 2000; 165: 685–692.

- [104] Seckl JR, Walker BR. Minireview: 11 $\beta$ -hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 2001; 142: 1371-1376.
- [105] Albiston AL, Obeyesekere VR, Smith RE and Krozowski ZS. Cloning and tissue distribution of the human 11 $\beta$  -hydroxysteroid dehydrogenase type 2 enzyme *Molecular and Cellular Endocrinology* 1994; 105: R11–R17.
- [106] Mercer WR and Krozowski ZS. Localization of an 11 $\beta$ -hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. *Endocrinology* 1992; 130: 540–543.
- [107] Brown RW, Chapman KE, Edwards CRW and Seckl JR. Human placental 11 $\beta$ -hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* 1993; 132: 2614–2621.
- [108] Seckl JR, Morton NM, Chapman KE, Walker BR. Glucocorticoids and 11 $\beta$ -hydroxysteroid dehydrogenase in adipose tissue. *Recent Progress in Hormone Research* 2004; 59: 359-93.
- [109] Ge RS, Hardy DO, Catterall JE, Hardy MP. Developmental changes in glucocorticoid receptor and 11 $\beta$ -hydroxysteroid dehydrogenase oxidative and reductive activities in rat Leydig cells. *Endocrinology*. 1997;138: 5089-95.
- [110] Ge RS, Hardy MP. Initial predominance of the oxidative activity of type 11 $\beta$ -hydroxysteroid dehydrogenase in primary rat Leydig cells and transfected cell lines. *Journal of Andrology* 2000; 21: 303-310.
- [111] Monder C, Miroff Y, Marandici A, Hardy MP. 11 $\beta$ -dehydrogenase alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells. *Endocrinology* 1994; 134: 1199-1204.
- [112] Phillips DM, Lakshmi V, Monder C. Corticosteroid 11 $\beta$ -hydroxysteroid dehydrogenase in rat testis. *Endocrinology* 1989;125: 209-216.
- [113] Hales DB, Payne AH. Glucocorticoid-mediated repression of P450<sub>scc</sub> mRNA and de novo synthesis in cultured Leydig cells. *Endocrinology* 1989; 124: 2099–2104.
- [114] Payne AH, Sha LL. Multiple mechanisms for regulation of 3 $\beta$ -hydroxysteroid dehydrogenase/D5-D4-isomerase, 17 $\alpha$ -hydroxylase/C17-20 lyase cytochrome P450, and cholesterol side-chain cleavage cytochrome P450 messenger ribonucleic acid levels in primary cultures of mouse Leydig cells. *Endocrinology* 1991; 129: 1429–1435.
- [115] Gao HB, Tong MH, Hu YQ, Guo QS, Ge R, Hardy MP. Glucocorticoid induces apoptosis in rat Leydig cells. *Endocrinology* 2002; 143: 130–138.
- [116] Gao HB, Tong MH, Hu YQ, You HY, Guo QS, Ge RS, Hardy MP. Mechanisms of glucocorticoid-induced Leydig cell apoptosis. *Molecular & Cellular Endocrinology* 2003; 199: 153–163.
- [117] Guo-Xin Hu, Qing-Quan Lian, Han Lin, Syed A. Latif, David J. Morris, Matthew P. Hardy, and Ren-Shan Ge. Rapid mechanisms of glucocorticoid signaling in the Leydig cell. *Steroids* 2008; 73: 1018–1024.
- [118] Gao HB, Ge RS, Lakshmi A, Hardy MP. Hormonal regulation of oxidative and reductive activities of 11 $\beta$ -hydroxysteroid dehydrogenase in rat Leydig cells. *Endocrinology* 1997; 138: 156-161.



- [119] Jamieson PM, Walker BR, Hapman KE, Andrew R, Rossiter S, Seckl JR. 11 beta-hydroxysteroid dehydrogenase type 1 is a predominant 11 beta-reductase in the perfused rat liver. *Journal of Endocrinology* 2000; 165: 685-692.
- [120] Seckl JR, Walker BR. Minireview: 11 $\beta$ -hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 2001; 142: 1371-1376.
- [121] Latif SA, Shen M, Ge RS, Sottas CM, Hardy MP, Morris DJ. Role of 11 $\beta$ -OH-C(19) and C(21) steroids in the coupling of 11 $\beta$ -HSD1 and 17 $\beta$ -HSD3 in regulation of testosterone biosynthesis in rat Leydig cells. *Steroids* 2011; 76: 682-689.
- [122] Hu GX, Lin H, Sottas CM, Morris DJ, Hardy MP, Ge RS. Inhibition of 11beta-hydroxysteroid dehydrogenase enzymatic activities by glycyrrhetic acid in vivo supports direct glucocorticoid-mediated suppression of steroidogenesis in Leydig cells. *Journal of Andrology* 2008; 29: 345-51.
- [123] Parthasarathy C, Yuvaraj S, Ilangovan R, Janani P, Kanagaraj P, Balaganesh M, Natarajan B, Sittadjody S, Balasubramanian K. Differential response of Leydig cells in expressing 11beta-HSD type I and cytochrome P450 aromatase in male rats subjected to corticosterone deficiency. *Molecular & Cellular Endocrinology* 2009; 311:18-23.
- [124] Haider SG, Passia D, Rommert FFG. Histochemical demonstration of 11 $\beta$ -hydroxysteroid dehydrogenase as a marker for Leydig cell maturation in rat. *Acta Histochemica (Suppl)* 1990; 38: 203-207.
- [125] Monder C, Hardy MP, Blanchard RJ, Blanchard DC. Comparative aspects of 11 $\beta$ -hydroxysteroid dehydrogenase: development of a model for the mediation of Leydig cell function by corticosteroids. *Steroids* 1994; 59: 69-73.
- [126] Neumann A, Haider SG, Hilscher B. Temporal coincidence of the appearance of elongated spermatids and of histochemical reaction of 11 $\beta$ -hydroxysteroid dehydrogenase in rat Leydig cells. *Andrologia* 1993; 25: 263-269.
- [127] Schafers BA, Schlutius BG, Haider SG. Ontogenesis of oxidative reaction of 17 $\beta$ -hydroxysteroid dehydrogenase and 11 $\beta$ -hydroxysteroid dehydrogenase in rat Leydig cells, a histochemical study. *The Histochemical Journal* 2001; 33: 585-595.
- [128] Ge RS, Gao HB, Nacharaju VL, Gunsalus GL, Hardy MP. Identification of a kinetically distinct activity of 11 $\beta$ -hydroxysteroid dehydrogenase in rat Leydig cells. *Endocrinology* 1997;138: 2435-2442.
- [129] Brereton PS, Van Driel RR, Suhaimi FB, Koyama K, Dilley R, Krozowski Z. Light and electron microscopy localization of the 11 $\beta$ -hydroxysteroid dehydrogenase type I enzyme in the rat. *Endocrinology* 2001; 142: 1644-1651.
- [130] Hardy MP, Gao HB, Dong Q, Ge R, Wang Q, Chai WR, Feng X, Sottas C. Stress hormone and male reproductive function. *Cell & Tissue Research* 2005; 322:147-53.
- [131] Ge RS, Dong Q, Sottas CM, Chen H, Zirkin BR, Hardy MP. Gene expression in rat Leydig cells during development from the progenitor to adult stage: a cluster analysis. *Biology of Reproduction* 2005; 72: 1405-1415.
- [132] Hu GX, Lian QQ, Chen BB, Prasad PV, Kumar N, Zheng ZQ, Ge RS. 7 $\alpha$ -hydroxytestosterone affects 11 beta-hydroxysteroid dehydrogenase 1 direction in rat Leydig cells. *Endocrinology* 2010; 151: 748-54.
- [133] Koeva Y, Bakalska M, Atanassova N, Georgieva K, Davidoff M. 11 $\beta$  hydroxysteroid dehydrogenase type 2 expression in the newly formed Leydig cells after ethane

- dimethanesulphonate treatment of adult rats. *Folia Histochemica & Cytobiologica* 2007; 45: 381-6.
- [134] Bakalska M, Atanassova N, Angelova P, Koeva I, Nikolov B, Davidoff M. Degeneration and restoration of spermatogenesis in relation to the changes in Leydig cell population following ethane dimethanesulfonate treatment in adult rats. *Endocrine Regulations* 2001; 35: 211-217.
- [135] Bakalska M, Koeva I, Atanassova N, Angelova P, Nikolov B, Davidoff M. Steroidogenic and structural differentiation of new Leydig cell population following exposure of adult rats to ethane dimethanesulphonate. *Folia Biologica (Praha)* 2002; 48: 205-209.
- [136] Ge RS, Dong Q, Niu EM, Sottas CM, Hardy DO, Catterall JF, Latif SA, Morris DJ, Hardy MP. 11 beta-hydroxysteroid dehydrogenase 2 in rat Leydig cells: its role in blunting glucocorticoid action at physiological levels of substrate. *Endocrinology* 2005; 146: 2657-2664.
- [137] Hardy M and Schlegel P. Testosterone production in the aging male: Where does the slowdown occur? *Endocrinology* 2004; 145: 4439-40.
- [138] Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *Journal of Clinical Endocrinology & Metabolism* 2001; 86: 724-31.
- [139] Wang C, Hikim AS, Ferrini M, Bonavera JJ, Vemet D, Leung A, Lue YH, Gonzalez-Cadavid NF, Schwerdtloff RS. Male reproductive ageing: using the brown Norway rat as a model for man. *Novartis Found Symposium*. 2002; 242: 82-95.
- [140] Chen H, Huhtaniemi I, Zirkin BR. Depletion and repopulation of Leydig cells in the testes in aging Brown Norway rats. *Endocrinology* 1996; 137: 3447-52.
- [141] Zirkin BR and Chen H. Regulation of Leydig cell steroidogenic function during aging. *Biology of Reproduction* 2000; 63: 977-81.
- [142] Chen H, Luo L, Zirkin BR. Leydig cell structure and function during aging. In: Payne AH, Hardy MP, Russell LD, eds. *The Leydig cell*. Cache River Press, Vienna, IL; 1996: p221-30.
- [143] Schultz R, Isola J, Parvinen M, Honkaniemi J, Wikstrom AC, Gustafsson JA, Peltto-Huikko M. Localization of the glucocorticoid receptor in testis and accessory sexual organs of male rat. *Molecular & Cellular Endocrinology* 1993; 95: 115-20.
- [144] Monder C, White PC. 11 $\beta$  hydroxysteroid dehydrogenase. *Vitamines & Hormones* 1993; 47: 187-271.
- [145] Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CR. Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. *Journal of Clinical Endocrinology & Metabolism* 1995; 80 :3155-59.
- [146] Koeva Y, Bakalska M, Atanassova N, Georgieva K, Davidoff M. Age-related changes in the expression of 11beta-hydroxysteroid dehydrogenase type 2 in rat Leydig cells. *Folia Histochemica & Cytobiologica* 2009; 47: 281-287.
- [147] Sankar BR, Maran RR, Sudha S, Govindarajulu P, Balasubramanian K. Chronic corticosterone treatment impairs Leydig cell 11beta-hydroxysteroid dehydrogenase

- activity and LH-stimulated testosterone production. *Hormone & Metabolism Research* 2000; 32: 142-146.
- [148] Morita H, Cozza EN, Zhou MY, Gomez-Sanchez EP, Romero DG, Gomez-Sanchez CE. Regulation of the 11 beta-hydroxysteroid dehydrogenase in the rat adrenal. Decrease enzymatic activity induced by ACTH. *Endocrine* 1997; 7: 331-5.
- [149] Shimojo M, Condon J, Whorwood CB, Stewart PM. Adrenal 11 beta-hydroxysteroid dehydrogenase. *Endocrine Research* 1996; 22:771-80.
- [150] Shimojo M, Whorwood CB, Stewart PM. 11 beta-hydroxysteroid dehydrogenase in the rat adrenal. *Journal of Molecular Endocrinology* 1996; 17: 121-30.
- [151] Smith RE, Li KX, Andrews RK, Krozowski Z. Immunohistochemical and molecular characterization of the rat 11 beta-hydroxysteroid dehydrogenase type II enzyme. *Endocrinology* 1997; 138: 540-547.
- [152] Koeva YA, Bakalska MV, Petrova EI, Atanassova NN. 11beta hydroxysteroid dehydrogenase type 2 in the adrenal gland by testosterone withdrawal of adult rats. *Folia Medica (Plovdiv)* 2010; 52: 38-42.
- [153] Atanassova N, Koeva Y, Bakalska M, Pavlova E, Nikolov B, Davidoff M. Loss and recovery of androgen receptor protein expression in the adult rat testis following androgen withdrawal by ethane dimethanesulfonate. *Folia Histochemica et Cytobiologica* 2006; 44: 81-86.
- [154] Plecas B, Pesic VP, Mirkovic D, Majkic-Singh N, hristic M, Solarovic T. Opposite effects of dexamethasone and ACTH on the adrenal cortex response to ethane dimethanesulphonate (EDS). *Experimenatal Toxicology & Pathology* 2001; 53: 31-34.
- [155] Petrova E, Koeva Y, Bakalska M, Atanassova N, Davidoff M. Morphofunctional characteristics of rat adrenocorticytes after treatment with ethane dimethanesulphonate. *Jubilee Scientific Session of Medical University, Plovdiv, 2005, abstract book*; 164.
- [156] Stalvey JR. Inhibition of 3 beta-hydroxysteroid dehydrogenase- isomerase in mouse adrenal cells: a different effect of testosterone. *Steroids* 2002; 67: 721-31.
- [157] Michael AE & Cooke BA. A working hypothesis for the regulation of steroidogenesis and germ cell development in the gonads by glucocorticoids and 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD). *Molecular and Cellular Endocrinology* 1994; 100: 55-63.
- [158] Omura T & Morohashi K. Gene regulation of steroidogenesis. *Journal of Steroid Biochemistry and Molecular Biology* 1995; 53: 19-25.
- [159] Monder C & Lakshmi V. Evidence for kinetically distinct forms of corticosteroid 11 $\beta$ -hydroxysteroid dehydrogenase in rat liver microsomes. *Journal of Steroid Biochemistry* 1989; 32: 77-83.
- [160] Mercer W, Obeyeskere V, Smith R & Krozowski Z. Characterization of 11 $\beta$ HSD1B gene expression and enzyme activity. *Molecular and Cellular Endocrinology* 1993; 92: 247-251.
- [161] Yding Andersen C. Possible new mechanism of cortisol action in female reproductive organs: physiological implications of the free hormone hypothesis. *Journal of Endocrinology* 2002; 173: 211-217.

- [162] Yding Andersen C, Morineau G, Fukuda M, Westergaard LG, Ingerslev HJ, Fiet J & Byskov AG. Assessment of the follicular cortisol:cortisone ratio. *Human Reproduction* 1999; 14: 1563–1568.
- [163] Yong PYK, Thong KJ, Andrew R, Walker BR & Hillier SG. Development-related increase in cortisol biosynthesis by human granulosa cells. *Journal of Clinical Endocrinology and Metabolism* 2000; 85: 4728–4733.
- [164] Hillier SG & Tetsuka M. An anti-inflammatory role for glucocorticoids in the ovaries? *Journal of Reproductive Immunology* 1998; 39: 21–27.
- [165] Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the Southeastern United States: a prospective study. *Journal of Clinical Endocrinology & Metabolism* 1998; 83: 3078–3082.
- [166] Legro, R.S. & J.F. Strauss III. Molecular progress in infertility: polycystic ovary syndrome. *Fertility & Sterility* 2002; 78: 569–576.
- [167] Milutinović DV, Macut D, Božić I, Nestorov J, Damjanović S, Matić G. Hypothalamic-pituitary-adrenocortical axis hypersensitivity and glucocorticoid receptor expression and function in women with polycystic ovary syndrome. *Experimental Clinical Endocrinology & Diabetes*. 2011; 119: 636-43.
- [168] Diamanti-Kandarakis E, Xyrafis X, Boutzios G, Christakou C. Pancreatic beta-cells dysfunction in polycystic ovary syndrome. *Panminerva Medicine* 2008; 50: 315-25.
- [169] Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nature Reviews of Endocrinology* 2011;7 :219-31.
- [170] Waterworth DM, Bennett ST, Gharani N, McCarthy MI, Hague S, Batty S, Conway GS, White D, Todd JA, Franks S, Williamson R. Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. *Lancet* 1997; 349: 986–990.
- [171] Nelson VL, Legro RS, Strauss JF 3rd, McAllister JM.. Augmented androgen production is a stable phenotype of propagated theca cells from polycystic ovaries. *Molecular Endocrinology* 1999; 13: 946–957.
- [172] Nelson VL, Qin KN, Rosenfield RL, Wood JR, Penning TM, Legro RS, Strauss JF 3rd, McAllister JM. The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2001; 86: 5925–5933.
- [173] Wickenheisser JK, Quinn PG, Nelson VL, Legro RS, Strauss JF 3rd, McAllister JM. Differential activity of the cytochrome P450 17  $\alpha$ -hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. *Journal of Clinical Endocrinology and Metabolism* 2000; 85: 2304–2311.
- [174] J.F. Strauss III. Some New Thoughts on the Pathophysiology and Genetics of Polycystic Ovary Syndrome. *Annals of New York Academy of Sciences* 2003; 997: 42–48.
- [175] Svendsen PF, Madsbad S, Nilas L, Paulsen SK, Pedersen SB. Expression of 11 $\beta$ -hydroxysteroid dehydrogenase 1 and 2 in subcutaneous adipose tissue of lean and obese women with and without polycystic ovary syndrome. *International Journal of Obesity (Lond)* 2009; 33:1249-56.
- [176] Tsilchorozidou T, Honour JW, Conway GS. Altered cortisol metabolism in polycystic ovary syndrome: insulin enhances 5 $\alpha$ -reduction but not the elevated adrenal



- steroid production rates. *Journal of Clinical Endocrinology and Metabolism* 2003; 88: 5907-13.
- [177] Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, Lavery GG, Bedendo O, Ray DW, Laing I, Malunowicz E, White PC, Hewison M, Mason PJ, Connell JM, Shackleton CHL, Stewart PM. Mutations in the gene encoding 11 $\beta$ -hydroxysteroid dehydrogenase type 1 and hexose- 6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nature Genetics* 2003; 34: 434-439.
- [178] Phillipov G, Palermo M, Shackleton CH. Apparent cortisone reductase deficiency: a unique form of hypercortisolism. *J Clin Endocrinol Metab*, 1996; 81:3855-3860.
- [179] Rodin A, Thakkar H, Taylor NJ, Clayton R. Hyperandrogenism in polycystic ovary syndrome: evidence of dysregulation of 11 $\beta$ -hydroxysteroid dehydrogenase. *New English Journal of Medicine* 1994; 330: 460-465.
- [180] Gambineri A, Vicennati V, Genghini S, Tomassoni F, Pagotto U, Pasquali R, Walker BR. Genetic variation in 11beta-hydroxysteroid dehydrogenase type 1 predicts adrenal hyperandrogenism among lean women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2006; 91: 2295-302.
- [181] Hines GA, Smith ER, Azziz R. Influence of insulin and testosterone on adrenocortical steroidogenesis in vitro: preliminary studies. *Fertility & Sterility* 2001; 76: 730-735.
- [182] Andrew R, Phillips DIW, Walker BR. Obesity and gender influence cortisol secretion and metabolism in man. *Journal of Clinical Endocrinology and Metabolism* 1998; 83: 1806-1809.
- [183] San Milla'n JL, Botella-Carretero JL, Alvarez-Blasco F, Luque-Ramírez M, Sancho J, Moghetti P, Escobar-Morreale HF. A study of the hexose-6-phosphate dehydrogenase gene R453Q and 11 $\beta$ -hydroxysteroid dehydrogenase type 1 gene 83557insA polymorphisms in the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2005; 90: 4157-4162.
- [184] White PC. Genotypes at 11 $\beta$ -hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. *Journal of Clinical Endocrinology and Metabolism* 2005; 90: 5880-5883.
- [185] Draper N, Powell BL, Franks S, Conway GS, Stewart PM & McCarthy MI. Variants implicated in cortisone reductase deficiency do not contribute to susceptibility to common forms of polycystic ovary syndrome. *Clinical Endocrinology* 2006; 65: 64- 70.
- [186] Gambineri A, Tomassoni F, Munarini A, Stimson RH, Mioni R, Pagotto U, Chapman KE, Andrew R, Mantovani V, Pasquali R, Walker BR. A combination of polymorphisms in HSD11B1 associates with in vivo 11{beta}-HSD1 activity and metabolic syndrome in women with and without polycystic ovary syndrome. *European Journal of Endocrinology* 2011; 165: 283-92.
- [187] Mlinar B, Marc J, Jensterle M, Bokal EV, Jerin A, Pfeifer M. Expression of 11 $\beta$ hydroxysteroid dehydrogenase type 1 in visceral and subcutaneous adipose tissues of patients with polycystic ovary syndrome is associated with adiposity. *Journal of Steroid Biochemistry & Molecular Biology* 2011; 123: 127-32.
- [188] Reaven G. Metabolic syndrome — pathophysiology and implications for management of cardiovascular disease. *Circulation* 2002; 106: 286-288.

- [189] Walker B, Seckl J. Cortisol metabolism. In: Björntorp P, ed. *International Textbook of Obesity*. 2001, Chichester, UK: John Wiley and Sons; 241–268.
- [190] Morton NM, Seckl JR. 11 $\beta$ -hydroxysteroid dehydrogenase type 1 and obesity. *Frontiers in Hormone Research* 2008; 36: 146–64.
- [191] Livingstone DEW, Jones G, Smith K, Jamieson PM, Andrew R, Kenyon CJ, Walker BR. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology* 2000; 141: 560–563.
- [192] Westerbacka J, Yki-Järvinen H, Vehkavaara S, Häkkinen A, Andrew R, Wake D, Seckl J, Walker B. Body fat distribution and cortisol metabolism in healthy men: enhanced 5-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. *Journal of Clinical Endocrinology and Metabolism* 2003; 88: 4924–4931.
- [193] Livingstone DEW, Kenyon CJ, Walker BR. Mechanisms of dysregulation of 11  $\beta$  hydroxysteroid dehydrogenase type 1 in obese Zucker rats. *Journal of Endocrinol* 2000; 167: 533–539.
- [194] Mattsson C, Olsson T. Estrogens and glucocorticoid hormones in adipose tissue metabolism. *Current Medicinal Chemistry* 2007; 14:2918–24.
- [195] Andersson T, Söderström I, Simonyté K, Olsson T. Estrogen reduces 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in liver and visceral, but not subcutaneous, adipose tissue in rats. *Obesity (Silver Spring)*. 2010; 18: 470–5.
- [196] Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. *Science* 2001; 294: 2166–2170.
- [197] Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, Shinyama H, Sharp MGF, Fleming S. Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *Journal of Clinical Investigations*, 2003; 112: 83–90.
- [198] Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, Seckl JR & Mullins JJ. Metabolic syndrome without obesity: hepatic overexpression of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proceedings of the National Academy of Sciences USA* 2004; 101: 7088–7093.
- [199] Kotelevtsev YV, Holmes MC, Burchell A, Houston PM, Scholl D, Jamieson PM, Best R, Brown RW, Edwards CRW, Seckl JR & Mullins. 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity and stress. *Proceedings of the National Academy of Sciences USA*, 1997; 94: 14924–14929.
- [200] Morton NM, Holmes MC, Fievet C, Staels B, Tailleux A, Mullins JJ & Seckl JR. Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11 $\beta$  hydroxysteroid dehydrogenase type 1 null mice. *Journal of Biological Chemistry*, 2001; 276: 41 293–300.
- [201] Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ & Seckl JR. Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 $\beta$ -hydroxysteroid dehydrogenase type 1 deficient mice. *Diabetes* 2004; 53: 931–938.

- [202] Stimson RH, Walker BR. Glucocorticoids and 11beta-hydroxysteroid dehydrogenase type 1 in obesity and the metabolic syndrome. *Minerva Endocrinology* 2007; 32: 141-159.
- [203] Nair S, Lee YH, Lindsay RS, Walker BR, Tataranni PA, Bogardus C, Baier LJ & Permana PA. 11Beta-hydroxysteroid dehydrogenase type 1: genetic polymorphisms are associated with type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. *Diabetologia* 2004; 47: 1088-1095.
- [204] Franks PW, Knowler WC, Nair S, Koska J, Lee YH, Lindsay RS, Walker BR, Looker HC, Permana PA, Tatarani PA, Hanson RL. Interaction between an 11bHSD1 gene variant and birth era modifies risk of hypertension in Pima Indians. *Hypertension* 2004; 44: 681-688.
- [205] Morales MA, Carvajal CA, Ortiz E, Mosso LM, Artigas RA, Owen GI & Fardella CE. Possible pathogenetic role of 11 beta-hydroxysteroid dehydrogenase type 1 (11beta HSD1) gene polymorphisms in arterial hypertension. *Revista Médica de Chile*, 2008; 136: 701-710.
- [206] Rask E, Walker BR, Soderberg S, Livingstone DE, Eliasson M, Johnson O, Andrew R, Olsson T. Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. *Journal of Clinical Endocrinology & Metabolism* 2002; 87: 3330-3336.
- [207] Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CRW. Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. *Journal of Clinical Endocrinology & Metabolism* 1995; 80: 3155-3159.
- [208] Andrews RC, Rooyackers O, Walker BR. Effects of the 11beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes. *Journal of Clinical Endocrinology & Metabolism* 2003; 88: 285-291.
- [209] Sandeep TC, Andrew R, Homer NZ, Andrews RC, Smith K, Walker BR. Increased in vivo regeneration of cortisol in adipose tissue in human obesity and effects of the 11beta-hydroxysteroid dehydrogenase type 1 inhibitor carbenoxolone. *Diabetes* 2005; 54: 872-879.
- [210] Alberts P, Nilsson C, Selen G, Engblom LO, Edling NH, Norling S, Klingström G, Larsson C, Forsgren M, Ashkzari M, Nilsson CE, Fiedler M, Bergqvist E, Ohman B, Björkstrand E, Abrahmsen LB. Selective inhibition of 11bhydroxysteroid dehydrogenase type 1 improves hepatic insulin sensitivity in hyperglycaemic mice strains. *Endocrinology* 2003; 144: 4755-4762.
- [211] Hermanowski-Vosatka A, Balkovec JM, Cheng K, Chen HY, Hernandez M, Koo GC, Le Grand CB, Li Z, Metzger JM, Mundt SS, Noonan H, Nunes CN, Olson SH, Pikounis B, Ren N, Robertson N, Schaeffer JM, Shah K, Springer MS, Strack AM, Strowski M, Wu K, Wu T, Xiao J, Zhang BB, Wright SD, Thieringer. 11b-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. *Journal of Experimental Medicine* 2005; 202: 517-527.
- [212] Wamil M, Seckl JR. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 as a promising therapeutic target. *Drug Discovery Today* 2007; 12) :504-520.

- [213] Morton NM. Obesity and corticosteroids: 11 $\beta$ -hydroxysteroid type 1 as a cause and therapeutic target in metabolic disease. *Molecular & Cellular Endocrinology* 2010 25; 316: 154-64.
- [214] Morton NM, Ramage L & Seckl JR. Down-regulation of adipose 11 $\beta$ -hydroxysteroid dehydrogenase type 1 by high-fat feeding in mice: a potential adaptive mechanism counteracting metabolic disease. *Endocrinology* 2004;145: 2707–2712.
- [215] Harris A, Seckl J. Glucocorticoids, prenatal stress and the programming of disease. *Hormones & Behavior* 201; 59: 279-89.
- [216] Marciniak B, Patro-Małyśza J, Poniedziałek-Czajkowska E, Kimber-Trojnar Z, Leszczyńska-Gorzelak B, Oleszczuk J. Glucocorticoids in pregnancy. *Current Pharmacology & Biotechnology* 201; 12:750-757.
- [217] McTernan CL, Draper N, Nicholson H, Chalder SM, Driver P, Hewison M, Kilby MD, Stewart PM. Reduced placental 1 $\beta$ -hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. *Journal of Clinical Endocrinology & Metabolism* 2001; 86: 4979–4983.
- [218] Beitens IZ, Bayard F, Ances IG, Kowarski A, Migeon CJ. The metabolic clearance rate, blood production, interconversion and transplacental passage of cortisol and cortisone in pregnancy near term. *Pediatric Research* 1997; 37: 509–519.
- [219] Brown RW, Diaz R, Robson AC, Kotelevtsev Y, Mullins JJ, Kaufman MH, Seckl JR. Isolation and cloning of human placental 11 $\beta$  hydroxysteroid dehydrogenase-2 cDNA. *Biochemical Journal* 1996; 313: 1007–1017.
- [220] Benediktsson R, Calder AA, Edwards CRW, Seckl JR. Placental 11 $\beta$ -hydroxysteroid dehydrogenase type 2 is the placental barrier to maternal glucocorticoids: ex vivo studies. *Clinical Endocrinology* 1997; 46: 161–166.
- [221] Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nat Clinical Practice of Endocrinology and Metabolism* 2007; 3:479-488.
- [222] Edwards CRW, Benediktsson R, Lindsay R, Seckl JR. Dysfunction of the placental glucocorticoid barrier: a link between the foetal environment and adult hypertension? *Lancet* 1993; 341: 355–357.
- [223] Stewart PM, Rogerson FM, Mason JJ. Type 2 11 $\beta$ -hydroxysteroid dehydrogenase messenger RNA and activity in human placenta and fetal membranes: its relationship to birth weight and putative role in fetal steroidogenesis. *Journal of Clinical Endocrinology & Metabolism* 1995; 80: 885–890.
- [224] Murphy VE, Zakar T, Smith R, Giles WB, Gibson PG, Clifton VL. Reduced 11 $\beta$ -hydroxysteroid dehydrogenase type 2 activity is associated with decreased birth weight centile in pregnancies complicated by asthma. *Journal of Clinical Endocrinology & Metabolism* 2002; 87: 1660–1668.
- [225] Marchais-Oberwinkler S, Henn C, Möller G, Klein T, Negri M, Oster A, Spadaro A, Werth R, Wetzel M, Xu K, Frotscher M, Hartmann RW, Adamski J. 17 $\beta$ -Hydroxysteroid dehydrogenases (17 $\beta$ -HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. *Journal of Steroid Biochemistry & Molecular Biology* 2011; 125: 66-82.



- [226] Vihko P, Herrala A, Harkonen P, Isomaa V, Kaija H, Kurkela R, Pulkka A. Control of cell proliferation by steroids: the role of 17HSDs. *Molecular & Cellular Endocrinology* 2006; 248: 141–148.
- [227] Vihko P, Herrala A, Harkonen P, Isomaa V, Kaija H, Kurkela R, Li Y, Patrikainen L, Pulkka A, Soronen P, Torn S. Enzymes as modulators in malignant transformation. *Journal of Steroid Biochemistry & Molecular Biology* 2005; 93: 277–283.
- [228] Geissler W, Davis D, Wu L, Bradshaw K, Patel S, Mendonca B, Elliston K, Wilson J, Russell D, Andersson S. Male pseudohermaphroditism caused by mutations of testicular 17 $\beta$ -hydroxysteroid dehydrogenase 3. *Nature Genetics* 1994; 7: 34–39.
- [229] Rasiah KK, Gardiner-Garden M, Padilla FJ, Moller G, Kench JG, Alles MC, Eggleston SA, Stricker PD, Adamski J, Sutherland RL, Henshall SM, Hayes VM. HSD17B4 overexpression, an independent biomarker of poor patient outcome in prostate cancer. *Molecular & Cellular Endocrinology* 2009; 301: 89–96.
- [230] Jin Y, Penning TM. Aldo–keto reductases and bioactivation/detoxication, *Annual Review of Pharmacology & Toxicology* 2006; 47: 263–292.
- [231] Stanbrough M, Bubley GJ, Ros K, Golub TR, Rubi MA, Penning TM, Febbo PG, Balk SP. Increased expression of genes converting adrenal androgens to testosterone in androgen independent prostate cancer. *Cancer Research* 2006; 66: 2815–2825.
- [232] Biswas MG, Russell DW. Expression cloning and characterization of oxidative 17 $\beta$ - and 3 $\alpha$ -hydroxysteroid dehydrogenases from rat and human prostate. *Journal of Biological Chemistry* 1997; 272: 15959–15966.
- [233] Prehn C, Moller G, Adamski J. Recent advances in 17 $\beta$ -hydroxysteroid Dehydrogenases. *Journal of Steroid Biochemistry & Molecular Biology* 2009; 114, 72–77.
- [234] Haynes BP, Straume AH, Geisler J, A'Hern R, Helle H, Smith IE, Lønning PE, Dowsett M. Intratumoral estrogen disposition in breast cancer, *Clinical Cancer Research* 2010; 16: 1790–1801.
- [235] Fomitcheva J, Baker ME, Anderson E, Lee GY, Aziz N. Characterization of Ke 6, a new 17 $\beta$ -hydroxysteroid dehydrogenase, and its expression in gonadal tissues. *Journal of Biological Chemistry* 1998; 273: 22664–22671.
- [236] Maxwell MM, Nearing J, Aziz N. Ke 6 gene. Sequence and organization and aberrant regulation in murine polycystic kidney disease, *Journal of Biological Chemistry* 1995; 270: 25213–25219.
- [237] Su J, Lin M, Napoli JL. Complementary deoxyribonucleic acid cloning and enzymatic characterization of a novel 17 $\beta$ /3 $\alpha$ -hydroxysteroid/retinoid short chain dehydrogenase/reductase. *Endocrinology* 1999; 140: 5275–5284.
- [238] Froyen G, Corbett M, Vandewalle J, Jarvela I, Lawrence O, Meldrum C, Bauters M, Govaerts K, Vandeleur L, Esch H, Chelly J, Sanlaville D, Bokhoven H, Ropers HH, Laumonnier F, Ranieri E, Schwartz CE, Abidi F, Tarpey PS, Futreal PA, Whibley A, Raymond FL, Stratton MR, Fryns JP, Scott R, Peippo M, Sipponen M, Partington M, Mowat D, Field M, Hackett A, Marynen P, Turner G, Gecz J. Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation. *American Journal of Human Genetics* 2008; 82: 432–443.

- [239] Yang SY, He XY, Miller D. HSD17B10: a gene involved in cognitive function through metabolism of isoleucine and neuroactive steroids, *Molecular Genetics & Metabolism* 2007; 92: 36–42.
- [240] Brereton P, Suzuki T, Sasano H, Li K, Duarte C, Obeyesekere V, Haeseleer F, Palczewski K, Smith I, Komesaroff P, Krozowski Z. Pan1b (17 $\beta$ HSD11)- enzymatic activity and distribution in the lung, *Molecular & Cellular Endocrinology* 2001; 171: 111–117.
- [241] Day JM, Foster PA, Tutill HJ, Parsons MF, Newman SP, Chander SK, Allan GM, Lawrence HR, Vicker N, Potter BV, Reed MJ, Purohit A. 17 $\beta$ hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *International Journal of Cancer* 2008; 122: 1931–1940.
- [242] Luu-The V, Tremblay P, Labrie F. Characterization of type 12 17 $\beta$ hydroxysteroid dehydrogenase (17 $\beta$ -HSD12), an isoform of type 3 17 $\beta$ -hydroxysteroid dehydrogenase responsible for estradiol formation in women. *Molecular Endocrinology* 2006; 20: 437–443.
- [243] Horiguchi Y, Araki M, Motojima K. 17 $\beta$ -hydroxysteroid dehydrogenase type 13 is a liver-specific lipid droplet-associated protein. *Biochemical Biophysical Research Communications* 2008; 370: 35–238.
- [244] Lukacik P, Keller B, Bunkoczi G, Kavanagh KL, Lee WK, Adamski J, U. Oppermann. U Structural and biochemical characterization of human orphan DHRS10 reveals a novel cytosolic enzyme with steroid dehydrogenase activity. *Biochemical Journal* 2007; 402: 419–427.
- [245] Jansson AK, Gunnarsson C, Cohen M, Sivik T, Stal O. 17 $\beta$ -hydroxysteroid dehydrogenase 14 affects estradiol levels in breast cancer cells and is a prognostic marker in estrogen receptor-positive breast cancer. *Cancer Research* 2006; 66: 11471–11477.
- [246] Day JM, Tutill HJ, Purohit A & Reed MJ. Design and validation of specific inhibitors of 17 $\beta$ -hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. *Endocrine-Related Cancer* 2008; 15: 665–692.
- [247] Day JM, Foster PA, Tutill HJ, Parsons MF, Newman SP, Chander SK, Allan GM, Lawrence HR, Vicker N, Potter BV. 17 $\beta$ hydroxysteroid dehydrogenase type 1, and not type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *International Journal of Cancer*; 2008 122: 1931–1940.
- [248] Yang SY, He XY, Schulz H. Multiple functions of type 10 17 $\beta$ hydroxysteroid Dehydrogenase. *Trends in Endocrinology & Metabolism* 2005; 6: 167–175.
- [249] Vihko P, Herrala A, Harkonen P, Isomaa V, Kaija H, Kurkela R, Pulkka A. Control of cell proliferation by steroids: the role of 17HSDs. *Molecular & Cellular Endocrinology* 2006; 248: 141–148.
- [250] Vihko P, Herrala A, Harkonen P, Isomaa V, Kaija H, Kurkela R, Li Y, Patrikainen L, Pulkka A, Soronen P, Torn S. Enzymes as modulators in malignant transformation. *Journal of Steroid Biochemistry & Molecular Biology* 2005; 93: 277–283.
- [251] Peltoketo H, Luu-The V, Simard J, Adamski J. 17 $\beta$ -Hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. *Journal of Molecular Endocrinology* 1999; 23: 1–11.

- [252] Geissler W, Davis D, Wu L, Bradshaw K, Patel S, Mendonca B, Elliston K, Wilson J, Russell D, Andersson S. Male pseudohermaphroditism caused by mutations of testicular 17 $\beta$  hydroxysteroid dehydrogenase 3. *Nature Genetic* 1994; 7: 34–39.
- [253] Day MJ, Tutill HJ, Foster PA, Bailey HV, Heaton WB, Sharland CM, Vicker N, Potter BV, Purohit A, Reed MJ. Development of hormonedependent prostate cancer models for the evaluation of inhibitors of 17beta-hydroxysteroid dehydrogenase type 3, *Molecular & Cellular Endocrinology* 2009; 301: 251–258.
- [254] Neocleous V, Sismani C, Shamma C, Efstathiou E, Alexandrou A, Ioannides M, Argyrou M, Patsalis PC, Phylactou LA, Skordis N. Duplication of exons 3-10 of the HSD17B3 gene: A novel type of genetic defect underlying 17 $\beta$ -HSD-3 deficiency. *Gene* 2012; 499: 250-255.
- [255] Faienza MF, Giordani L, Delvecchio M, Cavallo L. Clinical, endocrine, and molecular findings in 17beta-hydroxysteroid dehydrogenase type 3 deficiency. *Journal of Endocrinological Investigations* 2008; 31: 85-91.
- [256] Penning TM, Byrns MC. Steroid hormone transforming aldo-keto reductases and cancer. *Annals of New York Academy of Sciences* 2009; 1155: 33–42.
- [257] Kissinger CR, Rejto PA, Pelletier LA, Thomson JA, Showalter RE, Abreo MA, Agree CS, Margosiak S, Meng JJ, Aust RM, Vanderpool D, Li B, Tempczyk-Russell A, Villafranca JE. Crystal structure of human ABAD/HSD10 with a bound inhibitor: implications for design of Alzheimer's disease therapeutics. *Journal of Molecular Biology* 2004; 342; 943–952.
- [258] Du Yan S, Zhu Y, Stern ED, Hwang YC, Hori O, Ogawa S, Frosch MP, Connolly ES Jr., Taggert RMc, Pinsky DJ, Clarke S, Stern DM, Ramasamy R. Amyloid beta-peptide-binding alcohol dehydrogenase is a component of the cellular response to nutritional stress. *Journal of Biological Chemistry* 2000; 275: 27100–27109.