Chapter 6

Hydrohysteroid Dehydrogenases – Biological Role and Clinical Importance – Review

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1. Introduction

Hydroxysteroid dehydrogenases (HSDs) belong to the NADPH/NAD+-dependent oxidoreductases, which interconvert ketones and the corresponding secondary alcohols. As their names imply, they catalyze the oxidoreduction in different positions of steroidal substrates (3α-, 3β-, 11β-, 17β-, 20α- and 20β-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 occured 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 5α-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 17β-HSD enzyme [10].

In this review, we summarise the data from the literatute and our own data on the main HSDs (11β-HSD, 3β-HSD 17β-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 deceases.

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 xydroxylation (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 (P450scc, *CYP11A1)*, cytochrome P450 17α-hydroxylase (P450c17, 17α-hydroxylase, 17-20 lyase, *CYP17A1*), P450 aromatase (aromatase, *CYP19A1*), 21α-hydroxylase (*CYP21A*), 11β-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: 3β-hydroxysteroid dehydrogenase (3β-HSD),11β-hydroxysteroid dehydrogenase (11β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-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 P450scc 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 P450c17, which catalyzes the conversion of the C21 steroids pregnenolone or progesterone to the C19 steroids dehydroepiandrosterone or androstenedione, respectively; 2) 3β-HSD (Δ5-Δ4 isomerase), which catalyzes the conversion of the Δ5 hydroxysteroids - pregnenolone or dehydroepiandrosterone to the Δ4 ketosteroids - progesterone or androstenedione, respectively; 3) 17β-HSD (17-ketosteroid reductase), which catalyzes the final step in the biosynthesis of testosterone [16].

Corticosteroids (mineralocorticoids and glucocorticoids, C-21 cabons) derive from progestagens (progesterone and 17α-OH progesterone) after hydroxylation of carbon-21 by the enzyme 21α-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 11β-hydroxylase (for corticosterone). These enzymes are nearly identical (they share 11β-hydroxylation and 18-hydroxylation functions). Aldosterone synthase is also able to perform 18-oxidation. 11β-hydroxysteroid dehydrogenase (11β-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 pathays/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 P450scc 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 3β-HSD and P450c17 catalyze the overall conversion of pregnenolone to androstenedione, the precursor of testosterone. This conversion can occur via one of two main pathways, either via Δ4 or Δ5 pathway and the preferred route is both species- and age-dependent. [14] (Figure 2.).

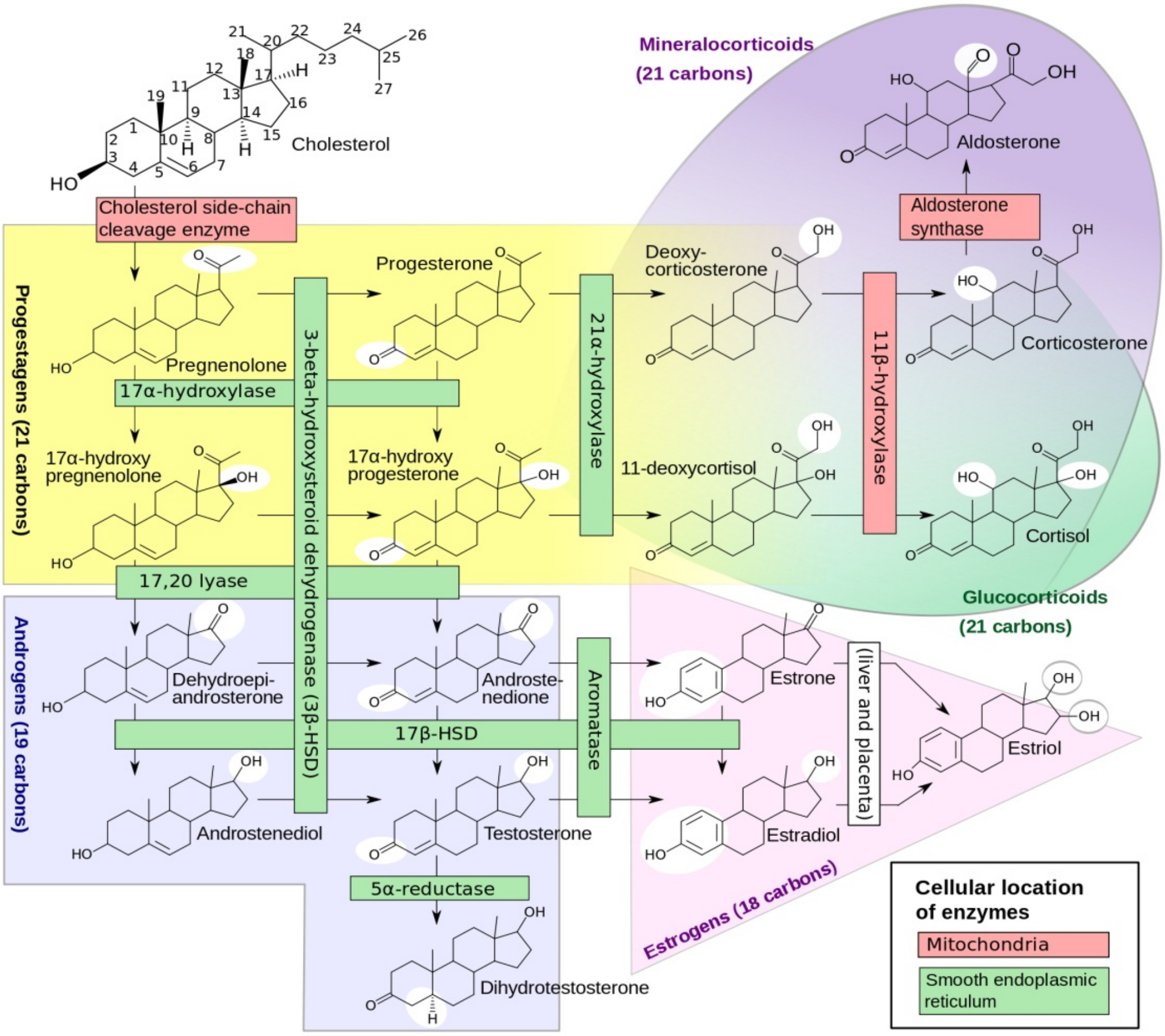

Figure 1. Pathways of human steroidogenesis [12].

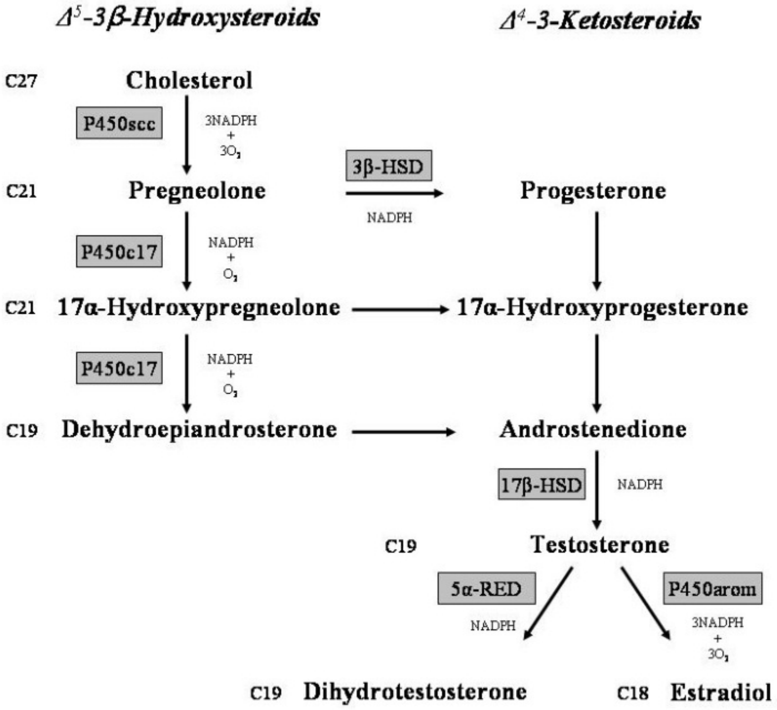

Figure 2. Steroid biosynthetic pathways as adapted to Payne, 2007 [16]

The Δ4 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 Δ5 pathway predominates in the adult and fetal testis because human P450c17 enzyme readily converts 17*α*-hydroxypregnenolone to dehydroepiandrosterone (DHEA), but has little emzyme activity when 17*α*-hydroxyprogesterone is the substrate. In the rat, P450c17 readily cleaves both the Δ4 and Δ5 C21 steroids, but in contrast to the human, it has a preference for the Δ4 pathway. In the mouse the Δ4 pathway dominates before puberty but in adult animals the Δ5 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. 3β-HSD gene family – function, tissues distribution, regulation and clinical importance

The 3β-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 3β-HSD controls the critical steroidogenic reactions in the adrenal cortex, gonads, placenta, and peripheral target tissues [31]. The 3β-HSD isoforms catalyze the conversion of the Δ5-3β-hydoxysteroids - pregnenolone, 17α-hydroxypregnenolone, and DHEA, to the Δ4-3-ketosteroids - progesterone, 17α-hydroxyprogesterone, and androstenedione, respectively. Two sequential reactions are involved in the conversion of the Δ5-3β-hydroxysteroid to a Δ4-3 ketosteroid. The first reaction is the dehydrogenation of the 3β-hydroxysteroid, requiring the coenzyme NAD^+, yielding the Δ5-3-keto intermediate, and reduced NADH. The reduced NADH, activates the isomerization of the Δ5-3- keto steroid to yield the Δ4-3-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 3β-HSD activity was detected to the smooth endoplasmic reticulum and mitochondrial cristae and later in the microsomal fraction suggesting that 3β-HSD is a membrane-associated enzyme [16]. Submitochondrial fractionation studies showed that 3β-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, 3β-HSD activity could be preferentially distributed to the mitochondria under certain physiological conditions [35, 36].

Isoforms: Structural studies of 3β-HSD family characterized several isoforms, products of distint 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) 3β-HSD, six in mouse, four in rat and three in hamster. Multiple 3β-HSD isoenzymes have been cloned from several other species, further illustrating that the 3β-HSD gene family is conserved in vertebrate species The human type I 3β-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 h*HSD3B1* and h*HSD3B2* 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 3β-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 17β-HSD activity specific to 5α-androstane-17β-ol steroids, thus suggesting a key role in controlling the bioavailibility 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 possition. [47].

To date, six distinct cDNAs encoding murine members of the 3β-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 - 3β-HSD types I, II and III function as dehydrogenase/isomerases, and are essential for the biosynthesis of active steroid hormones whereas 3β-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 3β HSD I is expressed in gonads and adrenal gland, whereas 3β-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, 3β-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 3β-HSD I has 84% identity to mouse VI, and 71% identity to human II [31, 50].

Tissue distribution: As 3β-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 3β-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 3β-HSD is not expressed to a high degree in the fetal cortex, P450c17 is expressed, thereby directing the steroidogenic pathway toward Δ5-hydroxysteroid (*i.e.*, DHEA) production. There is zone-specific steroid secretion pattern dependent on the relative expression levels of 3β-HSD, P450c17 and P450 21α-hydroxylase (P450c21) that serve as molecular markers of the adrenocortical developmental state [51, 52]. After birth, the coexpression of 3β-HSD and P450c21 leads to aldosterone production, whereas the coexpression of 3β-HSD and P450c17 results in production of cortisol. The expression of P450c17 along with low levels of 3β-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 Adrenocorticotropic hormone (ACTH), Epidermal Growth Factor (EGF), Fibroblast Growth Factors (FGFs), Insulin-like Growth Factors (IGFs), thyroid hormone (T3), Transforming Growth Factor-β (TGFβ) [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 3β-HSD and other steroidogenic enzymes *in vivo*.

Ovary: Ontological studies for 3β-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]. 3β-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 3β-HSD regulation in polycystic thecal cells although the mechanisms are unclear [58].

Preantral/antral *follicular* expression studies show 3β-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, 3β-HSD has been shown to have different expression patterns. In the rat, preantral, antral, and preovulatory rat follicles showed 3β-HSD expression in the theca, but no expression was seen in the granulosa layer [60]. In contrast to rodents, pigs, and primates, 3β-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 3β-HSD expression concomitantly with other steroidogenic enzymes. The role of prolactin (PRL) on primate 3β-HSD is unclear, although PRL was shown to be inhibitory. Interestingly in postmenopausal women 30% of circulating Δ4-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 3β-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 3β-HSD protein and mRNA levels in human granulosa-lutal cells, and this effect could be enhanced by insulin [66]. Although the direct control of 3β-HSD by PRL in humans has yet to be demonstrated, PRL has been shown to up-regulate 3β-HSD transcriptional activity *in vitro* [67]. During regression of CL (luteolysis) the expression of 3β-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 5α-Reductase (5α-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 3β-HSD as early as 18 wk of gestation. During gestation in human, 3β-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 3β-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 3β-HSD is highly applicable as a marker for visualization both, fetal and adult LC. The expression of 3β-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 form adult or fetal LC that is very helpful to study kinetic and differentiation pattern of LCs (Figure 4) [71]. Application of IHC for 3β-HSD is widely used by many authors in quantification studies of LC under normal and experimental/pathological conditions, especially those of hormonal manipulations [72]. 3β-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 3β-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, TGFβ, FGFs, Activin A) are also suggested to control the expression of 3β-HSD [31].

Peripheral tissues; Expression of 3β-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 3β-HSD activity [75], and 3β-HSD protein was seen in 36% of breast carcinoma samples tested [76, 77]. The 3β-HSD expressed in human placenta is the peripheral isoform, type I 3β-HSD, and it is under differential regulatory control than the adrenal/gonadal isoform, type II 3β-HSD [78, 79]. In the prostate epithelium 3β-HSD expression was colocalized with 17β-HSD type V in normal conditions. 3β-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 3β-HSD expression is presumed to be important in the metabolism and inactivation of steroids. 3β-HSD activity in human liver microsomes was shown to be three times higher for the reduction of DHT to 3Δ-DIOL than the reverse reaction [82]. The circulating levels of steroids might affect regulation of 3β-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 3β-HSD was confined to keretinocytes, co-expressed with 17β-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]. 3β-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, 3β-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]

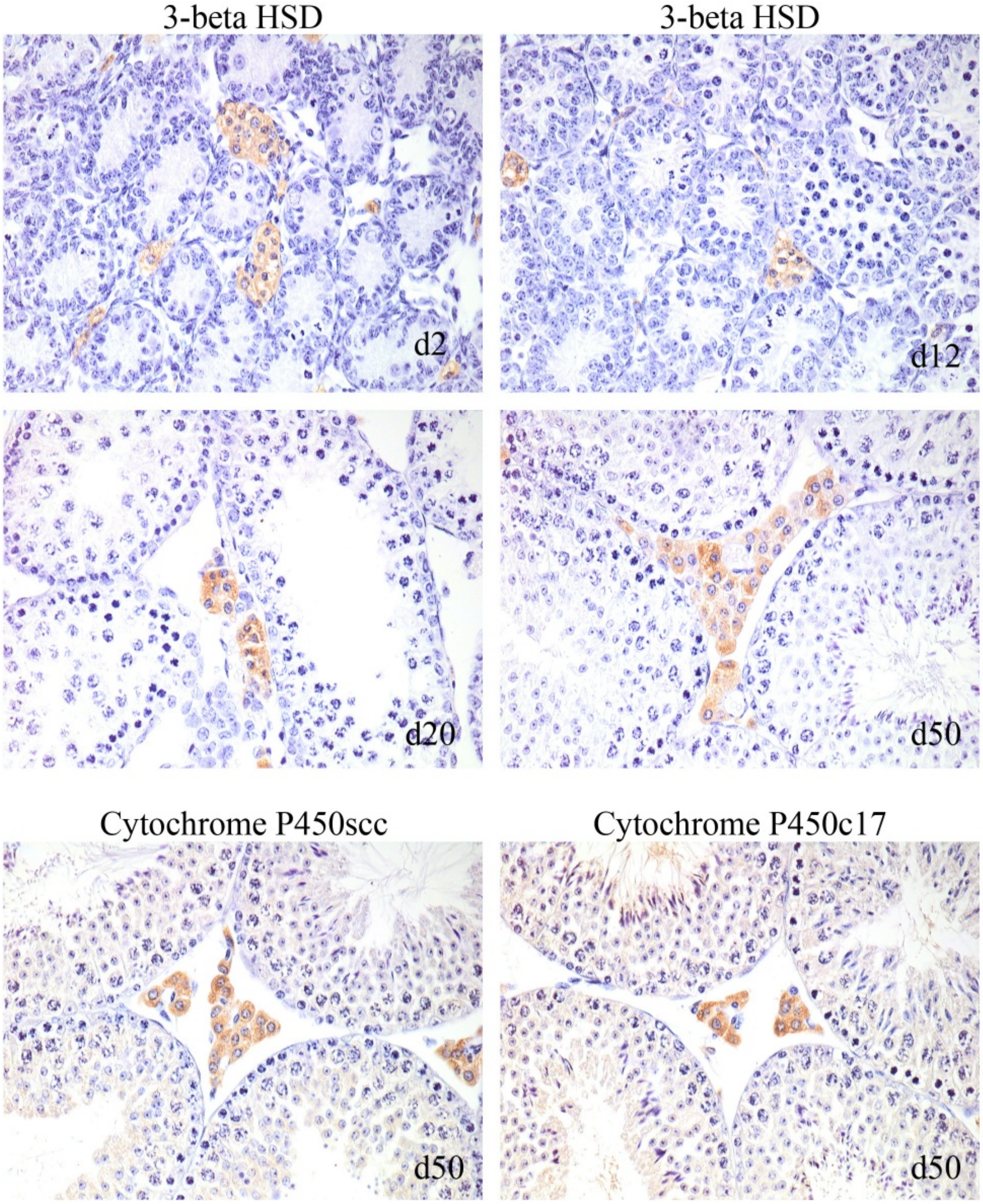

Figure 3. Immunoexpression of steroideogenic ezymes (3β-HSD, cytochrome P450scc and cytochrome P450c17 in the Leydig cells (DAB-brown) of postnatal mouse testis after birth to sexual maturity (d2-neonatal, d12-prepubertal, d20-pubertal, d50-adult) x400.

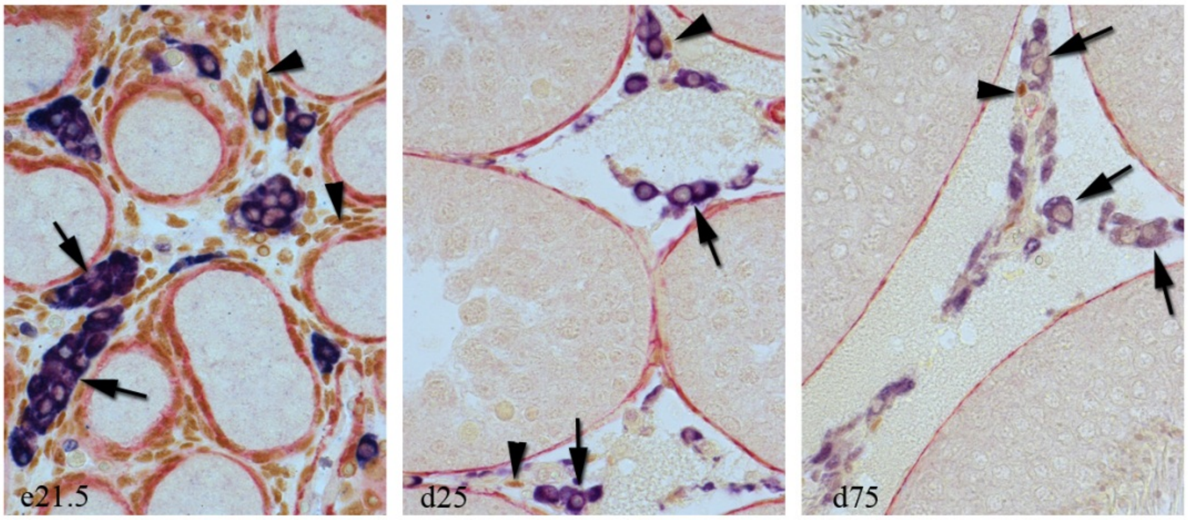

Figure 4. Triple immunostaining for 3β-HSD (blue), α-smooth muscle actin (red) and COUP TFII (brown) in fetal (embryonal day 21.5) and postnatal (pubertal-d25 and adult-d75) rat testes. Fetal and adult LCs (arrows) are clrearly distinguishable from presumptive ptogenitors cells (arrowheads) x400.

Regulation: The regulation of 3β-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 3β-HSD expression but mechanisms by which cAMP stimulate transcription of the *HSD3B2* gene are not clear yet. [31].

Gonadal expression of human 3β-HSD II and mouse 3β-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, P450scc, and 3β-HSD [92]. The exact mechanisms by which DAX-1 overexpression affects 3β-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 TGFβ 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 3β-HSD expression. For example, glucocorticoids stimulate the expression of 3β-HSD in adrenal cells [98], whereas androgens inhibit 3β-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 3β-HSD genetic deficiency:

Homozygous mutations in *HSD3B1* are lethal in human due to interruption of pregnancy before the end of the first trimester because 3β-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 3β-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 3β-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 11β-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 3β-HSD deficiency present hypospadias. On the other hand, the complete or partial inhibition of 3β-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 3β-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 Δ5-3β-hydroxy steroids such as pregnenolone (PREG), 17OH-PREG, and DHEA are elevated in affected individuals. An elevated ratio of Δ5/Δ4-steroids is considered to be the best biological parameter for the diagnosis of 3β- 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 3β-HSD deficiency. It is well recognized that plasma levels of 17OH-PROG and Δ4-DIONE and other Δ4-steroids are frequently elevated in 3β-HSD-deficient patients. Such observations are consistent with a functional type I 3β-HSD enzyme that is expressed in peripheral tissues. Moreover, the peripheral type I 3β-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. 11β-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 11β-hydroxysteroid dehydrogenases (11β-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 11β-HSD (Figure 5, [108]). To date, two isoforms of 11β-HSD have been identified: 1) 11β-HSD1 acts predominantly as an NADP(H)-dependent reductase that converts inactive circulating 11-ketosteroids, into active glucocorticoids generating active cortisol or corticosterone; 2) 11β-HSD2 is a high affinity NAD+-dependent enzyme that catalyses the inactivation of glucocorticoids [102-107]. Although the biochemistry of 11β-HSD is well established, the physiological significance of glucocorticoid metabolism by these enzymes is still not fully

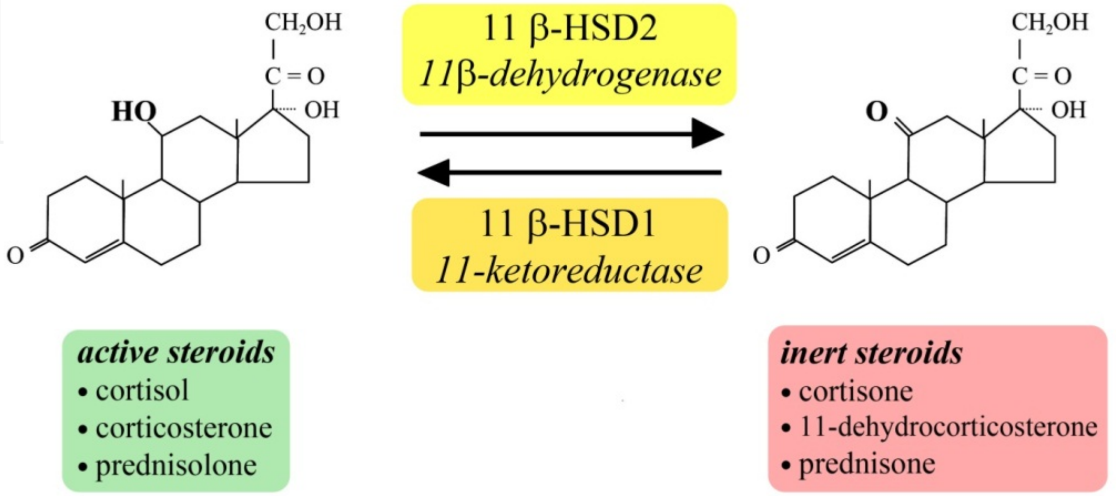

Figure 5. 11β-hydroxysteroid dehydrogenase (11β-HSD) (Adopted by Seckl et al., 2004 [108])

understood. The enzymatic inactivation of cortisol and corticosterone by 11β-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 11β-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 11β-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 11β-HSD in relation to metabolite syndrome.

5. 11β-hydroxysteroid dehydrogenase in developing testis- marker for differentiation of the Leydig cells

The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) is hypothesized to modulate LCs steroidogenesis by controlling the intracellular concentration of glucocorticoids. By doing so, 11β-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 11β-HSD1 [117]. Both isoforms of 11β-HSD are localized in testicular LCs [118-121]. Recent studies showed that reductase activity predominates in both human and rat type 1 11β-HSD [109]. In contrast, the other 11β-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 11β-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 11β-HSD1 in modulating intracellular corticosterone concentrations and, in turn, for a direct effect of glucocorticoids on LCs. On the other hand, 11β-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 11β-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 11β-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 11β-HSD in the rat LCs on 35 pnd. The developmental pathway of ALCs population is accompanied with an increase in the 11β-HSD activity and thus the enzyme can be used as a marker for steroidogenic differentiation of LCs [112,124,126,127]. Examination of 11β-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 11β-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 11β-HSD oxidative activity [109,125] and enzymatic behavior of 11β-HDS in LCs is not consistent with the presence of type 1 alone [127,128]. Developmental analysis of 11β-HSD in rat LCs revealed that 11β-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 11β-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 11β-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, 11β-HSD type 2 may also play a protective role in blunting the suppressive effects of glucocorticoids on LCs steroidogenesis. The inhibition of 11β-HSD1 predominantly lowered reductase activity whereas by inhibition of 11β-HSD2 alone, the oxidase activity was more prominently suppressed [131]. Recently, it has been reported that products such 7α-hydroxytestosterone significantly switched 11β-HSD1 oxidoreductase activities toward reductase in developing rat testis and thus regulates the direction of 11β-HSD1 activity in LCs [132]. It seems that the switch of 11β-HDS activity from reduction to oxidation during the transition from PLCs to ALCs [109] can be associated with the presence of 11β-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 11β-HSD oxidative and reductive activities [144]. When oxidation predominates over reduction, 11β-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 11β-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 11β-HSD during renewal of LCs population [133]. The quantitative immunohistochemical analysis of 11β HSD2 pattern after EDS treatment revealed progressive increases in the reaction intensity during postnatal development (on d 21after 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 11β-HSD2 expression are consistent with previous data about structural and functional maturation of the new population of LCs after EDS [134,135]. Therefore, 11β-HSD2 can be a useful marker for ALCs differentiation and the reaction intensity might be associated with increased 11β-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 11β-HSD2 gene that is in parallel with enhanced 11β-HSD2 enzyme activity during postnatal development [136]. Together with previous studies [126] the data from EDS model suggest the relationship between 11β-HSD and kinetics of spermatid differentiation and restoration of T production by new LCs population.

6. 11β-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 3β-HSD, a key marker for LCs steroidogenic activity but also through the inhibition of 11β-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 11β-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 11β-HSD type 2 in LCs with aging implies suppression in 11β-HSD oxidative capacity resulting in elevated inhibitory potency of corticosterone on T production [136]. The reduced expression of 11β-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 11β-HSD 2 oxidative activity by treatment with 11β-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 11β-HSD leading to impaired LCs steroidogenesis [147]. Therefore, the reduction of 11β-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)

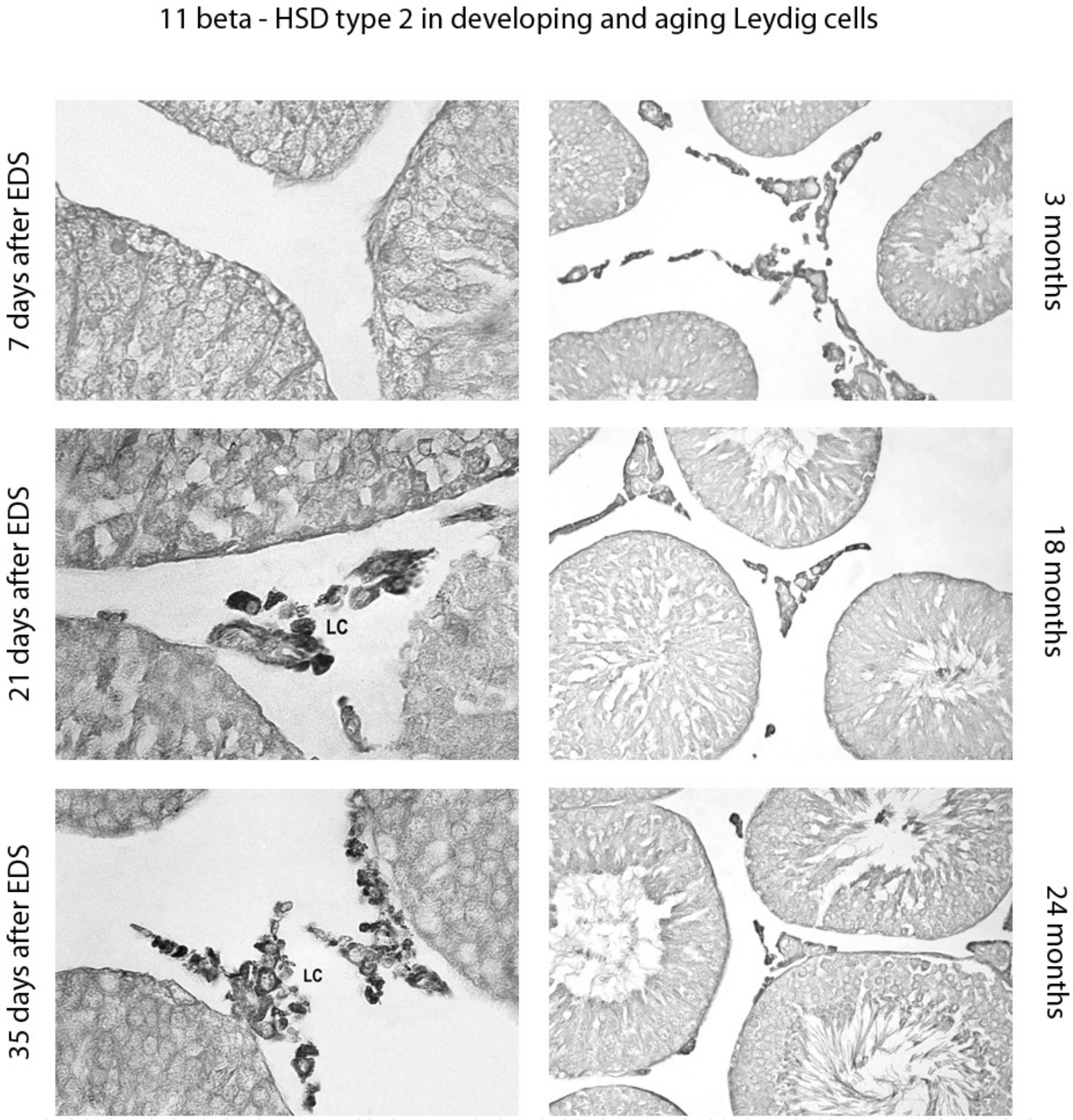

Figure 6. 11β-HSD type 2 in developing Leydig cells (LC)- 7, 21 and 35 days after EDS; and aging Leydig cells- 3, 18 and 24-months of age. x 400.

7. 11β-hydroxysteroid dehydrogenase in the adrenal gland - expression profile under conditions of testosterone withdrawal

As mentioned above, the enzyme11β-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 11β-HSD allows access of cortisol or corticosterone to the mineralcorticoid receptors where they act as mineralcorticoids [148]. Northern blot analyses revealed expression of mRNAs encoding both 11β-HSD1 and 11β-HSD2 in the whole rat adrenal gland. *In situ* hybridization of rat adrenal cortex and medulla demonstrated specific localization of 11β 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, 11β-HSD2 mRNA was more uniformly distributed in the cortex and was low/absent in the medulla [149, 150]. The expression of 11β-HSD2 has been demonstrated in rat adrenal gland by immunohistochemical and molecular analyses and the 11β-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 11β-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 11β-HSD2 expression correlated with the changes of serum T levels following the exposure after EDS [153]. The lowest 11β-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 11β-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 11β-dehydro product in an NAD+-dependent manner in adrenal gland [151]. Using enzymehistochemistry a strong reduction was found in the activity of $NADH_2$-cytochrome-C-reductase that is involved in NAD+-synthesis as a cofactor in the adrenal gland after EDS treatment of adult rats [155]. Immunohistochemical analysis revealed that the 11β-HSD2 expression pattern in adrenal gland of EDS treated rats [152] is very similar to the enzymehistochemical profile of $NADH_2$- cytochrome-C-reductase [155], supporting the view that 11β-HSD2 acts as high-affinity NAD+-dependent dehydrogenase in the rat adrenal gland [151]. On the other hand, the increase in the expression of 11β-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 11β-HSD2 (high-affinity NAD+- 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 11β-HSD2 in the adrenal gland under conditions of testosterone withdrawal. The EDS results bring additional elucidation on the functional significance of 11β-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 11β-HSD2 expression in adrenal gland and kinetics of restoration of testosterone production during renewal of testicular adult LCs population after EDS treatment. (Figure 7)

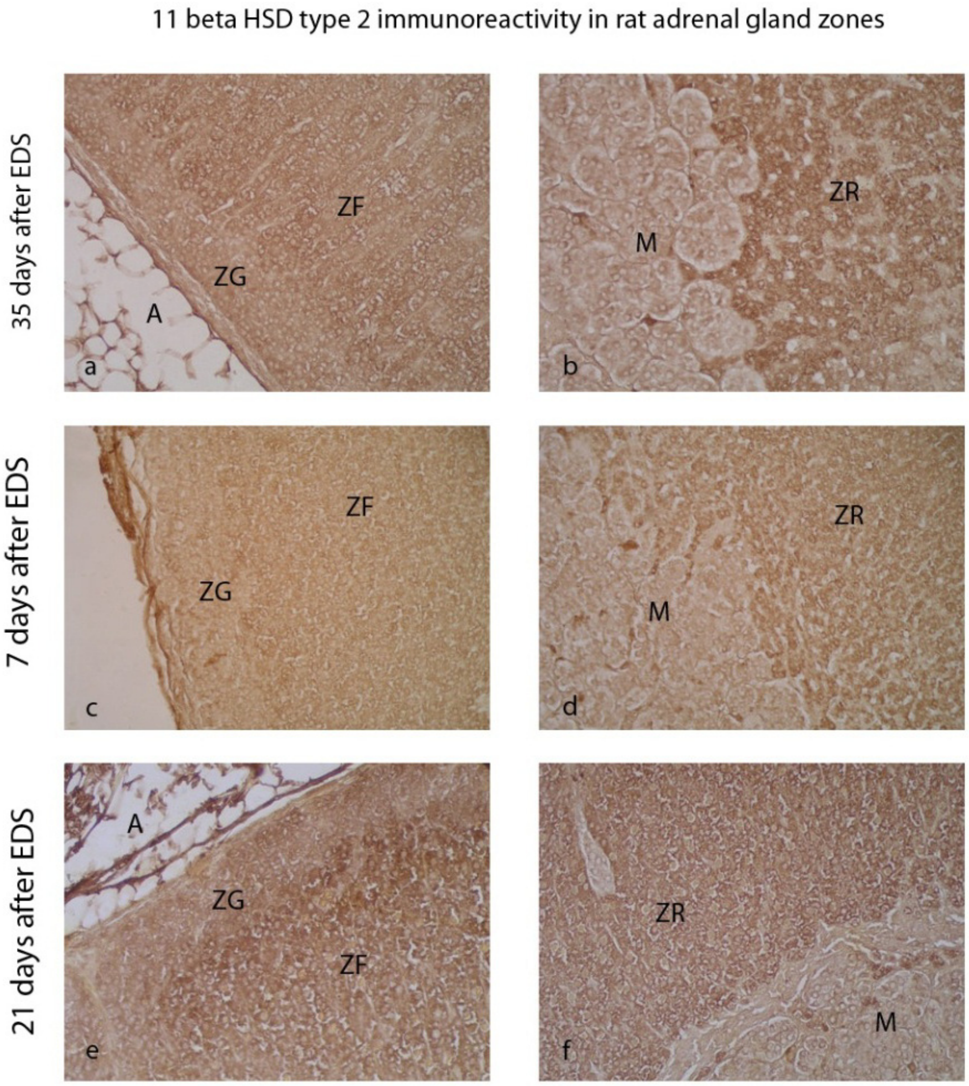

Figure 7. 11β-HSD2 immunoreactivity in rat adrenal gland zones. 35 days after EDS (a, b); 7 days after EDS (c, d); 21 days after EDS (e, f). 11β-HSD2- immunoreactivity in the zona fasciculata (ZF) and zona reticularis (ZR), and the adipocytes of adrenal capsula adipose (A). Less sensitive were the adrenocorticocytes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

8. 11β –hydroxysteroid dehydrogenase in the ovary – cellular localization/distribution and relation to Polycistic 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 11β-HSD enzymes play a crucial role in controlling the tissue concentration of cortisol. The two types of 11β-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 11β-HSD types 1 and 2 is well documented. 11β-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 11β-HSD type 1 is only seen in granulosa cells from preovulatory follicles [161]). As a result, developmentally regulated pattern of 11β-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 11β-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 11β-HSD1 but not of 11β-HSD2 mRNA expression [175]. Previous studies have described an increased 5alpha-reduction of cortisol and impaired regeneration of cortisol from cortisone by 11β-HSD1 in PCOS, supporting the concept of an altered cortisol metabolism in POCS [176].

In the rare syndrome of cortisone reductase deficiency, impaired ability of 11β-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 11β-HSD1, and female patients affected by cortisol 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 11β-HSD1 activity [179]. Gambineri et al., [180] reported that polymorphism, predicting lower peripheral regeneration of cortisol by 11β-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 11β-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. 11β –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 11β-HSD1 that converts inactive metabolite cortisone to active cortisol, thereby amplifying local glucocorticoid action [104]. 11β-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 11β-HSD1 in abdomenal adipose tissue [191]. In human subcutaneous abdominal adipose tissue, 11β-HSD1 activity is increased both *in vivo* and *in vitro* and the enhanced 11β-HSD1 activity in biopsies is accompanied by elevated 11β- HSD1 mRNA levels [108]). It is interesting to note, that increased subcutaneous adipose 11β-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 11β-HSD1 activity in obesity and metabolic syndrome are still not fully inderstood. 11β-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 11β-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 11β-HSD1 selectively in adipose tissue have been generated, using the adipocyte fatty acid binding protein (aP2) promoter [196, 197]. The adipose-selective 11β-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 11β-HSD1 in liver showed an attenuated metabolic syndrome with modest insulin resistance and hypertriglyceridemia, hypertension and fatty liver, but with normal body weight [198]. 11β-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 11β-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 11β-HSD1 expression and increased whole-body 11β-HSD1 activity, associated with increased prevalence of the metabolic syndrome. These findings strengthen the view that variations in 11β-HSD1 activity influence the metabolic profile and provide a new evidence that *HSD11B1* gene influence enzyme activity *in vivo* [186].

10. 11β-HSD and metabolite syndrome - clinical importance

Based on human data and genetic models, 11β-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, 11β-HSD1 expression is elevated in adipose tissue in obesity [206], whereas inhibition of 11β -HSD1 enhances insulin sensitivity and provides a new approach to treat type 2 diabetes [207-209]. Polymorphisms in the HSD11B1 gene that encodes 11β-HSD1 have been associated with type 2 diabetes [203] and hypertension [204, 205]. On the other hand, a polymorphism that predicts 11β-HSD1 deficiency may protect against obesity and its metabolic consequences because of impaired regeneration of cortisol in adipose tissue [180]. 11β-HSD1 inhibition is a tempting target for treatment of the metabolic syndrome and its complications. Selective 11beta-HSD1 inhibitors in rodents cause weight loss, improve insulin sensitivity and delay progression of cardiovascular disease [210-212]. Pharmacological inhibition of 11b-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, 11β-HSD1 gene knock-out (11β-HSD1-/-) 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 11β-HSD1 inhibitors to lower intracellular glucocorticoid levels and to treat both obesity and its metabolic complications.

11. 11β –hydroxysteroid dehydrogenase and pregnancy – role of 11b-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 11β-HSD2 that catalyses the rapid conversation of active cortisol and corticosterone to physiologically inert cortisone and corticosterone [219]. 11β-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 11β-HSD2 expression - progesterone, estrogen, hypoxia, infection and proinflammatory cytokines reduce placental 11β-HSD2 activity. Conversely, placental 11β-HSD2 activity is stimulated by glucocorticoids, retinoids and leptin [221]. Studies in rats and human indicate that the deficiency in placental 11β-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 11β-HSD have low birth weight. Intrauterine growth retardation in human is associated with increased fetal cortisol levels and reduced placental 11β-HSD2 activity [217]. Studies on prenatal exposure to 11β-HSD inhibitors such as glycyrrhetinic 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. 11β-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 11β-HSD2 is a key player in fetal programming [215, 216, 221].

12. 17β-HSD dehydrogenase and multifunstional izoforms: localization, function and relevance to clinical therapeutic strategies

17βHydroxysteroid dehydrogenases (17β-HSDs, 17HSD/KSRs) are NAD(H)- and/or NADP(H)-dependent enzymes that catalyze the oxidation and reduction of active 17β-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 afnity for their receptors in the 17β-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, 17β-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 17β-HSD3 and 17β-HSD14, 17β-HSD1 and 2 were thought to be exclusively converting sex steroids. However, the participation of the two latter enzymes (17β-HSD1 and 2) in retinoic acid metabolism recently was suggested. Other 17β-HSD types were already known to be multifunctional and some of them play important roles in different metabolic pathways.

17β-HSD7 is mainly involved in cholesterol synthesis, 17β-HSD4 is implicated in β-oxidation of fatty acids, 17β-HSD5 participates in both prostaglandin and steroid metabolism, and 17β-HSD12 is required in fatty acid elongation. 17β-HSD10 catalyzes the oxidation of short chain fatty acids. 17β-HSD6 and 9 play a role in retinoid conversion. For some 17β-HSDs, the physiological function is not yet clear. For several types of 17β-HSDs participation in the pathophysiology of human diseases has been postulated [225]. The specificity of each 17β-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 develpment	[10,226, 227]
3	HSD17B3	Steroid (androgen) synthesis	Pseudohermaphroditism in males associated with obesity, prostate cancer	[10,228]
4	HSD17B4	Fatty acid β-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, 3α-3β-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 MHBD 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 17β-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 multifunctionaloty 17β-HSD enzymes, the biological and clinical aspects of each isoform will be described separately.

17β-HSD type1: 17β-HSD1 catalyzes the activation of estrone (E1) to the most potent estrogen estradiol (E2), predominantly considered as an ezyme 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) 17β-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 17β-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 h17β-HSD1 is not fully estrogenen-specific but it possesses significant androgenic sctivity. The enzyme catalyses both oxidative (17-hydroxy to 17-keto) and reductive (17-keto to 17-hydroxy) 17b-HSD activity with a proper cofactor added in vitro. However, in cultured cells, the h17β-HSD1 has been shown to catalyse predominantly the reductive reaction [247]. Although h17β-HSD1 expression in various peripheral tissues is low, its catalytic efficacy is markedly higher than those measured for 17β-HSD7 and 17β-HSD12 [248, 242], suggesting an important role for 17β-HSD1 in peripheral E2 formation. Data from animal models further demonstrated the ability of h17β-HSD1 to enhance estrogen action in target tissues and its decrease after treating the mice with 17β-HSD1 inhibitors [10]. These data suggest that 17β-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 17β-HSD1 point out the pivotal role of 17β-HSD1 in breast cancer, ovarian tumor, endometriosis, endometrial hyperplasia and uterine leiomyoma [249, 250]. Consequently, inhibition of 17β-HSD1 is considered as a valuable therapeutic approach for treatment of these deseases. In vivo evaluation of 17β-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 17β-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 of17β-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 endometiosis. 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, 17β-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 17β-HSD1 inhibitor treatment. The data concerning the expression of 17β-HSD1 in normal and diseased human endometrium are not fully conclusive. However, in most of the studies, the 17β-HSD1 expression is detected in normal endometrium, endometriosis specimens and endometriotic cancer. Other 17β-HSD enzymes including 17β-HSD2, 17β-HSD5, 17β-HSD7 and 17β-HSD12 have also been detected in the endometrium under different pathological conditions like endometriosis and PCOS [10]. Collectively, the data suggest that 17β-HSD1 inhibition is one of the several possible approaches to reduce estrogen production both in eutopic and in ectopic endometrial tissue.

17β-HSD type-2: 17-HSD/KSR2 converts 17β-hydroxy forms of estrogens and androgens (estradiol, testosterone and 5α-dihydrotestosterone) to their less active 17-keto forms (estrone, androstenedione and 5α-androstanedione).The enzyme also possesses 20α-HSD activity, thereby activating 20α-hydroxyprogesterone to progesterone. The 17β-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 17β-HSD2 enzyme protects tissues from excessive steroid action [251]. 17β-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 17β-HSD2 expression in the placenta and in foetal liver and intestine, together with the observed oxidative 17β-HSD2activity, are the basis for the hypothesis, suggesting a role for the enzyme in lowering the sex steroid exposure of the foetus.

Phylogenic analyses have indicated that 17β-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 17β-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 17β-HSD2 for prenatal eye morphogenesis and eye development [10]. These TG mice overexpressing human 17β-HSD2 showed growth retardation, disrupted spermayogenesis, female masculinization, delayed eye opening, squint appearance of the eyes and some of these defects closely resembeled 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 17β-HSD2 provide evidence for the essential role of 17β-HSD2. Embryonic death in the HSD17B-KO mice is reported, related to lack of action of 17β-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 17β-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 17β-HSD2 inhibitors. Decrease in bone resorption and maintenance of bone formation was achieved in this experimental model.

17β-HSD type-3: 17-HSD/KSR3 17β-HSD3 converts Δ4-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 5α-androstanedione to 5α- dihydrotestosterone as well as estrone to estradiol [108]. Messender RNA for 17β-HSD3 are over-expressed in prostate cancer tissues. As T is known to be responsible for cell proliferation in androgen dependent diseases, 17β-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 17β-HSD3 inhibitors and strong suppression of tumor growth by 81% was found, suggesting that 17β-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 17β-HSD as rare mutation associated with 46XY disorder of sexual development [254]. Patients with 17β-HSD deficiency are usually classified as female at birth (although abdominal testes) but developed secondary male features at pubery with diminished virilization [255].

17β-HSD type-4: Among 17-HSD/KSRs, type 4 is an unique multifunctional enzyme consisting of 17-HSD/KSR-, hydratase- and sterol carrier 2-like domains. 17β-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 17β-HSD4 leads to disease known as Zellweger syndrome [251].

17β-HSD type-5: 17-HSD/KSR5 is also known as type 2 3α-HSD, and diferently from other 17-HSD/KSRs it belongs to the AKR (aldo-keto reductase) family. With other members of the AKR family (type 1 3α-HSD, type 3 3α-HSD and 20α-HSD), 17β-HSD5 shares 84%, 86% and 88% identity, respectively. Both human and mouse 17β-HSD5 catalyze the conversion of androstenedione to testosterone, and additionally possess 3α-HSD activity. Human 17β-HSD5 has been previously identified predominantly as 3α-HSD. Human, but not mouse, 17β-HSD5 also converts progesterone to 20α-dihydroprogesterone effectively. 17β-HSD5 appears to be involved in the formation of androgens in the testis and several peripheral tissues. Using specific probes and antibodies, human 17β-HSD5 has been localized in liver, adrenal, testis, basal cells of the prostate, and in prostatic carcinoma cell lines [251]. Recently, up-regulation of 17β-HSD5 was found in breast and prostate cancer [256].

17β-HSD type-6: 17-HSD/KSR6 is part of the catabolic cascade of 5α-dihydrotestosterone (DHT). The 17β-HSD6 shows low dehydrogenase activity with DHT, testosterone and estradiol and possesses a weak oxidative 3α-HSD activity. The 17β-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].

17β-HSD type-7: 17β-HSD7 is expressed in the developing follicles and in luteinized cells, being the enzyme of ovarian estradiol biosynthesis. Both rodent and human 17β-HSD7 catalyze exclusively the conversion of estrone to estradiol. The 17β-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, 17β-HSD7 mRNA has been detected in placental, mammary gland and kidney samples [251]. The 17β-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 17β-HSD7 in cholesterol biosynthesis was also suggested by the studies, showing a similar expression pattern of 17β-HSD7 and cholesterogenic enzymes during mouse embryonic development. Data from HSD17B7-KO mouse embryos evidently showed the essential role of 17β-HSD7 for cholesterol biosynthesis in vivo. The lack of 17β-HSD7 resulted in a marked blockage in foetal de novo cholesterol synthesis. Histological analysis revealed that the 17β-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 Tese data suggest a possible role of 17β-HSD7 in cholesterol biosynthesis in mice, while its role in E2 production in vivo needs further clarification [10].

17β-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 17βHSD8. In *in vitro* conditions, 17β-HSD8 converts most eficiently estradiol to estrone and, to some extent, it also catalyses oxidative reactions of androgens and the reduction from estrone to estradiol. The 17β-HSD8 is abundand in kidney, liver and gonads. Interestingly, in the ovary, 17β-HSD8 is present in cumulus cells and not in granulosa or luteal cells like 17βHSD1 and 7, respectively [251].

17β-HSD type-10: The 17β- -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 17β-HSD10 binds to amyloid-β peptide and appears to be up-regulated in patients suffering from this disease [225]. The mechanism by which 17β-HSD10 contributes to the pathology of AD is still not completely understood. The protein-protein interaction of 17β-HSD10 with amyloid-β appears to inhibit the enzymatic activity of 17β-HSD10. In vitro studies with a potent 17β-HSD10 inhibitor [257] have shown that inhibition of this enzyme can prevent its interaction with the amyloid-β peptide, suggesting 17β HSD10 as a potential target for the treatment of AD.Transgenic mice over-expressing human 17β-HSD10 suggesting that inhibition of 17β-HSD10 could protect from cerebral infarction and ischemia [258].

17β-HSD type-12: The mammalian 17β-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 17β-HSD12 share 40% sequence similarity with 17β-HSD3, and the data indicate that 17β-HSD12 is an ancestor of 17β-HSD3. In human and rodents, 17β-HSD12 is expressed universally and the highest expression of 17β-HSD12 is detected in tissues involved in the lipid metabolism, including the liver, kidney hearth, and skeletal muscle. In mice, the expression has also been detected in brown and white adipose tissue. 17β-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 17β-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 17β-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-derives), they failed to grow several mesoderm-derived structures. Therefore, the embryos at the age of E8.5–E9.5 were avoid 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 commont mechanisms operate in regulation of steroidogenesis, there are some differences/specificities between rodent and human, in particular the susceptibility of fetal testicular stereoidogenesis 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 dial with risk assessment of endocrine disruptors for human reproductive health. Species specific diffences in steroiodogenesis 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 desigh of new HSD inhibitors and development of new strategies for appropriate treatment of steroid-dependent deceases without use of invasive techniques.

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