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Hydroxysteroid Dehydrogenases – Localization, Function and Regulation in the Testis

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<http://dx.doi.org/10.5772/47740>

1. Introduction

Differentiation of the male phenotype including the outward development of secondary sex characteristics as well as the initiation and maintenance of spermatogenesis is stimulated by androgens (O'Shaughnessy et al., 2009; Verhoeven et al., 2010). There are two major androgens secreted by the testes: testosterone (T) and dihydrotestosterone (DHT). Weaker androgens: dehydroepiandrosterone (DHEA) and androstenedione are secreted in smaller amounts and converted metabolically to T and other androgens. Testosterone is the most abundant androgen. However, DHT is the most potent one.

As the result of intensive research over the last 20 years it has been confirmed that estrogens, produced by androgen aromatization, are also important in the regulation of male reproductive function (Carreau et al., 2003). In mice deficient for the estrogen receptor α gene (α ERKO) infertility, increased steroid acute regulatory protein (StAR) and 17β -hydroxysteroid dehydrogenase (17β -HSD) mRNA levels together with elevated T level have been found (Akingbemi et al., 2003; Eddy et al., 1996).

Specific receptors for androgens and estrogens have been found in both somatic and germ cells of the testis (Bilinska et al., 2000; Bilinska & Schmalz-Fraczek, 1999; Sierens et al., 2005; Wang et al., 2009). It has been confirmed that these receptors act as transcription factors regulating steroidogenesis at the transcription level. Furthermore, steroidogenesis requires the coordinated expression of related proteins and steroidogenic enzymes in response to hormonal stimulation. In Leydig cells luteinising hormone (LH) induce steroidogenesis by elaborating accumulation of intracellular cyclic adenosine monophosphate (cAMP), activation of protein kinase A (PKA) and expression of StAR resulting in subsequent T biosynthesis and secretion. Intratesticular T is maintained at constantly high levels. In the rat, endogenous T concentrations are the highest at stage VIII of the spermatogenic cycle (Parvinen, 1982). In addition, this stage together with stage VII have been found to be

In the male, Leydig cells which are present in the interstitium of the testis express all of the enzymes essential for the conversion of cholesterol to androgens and estrogens. The major pathways of steroid hormone synthesis are well established, and the sequence of the responsible steroidogenic enzymes has been elucidated (Payne & Hales, 2004). There are two major classes of steroidogenic enzymes: the cytochrome P450 heme-containing proteins and the hydroxysteroid dehydrogenases (HSDs). The first class contains: cytochrome P450 side chain cleavage (P450_{scc}), cytochrome P450 17 α -hydroxylase C17-C20 lyase (P450_{c17}) and cytochrome P450 aromatase (P450_{arom}), whereas the second includes both 3 β -hydroxysteroid dehydrogenase/ Δ 5- Δ 4-isomerase (3 β -HSD) and 17 β -hydroxysteroid dehydrogenase/ketosteroid reductase (17 β -HSD/KSR), (Payne, 2007).

The initial step of T biosynthesis is the conversion of the C27 cholesterol to the C21 pregnenolone (Figure 1). This reaction is catalyzed by the cytochrome P450_{scc}, which is located in the inner mitochondrial membrane. Pregnenolone diffuses across the mitochondrial membrane and is further metabolized by enzymes associated with the smooth

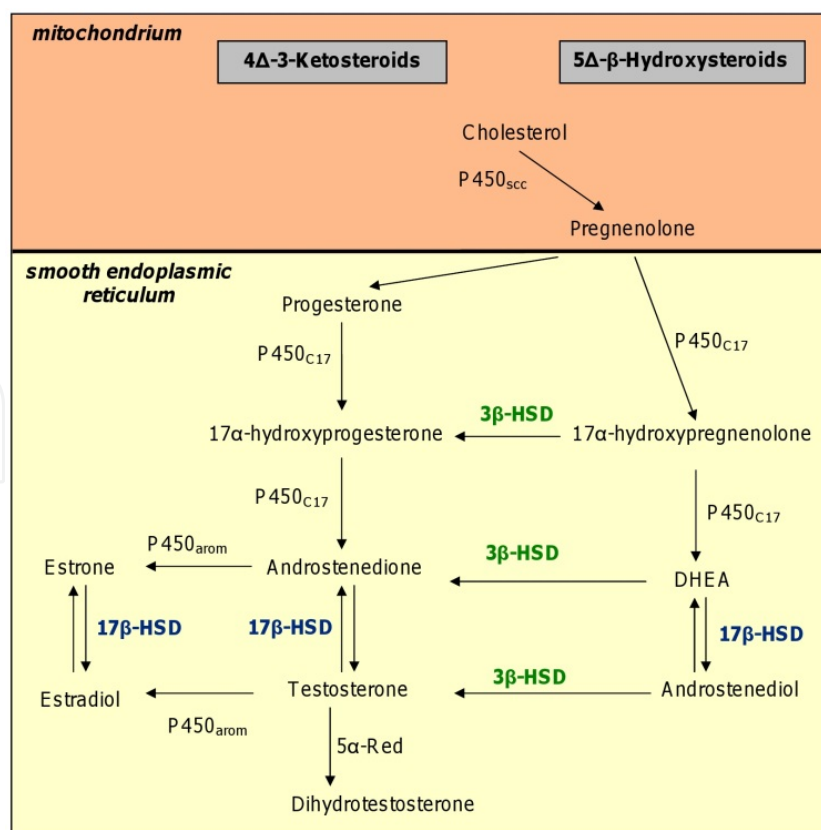


Figure 1. Steroid biosynthesis pathways in Leydig cell.

endoplasmic reticulum. P450c17 catalyzes the conversion of C21 pregnenolone or progesterone (P4) to the C19 dehydroepiandrosterone or androstenedione, respectively, while 3 β -HSD catalyzes the conversion of Δ 5-3 β -hydroxysteroids (pregnenolone or dehydroxypregnenolone, and DHEA, respectively) to the Δ 4-3-ketosteroids (P4, or 17 α -hydroxyprogesterone, and androstenedione, respectively). Depending on the animal species, biosynthesis of sex hormones proceeds down either one or both of the Δ 4 and Δ 5 pathways. In rodents, the Δ 4 pathway is primary whereas in primates, pigs and rabbits Δ 5 pathway is dominant (Fluck et al., 2003; Mathieu et al., 2002). In the final step of sex hormones biosynthesis, conversion of androstenedione into T, 17 β -HSD is involved. It was reported that the balance between these androgens depends on the type and activity of 17 β -HSD present (Simard et al., 2005).

3 β -HSDs are membrane-bound enzymes that are distributed in both mitochondrial and microsomal membranes (Payne & Hales, 2004; Pelletier et al., 2001). The relevance of dual localization of these HSDs is related to substrate accessibility (Simard et al., 2005). Coprecipitation studies have shown that, in the inner mitochondrial membrane, 3 β -HSD comprises a functional steroidogenic complex with P450_{scc}, which immediately provides substrates converted from cholesterol to the 3 β -HSD (Cherradi et al., 1995).

During the past decade, multiple isoforms of 3 β -HSDs have been isolated and characterized in human, mouse and rat tissues. Six, highly homologous in their amino acid sequence isoforms have been identified in the mouse, but only two of them: 3 β -HSD type I (3 β -HSD I), and 3 β -HSD type VI (17 β -HSD VI) are expressed in the testis. In human testis only 3 β -HSD I has been found (Payne & Hales, 2004).

Similarly to 3 β -HSDs, 17 β -HSDs are membrane-bound enzymes, and their soluble forms have also been reported. To date, 14 different types of 17 β -HSDs have been identified (Blanchard & Luu-The, 2007). Unlike 3 β -HSDs there is very little homology among the different 17 β -HSD enzymes. Only three types, 17 β -HSD type 3 (17 β -HSD 3), 17 β -HSD type 5 and 17 β -HSD type 12 (17 β -HSD 12), have been detected to be exclusively expressed in the testis. 17 β -HSD 3 converts androstenedione to T as well as it is an important partner of P450_{arom} involved in conversion of C18 steroid, estrone to E2 (Andersson et al., 1995). Recently, it has been confirmed for mice, humans and primates that 17 β -HSD 12 shares high homology and function with 17 β -HSD 3 (Blanchard & Luu-The, 2007; Liu et al., 2007).

The hydroxysteroid dehydrogenases belong to the same phylogenetic protein family, namely the short-chain alcohol dehydrogenase reductase superfamily. These enzymes are involved in the reduction and oxidation of steroid hormones requiring NAD⁺/NADP⁺ as acceptors and their reduced forms as donors of reducing equivalents. Studies have shown that mouse 3 β -HSD has different cofactor preference: 3 β -HSD I requires NAD⁺ while 3 β -HSD type IV and V requires NADP⁺ as cofactors. Interestingly, 17 β -HSD 3 prefers NADPH as a cofactor, and its primary activity is reductive. Studies have shown that a mutation in *HSD3B3* gene leads to decreased NADPH binding to tyrosine that has been identified as a critical residue for binding. Substitution of tyrosine with different amino acids resulted in alterations in cofactor preference switching from NADPH to NADH (Andersson et al., 1995; McKeever et al., 2002). In addition, Schäfers et al. (2001) have reported different cofactor preference for 17 β -HSD 3 on different days of postnatal development in rat.

Both 3β -HSD and 17β -HSD are well known Leydig cell-specific markers in different mammals, at different times of development and under different perturbation regimes (Bilinska, 1994; Hejmej et al., 2011b; Mendis-Handagama & Ariyaratne, 2001; Teerds et al., 2007). In previous studies, activity of HSDs in testis of various mammals was mostly detected using histochemical techniques (Badrinarayanan et al., 2006; Bilinska 1979, 1983, 1994; Hutson, 1989), (Figure 2). Nowadays the resolution of their localization increased with applying specific antibodies (Kotula-Balak et al., 2011; Pelletier et al., 1999; Pinto et al., 2010).

It has been reported that 3β -HSD type III (3β -HSD III) as well as 17β -HSD 3 and 17β -HSD type 10 are useful markers also for germ cells in rat, mouse, equine and black bear testis (Almeida et al., 2011; Ivell et al., 2003; O'Shaughnessy et al., 2000). Recently Scott et al. (2009) have indicated 17β -HSD 3 as a good marker for Sertoli cells in fetal mouse testis.

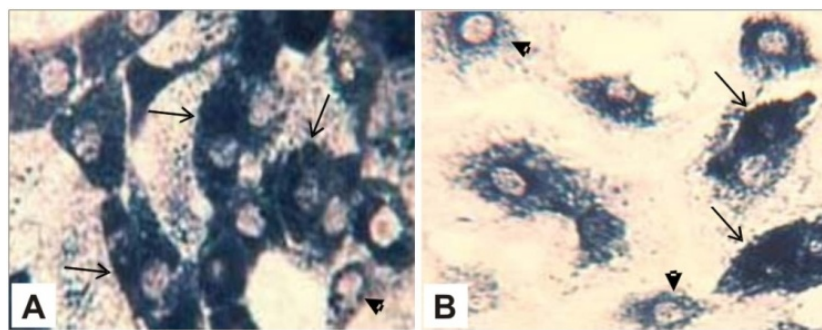


Figure 2. (A-B) Histochemical localization of 3β -HSD (A) and 17β -HSD (B) in cultured mouse Leydig cells. Note various intensity of the staining in the individual cells (arrows-strong staining, arrowheads-weak to moderate staining). Magnifications, $\times 320$.

3. Age-dependent activity of HSDs

In testis of mammals two morphologically and functionally different Leydig cell populations have been identified. One develops prenatally (fetal Leydig cells, FLCs) and the second arises postnatally (adult Leydig cells, ALCs), (Mendis-Handagama & Ariyaratne, 2001; Pinto et al., 2010). These two generations have different gene expression profiles, which indicate that they originate from separate types of stem cell (Dong et al., 2007; O'Shaughnessy et al., 2002b). Differentiation of FLCs is initiated by human chorionic gonadotropin (hCG), whereas development of ALCs is critically dependent on LH (O'Shaughnessy et al., 1998). After birth the population of FLCs decreases in size, although some fetal-type cells persist even in the adult testis. In the rat, FLCs that are arranged in large compact clusters and contain numerous lipid droplets. In ALCs the nuclei are large and cytoplasmic content is sparse with a few lipid droplets. The cytoplasm of Leydig cells of both populations contains abundant smooth endoplasmic reticulum (SER) and tubulovascular mitochondria, which are important organelles in biosynthesis of steroid hormones.

The FLCs are fully competent steroidogenically. It has been demonstrated that in rat, FLCs start to express LH receptors (LHR) and 3β -HSD I from fetal day (fd) 15.5 (Payne & Hales, 2004). However in mouse, 3β -HSD I expression has been detected shortly before fd 11

(Baker et al., 1999). Recently with the use both histochemical and immunohistochemical methods the presence of 3α -hydroxysteroid dehydrogenase (3α -HSD) and 17β -HSD has also been confirmed in FLCs of rat (Haider, 2004).

From fd 15.5 FLCs start actively producing T and its synthesis increases gradually (Habert & Picon, 1984). Expression of hormone receptors and enzymes in FLCs arise continuously during existence of this population in the testis. Interestingly, Ivell et al. (2003) have demonstrated that 17β -HSD type 10 (17β -HSD 10) starts to be expressed at the time when FLCs begin to involute. However, the pick of oxidative activity of these enzyme has been determined on postnatal day (pd) 16 (Schäfers et al., 2001).

Fetal population of Leydig cells is the primary source of T, androstenedione and DHT in both fetal and early postnatal testis (Ariyaratne & Mendis-Handagama, 2000; Huhtaniemi & Pelliniemi, 1992). Multiple studies have shown that T-producing capacity of FLC is significantly greater than that of ALC and is calculated to be even 87 pg per cell (Ariyaratne & Mendis-Handagama 2000; Huhtaniemi et al., 1982; Tapanainen et al., 1984).

During the neonatal-prepubertal period T is required for differentiation and morphogenesis of the male genital tract, activation of the hypothalamo-hypophyseal-testicular axis, completion of the testicular descent, masculinization of the brain, control of Sertoli cell number, initiation of spermatogenesis and formation of ALC precursors (Ariyaratne & Mendis-Handagama, 2000; Haider, 2004).

Steroidogenic capacity of FLCs is still high through the first postnatal week, although concentrations of circulating T are much lower due to decrease in number of these cells. Moreover, the inhibitory effects of Müllerian Inhibiting Substance (MIS) and transforming growth factor- β s (TGF- β s) on FLCs steroidogenic activity in postnatal testis have been described (Wu et al., 2007).

Testosterone production gradually increases to high levels with the development of ALCs (Benton, 1995; Chen et al., 2010; Hardy et al., 1989). The proliferation and differentiation of the adult population is regulated by an interplay of multiple regulatory factors, that can simulate, as well as inhibit, Leydig cells at each developmental stage. The development of ALCs is initiated around day 14 after birth and finishes around day 60. This process consists of multiple steps of proliferation and differentiation such as: proliferation of precursor cells; differentiation of precursor cells to Leydig cell progenitors, progenitors into newly formed adult Leydig cells, newly formed adult Leydig cells into immature adult Leydig cells; and finally, maturation of the immature adult Leydig cells to mature adult Leydig cells. In the rat, it has been reported that stem cells and mesenchymal precursor cells do not express steroidogenic enzymes however precursor cells acquire 3β -HSD III and other steroidogenic enzymes like cytochromes: P450scc and P450c17 prior to gain LHR (Hardy et al., 1989; Teerds et al., 2007; Zirkin, 2010). These cells have negligible amounts of 17β -HSD 3 while expression of steroid metabolizing enzymes 5α -reductase and 3α -HSD is high. Thus precursor cells produce androsterone as their main androgen product (O'Shaughnessy et al., 2000). Also expression of AR by early developmental stages of ALCs lineage is required for further transformations of these cells under androgen control (Ge & Hardy, 1997).

Differentiation of progenitors to newly formed adult Leydig cell is associated with the cell cytoplasm shape change from spindle shaped to polygonal. Newly formed Leydig cells move toward the central interstitium and locate near blood capillaries although they do not exclusively arrange in clusters. These cells express LHR and the levels of 3β -HSD VI, P450scc and P450c17 increase with the further steps of Leydig cell differentiation (Ariyaratne & Mendis-Handagama, 2000; Shan et al., 1993). It has been demonstrated that in mice *Hsd3b3* and *Hsd3b6*, remain fairly stable after birth but show a pubertal rise in expression around pd 20 (O'Shaughnessy et al., 2002)

5α -androstane- 3α and 17β -diol is synthesized as the predominant androgen with the emergent increase in activity of 17β -HSD 3 and in a continuous presence of 5α -reductase and 3α -HSD (Hardy et al., 1990). It is worth noting, that in immature ALCs 11β -hydroxysteroid dehydrogenase type 1 (11β -HSD 1) and 11β -hydroxysteroid dehydrogenase type 2 start to be expressed. In the rat testis, the presence of 11β -HSD 1 is in coincidence with the first appearance of elongated spermatids in the seminiferous tubules (Haider, 2004).

Adult Leydig cells are the dominant cell type of the Leydig cell lineage from pd 56 (Benton et al., 1995). Transformation of immature adult Leydig cells into mature adult Leydig cells is characterized by a significant increase in the average cell size and disappearance of cytoplasmic lipid droplets. The capacity to secrete T increases significantly in mature adult Leydig cells because of their enhanced responsiveness to circulatory LH due to the acquisition of higher numbers of LHR. During this time in the mouse testis, 3β -HSD VI becomes the predominant isoform of HSDs (Payne & Hales, 2004).

Additionally, the sharp decline in 5α -reductase activity overlaps. Shan et al. (1993) have reported that the mature Leydig cells by pd 90 produce 150 times more T than progenitors, and five times more than immature Leydig cells. Such high T levels are required for initiation, maintenance and regulation of the spermatogenesis. By day 90 the secretory capacity per ALC in rat has been estimated as 1.43 pg.

During puberty ALCs are particularly sensitive to androgens and expression of AR mRNA in this time is significant. Studies have shown that in the absence of AR, there is developmental failure of ALC maturation (O'Shaughnessy et al., 2010). However, there is well known phenomenon when ALCs destroyed by ethane dimethane sulphonate (EDS) administration can proliferate to regenerate the original population of Leydig cells (Teerds & Rijnities, 2007).

In aging human testis, both serum and intratesticular T concentrations progressively decline being in correlation to decreased LH level. In rat, these changes have been reported to be strain-dependent (Harman et al., 2001). In Brown Norway rats, the decrease in T level concomitantly with an increase in FSH level and unchanged LH level have been detected (Chen et al., 2002). Several studies have demonstrated that in men decrease in T level is associated with alterations in body composition, diminished energy, muscle strength and physical function, depressed mood and decreased cognitive function (Matsumoto, 2002). These age related changes result from the loss of steroidogenic capacity of the Leydig cells and/or reduction in their number (Chen et al., 2001, 2009). It has also been found that in

aging Leydig cells adenyl cyclase is maintained that results in the defect of the cAMP-LH signaling cascade. In addition, protein and mRNA levels of StAR have been significantly reduced, suggesting deficits in the transport of cholesterol to the inner mitochondrial membrane of aged Leydig cells. Moreover, the activity, protein level, and mRNA level of P450_{scc}, P450_{c17}, 3 β -HSD and 17 β -HSD have been found markedly reduced in old Leydig cells (Ivell et al., 2003; Luo et al., 1996; Midzak et al., 2009; Zirkin & Chen, 2000). Interestingly, these authors have demonstrated that long-term suppression of steroidogenesis by administration of T prevents or delays the reduced steroidogenesis that accompanies Leydig cell aging due to suppressing the production of the reactive oxygen species that are a by-product of steroidogenesis itself.

4. Regulation of HSDs function

4.1. Pituitary hormones and other peptides

Primary control of 3 β -HSD expression occurs through the activation by LH its receptor and the induction of the cAMP second messenger system (Simard et al., 2005). Recent findings from our own laboratory have revealed that bank vole Leydig cells treated with LH have increased steroidogenic capacity and T secretion (Gancarczyk et al., 2003). A profound hypogonadal effect and suppression of T production has been demonstrated in boars treated with deslorelin, an agonist of gonadotropin-releasing hormone (GnRH), (Kopera et al., 2008). In Leydig cells of treated boars very weak or lack of LHR and 3 β -HSD expression has been detected. In contrast, Lin et al. (2008) who treated mouse Leydig cells with GnRH agonists (I and II) have demonstrated that 3 β -HSD has been stimulated directly resulting in increase of T production. On the contrary, 17 β -HSD was not induced in treated cells.

Formation of cAMP activates steroidogenesis by temporally distinct manners either, acutely (minutes) due to StAR action or chronically (hours) related on P450_{scc}, P450_{c17}, 3 β -HSD and 17 β -HSD activities. The delivery of cholesterol into the inner mitochondrial membrane is the rate-determining step in steroidogenesis. Also the differences in the way that cultured Leydig cells respond to cAMP have been reported. In mouse Leydig cells, cAMP has stimulated T production which then suppressed 3 β -HSD at the mRNA level, whereas addition of cAMP to cultured rat Leydig cells increases 3 β -HSD activity and expression at both the mRNA and protein level after 24–72 h (Keeney & Mason 1992; Payne & Sha 1991).

Interestingly, expression of 3 β -HSD has been reported to be dependent upon steroidogenesis factor 1 (SF1). SF1 response element has been detected in the proximal promoter region of the human 3 β -HSD type II gene. Recently Scott et al. (2009) have confirmed that the mouse 3 β -HSD I gene promoter has three potential SF1 consensus binding sites. However, it is currently unknown whether SF1 regulates the expression of 17 β -HSD.

A series of studies show that pituitary hormone-prolactin (PRL) and thyroid hormones regulate activity of HSDs. In Leydig cells of hypophysectomized rats treated with PRL, a significant increase in number of 3 β -HSD immunopositive cells together with an increase in T and E2 concentrations was found (Dombrowicz et al., 1992; Manna et al., 2001). Also our

own studies have revealed that in bank voles treated with PRL, the levels of androgens and estrogens have increased markedly within the testis (Gancarczyk et al., 2006). These results point the role of PRL in promoting multiplication, differentiation and regulation of steroidogenic function of Leydig cells. Similar functions have been confirmed for thyroid hormones. In physiological levels these hormones have profoundly increased the number of mesenchymal precursors of ALCs and supported their further differentiation (Maran, 2003; Mendis-Handagama et al., 1998; Teerds et al., 1998).

4.2. Steroids

In Leydig cells, the action of P4, has been reported to be mediated only by non-classical receptors while the classical nuclear progesterone receptor has not been found in these cells (Oettel & Mucopadhyay 2004). The direct stimulatory or inhibitory effect of P4 on steroidogenesis in Leydig cells has been demonstrated, although its mode of action remains obscure (El-Hefnawy et al., 2000; Schwarzenbach et al., 2003). In rats, exposure in utero to subnormal levels of hydroxyprogesterone suppresses testicular steroidogenesis by decreasing the HSDs levels, which in turn suppresses the reproductive activity of the male (Pushpalatha et al., 2003). Studies have shown that in elderly men P4 levels increase within the testis and the spermatic vein, having a detrimental effect on Leydig cell steroidogenic function (El-Hefnawy & Huhtaniemi, 1998). It is interesting but still not resolved if and how P4 influences HSDs.

It has been reported that both endogenous and exogenous sex hormones are able to modify steroidogenesis at the level of 3β -HSD. In rats treated with an androgen antagonist, increased induction of hCG and 3β -HSD activity has been observed, whereas treatment with an androgen agonist decreased hCG induction and 3β -HSD activity, respectively (Ruiz de Galarreta et al., 1983). Similarly, T and DHT have inhibited 3β -HSD activity in adult rat and mouse Leydig cells (Simard et al., 2005). Recent findings by Kostic et al. (2011) have demonstrated that in androgenized rats, T upregulated P4 synthesis. In these animals prolonged treatment with high T doses caused significant increase of 3β -HSD mRNA and protein levels whereas no effect has been observed on 17β -HSD expression. Freeman (1985) has demonstrated that E2 inhibited P4 biosynthesis in a dose-dependent manner in Leydig cells via inhibition of the activity of 3β -HSD. Also studies *in vivo* on rats and bank voles treated with E2 have shown disturbances in sex hormones balance within the testis. Low T and high E2 levels in treated animals have been reported to affect spermatogenesis (Gancarczyk et al., 2004; Rao & Chinoy, 1986).

4.3. Testicular paracrine factors

It has already been accepted that the function and activity of the testis is regulated by many locally produced factors and by cell-cell interactions. The effects of cytokines and growth factors on HSDs expression has been reported to be diverse (for review see Herrmann et al., 2002). Tumor necrosis factor (TNF) and interleukin 1 (IL-1) inhibited 3β -HSD activity in mouse and rat Leydig cells. However, IL-1 only inhibited cAMP stimulated enzyme

synthesis, whereas TNF also reduced basal enzyme expression. In contrast, epidermal growth factor (EGF) increased activity and expression of 3β -HSD, which has also been demonstrated for transforming growth factor (TGF). Acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) inhibited 3β -HSD. In pig Leydig cells, bFGF has been reported to increase 17β -HSD expression (Sordoillet et al., 1992). Our own studies in bank voles have revealed that insulin like growth factor I (IGF-I) stimulates whereas IL-1 and interleukin 1α (IL- 1α) inhibit testis steroidogenic and spermatogenic function in sexually active males (Gancarczyk et al., 2006; Kmicikiewicz & Bilinska, 1997; Kmicikiewicz et al., 1999). Interesting results have been recently reported by Ivell et al. (2011) who demonstrated that 17β -HSD mRNA expression in mice testis is regulated by locally produced relaxin (RLN) dependently of animal age.

4.4. Photoperiod

In seasonal breeders the function of the pituitary-testicular axis undergoes annual cyclic variations. Multiple studies including our own have shown that photoperiod is an important factor regulating steroidogenesis. Changes in LH and FSH secretion depending on the light length are responsible for seasonal variations in size, structure and function of the testis (Bartke & Steger, 1992). Under laboratory conditions, bank voles kept in long light regime show higher testis weight and increased steroidogenic activity than animals exposed to short one (Bilinska et al., 2000, 2001; Tähkä et al., 1982). In several seasonal breeders, the serum and testicular concentrations of steroid hormones have exhibited seasonal fluctuations and are always lower in regressed animals (Frungieri et al., 1999; Hance et al., 2009; Kotula-Balak et al., 2003).

In addition, the Leydig cell morphology as well as localization and expression of HSDs have been found to change seasonally in the sika deer, black bear and northern fur seal (Hayakawa et al., 2010; Ibluchi et al., 2010; Tsubota et al., 1997, 2001). In black bears during their mating season, 17β -HSD 3 has been detected both in Leydig cells and in Sertoli cells. Moreover, in these animals expression of 3β -HSD was the highest in June. In Syrian hamster, specific melatonin receptors (mell1a) have been reported in Leydig cells via which melatonin down-regulated the gene expression of both 3β -HSD, and 17β -HSD (Frungieri et al., 2005).

4.5. Endocrine disrupting chemicals

A large body of information concerning the effects of endocrine disrupting compounds (EDCs) on Leydig cells steroidogenesis during fetal development and in adult mammals has been accumulated in the past decades. EDCs can disturb morphology and normal endocrine functions of the Leydig cells or oppose the actions of androgen through their estrogenic or anti-androgenic properties (Hejmej et al., 2011a). A number of compounds act directly on Leydig cells to diminish T production by interfering with the expression of steroidogenic enzymes, at the protein and/or mRNA level (Skakkebaek, et al., 2001).

Our recent results have shown that administration of the estrogenic compound, 4-*tert*-octylphenol (OP), to adult bank voles has caused the significant decrease of 3 β -HSD and increase of P450arom expression concomitantly with the alteration of the androgen/estrogen balance within the testis of sexually active animals (Hejmej et al., 2011b). Similar results have been reported by Victor-Costa et al. (2010) on rats treated with atriazine. These authors concluded that inhibition of 3 β -HSD function is one of the possible mechanism through which xenoestrogens disturb spermatogenesis. *In vitro* studies on Leydig cells obtained from various mammals have revealed decrease in the activity and expression of 3 β -HSD after OP, bisphenol A (BPA) and genistein administration (Hu et al., 2010; Kotula-Balak et al., 2011; Ye et al., 2011). Our study demonstrated that OP markedly disturbs morphology and steroidogenic function of the Leydig cells through direct effect on 3 β -HSD expression and localization (Kotula-Balak et al., 2011). In detail, treatment with high doses of OP (10^{-4} – 10^{-6} M) resulted in a reduced staining intensity and the staining was usually located near the nucleus, whereas in the low OP doses (10^{-7} and 10^{-8} M) it was mainly dispersed throughout the cytoplasm (Figure 3).

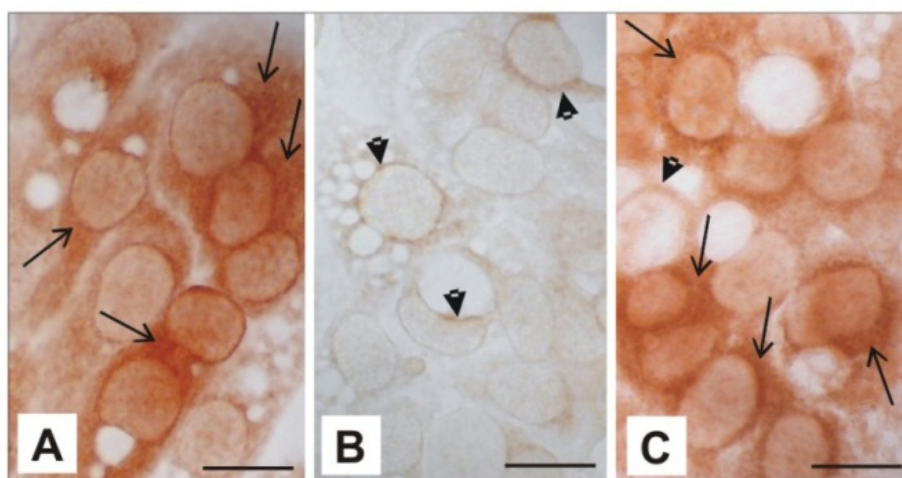


Figure 3. (A-C) Immunostaining for 3 β -HSD. Positive staining of various intensity is confined to the cytoplasm of Leydig cells (arrows). Note, clearly reduced staining for 3 β -HSD in Leydig cells treated with high OP dose (B). In many cells weak to moderate staining in the perinuclear region is visible (arrowheads). In Leydig cells treated with low OP dose (C) the intensity of immunostaining is similar to that of the control (A), (arrows). Only in a few cells staining in the perinuclear region is visible (arrowhead). Bars 20 μ m.

It is worth noting that the effect of EDCs on HSDs function can be diverse depending on the choice of animal species, age, routes of administration and dose levels. Studies of Pogrmic-Majkic et al. (2010) have shown that in rat Leydig cells atriazine stimulated 17 β -HSD, whereas other authors reported inhibition of these enzyme in rat and human microsomes treated with various xenoestrogens (Hu et al., 2010; Vaithinathan et al., 2008; Ye et al., 2011). Recently, it has also been found that antiandrogens such as tributyltin, triclosan and flutamide modified HSDs expression in Leydig cells and microsomes of various mammals (Kim et al., 2008; Kumar et al., 2009; McVey & Cooke, 2003; Ohno et al., 2005; Ohsako et al., 2003).

5. HSDs in pathological conditions

5.1. Temperature and 3β -HSD activity

In recent years, disorders of human male reproductive development increased in incidence (Sharpe & Skakkebaek, 1993; Toppariet al., 1996). Cryptorchidism and hypospadias are the two most common congenital malformations that comprise a testicular dysgenesis syndrome (TDS), which arises during fetal development and manifests in adulthood (Skakkebaek et al., 2001).

Several studies have shown that increased intratesticular temperature in cryptorchid testes affects spermatogenesis, resulting in either reduced fertility or infertility. Other evidences collected over the years have indicated that increased testicular temperature negatively influences the development and differentiation of Leydig cells causing impairments in sex hormones biosynthesis (Huff et al., 2001; Kotula-Balak et al., 2001; Pinart et al., 2000).

Our recent studies have shown that in cryptorchid horses disturbances in differentiation and/or maturation of Leydig cells may be related to altered intracellular communication. In these animals decreased immunoexpression of gap junction protein, connexin 43, in testicular cells were accompanied with reduced expression of LHR, 3β -HSD and disturbed androgen/estrogen balance (Hejmej et al., 2005, 2007; Hejmej & Bilinska, 2008), (Figure 4). Altered expression of these proteins and imbalance in sex hormones level detected in cryptorchid horses suggested their additional influence on morphology and function of undescended testis. Markedly reduced expression of 3β -HSD has been also reported in rats with experimentally induced cryptorchidism (Wisner & Gomes, 1978). No significant changes in T levels have been detected in patients with cryptorchidism as well as other mammalian species (Bilinska et al., 2003; Farrer et al., 1985; Illera et al., 2003; Kawakami et al., 1999; Ren et al., 2006; Ryan et al., 1986).

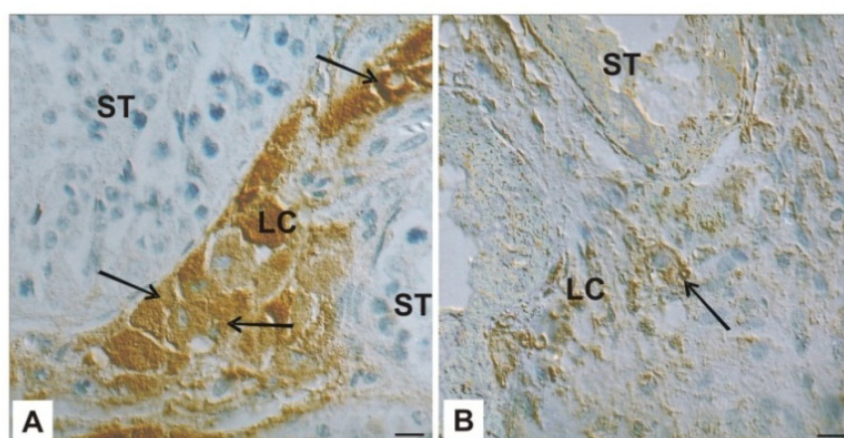


Figure 4. Immunohistochemical localization of 3β -HSD in testis of normal (A) and cryptorchid stallion (B). Counterstaining with Mayer's haematoxylin. The presence of 3β -HSD is confined to Leydig cells (arrows). Note a clearly weaker staining in the cryptorchid horse (A) than in the healthy stallion (B). LC- Leydig cells, ST-seminiferous tubules. Bars 20 μ m.

5.2. 3 β -HSD and 17 β -HSD deficiency

The development of the male internal and external genitalia in an XY fetus requires a complex interplay of many critical genes, enzymes and cofactors. In early fetal life, in the bipotential embryo, both Wolffian ducts and Müllerian ducts are present. Testosterone produced by FLCs acts on AR to stabilize the Wolffian ducts whereas MIS causes regression of Müllerian ducts (George et al., 2010). The formation of male external genitalia is induced by T and DHT.

Disruption in androgen production and/or action leads to disorder of sex development (DSD) also known as male pseudohermaphroditism. DSD is defined as a congenital condition in which development of chromosomal, gonadal or anatomical sex is atypical in such individuals (Hughes et al., 2006). 46, XY DSD is an autosomal recessive form of DSD which was first described in 1971 (Saez et al., 1971). Disturbances of androgen production can occur in all steps of T biosynthesis and its conversion into DHT.

Around 40 various mutations have already been described in *HSD3B2* gene. Mutation in this gene results in 3 β -HSD II deficiency and decreased T biosynthesis (Payne & Hales, 2004). Severe form of 3 β -HSD deficiency named congenital adrenal hyperplasia (CAH) impairs also steroidogenesis in adrenals (Moisan et al., 1999). In male patients, either perineal hypospadias or perineoscrotal hypospadias and ambiguous external genitalia or microphallus have been reported (Simard et al., 2005). Also, in these individuals gynecomastia has been observed as common at puberty. Serum levels of $\Delta 5$ steroids are elevated whereas levels of $\Delta 4$ steroids are slightly increased. In adulthood, males with 3 β -HSD II deficiency can reach normal levels of T due to the peripheral conversion of elevated $\Delta 5$ steroids by 3 β -HSD I and/or due to testicular stimulation by high LH levels. Most of the patients with mutation in *HSD3B2* gene are raised as males and display male social sex but there are some cases described where such individuals are castrated in childhood and treated as female (Mendonca et al., 2008). Males with 3 β -HSD II deficiency share common clinical features with patients deficient for 17 β -HSD 3 and 5 α -reductase 2.

Deficiency of the 17 β -HSD 3 can be caused by either homozygous or compound heterozygous mutations in the *HSD17B3* gene (Geissler et al., 1994). This autosomal recessive disorder manifests in males as undermasculinization characterized by hypoplastic-to-normal internal genitalia (epididymis, vas deferens, seminal vesicles, and ejaculatory ducts), but female external genitalia and the absence of a prostate (Boehmer et al., 1999; Lindqvist et al., 2001; Sinnecker et al., 1996; Ulloa-Aguirre et al., 1985).

At the time of puberty, there is a marked increase in plasma LH and, consequently, in testicular secretion of androstenedione. Mendonca et al. (2010) have found that significant amounts of the circulating androstenedione are converted to T in peripheral tissues by an unidentified member of the 17 β -HSD family, thereby causing virilization in many of these individuals. To date, 19 mutations in the *HSD17B3* gene have been found. Most of these patients are raised as girls during childhood but starts to display masculine behavior at puberty (Mendonca et al., 2008).

6. Conclusion

In Leydig cells, multiple factors regulate 3β -HSD and 17β -HSD function exerting very diverse effect. Nowadays further characterization of physiological and pathological conditions as well as endogenous and exogenous agents that can modify HSDs expression is becoming increasingly necessary. Especially, in light of recent reports indicating an increase in the incidences of developmental and functional disorders of the male reproductive tract. Exploration of the site and possible mechanisms of action of these agents in steroid biosynthesis is becoming important future research direction.

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Acknowledgement

This work was financially supported by the Ministry of Science and Higher Education, Grant N N303816640.

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