Abstract

**Background:** We have recently discovered that human type 12 17β-HSD (h17β-HSD12), a homolog of type 3 17β-HSD, is a new estrogen-specific 17β-hydroxysteroid dehydrogenase involved in the production of estradiol (E2). To further characterize this estradiol-producing enzyme, we have isolated the corresponding cDNA in the cynomolgus monkey (*Macaca fascicularis*), characterized its enzymatic activities and performed cellular localization using in situ hybridization.

**Results:** Using HEK-293 cells stably expressing *Macaca fascicularis* type 12 17β-HSD (mf17β-HSD12), we have found that the mf17β-HSD12 catalyzes efficiently and selectively the transformation of E1 into E2, in analogy with the h17β-HSD12. We have also quantified the mf17β-HSD12 mRNA expression levels in a series of *Macaca fascicularis* tissues using Quantitative RealTime PCR. The *Macaca fascicularis* 17β-HSD12 mRNA is widely expressed with the highest levels tissues found in the cerebellum, spleen and adrenal with moderate level observed in all the other examined, namely the testis, ovary, cerebral cortex, liver, heart, prostate, mammary gland, myometrium, endometrium, skin, muscle and pancreas. To gain knowledge about the cellular localization of the mf17β-HSD12 mRNA expression, we performed in situ hybridization using a 35S-labeled cRNA probe. Strong labeling was observed in epithelial cells and stromal cells of the mammary gland. In the uterus, the labeling is detected in epithelial cells and stromal cells of the endometrium.

**Conclusion:** These results strongly suggest that the *Macaca fascicularis* 17β-HSD12 is an essential partner of aromatase in the biosynthesis of estradiol (E2). It strongly suggests that in the estradiol biosynthesis pathway, the step of 17-ketoreduction comes after the step of the aromatization (the aromatization of 4-androstendione to estrone followed by the conversion of estrone into estradiol by estrogen specific 17β-HSDs) which is in contrast with the hypothesis suggesting that 4-androstenedione is converted to testosterone followed by the aromatization of testosterone.
**Background**

Seventeen \( \beta \)-hydroxysteroid dehydrogenases (17\( \beta \)-HSDs) are crucial enzymes involved in the formation of active sex steroids by the transformation of a keto into a hydroxyl-group at position 17. The best known 17\( \beta \)-HSD is type 3 17\( \beta \)-HSD (17\( \beta \)-HSD3) that is expressed in the testis where it transforms androstenedione (4-dione) into testosterone (T). Its deficiency is the cause of the well known male pseudohermaphroditism [4,5]. This enzyme is inactive for C18-steroids. An additional enzyme able to catalyze the transformation of 4-dione into T is type 5 17\( \beta \)-HSD [6,7]. Since 17\( \beta \)-HSD3 is not expressed in the ovary while 17\( \beta \)-HSD5 is present [8,9], and women deficient in 17\( \beta \)-HSD3 are asymptomatic [10], it is likely that 17\( \beta \)-HSD5 is the enzyme responsible for the formation of active androgens in the human ovary [11].

On the other hand, the most studied 17\( \beta \)-HSD is type 1 17\( \beta \)-HSD, probably because it is expressed abundantly in the placenta. In fact, 17\( \beta \)-HSD1 was the first 17\( \beta \)-HSD to be purified [12], cloned [13,14] and crystallized [15]. In intact cell in culture, the enzyme catalyzes almost exclusively the transformation of estrone (El) into estradiol (E2) [11,16]. The abundant co-expression of this estrogenic 17\( \beta \)-HSD as well as aromatase in the placenta suggests that the aromatization step precedes the 17\( \beta \)-HSDs step. This proposed mechanism is also in agreement with a higher affinity of aromatase for 4-dione than for testosterone. Other 17\( \beta \)-HSDs known to be able to metabolize estrogens. Thus types 7 and 12 17\( \beta \)-HSDs catalyze the formation of E2, types 2, 4, and 8 17\( \beta \)-HSDs that preferred NAD\(^+\) as cofactor are E2 inactivating enzymes [11].

Up to now, at least twelve isoforms of 17\( \beta \)-HSDs have been identified and some members of the 17\( \beta \)-HSDs family have been shown multifunctionality associated with cancer, metabolism diseases and neurodegenerative disorders, in addition to their roles in steroid metabolism [1-3]. Most of 17\( \beta \)-HSDs belong to the short-chain dehydrogenase reductase (SDR) superfamily except the type 5 17\( \beta \)-HSD, which belongs to the aldo-keto reductase (AKR) superfamily. A particular property of members of 17\( \beta \)-HSDs family is that they possess very different primary structures (an average of only approximately 20% amino acid identity) despite being highly specific for substrates having closely related structures. Additional regulation of 17\( \beta \)-HSDs activity is achieved by the specificity of tissue distribution of these 17\( \beta \)-HSDs, thus permitting each tissue to control intracellular steroid levels according to local needs. Such local intracellular formation of steroids in peripheral target tissues from the adrenal precursor dehydroepiandrosterone (DHEA) has been called intracrinology [17,18].

Recently, we have found that the h17\( \beta \)-HSD12, a homolog of type 3 17\( \beta \)-HSD, selectively catalyzes the formation of E2 [19]. To gain more knowledge about this potentially very important enzyme, we have isolated a corresponding enzyme in the cynomolgus monkey (Macaca fascicularis), and characterized its substrate specificity, mRNA tissue distribution and cellular localization.

**Results**

**Sequence of Macaca fascicularis 17\( \beta \)-HSD12**

We have isolated a coding sequence of Macaca fascicularis 17\( \beta \)-HSD12 (GenBank accession number AB169576) using Macaca fascicularis liver mRNA and PCR amplification. As illustrated in Fig. 1, amino acid sequence alignment of 17\( \beta \)-HSD12 between Macaca fascicularis and other species shows that the Macaca fascicularis sequence possesses 95%, 82%, 81%, 78% and 66% identity with human, cow, mouse, rat, and duck, respectively. In addition, this enzyme contains the conserved signatures of SDR family members, namely the putative YXXXK active center and the modified GXXGXL cofactor binding site.

**Substrate specificity of Macaca fascicularis 17\( \beta \)-HSD12**

We used the HEK-293 cells stably expressing \( \text{mf} \)17\( \beta \)-HSD12 to determine the substrate specificity of the enzyme in intact cells in culture without addition of exogenous cofactor. As shown in Fig. 2, in analogy with the h17\( \beta \)-HSD12, the \( \text{mf} \)17\( \beta \)-HSD12 catalyzes predominately the transformation of El into E2 while the transformation of 4-dione into T, E2 into El and T into 4-dione are not significant. Fig. 3 shows that the conversion of El into E2 increases proportionally with the incubation, thus indicating that our incubation conditions are appropriate and that time is a limiting step even after 50 h of incubation.

**Tissue distribution of Macaca fascicularis 17\( \beta \)-HSD12**

Using quantitative RealTime PCR, we examined the expressions level and tissue distribution of 17\( \beta \)-HSD12 mRNA in 16 Macaca fascicularis tissues, namely the adrenal gland, ovary, mammary gland, endometrium, myometrium, cerebral cortex, cerebellum, liver, pancreas, heart, testis, prostate, kidney, skin, muscle and spleen. As illustrated in Fig. 4, the \( \text{mf} \)17\( \beta \)-HSD12 is ubiquitously expressed with the highest level in the spleen, adrenal gland and cerebellum, and moderate levels all the other tissues examined. Macaca fascicularis 17\( \beta \)-HSD12, which is highly and widely expressed in gonadal as well as extragendal tissues is most probably a crucial enzyme involved in the biosynthesis of estradiol.

**Cell-specific distribution of Macaca fascicularis 17\( \beta \)-HSD12**

In order to obtain information about the cellular localization of 17\( \beta \)-HSD12 in estrogen-sensitive tissues, we performed in situ hybridization on the adult female Macaca
fascicularis mammmary gland and uterus using a 35S-labeled probe. The labelling was detected on both the epithelial cells of the alveoli and the stromal cells of the mammary gland (Fig. 5A). In the uterus, the hybridization signal was seen in the epithelial cells and in the stromal cells of the endometrium (Fig. 5C). In the uterine cervix, 17β-HSD12 mRNA was observed in the squamous epithelium and stromal cells (Fig. 5E). When the radiolabeled sense probes were used for hybridization in consecutive sections, only weak and diffuse labelling corresponding to background could be detected in the mammary gland, uterus and uterine cervix (Fig. 5B, D and 5F).

Discussion
In this report, we have shown that Macaca fascicularis 17β-HSD12 catalyzes selectively the transformation of E1 into E2, similar to that has been found for the corresponding human enzyme. The present data strongly suggest that 17β-HSD12 is most likely the key enzyme controlling the local conversion of E1 (low estrogenic activity) into E2 (the most potent natural estrogen). It is noteworthy that E1 could come from both sources, the local transformation of the precursor 4-dione by aromatase or from the circulation under the form of E1 and E1-S [20]. It is well recognized that most of peripheral cells possess all the necessary enzymatic machinery to transform the adrenal androgen precursors into E2 [17,18]. Cells that possess steroid sulfatase could use the sulfated precursor DHEA-S and E1-S to produce E2.

The mRNA tissue distribution analysis performed with quantitative RealTime PCR shows that the enzyme is distributed ubiquitously, thus suggesting its important role in the production of estradiol in a large number and pos-
sibly all peripheral target tissues. In contrast, 17β-HSD1 is more selectively expressed in the placenta [21] and in granulosa cells of the ovary [22,23]. The highest mRNA expression levels have been found in the cerebellum and spleen suggesting that this enzyme could play an important role in these tissues. However, in the tissues where a lower mRNA expression levels is observed, it could not mean that the role exerted by the enzyme is not important, but it reflects essentially the relatively fewer amount of cells expressing this enzyme compared to the total cells in the tissue.

Cloning of the cDNA of m17β-HSD12 has allowed us to study the cellular localization of the enzyme in the mammary gland and uterus. In the mammary gland, 17β-HSD12 mRNA expression was detected in both epithelial and stromal cells. This is in agreement with the action of estrogens in the development and proliferation of alveoli and lactogenesis in the mammary gland. In the uterus, the enzyme was found in both epithelial cells and stromal cells of the endometrium. These results strongly suggest that 17β-HSD12 is directly involved in the local synthesis of E2 in the estrogen target tissues.

Although twelve types of 17β-HSDs have been identified based on their specificity to catalyze the interconversion of the 17β-ketosteroids and 17β-hydroxysteroids, and named according to the chronological order of their characterization, the true physiological role of many of these enzymes remains to be determined. For example, the phenotype of Hsd17b2 knockout mice is not well explained by the known activities of 17β-HSD2 on sex steroids [24].

On the other hand, 17β-HSD4, which was originally identified as the estradiol-inactivating dehydrogenase from porcine endometrium [25,26], mainly participates in the β-oxidation of fatty acids [27]. Accordingly, mutations of the 17β-HSD4 gene lead to severe developmental defects resembling Zellweger syndrome [28], a rare hereditary disorder affecting infants, and usually results in death associated with unusual problems in prenatal development, an enlarged liver, high levels of iron and copper in the blood, and vision disturbances. Type 6 17β-HSD, an enzyme observed in the rat, most probably corresponds to type 9 17β-HSD in the mouse that catalyses the transformation of 5α-androstane-3α, 17β-diol (3α-diol) into androsterone (ADT). The corresponding human ortholog is not yet identified. On the other hand, type 7 17β-HSD...
is presently best characterized as a 3β-reductase involved in cholesterol metabolism [29], and the role of type 8 17β-HSD as an estradiol inactivating enzyme [11,30,31] is still not well defined. Types 10 and 11 17β-HSDs metabolize 5α-reduced steroids but their involvement in the metabolism of active steroids is unclear. Type 12 17β-HSD was identified as 3-ketoacyl-CoA reductase, involved in long chain fatty acid elongation with a very weak activity [32]. Recently, we have found that the H17β-HSD12 catalyzes selectively the formation of E2 [19].

Overall, among the twelve enzymes identified as 17β-HSDs, only four are most likely working as 17β-HSDs synthesizing active steroids: the types 3 and 5 17β-HSDs are involved in the transformation of C19-steroids (4-dione into T) while types 1 and 12 are involved in the conversion of E1 into E2. The selective activity of type 12 17β-HSD for the transformation of E1 into E2 in the Macaca fascicularis (this report) as well as the high mRNA expression levels of this enzyme in estrogen-sensitive tissues, including the mammary gland and uterus, strongly suggest that this enzyme is a crucial partner of aromatase in the biosynthesis of estradiol. Indeed, the presence of estrogen specific 17β-HSDs (types 1, 7 and 12 17β-HSDs) catalyzing the transformation of E1 to E2 and the higher affinity of aromatase for 4-dione than for T are strongly in favor of the pathway in which 4-dione is converted into E1 by aromatase followed by the transformation of E1 into E2 by estrogen specific 17β-HSDs. This pathway is in contrast with a generally believed pathway in which 4-dione is transformed to T by 17β-HSDs followed by the aromatization of T into E2. It strongly suggests that in the estradiol biosynthesis pathway, the step of 17-ketoreduction comes after the step of the aromatization (the aromatization of 4-androstenedione to estrone followed by the conversion of estrone into estradiol by estrogen specific 17β-HSDs). The higher affinity of aromatase for 4-androstenedione than for testosterone seems to agree with the present data.

**Conclusion**

These results strongly suggest that the *Macaca fascicularis* 17β-HSD12 is an essential partner of aromatase in the biosynthesis of estradiol (E2). It strongly suggests that in the estradiol biosynthesis pathway, the step of 17-ketoreduction comes after the step of the aromatization (the aromatization of 4-androstenedione to estrone followed by the conversion of estrone into estradiol by estrogen specific 17β-HSDs) which is in contrast with the hypothesis suggesting that 4-androstenedione is converted to testosterone followed by the aromatization of testosterone.

**Methods**

**Experimental animals**

The maintenance and handling of experimental animals followed the National Institute of Health Guidelines for the use and care of animals and was done under approval and supervision of the Comité de Protection des Animaux du CHUQ (CPAC). The tissues used were harvested from euthanized normal female and male cynomolgus monkeys, frozen in liquid nitrogen and stored at -70°C until analysis.

**RNA isolation and cDNA synthesis**

Total RNA was extracted from the *Macaca fascicularis* adrenal gland, ovary, mammary gland, endometrium, myometrium, cerebral cortex, cerebellum, liver, pancreas, heart, testis, prostate, kidney, skin, muscle and spleen by using Tri-Reagent RNA/DNA/Protein Isolation Reagent (Molecular Research Center Inc., Cincinnati, OH, USA). The extraction was performed according to the manufacturer's directions. Quantification was made by optical density at 260 nm. 5.0 µg of total RNA was converted to cDNA by incubation at 42°C for 1 h with 200 U of Superscript II RNase H-RT (Invitrogen, Burlington, Ont. Canada), 300 ng of oligo-dT18, 500 µM deoxynucleotide triphosphate, 10 mM dithiothreitol, and 34 U of porcine RNase inhibitor (Amersham Pharmacia) in a final volume of 50 µl. The resulting products were then treated with RNase A for 30 min at 37°C and purified thereafter with Qiaquick PCR purification kit (QIAGEN, Mississauga, Ontario).
Isolation of *Macaca fascicularis* 17β-HSD12 cDNA

Since ortholog genes between the human and monkey possess generally more than 90–95% identity, we designed oligonucleotide primers based on the human sequence before the initiation codon (forward primer 5'-GTA-GTG-AGG-CCT-AGT-GGA-AAG-CCA-TG-3') and after stop codon (reverse primer 5'-CAA-GTT-ACA-ATG-CAG-TTA-TCA-TGC-3'). The fragment containing the entire *mf* 17β-HSD12 open reading frame was obtained by reverse transcriptase PCR (RT-PCR) amplification from *Macaca fascicularis* liver mRNA. The resulting PCR product was directly subcloned into a Zero Blunt TOPO vector (Invitrogen, Burlington, Ont. Canada). Plasmid DNA was prepared using the Qiagen Mega kit (Qiagen, Chatsworth, CA, USA) following the manufacturer’s protocol. The integrity of the construct was verified by automated dideoxynucleotide DNA sequencing using the Big Dye Terminator v3.1 Cycle Sequencing (ABI Prism, applied biosystem, Forster city, CA). The cDNA fragment was further transferred into PCMVneo expression vector in order to prepare stable transfected HEK-293 cells for activity characterization.
Enzymatic activity was determined in intact HEK-293 cells stably expressing Macaca fascicularis 17β-HSD12 as previously described [16]. Enzymatic activities were determined with intact cells stably expressing m(17β-HSD12 in culture. Briefly, cells were plated in 6-well plates to approximately 5 × 10⁵/well in MEM. 0.1 μM of the [¹⁴C]-labeled steroids (Dupont Inc., Mississauga, Ont. Canada) were added to freshly changed culture medium and incubated for 20 h. Mock transfection was done with the plasmid DNA of the pCMVneo vector. After incubation, the steroids were extracted twice with 2 ml of ether. The organic phases were then pooled and evaporated to dryness. The steroids were dissolved in 50 μL of dichloromethane, applied to Silica gel 60 thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany), before separation by migration in the toluene/acetone (4:1) solvent system. Substrates and metabolites were identified by comparison with reference steroids, revealed by autoradiography and quantified using the Phospholmager System (Molecular Dynamics, Inc., Sunnyvale, CA).

mRNA expression by Quantitative RealTime PCR (Q_RTPCR)

Macaca fascicularis 17β-HSD12 was amplified using the gene-specific primers: 5’-CAG-GCT-TGG-CTG-GTC-TTG-AA-3’ and 5’-CAC-CAT-GCC-AGG-CAG-TAC-CAA-3’. cDNA corresponding to 30 ng of the initial total RNA was used to perform fluorescent-based RealTime PCR quantification using the LightCycler RealTime PCR apparatus (Hoffman-La Roche Inc. Nutley, NJ) as described [33]. The conditions for the PCR reactions were: denaturation at 94°C for 15 sec, annealing at 50°C for 10 sec and elongation at 72°C for 35 sec. The data were normalized using the mRNA expression levels of the Macaca fascicularis housekeeping gene glucose-6-phosphate dehydrogenase (G6PDH) as internal standard. The mRNA expression levels are expressed as number of copies/μg total RNA using a standard curve of Cp versus logarithm of the quantity. The standard curve is established using known cDNA amounts of 0, 10², 10³, 10⁴, 10⁵ and 10⁶ copies of cDNA of glucose-6-phosphate dehydrogenase (forward primer: 5’-GGC-TGG-AAC-CGC-ATC-ATT-GTG-GA-3’ and reverse primer: 5’-GGC-GAT-GTT-GTC-CCG-GTT-CCA-GA-3’) and a LightCycler 3.5 program provided by the manufacturer (Roche Inc., Nutley, NJ). All sample were run in duplicates and quantification of each target gene expression was done two or three times. Results are expressed as mean ± SEM.

In situ hybridization

Recombinant plasmid pCRII-TOPO (Invitrogen, Burlington, Ont. Canada), containing a 331 bp Macaca fascicularis 17β-HSD12 fragment located at position 1–330 bp downstream from the ATG start codon was obtained by amplification using polymerase chain reaction. In situ hybridization with the antisense and sense 35S-labeled cRNA probes was performed as previously described.
Statistics

Results are given as mean ± SEM of two or three experiments. Data were analyzed by student's t-test for two columns. The differences was considered significant if p < 0.05.

Abbreviations

17β-estradiol
E2 estradiol
El-S estrone sulfate
El estrone
DHEA-S dehydroepiandrosterone sulfate
DHEA dehydroepiandrosterone
ADT androsterone
4-dione androstenedione

Authors' contributions

HL has participated in the design of the study and in drafting the manuscript; she has carried out the molecular biology manipulations, enzymatic assay, and the cell cultures. SFZ and GP carried out the in situ hybridization. VB carried out the partial enzymatic assays. FL has participated in careful reading of the manuscript. VLT conceived the study and was implicated in the redaction of the article. All authors read and approved final manuscript.

Acknowledgements

We would like to thank Nathalie Paquet, Guy Reimnitz and Mélanie Robitaille for their skilful technical assistance. This work was supported by CIHR grant to V.L.T. and F.L.

References

1. Mindnich R, Moller G, Adamski J: The role of 17 beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 2004, 218(1–2):7-20.
2. Moeller G, Adamski J: Multifunctionality of human 17beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 2006, 248(1–2):47-55.
3. Yan SD, Fu J, Soto C, Chen X, Zhu H, Al-Mohanna F, Collison K, Zhu A, Stern E, Saido T, et al: An intracellular protein that binds amyloid-beta peptide and mediates neurotoxicity in Alzheimer's disease. Nature 1997, 389(6652):689-695.
4. Imai H, Tamaoki B: Testicular 17 beta-hydroxysteroid dehydrogenase:molecular properties and reaction mechanism. Steroids 1986, 48(1–2):1-26.
5. Geissler W, Davis DL, Wu L, Bradshaw KD, Patel S, Mendonca BB, Ellisone KO, Wilson JD, Russell DW, Andersson S: Male pseudohermaphroditism caused by mutations of testicular 17 beta-hydroxysteroid dehydrogenase [3 see comments]. Nat Genet 1994, 7(1):34-39.
6. Dufort I, Rheault P, Huang XF, Soucy P, Luu-The V: Characteristics of a highly labile human type 5 17beta-hydroxysteroid dehydrogenase. Endocrinology 1999, 140(2):568-574.
7. Qiu W, Zhou M, Labrie F, Lin SX: Crystal structures of the multispecific 17beta-hydroxysteroid dehydrogenase type 5: critical androgen regulation in human peripheral tissues. Mol Cell Endocrinol 2004, 180(7):1798-1807.
8. Qin KN, Rosenfield RL: Expression of 17 beta-hydroxysteroid dehydrogenase type 5 in human ovary: a pilot study. J Soc Gynecol Invest 2000, 7(1):61-64.
9. Pelleiter G, Luu-The V, Tetz T, Labrie F: Immunocytochemical localization of type 5 17beta-hydroxysteroid dehydrogenase in human reproductive tissues. J Histochem Cytochem 1999, 47(6):731-738.
10. Mendonca BB, Arnhold JJ, Bloise W, Andersson S, Russell DW, Wilson JD: 17Beta-hydroxysteroid dehydrogenase 3 deficiency in women. J Clin Endocrinol Metab 1999, 84(2):802-804.
11. Luu-The V: Analysis and characteristics of multiple types of human 17beta-hydroxysteroid dehydrogenase. J Steroid Biochem Mol Biol 2001, 76(1–5):143-151.
12. Karavolas HJ, Baedecker ML, Engel LL: Human placental 17 beta-estradiol dehydrogenase. V. Purification and partial characterization of the diphosphopyridine nucleotide (triphosphopyridine nucleotide)-linked enzyme. J Biol Chem 1970, 245(19):4948-4952.
13. Feltokoto H, Isomaa V, Maentausta O, Vihko R: Complete amino acid sequence of human placental 17 beta-hydroxysteroid dehydrogenase deduced from cDNA. FEBS Lett 1988, 239(1):73-77.
14. Luu The V, Labrie C, Zhao HF, Couet J, Lachance Y, Simard J, Leblanc G, Cote J, Berube D, Gagne R, et al: Characteristics of cDNAs for human estradiol 17 beta-dehydrogenase and assignment of the gene to chromosome 17: evidence of two mRNA species with distinct 5' termini in human placenta. Mol Endocrinol 1989, 3(8):1301-1309.
15. Ghosh D, Pleterov VZ, Zhu DW, Wawrzak Z, Dux W, Pangborn W, Labrie F, Lin SX: Structure of human estrogenic 17 beta-hydroxysteroid dehydrogenase at 2.20 A resolution. Structure 1999, 5(5):503-513.
16. Luu-The V, Zhang Y, Poirier D, Labrie F: Characteristics of human types 1, 2 and 3 17 beta-hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition. J Steroid Biochem Mol Biol 1995, 55(5–6):S81-587.
17. Labrie F: Intracrinology. Mol Cell Endocrinol 1991, 78(3):C113-118.
18. Labrie F, Luu-The V, Labrie C, Belanger A, Simard J, Lin SX, Pelleiter G: Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and [34,35]. After hybridization, the sections were dehydrated and coated with liquid photographic emulsion (Kodak-NTB2; diluted 1:1 with water). After 27–36 days of exposure, the sections were processed and counterstained with haematoxylin.

Page 8 of 9
(page number not for citation purposes)
their precursor dehydroepiandrosterone. *Endocr Rev* 2003, 24(2):152-182.

19. Luu-The V, Tremblay P, Labrie F. Characterization of type 12 17 beta-hydroxysteroid dehydrogenase, an isofrom of type 3 17 beta-hydroxysteroid dehydrogenase responsible for estradiol formation in women. *Mol Endocrinol* 2005, 20(2):437-443.

20. Belanger A, Candas B, Dupont A, Cusan L, Diamond P, Gomez JL, Labrie F. Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men. *J Clin Endocrinol Metab* 1994, 79(4):1086-1090.

21. Luu-The V, Labrie C, Zhao HF, Couet J, Lachance Y, Simard J, Cote J, Leblanc G, Lagace L, Berube D, et al. Purification, cloning, complementary DNA structure, and predicted amino acid sequence of human estradiol 17 beta-dehydrogenase. *Ann N Y Acad Sci* 1990, 595:40-52.

22. Ghersevitch SA, Poutanen MH, Martikainen HK, Viikko RK. Expression of 17 beta-hydroxysteroid dehydrogenase in human granulosa cells: correlation with follicular size, cytochrome P450 aromatase activity and oestradiol production. *J Endocrinol* 1994, 143(1):139-150.

23. Sawetawan C, Milewich L, Word RA, Carr BR, Rainey WE. Compartmentalization of type 1 17 beta-hydroxysteroid oxidoreductase in the human ovary. *Mol Cell Endocrinol* 1994, 99(2):161-168.

24. Rantakari P, Strauss L, Pakarinen P, Poutanen M. Hydroxysteroid (17-beta) Dehydrogenase 2 Knockout Mice Display Embryonic Lethality and Growth Retardation, and Suffer from Severe Hydrocephalus and Polycystic Kidneys. In The Endocrine Society’s 87th Annual Meeting San Diego: The Endocrine Society; 2005.

25. Leenders F, Adamski J, Husen B, Thole HH, Jungblut PW. Molecular cloning and amino acid sequence of the porcine 17 beta-estradiol dehydrogenase. *Eur J Biochem* 1994, 222(1):221-227.

26. Adamski J, Husen B, Marks F, Jungblut PW. Purification and properties of oestradiol 17 beta-dehydrogenase extracted from cytoplasmic vesicles of porcine endometrial cells. *Biochem J* 1992, 288(Pt 2):375-381.

27. Hiltunen JK, Wenzel B, Beyer A, Erdmann R, Fossa A, Kunau WH. Peroxisomal multifunctional beta-oxidation protein of Saccharomyces cerevisiae. Molecular analysis of the fox2 gene and gene product. *J Biol Chem* 1992, 267(10):6646-6653.

28. Grunsven EG, van Berk M, de Klerk JB, Adamski J, Lemoine H, Clayton PT, Cuebas DA, Wanders RJ. Peroxisomal D-hydroxyacyl-CoA dehydrogenase deficiency: resolution of the enzyme defect and its molecular basis in bifunctional protein deficiency. *Proc NatlAcad Sci USA* 1996, 93(5):2128-2133.

29. Marijanovic Z, Lauher D, Moller G, Gege C, Husen B, Adamski J, Breiding R. Closing the gap: identification of human 3-keto-steroid reductase, the last unknown enzyme of mammalian cholesterol biosynthesis. *Mol Endocrinol* 2003, 17(9):1715-1725.

30. Fomitcheva J, Baker ME, Anderson E, Lee GY, Aziz N. Characterization of Ke 6, a new 17beta-hydroxysteroid dehydrogenase, and its expression in gonadal tissues. *J Biol Chem* 1998, 273(35):22664-22671.

31. Pelletier G, Luu-The V, Li S, Labrie F. Localization of type 8 17beta-hydroxysteroid dehydrogenase mRNA in mouse tissues as studied by in situ hybridization. *J Histochem Cytochem* 2005, 53(10):1257-1271.

32. Moon YA, Horton JD. Identification of two mammalian reductases involved in the two-carbon fatty acylation cascade. *J Biol Chem* 2003, 278(9):7335-7343.

33. Luu-The V, Pelletier G, Labrie F. Quantitative appreciation of steroidogenic gene expression in mouse tissues: new roles for type 2 Salpah-reductase, 20alpha-hydroxysteroid dehydrogenase and estrogen sulfotransferase. *J Steroid Biochem Mol Biol* 2005, 93(3-5):269-276.

34. Givaltos L, Li S, Pelletier G. Age-related decrease in the hypothalamic CRH mRNA expression is reduced by dehydroepiandrosterone (DHEA) treatment in male and female rats. *Brain Res Mol Brain Res* 1997, 48(1):107-114.

35. Pelletier G, Dupont E, Simard J, Luu-The V, Belanger A, Labrie F. Ontogeny and subcellular localization of 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD) in the human and rat adrenal, ovary and testis. *J Steroid Biochem Mol Biol* 1992, 43(5):451-467.