BIOLOGY OF REPRODUCTION **68,** 2092–2099 (2003)
Published online before print 22 January 2003.
DOI 10.1095/biolreprod.102.012476

Characterization of 17β-Hydroxysteroid Dehydrogenase Type 7 in Reproductive Tissues of the Marmoset Monkey[1]

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ABSTRACT

In contrast to the known rodent enzymes, the physiological significance of 17β-hydroxysteroid dehydrogenase type 7 (17HSD7) and its presumed function in reproductive biology is not well understood in primates. As a first step, we recently cloned the complete coding regions of human and marmoset monkey (*Callithrix jacchus*) 17HSD7 (cj17HSD7). In the present work the complete cDNA of marmoset 17HSD1 (cj17HSD1), including the proximal promoter region, and a partial sequence of marmoset aromatase (cjARO) were sequenced in order to compare the expression of these estradiol synthesizing enzymes with that of 17HSD7 in a primate model and to identify tissues where 17HSD7 might participate in the pathway of estradiol synthesis. The gene structures of cj17HSD1 and cj17HSD7 were determined and proved to be very similar to the human orthologues. Northern hybridization showed that cjARO mRNA seems to be coexpressed preferably with cj17HSD1 in placenta, whereas in other tissues it is expressed in parallel only with cj17HSD7. Especially in corpora lutea, the cj17HSD7 transcript is detectable throughout the luteal phase of the ovarian cycle and increases during pregnancy, in parallel with the transcript of aromatase. Results were confirmed by immunoblots and immunohistochemistry using new polyclonal antisera directed against cj17HSD7 and cjARO protein. The enzymatic conversion of estrone to estradiol was assessed in marmoset corpora lutea. The pattern of coexpression with aromatase supports the hypothesis that luteal 17HSD7 complements placental 17HSD1, ensuring continued estradiol synthesis throughout pregnancy in primates.

corpus luteum function, estradiol, female reproductive tract, ovulatory cycle, pregnancy

INTRODUCTION

In mammals, the interconversion of ketosteroids and their respective hydroxysteroids is performed by a group of enzymes called 17β-hydroxysteroid dehydrogenases (17HSDs). They are numbered chronologically in the order they were identified, and so far 11 different members of this protein family were reported [review, 1–3]. Not all of them are primarily involved in reproductive physiology. Different 17HSDs are specialized either for reduction of ketosteroids—such as 17HSDs 1, 3, and 7—or for oxidation of hydroxysteroids—such as 17HSDs 2, 4, and 8. Of the human enzymes, two are capable of catalyzing the last step in estradiol synthesis and are therefore involved in the control of estrogen action. These are 17β-hydroxysteroid dehydrogenase type 1 (17HSD1) and 17β-hydroxysteroid dehydrogenase type 7 (17HSD7).

17HSD1 was the first of the 17HSD enzymes identified and purified [4, 5]. It is a cytosolic enzyme and has a different substrate specificity in rodents and humans. It converts only estrogens in humans, but it can convert androgens equally well as estrogens in rodents. 17HSD1 is known to be expressed in granulosa cells of developing ovarian follicles of probably all mammals [6–8] and, in addition, in the syncytiotrophoblast of the placenta in primates and some other species [9, 10]. 17HSD1 is thought to be responsible for the preovulatory surge of estradiol arising from the granulosa cells of the dominant follicle and for fetal estradiol supply from the placenta during primate pregnancy [11, 12].

17HSD7 was first identified in the corpus luteum (CL) of the rat ovary as a microsomal prolactin receptor-associated protein, which is upregulated during pregnancy [13, 14]. Its enzymatic properties as an estrogenic 17HSD were discovered later in mice [15]. Results from experiments with mice and rats clearly indicated a specific role for the maintenance of pregnancy, but maintenance of pregnancy and control of CL function are very different in primates and rodents [12, 16]. In rodents, estradiol synthesis takes place exclusively in the CL throughout pregnancy, and the placenta is not a steroidogenic tissue. In humans and nonhuman primates, estradiol synthesis takes place in the CL only during a period at the beginning of pregnancy; later it takes place mainly in the placenta, i.e., there is a luteoplacental shift. According to these species-specific differences, a different regulation of 17HSD7 function might be expected.

The human 17HSD7 cDNA was recently cloned and the genomic structure was determined [17]. The human protein has not been examined yet. The common marmoset monkey has been proven as a suitable animal model to investigate the role of 17HSDs in primate reproductive biology [18, 19]. The aim of our study was to elucidate the role of 17HSD7 compared with that of 17HSD1 in primate reproductive tissues and especially during primate pregnancy where estradiol is known to be a prerequisite of placental and fetal development [20]. There is some phylogenetic evidence that 17HSD7 may have an ancient function in cholesterogenesis apart from its function in estradiol synthesis [21]. For this reason, the expression of aromatase (ARO) was monitored in parallel to 17HSD1 and 17HSD7 in this study to make sure that estrone as a substrate for estradiol synthesis can be provided in a particular tissue.

[1]Supported by Deutsche Forschungsgemeinschaft (Ei333/6-3).
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Received: 17 October 2002.
First decision: 13 November 2002.
Accepted: 8 January 2003.

ISSN: 0006-3363. http://www.biolreprod.org

MATERIALS AND METHODS

Animals

Adult female and male marmoset monkeys (*Callithrix jacchus*) were housed pairwise in a controlled environment at the German Primate Center, Göttingen, Germany. Ovarian cycles were controlled by $PGF_{2\alpha}$-induced luteolysis, and cycling or state of pregnancy were monitored as described earlier by regular assessment of serum progesterone [22]. The presence of corpora lutea and initiation of pregnancy were additionally confirmed by ultrasound examination of the trained animals (Esaote ultrasound system, Esaote Biomedica, Munich, Germany) [23, 24]. CL were obtained by luteectomy and placental tissue from Cesarean sections. For the determination of tissue-specific expression, various organs were collected from healthy animals that were culled as part of the colony management program. Tissues were immediately frozen in liquid nitrogen and stored at −80°C.

All experimental protocols received approval of the local animal experiment committee.

Cloning and Sequencing of cDNA

Total RNA was extracted from marmoset placenta using an RNeasy Midikit (Qiagen, Hilden, Germany) and digested by DNase I (Promega, Mannheim, Germany) for 30 min at 37°C. Complementary DNA was synthesized by SuperscriptII reverse transcriptase with oligo$(dT)_{12–18}$ primers (Invitrogen Life Technologies, Karlsruhe, Germany) and 2 μg of template RNA. Marmoset-specific polymerase chain reaction (PCR) fragments were generated by PCR using Turbo Pfu polymerase (Stratagene, Heidelberg, Germany). Primers were derived from the following human sequences: For 17HSD1 [25], forward, 5′-CAG CTG GAC GTA AGG GAC TC-3′; reverse, oligo (dT) anchor primer (39 bp) from 5′/3′RACE Kit (Roche Molecular Biochemicals, Mannheim, Germany), product length 1219 bp; and forward, 5′-GGC CTG CAC TTG GCC GTA CG-3′; reverse, 5′-GGC CTG CAG CAT CCG CAC AG-3′; product length 330 bp. For 17HSD7 [17], forward, 5′-CTG CTG AGG TCA CCA TTG TA-3′; reverse, 5′-CCA GAT GAG CTG AGA TGG AT-3′; product length 344 bp. For ARO [38], forward, 5′-TAA CAT CAC CAG CAT CGT GCC-3′; reverse, 5′-CCG AAT CGA GAC CTG TAA TG-3′; product length 318 bp). The fragments were cloned into the vector PCR-Script (PCR-Script Amp Cloning Kit, Stratagene). Sequencing reactions were performed by Sequiserve, Vaterstetten, Germany.

Determination of Gene Structures

A genomic cosmid library with 1.5-fold coverage and an average insert length of 32 kb was obtained from the resource center Primary Database of the German Human Genome Project at the Max Planck Institute for Molecular Genetics (Berlin, Germany). The library was screened with randomly primed [^{32}P]dCTP-labeled probes corresponding to the full length of the coding sequences of 17HSD1 or 17HSD7, respectively. After hybridization, filters were washed and exposed to Kodak X-Omat film (Kodak GmbH, Stuttgart, Germany) for 12 h at room temperature as described [17]. Four positive clones were detected for each gene. Two 17HSD1 clones and one 17HSD7 clone could be verified by PCR using primers in the 3′ region of the cDNA.

Northern Hybridization

For Northern analysis total RNA was extracted from various marmoset tissues as indicated in the figure legends using the RNeasy Midikit (Qiagen). Marmoset-specific digoxigenin-labeled riboprobes were prepared by in vitro transcription with T3-polymerase (DIG RNA labeling kit, Roche) from specific cDNA fragments cloned as described above. Total RNA (5 μg/lane) was separated on 1% formaldehyde agarose gels and transferred to positively charged nylon membranes(according to the DIG User's Manual, Roche). Prehybridization and hybridization were conducted at 68°C. The membranes were subjected to stringent washing and chemiluminescent detection with the substrate CDP Star (Roche). Densitometric analysis was performed by digitalization of x-ray films using the software Quantity One 4.0 and the Gel Doc video documentation system (Biorad, Munich, Germany).

Antibody Preparation

Polyclonal antipeptide antibodies directed against 17HSD7 and ARO were generated by immunization of rabbits (Pineda Antikörper Service, Berlin, Germany). The synthetic peptides [NH2-C]KMDLDEDTAEKFYQK[-CONH2] corresponding to amino acids 301–315 of marmoset and human 17HSD7 and [NH2-C]KPNEFTLENFAKNVPYR[-CONH2] corresponding to amino acids 409–425 of human ARO were coupled to keyhole limpet hemocyanin and were used as antigens. All antisera were purified by affinity chromatography.

Specificity of the antisera was tested by immunoblot analysis. The antibody directed against ARO was validated by direct comparison with the specificity of a commercially available antibody (Biozol Diagnostika, Eching, Germany, originally described by [26]) on microsomal extracts of marmoset placenta.

Immunoblotting

Bacterial lysates were obtained from *E. coli* BL21 CodonPlus (DE3)-RP (Stratagene) expressing fusion proteins of glutathione S-transferase with human 17HSD7, human 17HSD1, mouse 17HSD7, or glutathione S-transferase alone, respectively. The cells were lysed by treatment with 7.5 U/μl rLysozyme (Novagen, Schwalbach/Taunus, Germany) in PBS/5% glycerol followed by three cycles of freezing and thawing and treatment with Benzonase (Merck Eurolabs, Darmstadt, Germany) according to the manufacturer's instructions.

Microsomal protein extracts were prepared by disruption of marmoset tissues in PBS using an Ultraturrax homogenizer. Homogenates were centrifuged at 600 × *g* to remove remaining tissue debris. The 600 × *g* supernatant was subjected to centrifugation at 40 000 × *g* for 2 h. The 40 000 × *g* pellet was resuspended in PBS and aliquots were used for protein determination [27] and immunoblots.

For immunoblots, denaturing/loading buffer (100 mM Tris, pH 6.8, 100 mM DTT, 2% SDS, 10% glycerol, 0.01% bromophenol blue) was added to the aliquots. Samples were subjected to denaturing PAGE on 10% gels and subsequent semidry blotting onto nitrocellulose membranes (Amersham Biosciences, Freiburg, Germany) according to standard procedures (Biorad Mini-Protean 3 Cell, Instruction Manual). Membranes were blocked with PBS/0.05% Tween20 and incubated overnight at 4°C with primary antibody (17HSD7 1:2000, ARO 1:500). In negative controls the primary antibody was blocked by 1 h preincubation at room temperature with an excess concentration of the peptide that had been used for immunization.

Bound antibodies were visualized using a goat anti-rabbit IgG coupled to alkaline phosphatase in a dilution of 1:10 000 and nitroblue tetrazolium/bromo-chloro-indolyl phosphate (Sigma Aldrich, Taufkirchen, Germany) as substrate.

Immunohistochemistry

Tissues were fixed with 4% paraformaldehyde and embedded in paraffin. Five-micrometer sections were deparaffinized, hydrated, and stained according to the DAKO Envision protocol (DAKO Diagnostika, Hamburg, Germany) using a dilution of 1:500 or 1:1000 of the primary antibody and using diaminobenzidine as the substrate for visualization of the immune reaction. In negative controls the primary antibody was blocked by 1 h preincubation at room temperature with an excess concentration of the peptide that had been used for immunization.

Staining was visualized with an Axiophot microscope (Zeiss, Oberkochen, Germany) using Openlab 3.0 digital image analysis (Improvision, Coventry, U.K.).

HPLC Analysis of Enzyme Activity

Frozen CLs were thawed on ice and homogenized by ultraturrax in 0.5 ml phosphate buffered saline. Conversion of estradiol to estrone was measured by HPLC analysis as described by Adamski et al. [28]. Determination of protein content was performed according to Markwell et al. [27].

Statistical Analyses

For comparison of reductive and oxidative enzyme activities in CL, a paired Student *t*-test was performed.

RESULTS

Sequence Analysis of Marmoset Estradiol Synthesizing Enzymes

The cDNAs from the complete coding region and the proximal promoter region of marmoset 17HSD1 (cj17HSD1) and

TABLE 1. Percent homology (cDNA/protein) of the cloned marmoset-specific cDNAs and the deduced amino acid sequences compared with the known cDNAs of other species (human, [25], [17], [44]; rat, [45], [13], [46]; mouse, [47], [15], [48]).

	cj17HSD1 (1160 bp)	cj17HSD7 (971 bp)	ARO (278 bp)
Accession number of marmoset sequence	AF272013	AF263468	AY034779
Human	89/85	95/95	96/94
Rat	78/78	83/81	86/76
Mouse	78/79	81/78	87/79

a partial sequence of marmoset ARO (cjARO) were obtained by RT-PCR from marmoset placental RNA. They were cloned and the sequence homologies to other species were determined (Table 1). Marmoset 17HSD7 (cj17HSD7), which had been cloned earlier [18], was also included in the table.

Marmoset 17HSD1 is remarkably less conserved than cj17HSD7. The coding region of cj17HSD1 is 46 nucleotides shorter than the human cDNA owing to a deletion in exon 6. Consequently, the deduced amino acid sequence is shortened by 14 residues.

In the coding region of cj17HSD7 there is a deletion of only two nucleotides, which causes the generation of an earlier stop codon than in the human orthologue. The deduced amino acid sequence is 11 residues shorter than in the human.

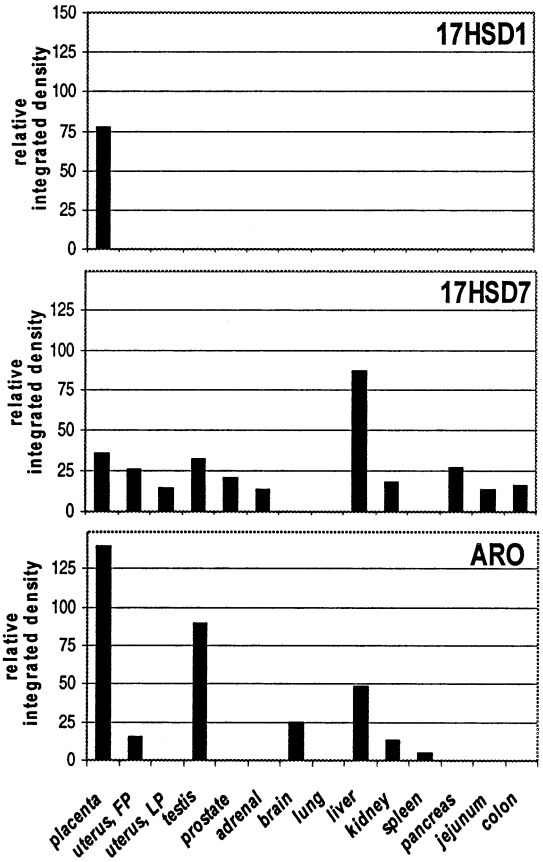

FIG. 1. Semiquantitative analysis of Northern blots with total RNA (5 μg/lane) extracted from various marmoset tissues, as indicated, showing the tissue-specific expression and coexpression of marmoset 17β-hydroxysteroid dehydrogenase type 7 (cj17HSD1), cj17HSD7, and aromatase. Integrated density was calculated in relation to the expression level of β-actin in the same samples from one typical experiment. FP, Proliferative phase; LP, secretory phase.

The proximal promoter region of cj17HSD1 was determined from position −1 to −361 and contained essentially the same regulatory elements as the human orthologue (data not shown). Overall homology of the promoter region amounts to 93%.

Intron-exon organization of cj17HSD1 and cj17HSD7 genes were obtained by screening a genomic cosmid library. Introns of 17HSD7 were not sequenced, but the sizes were determined using intron-spanning PCR primers. There is again a close resemblance to the human orthologues, with the exception of intron 8 from cj17HSD7. It has a length of 8.0 kb in the human gene and it spans only 3.0 kb in the marmoset gene. Unfortunately, no clone was identified in the cosmid library, which contained the complete cj17HSD7 gene. Therefore, the promoter region and exons 1 and 2 of this gene could not be analyzed in this study.

Tissue Distribution of 17HSD7 Compared with 17HSD1 and Aromatase

The overall tissue distribution of 17HSD7 mRNA was compared with that of 17HSD1 and ARO and quantified by densitometric analysis relative to β-actin expression in northern blots using marmoset-specific RNA-probes (Fig. 1). Apart from placenta, all tissues were obtained from adult, nonpregnant animals.

As shown earlier [18], cj17HSD1 is represented by two transcripts of 2.3 and 1.3 kb in size. Only the smaller transcript that corresponds to the expression of an active enzyme [29] was quantified and was found exclusively in placenta. The mRNA sizes of cj17HSD7 transcripts are approximately 2.8 and 1.4 kb in the marmoset [18]. The density of these two transcripts showing a broader tissue distribution than cj17HSD1 mRNA was calculated by the addition of both values. Marmoset 17HSD7 mRNA was expressed in steroidogenic tissues like placenta or testes, but the strongest signal was observed in liver. Marmoset ARO mRNA was coexpressed with 17HSD7 in placenta, uterus (proliferative phase), testis, liver, and kidney.

To confirm these results at the level of protein expression, a polyclonal antiserum directed against 17HSD7 was generated and its specificity was proven by immunoblot analysis (Fig. 2). Different fusion proteins with glutathione S-transferase were overexpressed in *E. coli*. The antiserum reacted with a fusion protein of human or murine 17HSD7, but not with a fusion protein of human 17HSD1 and glutathione S-transferase or glutathione S-transferase alone.

In microsomal extracts from different marmoset tissues, protein bands of approximately 30, 60, and 80 kDa were detected (Fig. 3). The immunoreaction with the 30- and 60-kDa protein could be inhibited by preabsorption of the antiserum with the purified peptide antigen. Interestingly, there was no specific protein signal in placenta and uterus, although a moderate signal for the respective mRNA was shown to be present in the Northern blot analysis (Fig. 1).

Expression of Estradiol Synthesizing Enzymes in Reproductive Tissues During Ovarian Cycle and Pregnancy

The time-related expression pattern of estradiol synthesizing enzymes was studied in three tissues that are involved in the maintenance of pregnancy, i.e., in CL, uterus, and placenta. Marmoset pregnancy lasts approximately 144 days [30]. Total RNA from late luteal phase (Day 13/14)

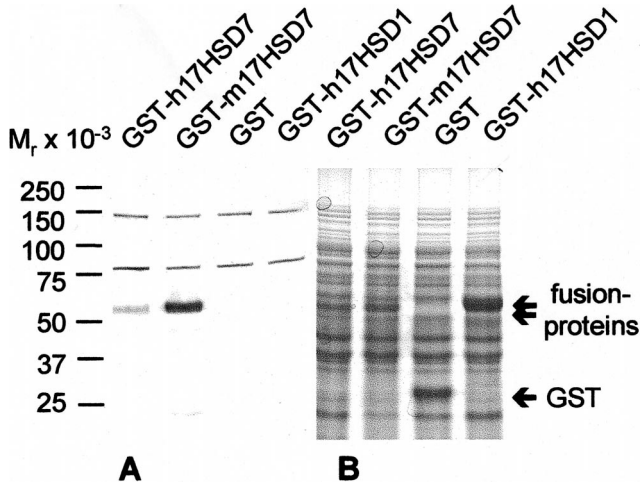

FIG. 2. Specificity of the antiserum used in immunoblots and immunohistochemistry. Proteins were expressed as fusion proteins with glutathione S-transferase in *E. coli*. Equal amounts of protein were loaded on blot (**A**) and Coomassie stained gel (**B**). hHSD7, Human 17β-hydroxysteroid dehydrogenase type 7 (17HSD7); mHSD7, murine 17HSD7; hHSD1, human 17HSD type 1 (17HSD1); GST, glutathione S-transferase.

and Days 28/29, 38, 40, 98, and 135 of pregnancy (near term) was subjected to Northern blot analysis (Fig. 4). In all tissues examined, cj17HSD1 mRNA was detected almost exclusively in the placenta, even as early as Day 38, and only faintly in one of the two CL from Day 38. In contrast to that, the strongest signal for cj17HSD7 mRNA was observed in the CL. It increased relative to β-actin mRNA in the CL during the first part of pregnancy before the luteo-placental shift in marmoset monkeys [31]. After that, at Day 98, cj17HSD7 expression was reduced. In contrast to the expression pattern reported for the rat [14], cjHSD7 was additionally expressed at a lower level in the pregnant uterus and even lower in placenta. Marmoset ARO was expressed in CL and placenta at all times. Thus in both tissues, CL and placenta, the two steps of estradiol synthesis, aromatization and reduction, can be performed. ARO is expressed in all estradiol synthesizing tissues, CL and placenta, whereas cj17HSD1 and cj17HSD7 are never expressed at the same level in a given tissue. Compared with its expression level in uterus and placenta, cj17HSD7 mRNA is most prominent in the CL of pregnancy.

The coexpression of cj17HSD7 and cjARO was confirmed by immunoblots of CL samples from different time points of pregnancy (Fig. 5). The expression of both proteins increases up to Day 98 of pregnancy and fades after delivery.

Immunolocalization of cj17HSD7

Cell type-specific expression of cj17HSD7 was examined by immunohistochemistry on paraffin sections (Fig. 6). In the ovary the protein was assigned exclusively to luteal cells. No cj17HSD7 protein was detected in follicles (data not shown). A moderate immunoreaction was further observed in the placental villi, in Leydig cells, and in specific cells of the tubular compartment of the testis, presumably Sertoli cells, as well as in epithelial cells of the epididymis. Preabsorption with the immunizing peptide abolished the immunoreaction completely.

Enzymatic Interconversion of Estradiol and Estrone in Marmoset CL

As only 17HSD7 mRNA and protein, but not 17HSD1 mRNA, were shown to be present in marmoset CL (Fig. 4), any estradiol synthetic enzyme activity in this tissue should be due to 17HSD7. A limited amount of luteal tissue was available for the assessment of this enzymatic activity. Triplicate samples from two CLs of the midluteal phase and from Day 45 of pregnancy were examined. Interconversion of estradiol and estrone was readily detectable. Reductive activity (estradiol synthesis) was 1.5- to 3-fold higher than oxidative activity (30.9 ± 8.1 μU/mg protein reduction vs. 10.0 ± 1.5 μU/mg protein oxidation in the midluteal phase CL, and 18.9 ± 5.8 μU/mg protein reduction vs. 13.2 ± 3.7 μU/mg protein oxidation in the CL from Day 45 of pregnancy, $P < 0.05$).

DISCUSSION

In our recent work the complete coding region of cj17HSD7 and a partial cDNA sequence of cj17HSD1 had been cloned [18]. The sequences revealed a high similarity between marmoset and human enzymes. The two estradiol synthesizing enzymes showed a different tissue distribution. The present study was intended to verify expression and activity of 17HSD7 in reproductive tissues of primates.

The coding region of cj17HSD1 was cloned completely, and the gene structures of cj17HSD1 and cj17HSD7 were analyzed. Compared with the human enzymes, again no major differences were detected in the marmoset, indicating the same structure-function relationships in the two species. However, 17HSD7 proved to be more conserved than 17HSD1.

A new specific polyclonal antibody was validated and enabled us to perform the first detailed study of 17HSD7 protein expression in primate tissues. Earlier Northern blot analysis of mouse and marmoset tissues as well as RT-PCR

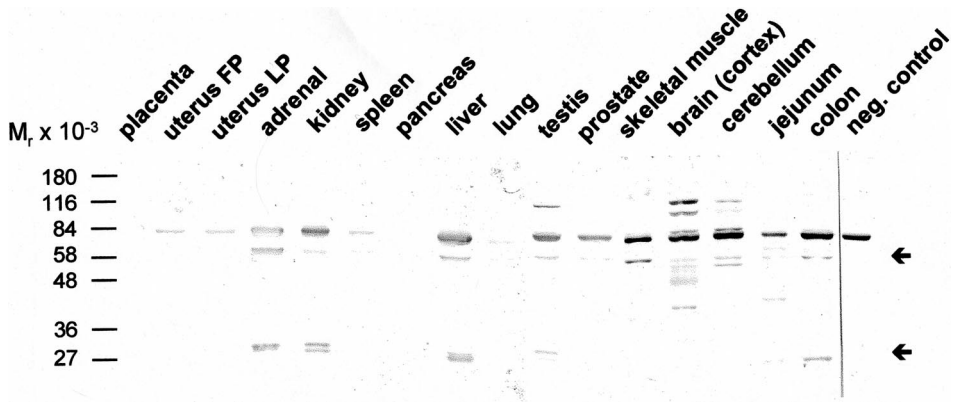

FIG. 3. Immunoblot with microsomal proteins (10 μg/lane) extracted from various marmoset tissues as indicated. Only nonspecific signals were seen using antiserum preabsorbed with the immunizing peptide (negative control, kidney). Arrows point at the positions of specific bands.

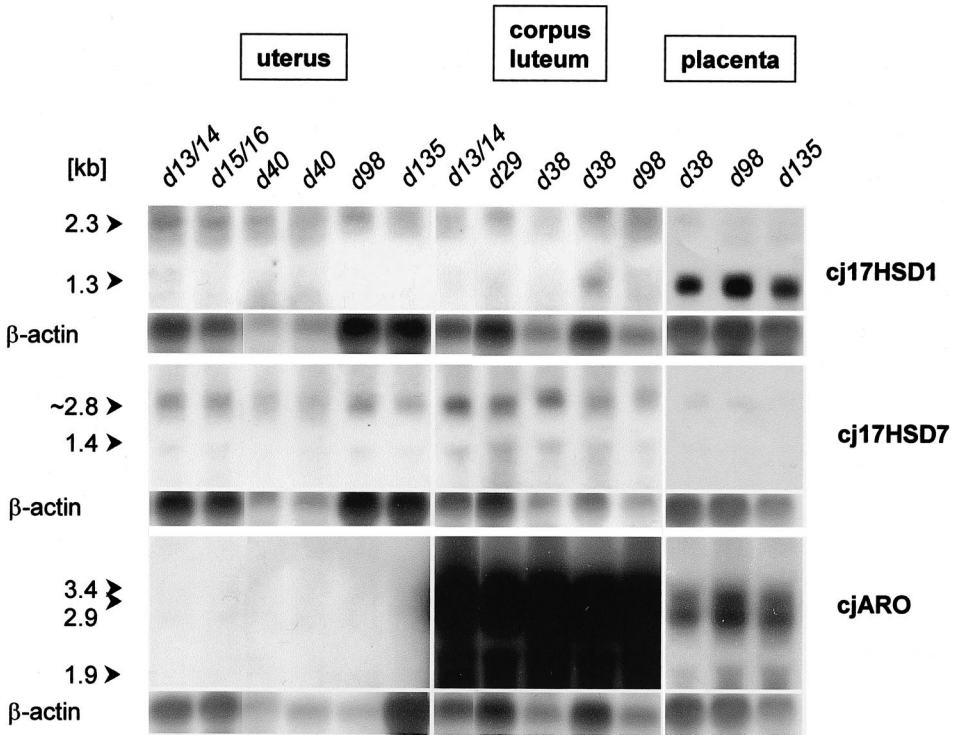

FIG. 4. Northern blot analysis of total RNA (5 μg/lane) extracted from marmoset tissues involved in the maintenance of pregnancy at different time points of the luteal phase and pregnancy as indicated. Specific probes for marmoset 17β-hydroxysteroid dehydrogenase type 1 (cj17HSD1), 17HSD7 (cj17HSD7), and aromatase (cjARO) were applied. Hybridization with a β-actin probe was used to check for even RNA loading and RNA degradation.

analysis of human tissues [15, 17, 18] had suggested a broader tissue distribution than originally assumed by immunological studies in rat [32]. Indeed, we detected cj17HSD7 protein in liver, kidney, reproductive tissues, adrenal, and intestine.

In this study the expression of ARO, which provides estrone as the substrate of 17HSD1 or 17HSD7, was examined in addition to the two 17HSDs. It was assumed that tissues expressing ARO together with 17HSD1 or 17HSD7 are indeed involved in estradiol synthesis. A look at the complete estrogenic pathway was considered as an important aspect in the elucidation of 17HSD7 function, because some phylogenetic evidence was presented that gave rise to speculations whether this enzyme might have an additional function in cholesterol synthesis [21]. Our study provides further arguments for this notion, showing the strongest expression of cj17HSD7 in liver accompanied only by a comparably weak expression of ARO. Moreover, exactly those gonadal cell types immunopositive for 17HSD7—luteal cells and Leydig cells—have been identified as sites of de novo cholesterogenesis [33]. Nevertheless, cj17HSD7 is always coexpressed with ARO in the gonads, and its protein expression is tightly regulated like that of ARO in the CL of pregnant animals.

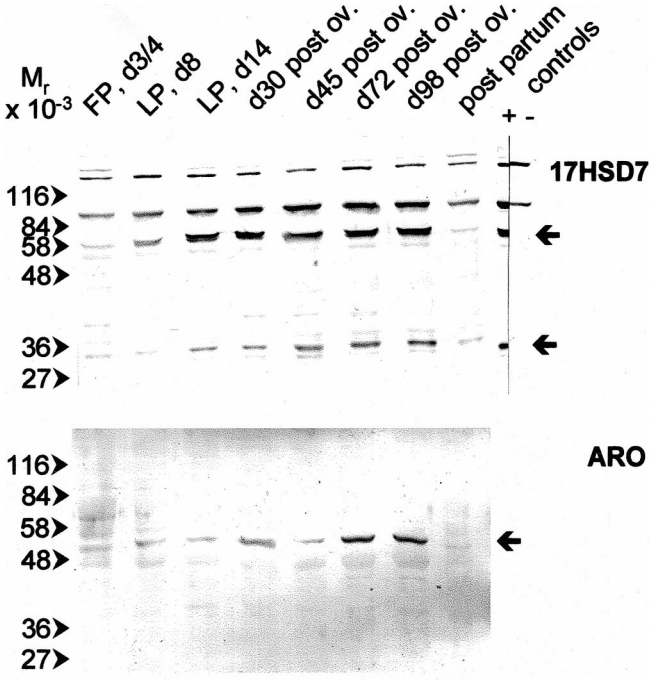

FIG. 5. Immunoblot analysis of marmoset corpus luteum (CL) homogenates from different time points of the luteal phase and pregnancy. No specific signal was seen using antiserum preabsorbed with the immunizing peptide (negative control). FP, Follicular phase; LP, luteal phase; post ov, postovulation.

According to our results, estradiol synthesis in the CL of pregnant animals appears as one of the key functions of 17HSD7 not only in rodents, but also in primates in spite of different control mechanisms of CL function and steroidogenic function of the placenta in these species. Reductive estrogenic enzyme activity in CLs of marmoset monkeys must be assigned to cj17HSD7, because it is the only estradiol synthesizing enzyme detectable in their CL. Similarly, 17HSD1 was not found in the CL of cycling or pregnant rats [7, 34]. It is true that also in the human a weak expression of 17HSD1 was shown in luteinized granulosa cells originating from patients treated for in vitro fertilization [6, 35, 36], but these cells were retrieved shortly before ovulation and did not necessarily represent a physiological state of luteal cells. In immunohistochemistry on sections of human ovaries from the midluteal phase, only a weak immunoreaction specific for 17HSD1 could be detected [6]. The primate model examined in this work offered the possibility to examine CL from different and defined time points of the ovarian cycle and pregnancy. In

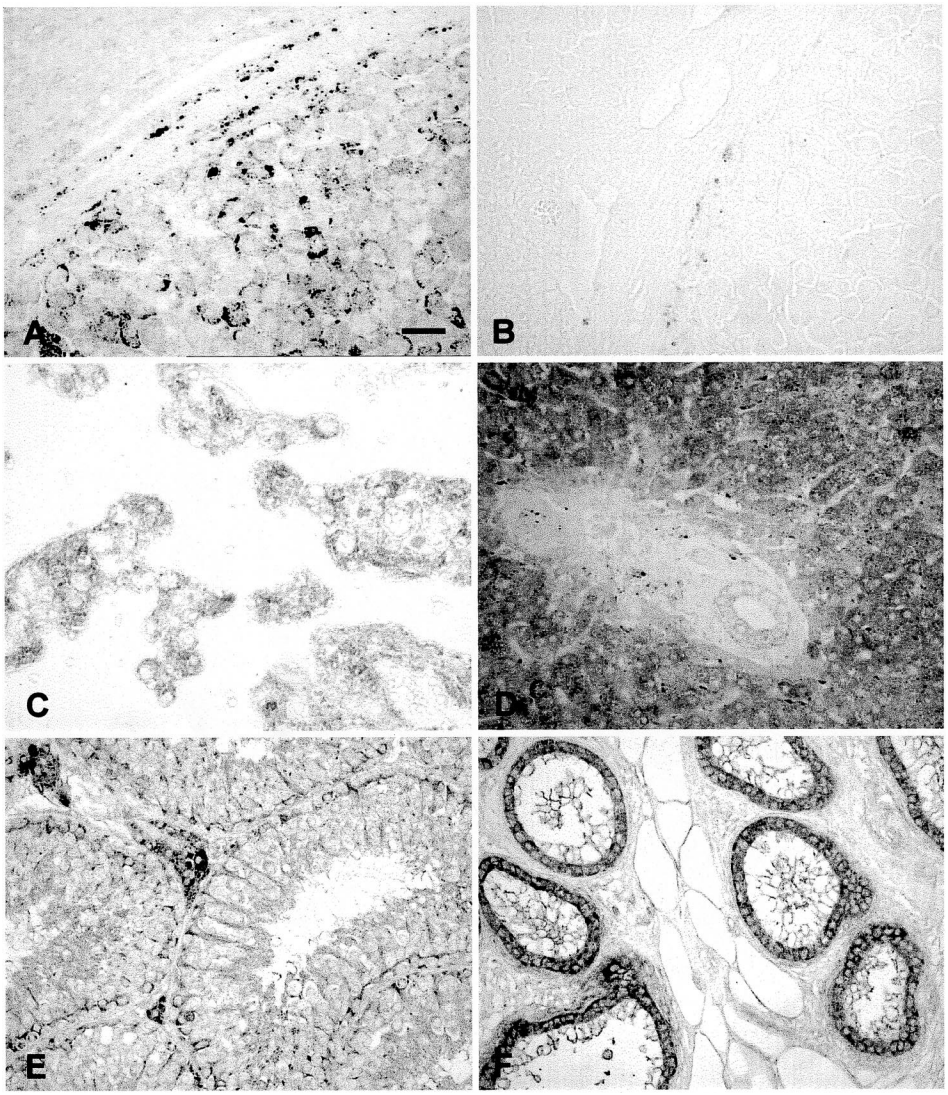

FIG. 6. Immunolocalization of marmoset 17β-hydroxysteroid dehydrogenase type 7 (cj17HSD7) in paraffin sections from marmoset tissues. **A**) Corpus luteum and surrounding ovarian tissue from the luteal phase of the ovarian cycle. **B**) Negative control: the same corpus luteum as in (**A**), antiserum preabsorbed with the immunizing peptide. **C**) Placental villi. **D**) Liver. **E**) Testis. **F**) Epididymis. Scale bar (50 μm) in (**A**) refers to all sections.

the marmoset monkey only 17HSD1 of placental origin seems to complement luteal 17HSD7 in performing the last step in estradiol synthesis after the luteo-placental shift in steroidogenesis.

It was suggested that 17HSD7 may have an important function during implantation because it could be localized specifically at the implantation site in mouse uterus by in situ hybridization [37]. Marmoset uteri were processed in toto (endometrium and myometrium) in this study, thus diluting endometrium-specific gene products that are expressed at low levels or only locally at distinct areas like implantation sites. This may be the reason why only a moderate expression of cj17HSD7 mRNA and no expression of cj171HSD1 or cjARO was observed in all uteri examined, irrespective of the time point chosen. Further studies are needed to prove a role of 17HSD7 in primate implantation.

In view of its broad tissue distribution, 17HSD7 is also a candidate enzyme for extragonadal estrogen synthesis. In humans and most higher primates the zona reticularis of the adrenal cortex secretes androgen precursors into the circulation, which can be converted to estradiol in peripheral, extragonadal tissues [38–42]. In New World monkeys such as *C. jacchus*, high circulating levels of dehydroepiandrosterone as an androgen precursor of extragonadal estradiol synthesis are maintained by the gonads [43]. This increased presence of circulating steroid precursors enables primates to take advantage of local tissue-specific estradiol synthesis.

In conclusion, the regulated coexpression pattern of cj17HSD7 and cjARO together with the estrogenic activity in CL tissue demonstrated in this study speak in favor of a genuine function of 17HSD7 in estradiol synthesis. On the other hand enzyme protein expression was also evident in nonreproductive tissues. Thus the proposed function in cholesterogenesis remains conceivable.

ACKNOWLEDGMENTS

We are grateful to Angelika Jurdzinski and Alexandra Marten for their help during tissue collection and staging of animals.

REFERENCES

1. Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenase. Endocrine Rev 1997; 18:281–305.
2. Peltoketo H, Luu-The V, Simard J, Adamski J. 17beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase family: nomenclature and main characteristics of the 17HSD/KSR enzymes. J Mol Endocrinol 1999; 23:1–11.
3. Adamski J, Jakob FJ. A guide to 17beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 2001; 171:1–4.
4. Engel LL, Groman EV. Human placental 17beta-estradiol dehydro-

genase: characterization and structural studies. Recent Prog Horm Res 1974; 30:139–169.
5. Strickler RC, Tobias B, Covey DF. Human placental 17beta-estradiol dehydrogenase and 20alpha-hydroxysteroid dehydrogenase. Two activities at a single enzyme active site. J Biol Chem 1981; 256:316–321.
6. Sawetawan C, Milewich L, Word RA, Carr BR, Rainey WE. Compartmentalization of type 1 17beta- hydroxysteroid oxydoreductase in the human ovary. Mol Cell Endocrinol 1994; 99:161–168.
7. Ghersevich SA, Poutanen MH, Rajaniemi HJ, Vikho RK. Expression of 17beta-hydroxysteroid dehydrogenase in the rat ovary during follicular development and luteinization induced with pregnant mare serum gonadotropin and human chorionic gonadotropin. J Endocrinol 1994; 140:409–417.
8. Zhang Y, Word RA, Fesmire S, Carr BR, Rainey WE. Human ovarian expression of 17beta-hydroxysteroid dehydrogenase types 1, 2 and 3. J Clin Endocrinol Metab 1996; 81:3594–3598.
9. Fournet-Dulguerov N, MacLusky NJ, Leranth CZ, Todd R, Mendelson CR, Simpson ER, Naftolin F. Immunohistochemical localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta. J Clin Endocrinol Metab 1987; 65:757–764.
10. Takeyama J, Sasano H, Suzuki T, Iinuma K, Nagura H, Andersson S. 17beta-Hydroxysteroid dehydrogenase types 1 and 2 in human placenta: an immunohistochemical study with correlation to placental development. J Clin Endocrinol Metab 1998; 83:3710–3715.
11. Peltoketo H, Vihko P, Vihko R. Regulation of estrogen action: role of 17beta-hydroxysteroid dehydrogenases. Vitam Horm 1999; 55:353–398.
12. Peltoketo H, Nokelainen P, Piao Y, Vihko R, Vihko P. Two 17beta-hydroxysteroid dehydrogenases (17HSDs) of estradiol biosynthesis: 17HSD type 1 and type 7. J Steroid Biochem Mol Biol 1999; 69: 431–439.
13. Duan WR, Linzer DIH, Gibori G. Cloning and characterization of an ovarian-specific protein that associates with the short form of the prolactin receptor. J Biol Chem 1996; 271:15602–15607.
14. Duan WR, Parmer TG, Albarracin CT, Zhang L, Gibori G. PRAP, a prolactin receptor associated protein: its gene expression and regulation in the corpus luteum. Endocrinology 1997; 138:3216–3221.
15. Nokelainen P, Peltoketo H, Vihko R, Vihko P. Expression cloning of a novel estrogenic mouse 17beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase (m17βHSD7), previously described as a prolactin receptor-associated protein (PRAP) in rat. Mol Endocrinol 1998; 12:1048–1059.
16. Stouffer RL. Corpus luteum of pregnancy. In: Knobil E, Neill DE (eds.), Encyclopedia of Reproduction, vol. 1. San Diego, CA: Academic Press; 1999:709–717.
17. Krazeisen A, Breitling R, Imai K, Fritz S, Möller G, Adamski J. Determination of cDNA, gene structure and chromosomal localization of the novel human 17beta-hydroxysteroid dehydrogenase type 7. FEBS Lett 1999; 460:373–379.
18. Schwabe I, Husen B, Einspanier A. Expression of the estradiol-synthesizing 17beta-hydroxysteroid dehydrogenases type 1 and type 7 in the nonhuman primate *Callithrix jacchus*. Mol Cell Endocrinol 2001; 171:187–192.
19. Husen B, Adamski J, Rune GM, Einspanier A. Mechanisms of estradiol inactivation in primate uterus. Mol Cell Endocrinol 2001; 171: 179–186.
20. Pepe GJ, Albrecht ED. Regulation of functional differentiation of the placental villous syncytiotrophoblast by estrogen during primate pregnancy. Steroids 1999; 64:624–627.
21. Breitling R, Krazeisen A, Möller G, Adamski J. 17beta-hydroxysteroid dehydrogenase type 7—an ancient 3-ketosteroid reductase of cholesterogenesis. Mol Cell Endocrinol 2001; 171:199–204.
22. Einspanier A, Nubbemeyer R, Schlote S, Schumacher M, Ivell R, Fuhrmann K, Marten A. Relaxin in the marmoset monkey: secretion pattern in the ovarian cycle and early pregnancy. Biol Reprod 1999; 61:512–520.
23. Oerke AK, Einspanier A, Hodges JK. Detection of pregnancy and monitoring patterns of uterine and fetal growth in the marmoset monkey (*Callithrix jacchus*) by real-time ultrasonography. Am J Primatol 1995; 36:1–13.
24. Oerke AK, Einspanier A, Hodges JK. Noninvasive monitoring of follicle development, ovulation and corpus luteum formation in the marmoset monkey (*Callithrix jacchus*) by ultrasonography. Am J Primatol 1996; 39:99–113.
25. Peltoketo H, Isomaa V, Mäentausta O, Vihko R. Complete amino acid sequence of human placental 17beta-hydroxysteroid dehydrogenase deduced from cDNA. FEBS Lett 1988; 239:73–77.
26. Turner KJ, Macpherson S, Millar MR, McNeilly AS, Williams K, Cranfield M, Groome NP, Sharpe RM, Fraser HM, Saunders PT. Development and validation of a new monoclonal antibody to mammalian aromatase. J Endocrinol 2002; 172:21–30.
27. Markwell MA, Haas SM, Tolbert NE, Bieber LL. Protein determination in membrane and lipoprotein samples: manual and automated procedures. Methods Enzymol 1981; 72:296–303.
28. Adamski J, Sierralta WD, Jungblut PW. Harvesting and separation of two populations of lysosomes from porcine endometrium. Eur J Cell Biol 1988; 45:238–245.
29. Miettinen MM, Mustonen MV, Poutanen MH, Isomaa VV, Vihko RK. Human 17beta-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression. Biochem J 1996; 314:839–845.
30. Hearn JP. The common marmoset (*Callithrix jacchus*). In: Hearn JP (ed.), Reproduction in New World Primates, New Models in Medical Science. Lancaster, UK: MTP Press Ltd.; 1983:181–215.
31. Hodges JK, Henderson C, Hearn JP. Relationship between ovarian and placental steroid production during early pregnancy in the marmoset monkey (*Callithrix jacchus*). J Reprod Fertil 1983; 69:613–621.
32. Parmer TG, McLean MP, Duan WR, Nelson SE, Albarracin CT, Khan I, Gibori G. Hormonal and immunological characterization of the 32 kilodalton ovarian-specific protein. Endocrinology 1992; 131:2213–2221.
33. Royo T, Pedragosa MJ, Ayte J, Gil-Gomez G, Vilaro S, Hegardt FG. Testis and ovary express the gene for the ketogenic mitochondrial 3-hydroxy-3-methyglutaryl-CoA synthase. J Lipid Res 1993; 34:867–874.
34. Akinola LA, Poutanen M, Vihko R, Vihko P. Expression of 17beta-hydroxysteroid dehydrogenase type 1 and type 2, P450 aromatase, and 20alpha-hydroxysteroid dehydrogenase enzymes in immature, mature, and pregnant rats. Endocrinology 1997; 138:2886–2892.
35. Ghersevich SA, Poutanen MH, Martikainen HK, Vihko 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; 143:139–150.
36. Tremblay Y, Ringler GE, Morel Y, Mohandas TK, Labrie F, Strauss JF 3rd, Miller WL. Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen-q25. J Biol Chem 1989; 264:20458–20462.
37. Nokelainen P, Peltoketo H, Mustonen M, Vihko P. Expression of mouse 17beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase type 7 in the ovary, uterus, and placenta: localization from implantation to late pregnancy. Endocrinology 2000; 141:772–778.
38. Martel C, Rheaume E, Takahashi M, Trudel C, Couet J, Luu-The V, Simard J, Labrie F. Distribution of 17beta-hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. J Steroid Biochem Mol Biol 1992; 41(3–8):597–603.
39. Martel C, Melner MH, Gagné D, Simard J, Labrie F. Widespread tissue distribution of steroid sulfatase, 3beta-hydroxysteroid dehydrogenase/delta4-delta5 isomerase (3beta-HSD), 17beta-HSD, 5alpha-reductase and aromatase activities in the rhesus monkey. Mol Cell Endocrinol 1994; 104:103–111.
40. Conley AJ, Bird IM. The role of cytochrome P450 17alpha-hydroxylase and 3beta-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the delta5 and delta4 pathways of steroidogenesis in mammals. Biol Reprod 1997; 56:789–799.
41. Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, Belanger A. The key role of 17beta-hydroxysteroid dehydrogenases in sex steroid biology. Steroids 1997; 62:148–158.
42. Labrie F, Belanger A, Luu-The V, Labrie C, Simard J, Cusan L, Gomez J, Candas B. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging. Steroids 1998; 63:322–328.
43. Levine J, Wolfe LG, Schiebinger RJ, Loriaux DL, Cutler GB Jr. Rapid regression of fetal adrenal zone and absence of adrenal reticular zone in the marmoset. Endocrinology 1982; 111:1797–1802.
44. Simpson ER, Evans CT, Corbin CJ, Powell FE, Ledesma DB, Mendelsohn CR. Sequencing of cDNA inserts encoding aromatase cytochrome P-450 (P-450AROM). Mol Cell Endocrinol 1987; 52:267–272.
45. Ghersevich S, Nokelainen P, Poutanen M, Orava M, Autio-Harmainen H, Rajaniemi H, Vihko R. Rat 17 beta-hydroxysteroid dehydrogenase type 1: primary structure and regulation of enzyme expression in rat

ovary by diethylstilbestrol and gonadotropins in vivo. Endocrinology 1994; 135:1477–1487.
46. Hickey GJ, Krasnow JS, Beattie WG, Richards JS. Aromatase cytochrome P450 in rat ovarian granulosa cells before and after luteinization: adenosine 3′,5′-monophosphate-dependent and independent regulation. Cloning and sequencing of rat aromatase cDNA and 5′ genomic DNA. Mol Endocrinol 1990; 4:3–12.
47. Nokelainen P, Puranen H, Peltoketo M, Orava M, Vikho P. Molecular cloning of mouse 17beta-hydroxysteroid dehydrogenase type 1 and characterization of enzyme activity. Eur J Biochem 1996; 236:482–490.
48. Terashima M, Toda K, Kawamoto T, Kuribayashi I, Ogawa Y, Maeda T, Shizuta Y. Isolation of a full-length cDNA encoding mouse aromatase P450. Arch Biochem Biophys 1991; 285:231–237.