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Cell Type-Specific Expression of 17β-Hydroxysteroid Dehydrogenase Type 2 in Human Placenta and Fetal Liver*

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ABSTRACT

The enzymatic actions of the 17β-hydroxysteroid dehydrogenase (17βHSD) isozymes are crucial in steroid hormone metabolism/physiology. The type 1 isozyme catalyzes the conversion of the biologically inactive C_{18} steroid, estrone, to the active estrogen, 17β-estradiol, and the enzyme is predominantly expressed in the syncytiotrophoblast of the placenta and the granulosa cells of the ovary. 17βHSD type 2 is highly expressed in placenta, liver, and secretory endometrium and catalyzes the conversion of bioactive estrogens and androgens to biologically inactive 17-ketosteroid counterparts. The expression pattern of 17βHSD type 2 protein was determined in human term placenta and fetal liver by immunohistochemical analysis using monoclonal antibodies directed against distinct epitopes of the 17βHSD type 2 protein. In placenta, the protein was detected in the endothelial cells of fetal capillaries, but not in cytotrophoblasts or syncytiotrophoblast. There was dichotomous immunostaining seen among pairs of cotyledonary vessels and chorionic vessels. In the liver, on the other hand, staining was detected in the hepatocytes, but not in the cells lining blood vessels. We conclude that the cell type-specific localization of 17βHSD type 2 is in accord with the proposed physiological role of the enzyme, namely to protect tissues, in this case the fetus, from bioactive estrogen and androgen. (*J Clin Endocrinol Metab* **82:** 3872–3878, 1997)

A UNIQUE feature of human pregnancy is that the placenta throughout gestation produces large quantities of bioactive estrogens, *i.e.* 17β-estradiol and estriol. 17β-Estradiol is synthesized in the syncytiotrophoblast of the placenta primarily from the fetal adrenal C_{19} steroid precursor dehydroepiandrosterone sulfate (1). A majority of the estriol produced in human pregnancy is formed by the placental conversion of fetal plasma 16α-hydroxydehydroepiandrosterone sulfate to estriol (1, 2). The site of 16α-hydroxydehydroepiandrosterone sulfate formation is the fetal liver by the conversion of dehydroepiandrosterone sulfate (3).

For more than 35 yr, controversy has existed concerning the estrogen secretory products of the human placenta. Gurpide and co-workers (4) showed 30 yr ago that more than 90% of the 17β-estradiol and estriol synthesized in trophoblasts enters the maternal circulation, and it is generally agreed that 17β-estradiol and estriol, but not estrone, are secreted directly into maternal blood in the intervillous space (5). In contrast, there is a sizable umbilical vein-umbilical artery gradient for estrone, suggesting that estrone enters the fetal circulation from the syncytiotrophoblast (6, 7). Steroids that leave trophoblasts toward the fetal blood compartment, however, do not enter fetal blood directly, but must first enter the intravillous tissue and then traverse the wall of the fetal capillaries (8).

The interconversion of 17β-estradiol and estrone is catalyzed by different isozymes of 17β-hydroxysteroid dehydrogenase (17βHSD) (9). 17βHSD type 1 catalyzes the reduction of estrone to 17β-estradiol, whereas 17βHSD type 2 catalyzes the oxidation of 17β-estradiol to estrone. Other steroid substrates for the type 2 isozyme are the bioactive androgens, testosterone and dihydrotestosterone (10). Both isozymes are expressed in high levels in placental tissue (10–13), and immunohistochemical analysis of human placenta has shown that cytochrome P-450 aromatase (converts androstenedione to estrone) and 17βHSD type 1 are both confined to the syncytiotrophoblast (14). The cellular localization of 17βHSD type 2 in placenta and other tissues, however, is unknown.

To gain further insight into the physiological role of 17βHSD type 2 and to address again the secretion of 17β-estradiol (or estrone) from syncytiotrophoblast, we investigated the cell type-specific expression of the enzyme by ribonucleic acid (RNA) blotting and immunohistochemical staining of human placenta and fetal liver. The results indicate that 17βHSD type 2 is localized in the endothelial cells of the fetal capillaries and larger vessels of the placenta and in the hepatocytes of fetal liver.

Materials and Methods

Human tissues

Human fetal liver (14–18 weeks gestation) was obtained at the time of elective abortion of pregnancy for reasons other than liver disease. The consent forms and protocols for collecting human fetal liver and term placenta were approved by the institutional review board of the University of Texas Southwestern Medical Center. Human tissues for RNA

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isolation and immunoblotting were immediately snap-frozen in liquid nitrogen and stored at −80 C. Tissues for immunohistochemical analysis, embedded in OCT compound (Sakura Finetek, Torrance, CA), were snap-frozen in 2-methylbutane cooled in liquid nitrogen, and stored desiccated at −80 C.

RNA blotting

Total RNA was purified from frozen tissue using the RNA STAT-60 System (Tel-Test "B," Friendswood, TX) according to the manufacturer's instructions. Ten micrograms of total RNA was size-fractionated by electrophoresis, transferred to a nylon membrane, hybridized with a ^{32}P-labeled 17βHSD type 2 complementary DNA (cDNA) probe, washed under high stringency conditions, and exposed to x-ray film as previously described (13).

Production of recombinant 17βHSD type 2 fusion protein

To generate a 17βHSD type 2 cDNA suitable for subcloning into the bacterial expression vector pQE-30 (Qiagen, Chatsworth, CA), the following two primers were used in a PCR amplification with the human 17βHSD type 2 cDNA (10) as template: 5′-oligonucleotide, GCGC*GGATCC*AGCACTTTCTTCTCGGACACA (corresponding 5′→3′ to the *Bam*HI cloning site and sequence for 17βHSD type 2); and 3′-oligonucleotide, GCGC*AAGCTT*CTAGGTGGCCTTTTTCTTGTA (corresponding 3′→5′ to the *Hin*dIII cloning site and 17βHSD type 2 sequence). The amplified PCR product was digested with *Bam*HI and *Hin*dIII and inserted into pQE-30, designated pQE30–17βHSD2. The resulting pQE30–17βHSD2 recombinant plasmid encodes a fusion protein of 398 amino acids. The carboxy-terminal 386 amino acids comprise the 17βHSD type 2 protein (amino acids 2–387) and the amino-terminal 12 amino acids includes an initiator methionine and six consecutive histidine residues. PQE30–17βHSD2 was transformed into *Escherichia coli* host strain M15(pREP4) (Qiagen, Chatsworth, CA), grown to midlog phase in 1 l Luria Bertoni broth cultures at 37 C, and induced with isopropylthio-β-galactoside (2 mmol/L) for 3 h. The cells were collected by centrifugation and processed by a detergent washing procedure as described previously (15). The final inclusion body pellet was dissolved by a 30-s homogenization using a Brinkmann Polytron (Brinkmann, Westbury, NY) at maximum setting in a buffer containing 1.5% (wt/vol) sodium *N*-lauroylsarcosine and 10 mmol/L Tris-HCl (pH 8.0) and dialyzed against PBS overnight at 4 C. This fraction contained 17βHSD type 2 fusion protein with a purity of approximately 90% as revealed by SDS-polyacrylamide gel electrophoresis.

Production of monoclonal antibodies

The monoclonal antibody, mAb-C2–12 (subclass IgG1/κ), directed against 17βHSD type 2, was produced by immunizing mice with a synthetic carboxy-terminal peptide, [C]RALRMPNYKKKAT, corresponding to amino acids 375–387 in 17βHSD type 2. The peptide was coupled to keyhole limpet hemocyanin using *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (Pierce, Rockford, IL) (16). Mice were immunized with 25 μg coupled peptide in RIBI Adjuvant System (Ribi Immunochem Research, Hamilton, MT); 3 days after the second boost, the spleen was dissected out, and spleen cells were mixed with the myeloma cell line X.63-Ag 14 at a ratio of 4:1 and fused with 50% polyethylene glycol 1500 as previously described (17). Production of the monoclonal antibody mAb-EC2–14 (subclass IgG3/κ) and hybridoma screening were performed according to a method previously described to produce monoclonal antibodies directed against bacterially produced proteins (15). Ig was purified by column chromatography using protein G-agarose (Sigma Chemical Co., St. Louis, MO) (16).

Immunoblotting

Transfection of human embryonic kidney 293 cells with an expression plasmid (pCMV-17βHSD2) encoding the full-length 17βHSD type 2 protein or vector (pCMV) was performed as previously described (10). Forty-eight hours after transfection, the cells were harvested and frozen at −80 C as a pellet. Immunoblot analysis of homogenates of human tissues and transfected 293 cells was performed with peroxidase-conjugated antimouse IgG using the Enhanced Chemiluminescence (ECL) Western Blotting Detection System kit (Amersham, Arlington Heights, IL) as described previously (15).

Immunohistochemistry

Eight-micron cryosections were fixed with acetone for 10 min at room temperature, then immunoperoxidase staining was performed using the ABC Vectastain detection system (Vector Laboratories, Burlingame, CA) as described previously (18). The monoclonal antibodies mAb-C2–12, mAb-EC2–14, antihuman CD34 (IOM 34, Amac, Westbrook, ME), mouse IgG1 (MOPS 21, Sigma), and mouse IgG3 (FLOP 21, Sigma) were used at 10 μg/mL. All antibodies were diluted in phosphate-buffered saline except mAb-EC2–14, for which the NaCl concentration was increased to 500 mmol/L to prevent nonspecific binding. Color development (red) was performed with 3-amino-9-ethyl carbazole, and the sections were counterstained with hematoxylin. Sections were observed on a Leitz Laborlux-F brightfield microscope (Optronicf model ZI-470, Leitz, Rockleigh, NJ) using the Macintosh Quadra equipped with a Raster Opf Z4 MXTV frame grabber board. Photographs were taken using a Kodak XLF 8600 disub printer and Kodak ektatherm XLF print media (Eastman Kodak, Rochester, NY).

Results

A blot in which dissected human placental tissues and fetal liver tissue RNA were hybridized with a 17βHSD type 2 cDNA probe is presented in Fig. 1. The dissections included regional components of the placental vasculature. The chorionic arteries and veins on the placental surface branch to enter the cotyledons as stem vessels. These include the major truncus chori that branch into primary, secondary, and tertiary rami, which ramify into the sinusoidal network of the terminal villi (8). High levels of 17βHSD type 2 messenger RNA (mRNA; ~1.5 kb) were detected in placental villous tissue, tertiary vascular rami, whole placenta, and fetal liver. Very low levels of 17βHSD type 2 mRNA were observed in chorionic vein, chorionic plate, truncus chori, and primary/secondary rami. There was no evidence for significant levels of 17βHSD type 2 mRNA in decidua parietalis, chorion laeve, placental amnion membrane, reflected amnion membrane, chorionic artery, or fetal blood.

Monoclonal antibodies that recognize distinct epitopes of 17βHSD type 2, designated mAb-C2–12 and mAb-EC2–14, were generated for immunoblotting and immunohistochemical analysis of 17βHSD type 2 protein in various human tissues. An immunoblot in which protein extracts from whole placenta and fetal liver were incubated with the two antibodies is shown in Fig. 2. A protein of 45,000 daltons is specifically detected by mAb-C2–12 and mAb-EC2–14 in placenta and fetal liver as well as in transfected 293 cells expressing recombinant 17βHSD type 2 protein. The recognition of 17βHSD type 2 was abolished by preincubating mAb-C2–12 with the antigenic carboxy-terminal peptide; however, this peptide was unable to abolish binding of mAb-EC2–14 to the enzyme (data not shown).

We then determined which cell types express the 17βHSD type 2 enzyme in term placenta by immunohistochemical staining (Fig. 3). Cryosections from eight placental tissues, including the chorionic plate and vessels, as well as corresponding umbilical cords were immunostained with mAb-C2–12 and mAb-EC2–14. The expression pattern was identical in all eight specimens. Strong positive immunostaining was detected in the endothelial cells lining the capillaries and sinusoids in placental villi when sections were incubated

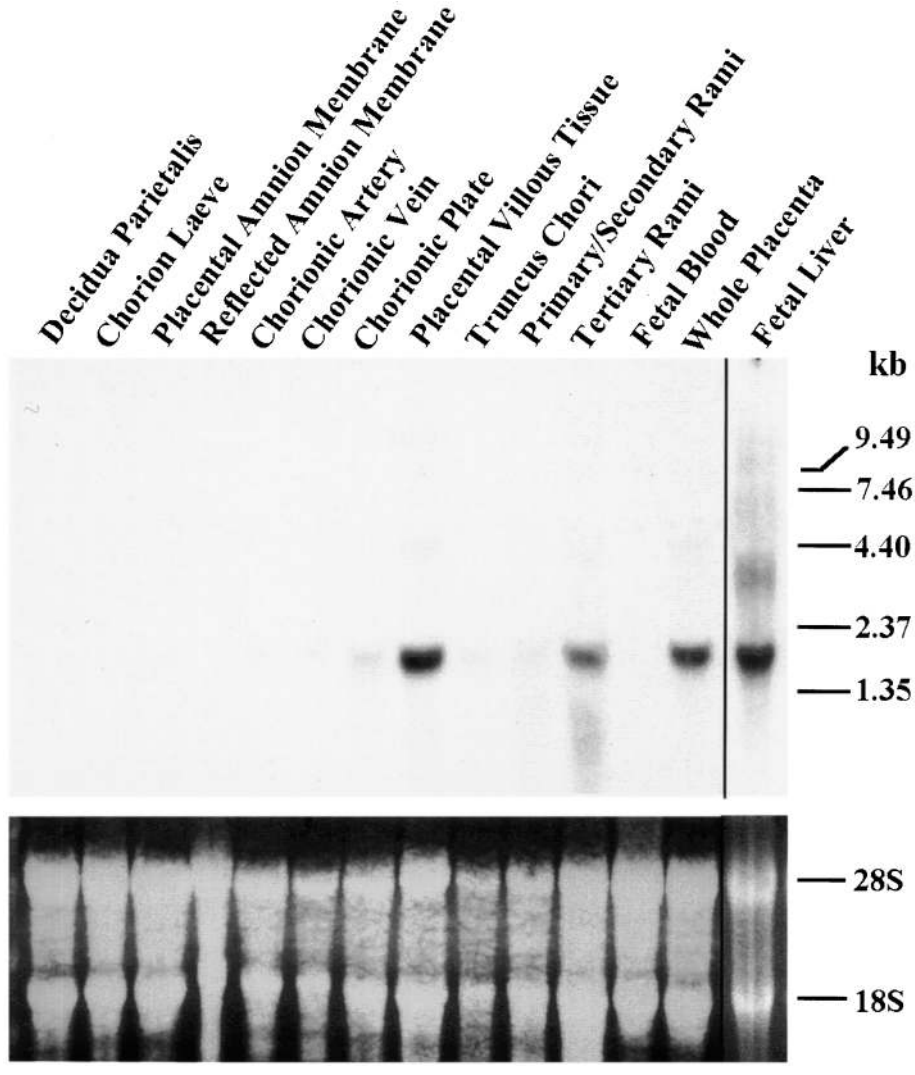

FIG. 1. Blot hybridization of RNA extracted from human placental tissues and fetal liver with a 17βHSD type 2 cDNA probe. Twenty micrograms of total RNA from the indicated tissues were subjected to blot hybridization on a nylon membrane as described in *Materials and Methods*. The membrane was hybridized with a full-length 17βHSD type 2 cDNA probe (2×10^6 cpm/mL) and subjected to autoradiography with intensifying screens for 18 h at −80 C. The ethidium-stained gel is presented in the *bottom panel*. The positions to which RNAs of known size migrated are shown on the *right*.

with mAb-C2–12 (Fig. 3A) or mAb-EC2–14 (Fig. 3B). An identical staining pattern was seen in adjacent sections stained for CD34, which is expressed by endothelial cells, confirming the identity of the cells containing 17βHSD type 2 immunoreactivity (data not shown). In contrast, no staining was seen in the trophoblast lining the placental villi. Pairs of cotyledonary vessels in the stem villi showed positive staining in the endothelial cells of one vessel only when incubated with mAb-C2–12 (Fig. 3C) and mAb-EC2–14 (Fig. 3D). The integrity of endothelial cells in both vessels was confirmed by incubating adjacent sections of tissue with an antihuman CD34 antibody (data not shown). Chorionic plate samples were taken with either a chorionic vein or artery, based on anatomical criteria, and the presence of endothelial cells was confirmed by anti-CD34 staining. Endothelial cells lining the chorionic veins of the chorionic plate also stained positive with mAb-C2–12 (Fig. 3E) and mAb-EC2–14 (data not shown), but those of chorionic arteries did not (Fig. 3F). In addition, no immunostaining was observed in endothelial cells lining the umbilical artery, umbilical vein, and the junction between chorionic vein and the umbilical vein or in decidua parietalis, amnion membrane, or chorionic plate (data not shown).

To ascertain which cell types express the 17βHSD type 2 enzyme in fetal liver, multiple sections from four fetuses were incubated with the monoclonal antibodies (Fig. 4). Positive immunostaining was detected in hepatocytes when sections were incubated with mAb-C2–12 (Fig. 4, A–C) and mAb-EC2–14 (Fig. 4D). Staining was found to be most intense in hepatocytes surrounding the portal tract and central vein compared with midzonal regions when sections were incubated (Fig. 4, C and D). No staining was observed in hematopoietic cells or endothelial cells lining the portal tract, central vein, or liver sinusoids (Fig. 4, C and D) or bile ductules. The integrity of endothelial cells was confirmed by incubating tissue sections with an antihuman CD34 antibody (data not shown). Consistently, no immunostaining was observed when irrelevant monoclonal antibodies of subclass IgG1 (Fig. 4E) or IgG3 (Fig. 4F) were incubated with placenta or liver sections. Also, the recognition of 17βHSD type 2 was abolished by preincubating mAb-C2–12 with the antigenic carboxy-terminal peptide; however, this peptide was unable

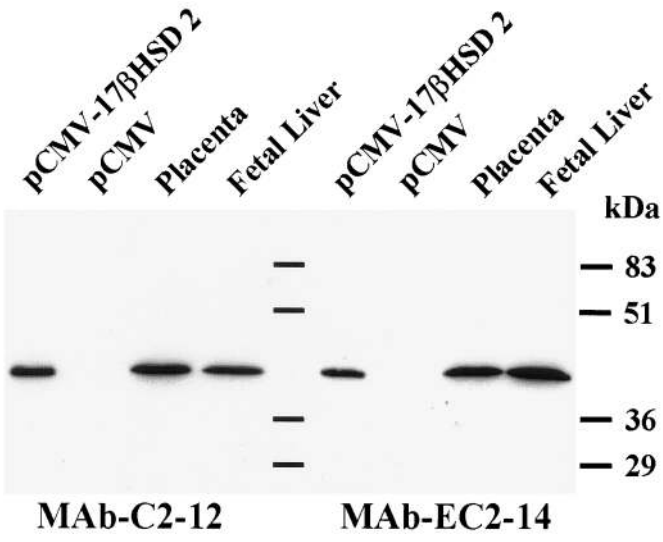

FIG. 2. Detection of 17βHSD type 2 by immunoblotting. Aliquots (5 μg protein) of extracts of whole placenta and fetal liver were electrophoresed on SDS-polyacrylamide gels and subjected to immunoblotting with monoclonal antibodies mAb-C2–12 (*left*) and mAb-EC2–14 (*right*). pCMV-17βHSD2, Cultured 293 cells (0.5 μg protein) transfected with 17βHSD type 2 expression plasmid (positive control). pCMV, Cultured 293 cells transfected with pCMV vector (negative control). The positions of prestained molecular size markers are shown on the *right*.

to abolish binding of mAb-EC2–14 to the enzyme (data not shown).

Discussion

In this study, RNA blotting and immunohistochemical analyses were used to determine the cell type-specific expression pattern of the 17βHSD type 2 enzyme in human term placenta and fetal liver. In placenta, the 17βHSD type 2 protein is confined to endothelial cells lining the fetal capillaries, cotyledonary, and chorionic vessels. In initial experiments, a placenta was dissected into 12 different tissue specimens. 17βHSD type 2 mRNA was detected in RNA from tertiary rami of the chorionic vessels and in villous tissue partially depleted of fetal cotyledonary vessels, and weak signals were detected in RNA from the chorionic plate, truncus branches of the fetal chorionic vessels, and primary/secondary rami of these vessels. This pattern of expression correlates with the relative amount of vascular elements present in the different samples, but even these dissected tissues contained multiple cell types potentially expressing the enzyme.

To define further the cell type(s) in which 17βHSD type 2 is localized, immunohistochemical analysis of human placenta was conducted. The validation of a positive signal in a particular tissue (cell) by immunohistochemical analysis may be problematic if a given antibody cross-reacts with an irrelevant protein with a similar epitope. Consequently, to address this important issue we generated monoclonal antibodies directed against different epitopes of 17βHSD type 2 protein to determine whether the same pattern would be expressed with both antibodies. mAb-C2–12 is directed against a synthetic carboxy-terminal peptide, and mAb-EC2–14 is directed against a bacterially produced 17βHSD type 2 fusion protein. That the monoclonal antibodies recognize different epitopes of 17βHSD type 2 protein was confirmed by two different experiments. First, mAb-EC2–14 could not bind to the carboxy-terminal peptide in an enzyme-linked immunosorbent assay, whereas mAb-C2–12 bound to the peptide; second, free carboxy-terminal peptide could not abolish binding of mAb-EC2–14 to 17βHSD type 2 protein in immunoblotting and immunohistochemical experiments, whereas the free peptide could abolish binding of mAb-C2–12 to the protein. We were unable to test whether binding of mAb-EC2–14 and mAb-C2–12 to 17βHSD type 2 could be abolished by preincubating the antibodies with the fusion protein because the 17βHSD type 2 fusion protein is purified from the inclusion body fraction of *E. coli* cells and, thus, is soluble only under strongly denaturing conditions that result in denaturation of the antibodies. Using the two monoclonal antibodies, we demonstrated that the endothelial cell is the cell type in placenta that exclusively expresses the 17βHSD type 2 enzyme.

The finding of high levels of oxidative 17βHSD type 2 enzyme in endothelial cells of capillaries and sinusoids in terminal villi provides an explanation as to why estrone is the major C_{18} steroid present in fetal umbilical venous blood. Furthermore, our results explain the observation by Tremblay and co-workers (19), that the mRNA encoding 17βHSD type 1, but not 17βHSD type 2, was expressed in primary cultures of trophoblasts, in contrast to whole placenta and fetal cotyledons, that contained large amounts of both mRNAs.

The 17βHSD type 2 protein is, however, not observed in all endothelial cells of the placenta. In contrast to high levels of type 2 protein in endothelial cells of capillaries and sinusoids in terminal villi, no immunoreactive 17βHSD type 2 could be demonstrated in endothelial cells lining the chorionic artery of the chorionic plate, umbilical artery, and umbilical vein. Furthermore, a dichotomy of expression is observed in the endothelial cells of cotyledonary vessels in the stem villi. This spatial gradient of 17βHSD type 2 immunostaining in endothelial cells (*i.e.* high levels in terminal villi, the site of feto-maternal exchange) to no detectable protein in the endothelial cells lining large fetal placental vessels, especially those leading into the placenta, is in concordance with one proposed physiological role of the enzyme, namely to protect the fetus from placental and maternally derived bioactive estrogen and androgen.

The finding that the 17βHSD type 2 enzyme is confined to the hepatocytes in fetal liver supports the hypothesis that its physiological role is to inactivate steroids. The majority of steroid metabolism takes place in the hepatocytes, the major cell type in the liver. Other enzymes confined to the hepatocyte and relevant to steroid metabolism include steroid 5α/β-reductases, 3α/β-hydroxysteroid dehydrogenases, cytochromes P-450, UDP-glucuronosyltransferases, and steroid sulfotransferases. The higher level of 17βHSD type 2 protein in hepatocytes surrounding the portal tract and central vein compared with midzonal regions is in contrast to expression of a drug-inactivating cytochrome P-450 enzyme, P-450 IIIA, showing uniform expression in all hepatocytes regardless of intralobular localization in fetal liver (20). In-

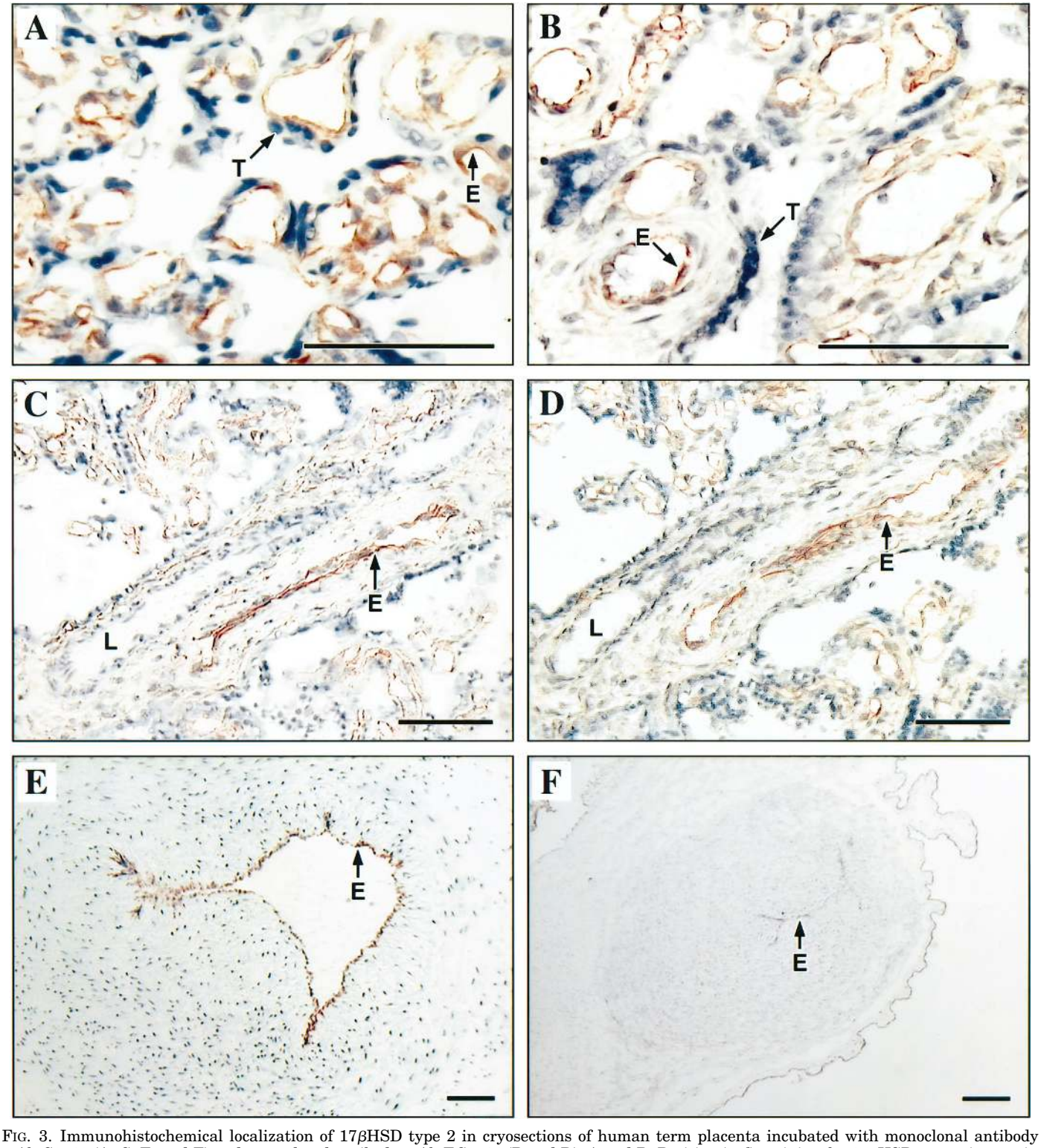

FIG. 3. Immunohistochemical localization of 17βHSD type 2 in cryosections of human term placenta incubated with monoclonal antibody mAb-C2–12 (A, C, E, and F) and monoclonal antibody mAb-EC2–14 (B and D). A and B, Positive (*red*) staining for 17βHSD type 2 is seen in the endothelial cells (E) lining the sinusoids and capillaries in terminal villi, with no staining observed in the trophoblast (T). C and D, Positive staining is seen in the endothelial cells of some vessels of the stem villi, whereas others are negative. L, Lumen. E, Positive staining is seen in endothelial cells lining the chorionic vein of the chorionic plate. F, No staining for 17βHSD type 2 is seen in the endothelial cells lining the chorionic artery of the chorionic plate. The *blue* color is hematoxylin counterstaining. *Bars* = 100 μm.

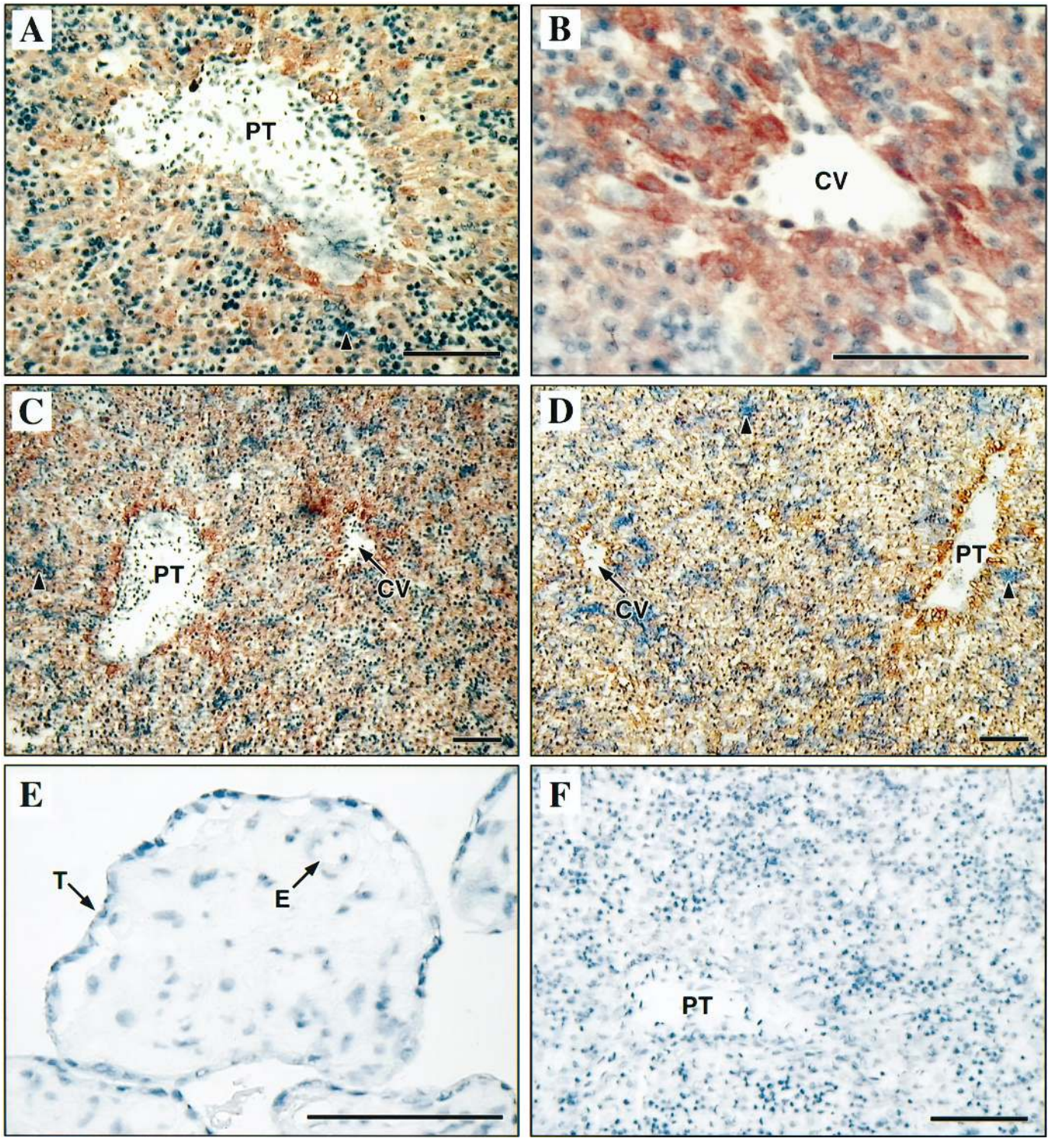

FIG. 4. Immunohistochemical localization of 17βHSD type 2 in cryosections of human fetal liver incubated with monoclonal antibody mAb-C2–12 (A–C) and monoclonal antibody mAb-EC2–14 (D). Positive (*red*) staining for 17βHSD type 2 is seen in A) hepatocytes surrounding the portal tract (PT) and B) central vein (CV). C and D demonstrate stronger staining of hepatocytes surrounding the portal tract (PT) and central vein (CV) than those at the midzonal region. No immunostaining was observed in endothelial cells lining the vessels of the portal tract, central vein, and sinusoids or in the cluster of hematopoietic cells (*arrowheads*). E, No staining was detected in the endothelial cells (E) of placental terminal villi when sections were incubated with an irrelevant antibody (IgG3). F, No staining was observed in hepatocytes of fetal liver when sections were incubated with an irrelevant monoclonal antibody (IgG1). The *blue* color is hematoxylin counterstaining. *Bars* = 100 μm.

terestingly, during postnatal and adult life, expression of P-450 IIIA was restricted to hepatocytes surrounding central vein and midzonal regions (20). Hence, it is conceivable that hepatocyte expression of 17βHSD type 2 also may shift after birth. This issue is important to an understanding of the metabolic pathway of a particular steroid, *i.e.* whether the steroid is metabolized as it traverses from blood (portal vein and hepatic artery) to blood (central vein) as a result of a spatial gradient of expression of particular steroid-metabolizing enzymes or simply because the substrate specificities of the various metabolizing enzymes in the hepatocytes are determining the pathway.

Acknowledgments

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Erratum

In the September issue (*Journal of Clinical Endocrinology and Metabolism* **82**: 2771–2776, 1997), Clinical Review 90, "Leptin and Clinical Medicine: A New Piece in the Puzzle of Obesity" by George A. Bray and David A. York, contains an error. On page 2773, the first sentence of the second paragraph of section IV reads, "Plasma leptin levels are higher in men than in women." As all of the cited references concur, there **are higher leptin levels in women than in men.** The authors regret their misstatement.