Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 87 (2003) 241–246
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Expression of P450 aromatase and 17β-hydroxysteroid dehydrogenase type 1 at fetal–maternal interface during tubal pregnancy

Yan Li [b], Li Qin [a], Zhi-Jie Xiao [a], Yan-Ling Wang [a], Riitta Herva [c], Jin-Hua Leng [d], Jing-He Lang [d], Veli Isomaa [b], Yun-Shang Piao [a,*]

[a] *State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China*
[b] *Biocenter Oulu and Research Center for Molecular Endocrinology, WHO Collaborating Centre, University of Oulu, FIN-90014 Oulu, Finland*
[c] *Department of Pathology, University of Oulu and Oulu University Hospital, FIN-90014 Oulu, Finland*
[d] *Department of Gynecology and Obstetrics, Peking Union Medical University, Beijing 100730, China*

Received 5 May 2003; accepted 30 September 2003

Abstract

Steroidogenesis in the placenta has been studied widely, but little is known about steroid metabolism in ectopic pregnancy. Previous studies have indicated that trophoblast invasion and placentation in the uterus and the fallopian tube may be controlled by similar mechanisms. As far as 17β-estradiol (E_2) production is concerned, it has been well demonstrated that its biosynthesis in the placenta involves the action of P450 aromatase (P450arom) and 17β-hydroxysteroid dehydrogenase type 1 (17HSD1). The purpose of this study was to characterize the expression pattern of P450arom and 17HSD1 at the fetal–maternal interface, particularly in various trophoblast cells, in tubal pregnancy. Using in situ hybridization, P450arom mRNA was localized in syncytiotrophoblast (ST) cells, which are mainly responsible for hormone production during pregnancy, whereas no signal was detected in villous cytotrophoblast (VCT), column CT and extravillous CT (EVCT) cells. Immunohistochemical assays revealed that 17HSD1 is present in ST cells, a large portion of EVCT cells and 20% of column CT cells. On the other hand, no expression of 17HSD1 was detected in VCT cells. In addition, 17HSD1 was found in epithelial cells of the fallopian tube. Interestingly, the expression level of 17HSD1 in fallopian tube epithelium during tubal pregnancy was significantly higher than that during normal cycle. Our data provide the first evidence that normal and tubal pregnancies possess identical expression of P450arom and 17HSD1 in ST cells and therefore, similar E_2 production in the placenta. Further, the association of 17HSD1 with EVCT cells indicates that 17HSD1 perhaps play a role in trophoblast invasion. Finally, increased expression of 17HSD1 in epithelial cells of fallopian tube may lead to a local E_2 supply sufficient for the maintenance of tubal pregnancy.

Keywords: Placenta; Trophoblast cells; Tubal pregnancy; Estrogen biosynthesis

1. Introduction

The human placenta is a unique organ for the maintenance of pregnancy. Its important functions include hormone supply for the maternal body and fetal development. In addition to the synthesis of placenta-specific hormones, such as human chorionic gonadotropin and placental lactogen [1,2], the placenta plays a critical role in producing progesterone and estrogens throughout gestation [3]. In the human, placental trophoblasts undergo differentiation towards either the villous or the extravillous trophoblast pathway at the initiation of implantation [4,5]. Villous trophoblasts cover the floating villi (FV), which are responsible for hormone production and fetal–maternal material exchange. In early placenta, FV are composed of fibrovascular stroma coated by double-layered trophoblast epithelium. The inner layer is constructed of villous cytotrophoblast (VCT) cells, while the outer layer consists of syncytiotrophoblast (ST) cells, the multinucleated giant cells that are differentiated from VCT cells.

During early nidation, extravillous CT (EVCT) cells originating from the CT columns invade into the maternal uterine wall and anchor the FV to the uterus [5–7]. During the 3rd postmenstrual week, anchoring villi (AV) arise from the CT columns, with their tips formed of CT solid columns [6]. The AV not only fix the placenta to the uterus, but also supply for the proliferation of migratory EVCT cells that colonize the maternal interstitium and arteries

* Corresponding author. Tel.: +86-10-62631832; fax: +86-10-62529248.
E-mail address: piaoys@panda.ioz.ac.cn (Y.-S. Piao).

0960-0760/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2003.09.013

during the first 18 weeks of pregnancy [8,9]. Accordingly, the establishment of AV and their consequent invasion into the uterus are essential for implantation and placentation, and therefore significantly contribute to the progression of pregnancy.

In the placenta, ST cells of FV are mainly accountable for the production of steroid hormones, such as progesterone and estrogens. The biosynthesis of estrogens in human placenta involves the action of P450 aromatase (P450arom) and 17β-hydroxysteroid dehydrogenase type 1 (17HSD1). P450arom catalyzes the aromatization of androgens to estrogens, for example, androstenedione (A-dione) to estrone (E_1) and testosterone to 17β-estradiol (E_2), whereas 17HSD1 mainly governs the reduction of E_1 to E_2. Since the biological potency of E_2 is approximately 100-fold compared to that of E_1, the physiologically active molecule of estrogens is hence E_2 [10]. Both P450arom and 17HSD1 are abundantly expressed in ST cells [11], in line with the role of ST cells in endocrine function. Cytosolic 17HSD1 has been found in the nuclei of ST [11,12], implying that it may also play a role in intranuclear conversion of E_1 to E_2. Our previous studies have revealed that 17HSD1 expression in placental cells is coordinated by a proximal promoter, a silencer and a cell-specific enhancer at the 5′-flanking region of the gene encoding 17HSD1 [13–15]. Also, it has been shown that CT cells freshly isolated from full-term placenta contain 17HSD1 [16]. In addition, 17HSD1 was immunolocalized in invasive CT cells of full-term placenta [17]. Our recent study demonstrated that cultured CT cells purified from first-trimester placenta express both P450arom and 17HSD1 and are capable of converting dehydroepiandrosterone and A-dione to E_2 [18]. However, it is not clear whether these two steroidogenic enzymes are present in various CT cells of early placenta in vivo.

Steroidogenesis in the placenta has been studied for several decades, but attention has mostly been paid to intrauterine pregnancy. So far, little is known about whether ectopic pregnancy, for example tubal pregnancy, involves normal placenta-like mechanisms controlling the biosynthesis of steroid hormones. In the present study, the expression of P450arom and 17HSD1 in placental trophoblasts during tubal pregnancy has been investigated for the first time. In addition, the existence of 17HSD1 in the fallopian tube was examined.

2. Materials and methods

2.1. Chemicals and reagents

The isotope [α-^{35}S]CTP (1300 Ci/mmol) was obtained from DuPoint–New England Nuclear (Boston, MA, USA). T7 and SP6 RNA polymerases, AMV reverse transcriptase, Taq DNA polymerase, RNasin and restriction enzymes were products of Promega Co. (Madison, WI, USA). Proteinase K and tRNA were purchased from Boehringer Mannheim (Mannheim, Germany). Paraformaldehyde (PFA) and bovine serum albumin (BSA) were from Sigma Chemical Co. (St. Louis, MO, USA). Rabbit polyclonal antibody against cytokeratin was provided by ZYMED (San Francisco, CA, USA). Rabbit antiserum against 17HSD type 1 has been described in our previous studies [19,20].

2.2. Tissue specimens

Twenty-three tissue specimens (within 10 weeks of gestation) of the fetal–fallopian tube compartment were obtained from tubally pregnant women scheduled for surgery at Oulu University Hospital (Oulu, Finland). The definition of tubal pregnancy was based on ultrasonic and pathological examinations. These samples had histologically normal placental tissues and fetal–maternal interface. Samples of human fallopian tube tissue of the mid-proliferative phase ($n = 3$) and the mid-secretory phase ($n = 4$) were collected from normally cycling women (age: 30–40 years) who had undergone hysterectomy because of uterine myoma at Peking Union Hospital (Beijing, China). All the specimens were collected according to the basic principles of Helsinki declaration. The project was approved by local ethical committees.

The tissue specimens were washed twice with phosphate-buffered saline (PBS) and fixed in 4% PFA at 4 °C overnight. The samples were then dehydrated in xylene and then ethanol, and embedded in paraffin (solidification point: 51–53 °C). Six μM sections were cut and collected on Super Frost+ glass slides (Menzel-Gläser, Germany). For pathological diagnosis, the sections were stained with hematoxylin and eosin after dewaxation with xylene.

2.3. In situ hybridization

A 444-bp cDNA fragment of human P450arom (nt 166–609) was amplified by reverse transcription polymerase chain reaction (RT-PCR) from total RNA of human placenta. The primers used were 5′-ATACCAGGTCCTGGCTACTG-3′ (sense) and 5′-GGTGTCCAGCATG-3′ (antisense). The amplified fragment was constructed into pGEM-T Easy Vector (Promega) and verified by sequencing. Sense and antisense [α-^{35}S]CTP-labeled cRNA probes were transcribed with T7 or SP6 RNA polymerase. The in situ hybridization was performed as previously described [21,22].

2.4. Immunohistochemistry

Paraffin sections were deparaffinized with xylene and rehydrated in a descending ethanol series. Then they were retrieved in 10 mM citrate buffer (pH 6.0) at 95 °C for 15 min. To eliminate endogenous peroxidase activity, the samples were treated with 1% hydrogen peroxide for 15 min. After being washed three times with 50 mM Tris–hydrochloride buffer (TBS), the sections were incubated with antiserum against 17HSD type 1 (1:200) or antibody against

cytokeratin (1:600) at 4 °C overnight. The subsequent procedures were carried out by using the DAKO ENVISION system (DAKO Co., Carpinteria, CA, USA) according to the instructions provided by the manufacturer. Counterstaining with hematoxylin was performed before mounting the slides.

3. Results

3.1. Expression of P450arom mRNA in trophoblast cells during tubal pregnancy

In situ hybridization demonstrated that P450arom mRNA is expressed in the ST cells of all FV. In contrast, the signal was not detected in any kind of CT cells, including VCT, column CT, and EVCT cells (Fig. 1).

3.2. Expression of 17HSD1 in trophoblast cells during tubal pregnancy

Immunohistochemical analysis showed 17HSD1 to be present in the cytoplasm and nuclei of the ST cells of FV (Fig. 2C and D). Similar localization of 17HSD1 was also found in a large portion of EVCT cells, which had invaded into the fallopian tube interstitium (Fig. 2F). Further, immunostaining of cytokeratin, a marker for epithelial cells, confirmed the identity of EVCT cells (Fig. 2E). On the other hand, no signal of 17HSD1 was observed in VCT cells (Fig. 2C). Seventy columns were analyzed and 80% of them did not express 17HSD1 (Fig. 2D).

3.3. Expression of 17HSD1 in the fallopian tube

Immunostaining was performed to examine 17HSD1 expression in the fallopian tube. Faint signal of 17HSD1 could be detected in the epithelial cells of the fimbriae at the mid-proliferative and mid-secretory stages (Fig. 3B and C). Interestingly, increased expression of 17HSD1 in fallopian tube epithelium was found during tubal pregnancy. An expression level clearly higher than that observed in normally cycling women was seen in four out of 23 tubal pregnancy specimens (Fig. 3D), while high expression was seen in six specimens (Fig. 3E), and strong expression was observed in the remaining 13 specimens (Fig. 3F).

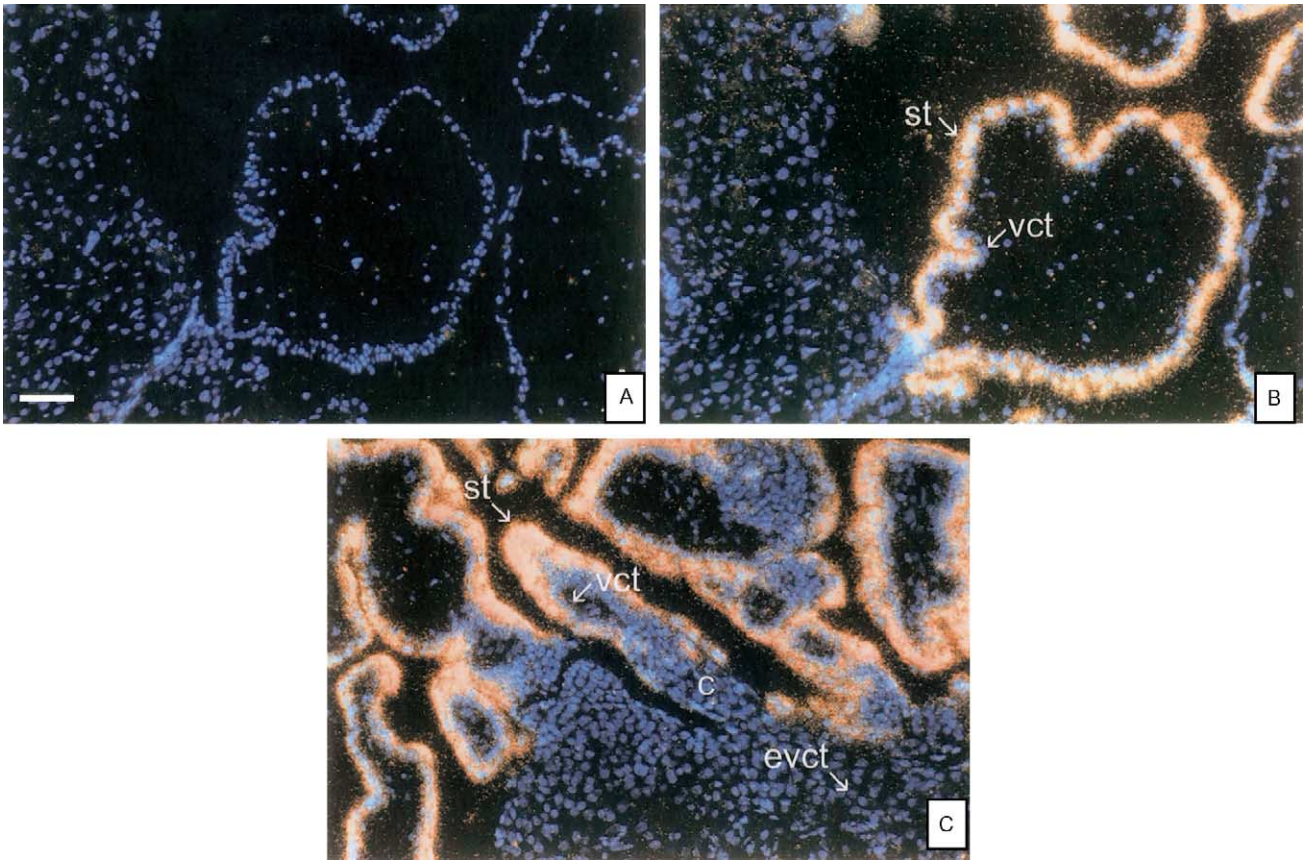

Fig. 1. Expression of P450arom mRNA at the fetal–maternal interface during tubal pregnancy was analyzed by in situ hybridization. (A) Negative control using a sense probe. (B and C) Detection of P450arom mRNA in ST, VCT, column CT and EVCT cells using an antisense probe. The column is represented by c (in C), and the magnification is 100× (scale bar shown in $A = 100\,\mu m$).

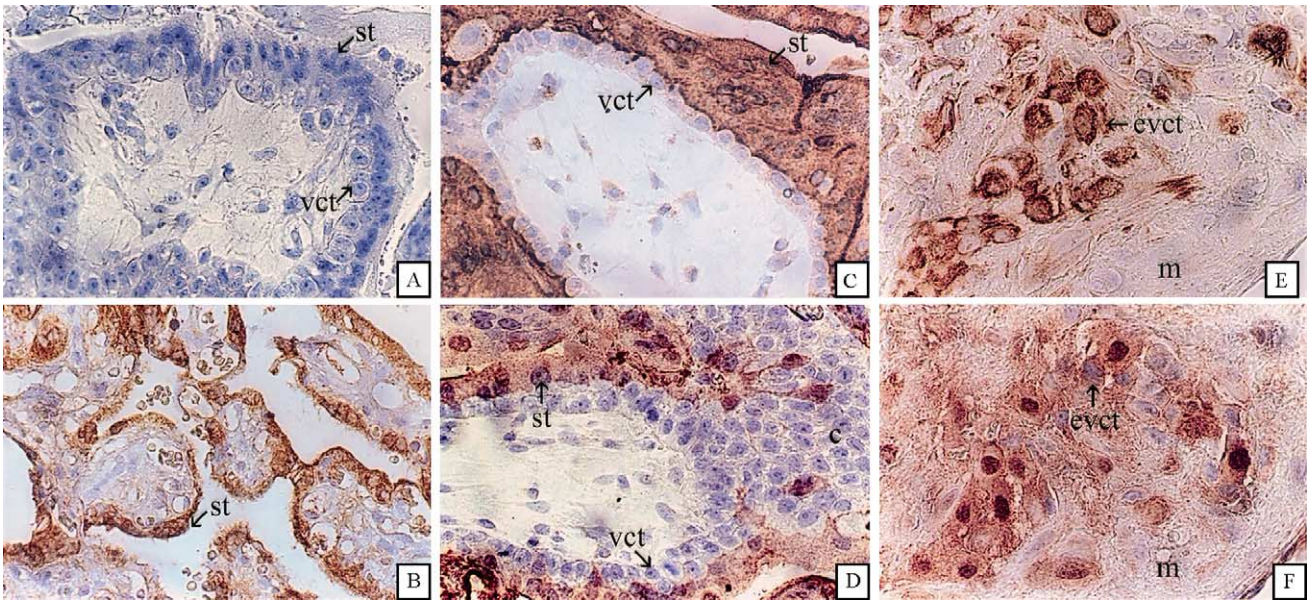

Fig. 2. Expression of 17HSD1 protein at the fetal–maternal interface during tubal pregnancy was analyzed by immunohistochemistry. (A) Negative control. (B) Positive control using human full-term placenta. (C and D) Detection of 17HSD1 in ST, VCT and column CT cells. (E) Immunostaining of cytokeratin in EVCT cells. F. Detection of 17HSD1 in EVCT cells. The column and maternal tissue are represented by c (in D) and m (in E and F), respectively. The magnification is 400×.

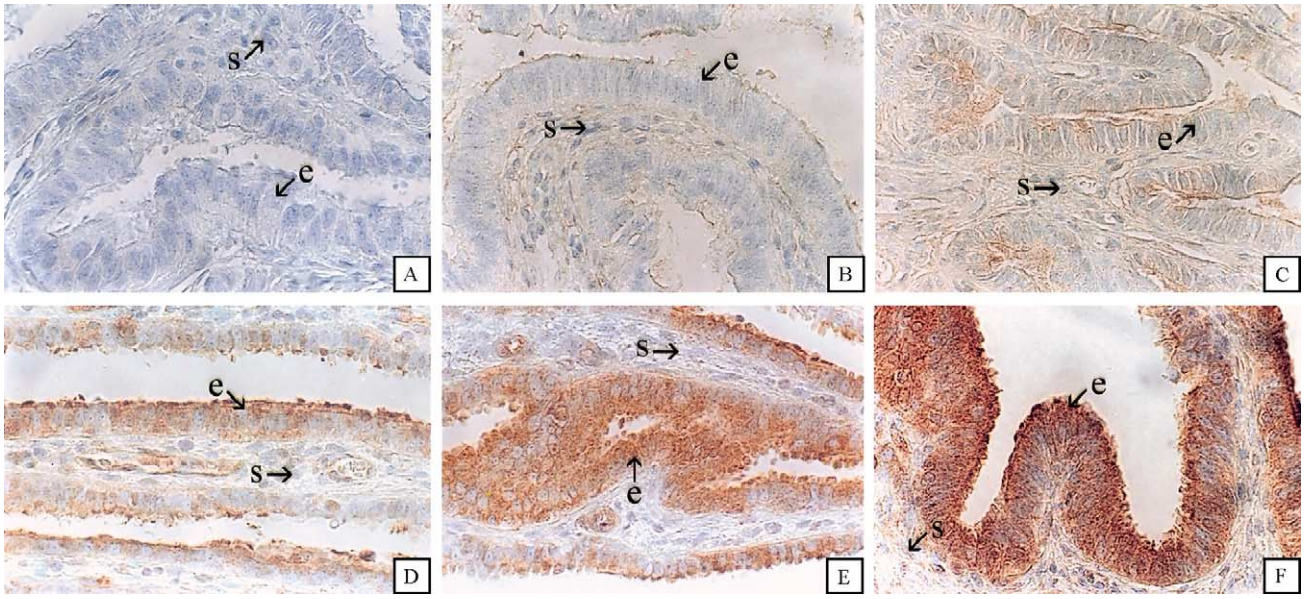

Fig. 3. Immunolocalization of 17HSD1 in the fimbriae of the fallopian tube. (A) Negative control. (B) Detection of 17HSD1 during mid-proliferative phase. (C) Detection of 17HSD1 during mid-secretory phase. (D–F) Detection of 17HSD1 during tubal pregnancy. Epithelial and stromal cells are indicated with e and s, respectively. The magnification is 400×.

4. Discussion

Ectopic implantation of the embryo in the fallopian tube results in tubal pregnancy. It has been pointed out that trophoblast cells in tubal pregnancy appear morphologically and physiologically similar to those found at normal intrauterine implantation sites [23]. Also, the placentation occurring in the fallopian tube is identical to that in the uterus [24]. Thus, tubal pregnancy provides a unique model for studying the function of trophoblast cells during early gestation. The advantage of using this model is that early placenta samples with fetal–maternal interface can be used and various types of CT cells can thus be easily obtained.

Chorionic villi are functional units of the placenta and contribute the dominant major parts of FV [6]. In the present study, P450arom and 17HSD1 were exclusively identified in

the ST cells of FV during tubal pregnancy. The data are in agreement with earlier findings showing that P450arom and 17HSD1 are present in ST cells [11,17], which further supports the assumption that ST cells are the main cell type for placental steroid hormone biosynthesis. On the other hand, our results indicate that the occurrence of tubal pregnancy is unlikely to affect the production of estrogens in the placenta. Moreover, it can be suggested that the intrauterine environment may not be essential for the biosynthesis of E_2 in the placenta, as the fallopian tube interstitium interacting with trophoblasts differs from the endometrium.

Our recent study clearly showed that P450arom and 17HSD1 are expressed in cultured CT cells isolated from normal human placenta of the first trimester [18]. In the cell culture system of the above investigation, VCT cells counted for the major portion of the cell population, because only the tips of chorionic villi were collected for the purification of CT cells. Differently, neither P450arom nor 17HSD1 were found in VCT cells during tubal pregnancy with in situ hybridization or immunostaining methods used in the present study. The divergent expression of P450arom and 17HSD1 in VCT cells under in vivo and in vitro conditions are difficult to interpret on the basis of our current knowledge. One explanation is that local factor(s) may modulate the function of VCT cells in vivo and, as a result, inhibit the expression of the two enzymes.

The present study demonstrates that P450arom is not present in any type of CT cells, indicating that ST cells are the only trophoblast cell population possessing a complete placental E_2 biosynthesis pathway, in which fetal and maternal-derived androgens are used as precursor molecules. In this study, 17HSD1 was identified in a large portion of EVCT cells that invaded into the interstitium of the fallopian tube during implantation, which occur at early stage of pregnancy. On the contrary, this enzyme was not found in VCT cells and 80% of column CT cells. Thus, the close contact of CT cells in vivo might play a role in switching off the 17HSD1 gene in CT cells. In accordance with a recent report showing that 17HSD1 is expressed in the invasive CT cells of full-term placenta [17], it seems obvious that the action of invasive CT cells, either during implantation or at the final stage of pregnancy, is associated with the function of 17HSD1. However, the physiological significance of this phenomenon and hence the local conversion of E_1 to E_2 in invasive CT cells remains to be further clarified. Nevertheless, it is likely that 17HSD1 may play a role in trophoblast invasion.

The in situ hybridization performed in this study failed to reveal P450arom in the fallopian tubes of either tubally pregnant or normally cycling women (data not shown). On the other hand, 17HSD1 could be detected for the first time in the epithelial cells of the fallopian tube by immunohistochemistry. The fallopian tube comprises the isthmus connected to the uterus, the ampulla close to the ovary, and the fimbriae between them. The fimbriae is the site where the fertilization and most of the cases of tubal pregnancy occur. An interesting finding in the present study was that a significantly increased amount of 17HSD1 was detected in the epithelial cells of the fimbriae during tubal pregnancy compared to normal fallopian tube specimens, which may result in a sufficient local E_2 supply for the maintenance of tubal pregnancy.

Acknowledgements

This work was supported by the Special Funds for Major State Basic Research Project (G1999055903), Chinese Academy of Sciences Knowledge Innovation Program (KSCX-2-SW-201 and KSCX3-IOZ-07), the Sigrid Jusélius Foundation, the Finnish Cancer Foundation, and the Centre for International Mobility, Finland, CIMO (MM/HA1 154).

References

[1] J.L. Jameson, A.N. Hollenberg, Regulation of chorionic gonadotropin gene expression, Endocr. Rev. 14 (1993) 203–221.
[2] S. Handwerger, Clinical counterpoint: the physiology of placental lactogen in human pregnancy, Endocr. Rev. 12 (1991) 329–336.
[3] E.R. Simpson, P.C. MacDonald, Endocrine physiology of the placenta, Ann. Rev. Physiol. 43 (1981) 163–188.
[4] P. Kaufmann, I. Scheffen, Placental development, in: R.A. Polin, W.W. Fox (Eds.), Fetal and Neonatal Physiology, Saunders, Philadelphia, 1992, pp.47−56.
[5] Y.W. Loke, A. King, Human trophoblast development, in: Human Implantation: Cell Biology and Immunology, Cambridge University Press, Cambridge, 1995, pp. 32−62.
[6] E.J. Popek, Normal anatomy and histology of the placenta, in: S.H. Lewis, E. Ferrin (Eds.), Pathology of the Placenta, second ed., Churchill Livingstone, Philadelphia, 1999, pp. 49−68.
[7] A. Tarrade, R. Lai-Kuen, A. Malassine, V. Tricottet, P. Blain, M. Vidaud, D. Evain-Brion, Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta, Lab. Invest. 81 (2001) 1199–1211.
[8] R. Pijnenborg, G. Dixon, W.B. Robertson, I. Brosens, Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy, Placenta 1 (1980) 3–19.
[9] R. Pijnenborg, Trophoblast invasion, Reprod. Med. Rev. 3 (1994) 53–73.
[10] M.H. Johnson, B.J. Everitt, Essential Reproduction, fourth ed., Blackwell, Oxford, 1992, p. 29.
[11] N. Fournet-Dulguerov, N.J. MacLusky, C.Z. Leranth, R. Todd, C.R. Mendelson, E.R. Simpson, F. Naftolin, Immunohistochemical localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta, J. Clin. Endocrinol. Metab. 65 (1987) 757–764.
[12] E. Dupont, F. Labrie, V. Luu-The, G. Pelletier, Localization of 17β-hydroxysteroid dehydrogenase throughout gestation in human placenta, J. Histochem. Cytochem. 39 (1991) 1403–1407.
[13] Y.S. Piao, H. Peltoketo, J. Oikarinen, R. Vhiko, Coordination of transcription of the human 17β-hydroxysteroid dehydrogenase type 1 gene (EDH17B2) by a cell-specific enhancer and a silencer: identification of a retinoic acid response element, Mol. Endocrinol. 9 (1995) 1633–1644.
[14] Y.S. Piao, H. Peltoketo, P. Vihko, R. Vihko, The proximal promoter region of the gene encoding human 17β-hydroxysteroid dehydrogenase type 1 contains GATA, AP-2 and Sp1 response elements: analysis of promoter function in choriocarcinoma cells, Endocrinology 138 (1997) 3417–3425.

[15] S. Leivonen, Y.S. Piao, H. Peltoketo, P. Numchaisrika, R. Vihko R, P. Vihko, Identification of essential subelements in the hHSD17B1 enhancer: difference in function of the enhancer and that of the hHSD17BP1 analog is due to −480C and −486G, Endocrinology 140 (1999) 3478–3487.
[16] C. Beaudoin, C.H. Blomquist, Y. Tremblay, Gene expression of 17β-hydroxysteroid dehydrogenase type 2 isozyme in primary cultures of human trophoblasts predicts different mechanisms regulating type 1 and type 2 enzymes, Endocrinology 136 (1995) 3807–3814.
[17] M. Bonenfant, P.R. Provost, R. Drolet, Y. Tremblay, Localization of type 1 17β-hydroxysteroid dehydrogenase mRNA and protein in syncytiotrophoblasts and invasive cytotrophoblasts in the human term villi, J. Endocrinol. 165 (2000) 217–222.
[18] S.J. Zhu, Y. Li, H. Li, Y.L. Wang, Z.J. Xiao, P. Vihko, Y.S. Piao, Retinoic acids promote the action of aromatase and 17β-hydroxysteroid dehydrogenase type 1 on the biosynthesis of 17β-estradiol in placental cells, J. Endocrinol. 172 (2002) 31–43.
[19] O. Mäentausta, R. Sormunen, V. Isomaa, V.P. Lehto, P. Jouppila, R. Vihko, Immunohistochemical localization of 17β-hydroxysteroid dehydrogenase in the human endometrium during the menstrual cycle, Lab. Invest. 65 (1991) 582–587.
[20] Y.S. Piao, H. Peltoketo, A. Jouppila, R. Vihko, Retinoic acids increase 17β-hydroxysteroid dehydrogenase type 1 expression in JEG-3 and T47D cells, but the stimulation is potentiated by epidermal growth factor, 12-*O*-tetradecanoylphorbol-13-acetate, and cyclic adenosine 3′,5′-monophosphate only in JEG-3 cells, Endocrinology 138 (1997) 898–904.
[21] A. Chotteau-Lelievre, X. Desbiens, H. Pelczar, P.A. Defossez, Y. de Launoit, Differential expression patterns of the PEA3 group transcription factors through murine embryonic development, Oncogene 15 (1997) 937–952.
[22] M. Mustonen, M. Poutanen, A. Chotteau-Lelievre, Y. de Launoit, V. Isomaa, S. Vainio, R. Vihko, P. Vihko, Ontogeny of 17β-hydroxysteroid dehydrogenase type 2 mRNA expression in the developing mouse placenta and fetus, Mol. Cell. Endocrinol. 134 (1997) 33–40.
[23] U. Earl, M. Wells, J.N. Bulmer, Immunohistochemical characterisation of trophoblast antigens and secretory products in ectopic tubal pregnancy, Int. J. Gynecol. Pathol. 5 (1986) 132–142.
[24] S. Randall, C.H. Buckley, H. Fox, Placentation in the fallopian tube, Int. J. Gynecol. Pathol. 6 (1987) 132–139.