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Biochemical Characterization of 20α-Hydroxysteroid Dehydrogenase

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ABSTRACT

In this review, we have tried to summarize the evidence and molecular characterization indicating that 20α-hydroxysteroid dehydrogenase (20α-HSD) is a group of the aldo-keto reductase (AKR) family, and it plays roles in the modulation and regulation of steroid hormones. This enzyme plays a critical role in the regulation of luteal function in female mammals. We have studied the molecular expression and regulation of 20α-HSD in cows, pigs, deer, and monkeys. The specific antibody against bovine 20α-HSD was generated in a rabbit immunized with the purified recombinant protein. The mRNA expression levels increased gradually throughout the estrous cycle, the highest being in the corpus luteum (CL) 1 stage. The mRNA was also specifically detected in the placental and ovarian tissues during pregnancy. The 20α-HSD protein was intensively localized in the large luteal cells and placental cytotrophoblast villus, glandular epithelial cells of the endometrium, syncytiotrophoblast of the placenta, the isthmus cells of the oviduct, and the basal part of the primary chorionic villi and chorionic stem villus of the placenta and large luteal cells of the CL in many mammalian species. Further studies are needed to determine the functional significance of the 20α-HSD molecule during ovulation, pregnancy, and parturition. This article will review how fundamental information of these enzymes can be exploited for a better understanding of the reproductive organs during ovulation and pregnancy.

(Key words : 20α-HSD, Reproductive tissues, Ovary, Placenta, testis)

INTRODUCTION

In steroid hormone target tissues, pairs of hydroxysteroid dehydrogenases (HSDs) co-exist; they interconvert potent steroid hormones with their cognate inactive metabolites (Penning *et al*., 2011). In all mammalian species, progesterone is essential for the preparation and maintenance of pregnancy, if it occurs. Progesterone primes the endometrium for possible implantation and inhibits uterine contraction until birth (Naidansuren *et al*., 2011). Aldo-keto reductases (AKRs) belong to a superfamily of NADPH-dependent reductases that act on a wide range of substrates, including simple carbohydrates, steroid hormones, and endogenous prostaglandins (Jez *et al*., 1997). The 20α-HSD enzyme is a member of the AKR family (Liu *et al*., 2007) (Fig. 1).

The enzyme 20α-HSD predominantly converts progesterone to its biologically inactive form, 20α-hydroxyprogesterone (20α-OHP) and has an important role in the termination of pregnancy and the initiation of parturition (Seong *et al*., 2002; Naidansuren *et al*., 2011; 2012)

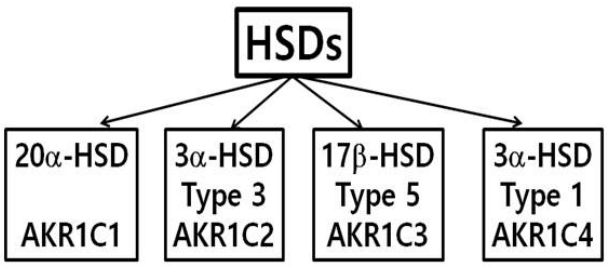

Fig. 1. Properties of aldo-keto reductases (AKRs). HSD family divided into 4 groups including 20α-HSD (AKR1C1), 3α-HSD (AKR1C2), 17b-HSD (AKR1C3), and 3α-HSD (AKR1C4) (Penning, 2011).

(Fig. 2). Progesterone production in the rodent corpus luteum (CL) is regulated by hormones, including prolactin and prostaglandin $F_2\alpha$ (Stocco *et al*., 2001). Prolactin suppresses 20α-HSD expression (Park *et al*., 2018), and progesterone secretion is maintained in the first half of pregnancy, whereas prostaglandin $F_2\alpha$ stimulates 20α-HSD expression at the end of pregnancy (Albarracin *et al*., 1994; Stocco *et al*., 2000).

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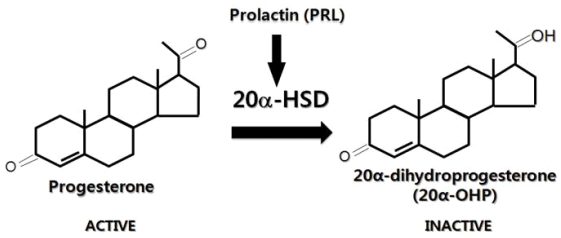

Fig. 2. 20α-HSD catalyzes the conversion of progesterone to 20α-OHP. Prolactin represses 20α-HSD during pregnancy.

The corpus luteum (CL) is the main source of progesterone throughout gestation in mammalians (Meyer, 1994; Seong *et al*., 2017). The concentration of progesterone in tissues increases from 6 months of gestation to parturition (Tsumagari *et al*., 1994). The placenta only contributes marginally and temporarily to the peripheral maternal plasma concentrations of progesterone, which are primarily associated with luteolysis (Schuler *et al*., 2006).

We previously showed that 20α-HSD was highly expressed in ovarian and placental tissues during the estrous cycle and pregnancy (Naidansuren *et al*., 2011, 2012; Kim *et al*., 2014; Seo *et al*., 2011; Nanjidsuren *et al*., 2011, 2014; Nanjidsuren and Min, 2014). Thus, this article will review some of the biochemical characterization of 20α-HSD in reproductive tissues.

CLONING OF MAMMALIAN 20α-HSD GENE

Bovine 20α-HSD encodes a putative protein of 323 amino acids including a 969-bp open reading frame, which has been cloned in bovine placental and ovarian tissues (Naidansuren *et al*., 2011). In pigs, 20α-HSD cDNA is 957 bp in length and encodes a protein of 319 amino acids (Seo *et al*., 2011). The full cDNA sequence of deer 20α-HSD was cloned; it consists of an open reading frame encoding 323 amino acids and consisting of 969 bp (Naidansuren *et al*., 2012). The mouse and rat 20α-HSD cDNAs in the corpus luteum (CL) have been determined by cloning (Ishida *et al*., 1999; Miura *et al*., 1994). The monkey and goat 20α-HSD cDNA was also cloned in the ovary and placenta (Higaki *et al*., 2002; Jayasekara *et al*., 2004). The 20α-HSD promoter region was cloned and characterized in mice, rats, and monkeys (Hirabayashi *et al*., 2004; Zhong *et al*., 1998; Najidsuren and Min, 2014).

According to a homology search, the nucleotide sequence of bovine 20α-HSD cDNA showed high homology to the 20α-HSD cDNA of other animals (deer 96%, goat 96%, human 84%, rabbit 83%, rat 81% and mouse 81%) (Table 1). Phylogenetic tree analysis showed that bovine 20α-HSD clusters with a high bootstrap in the lineage of goats, sharing the highest homology with goat 20α-HSD (Fig. 3).

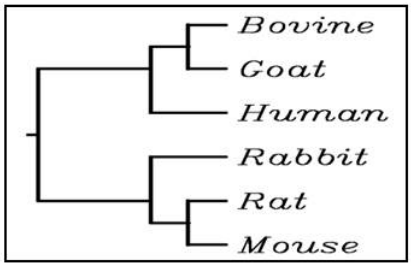

Fig. 3. The phylogenetic tree of the bovine 20α-HSD amino acid sequences from other vertebrate species. The other 20α-HSD amino acid sequences were obtained from GenBank (Naidansuren *et al*., 2011).

Table 1. Homology analysis of 20α-HSD

		Nucleotides (%)							
		Goat	Mouse	Rat	Human	Rabbit	Pig	Deer	Bovine
Amino acids (%)	Goat		75	74	80	78	80	87	93
	Mouse	67		94	79	80	76	80	78
	Rat	66	93		78	80	76	76	76
	Human	76	72	68		84	81	81	82
	Rabbit	73	74	69	80		79	78	79
	Pig	81	68	66	76	76		83	83
	Deer	96	69	67	76	73	80		90
	Bovine	96	68	66	76	74	81	96	

EXPRESSION OF 20α-HSD mRNA

Bovine 20α-HSD mRNA was expressed in the ovaries during estrous cycle, placental tissues during pregnancy, and cultured bovine CL cells (Naidansuren *et al*., 2011). Its mRNA was first detected 24 h after culture and increased 120 h after culture. Northern blot analysis was performed, and 20α-HSD mRNA was not detected in the CL of the corpus hemarrhagicum (CH)2, CH3, and CL3 stages, but was strongly detected in the CL2 and CL1 stages. Bovine 20α-HSD mRNA was also strongly detected in the pre-parturition placenta. Its expression was reported on days 30, 60, and 90 of pregnancy (Kim *et al*., 2014). The amounts showed strong amplification in the ovary, which those in the placenta and endometrium were slightly lesser.

The results of RT-PCR and real-time PCR for porcine samples showed that 20α-HSD was expressed on day 5, 10, 12, and 15 of the estrous cycle and day 0~60 of pregnancy in the ovary (Seo *et al*., 2011). It was also reported that 20α-HSD mRNA was specifically detected in the uterus on day 30 of the pregnancy. 20α-HSD mRNA was also detected in high in the placenta on day 30 of pregnancy (Nanjidsuren *et al*., 2014), it was significantly expressed in the ovary during pre-parturition rather than on days 30 and 60 of pregnancy.

Deer 20α-HSD mRNA was expressed in the placenta and ovary, and fetal skin on days 30 of pregnancy (Naidansuren *et al*., 2012). The results by the northern blot analysis showed a stronger expression at 60 and 70 days, than at 30 days of pregnancy. Goat 20α-HSD mRNA was expressed in the placenta and the intercaruncular part of the uterus during mid-to-late pregnancy but was not expressed in the adrenal gland, liver, or spleen during pregnancy (Jayasekara *et al*., 2004). Its expression was low or at a minimum level in the placenta on day 40 of pregnancy, but it was increased by day 90 and remained high until parturition (Jayasekara *et al*., 2005).

In case of primates, 20α-HSD expression was high in the ovary during pre-ovulation (Nanjidsuren *et al*., 2011). It was also expressed in the oviduct and placenta at pre-parturition. After northern blot analysis, monkey 20α-HSD mRNA level was strongly detected in the ovarian tissue. This is because the progesterone level was the lowest at the time of ovulation. Higaki *et al*.(2002) reported the expression of monkey 20α-HSD mRNA in the liver, intestine, adrenal glands, and kidneys. Another group showed high expression levels of 20α-HSD in the stomach, liver, kidneys, and mammary glands, and moderate levels in the ovary, adrenal glands, and colon (Liu *et al*., 2007). Human 20α-HSD (AKR1C1) is highly expressed in the liver, mammary glands, and brain, whereas the expression is lower in the prostate, testes, and uterus and is remarkably low in the adrenal glands (Zhang *et al*., 2000). Using PCR, its expression was detected expression not only in the ovary, uterus, and placenta, but also in many other tissues such as the heart, liver, adrenal glands, kidneys, muscles, peripheral blood lymphocytes, and testes (Nishizawa *et al*., 2000). Other groups reported that human AKR1C1 mRNA expression is highest in the lungs, followed by the liver, testes, mammary glands, endometrium, and brain (Rizner *et al*., 2006). Several classes of AKR1C1 inhibitors have been identified, including benzodiazepines, benzofuranes, steroid carboxylates, flavones, and derivatives of pyrimidine, anthranilic acid, and cyclopentane (El-Kabbani *et al*., 2011).

LOCALIZATION OF 20α-HSD PROTEIN

Bovine 20α-HSD protein level was the highest in the CL undergoing luteolysis throughout the estrous cycle (Naidansuren *et al*., 2011). It was localized in the large luteal cells during early pregnancy (Kim *et al*., 2014), and was especially intense in the CL of the ovaries at the terminal stage of the estrous cycle. Notably, the 20 α-HSD was mainly expressed in the trophoblast villus of the placenta, and staining was weaker in the glandular epithelial cells of the endometrium.

Porcine 20α-HSD protein was also localized in the large luteal cells on days 2 and 5 of the early estrous cycle (Seo *et al*., 2011). Its mRNA was strongly localized in the luteal cells of ovary at before parturition. However, its signal was not detected in the small luteal cells. The 20α-HSD protein was strongly localized in the trophoblast villus of the placenta on day 30 of pregnancy (Nanjidsuren *et al*., 2014). A weak signal of the protein was also detected in the glandular epithelial cells of the uterus. The deer 20α-HSD protein was expressed at a higher level in the placenta than in the ovary during early pregnancy, suggesting that 20α-HSD plays a pivotal function in the placenta in deer (Naidansuren *et al*., 2012). It was localized specifically in the basal part of primary chorionic villi and chorionic stem villus of the placenta. Additionally, the 20α-HSD protein was intensively localized in the large luteal cells and particularly intense in the corpus luteum of the ovaries during pregnancy.

Monkey 20α-HSD protein was localized in the isthmus cells of the muscularis layer of the oviduct and in the syncytial villi of syncytio trophoblast (Nanjidsuren *et al*., 2011).

In the rat, immunoactivity of the 20α-HSD protein

was revealed in decidual cells and trophoblastic giant cells on day 10 and spongiotrophoblasts and visceral yolk sac cells on day 21 of pregnancy (Shiota *et al.*, 1993). The 20α-HSD activity slowly decreased from days 5 to 18 of pregnancy, but rapidly increased in the ovary during parturition (Seong *et al.*, 2002). On the other hand, levels of placental cytosolic 20α-HSD were high from days 8 to 10 of pregnancy, not detectable from days 11 to 20 of pregnancy, and again, very high at the time of parturition. Thus, expression of 20α-HSD in the placental tissues is reported to be related the number of fetuses that survived in the specific time at which spontaneous fetus loss occurs. We also reported that mouse 20α-HSD was strongly expressed in the testes after parturition (Part *et al.*, 2018).

REGULATION OF 20α-HSD PROMOTER

A reporter assay, using reporter constructs of various lengths of the 5'-flanking region, revealed that the region between −83 and 60 bp upstream of the transcription start sites was essential for transcriptional activity in the mouse 20α-HSD promoter (Hirabayashi *et al.*, 2004).

Analysis of the 5'-flanking region of rat 20α-HSD revealed a single putative cyclic AMP-responsive element (CRE) at −2126 to −2118, one Nur77-binding site at −1639 to −1531, and another perfect but inverted Nur77-binding site located further upstream. Two putative prolactin response elements, which have been previously shown to bind Stat5/Stat3, were identified at position −87 to −77 and −1608 to −1600 (Zhong *et al.*, 1998). A rapid increase in the Nur77 mRNA level was observed in mice corpora lutea just before parturition, at a time when 20α-HSD is expressed (Stocco *et al.*, 2000). They also reported that Nur77 plays an important role in maintaining the ovarian physiology by mediating the prostaglandin $F_2\alpha$ induction of 20α-HSD (Stocco *et al.*, 2002). Decidual prolactin plays an important role in pregnancy by repressing the IL-6 and 20α-HSD' expression in the decidua (Bao *et al.*, 2007). Thus, prolactin signals through the Jak2/Stat5 pathway to down-regulate 20α-HSD expression in the decidua.

In monkey 20α-HSD, the promoter region (2,002 bp) included several putative binding sites for different transcription factors like Ap-1, Sp-1, Oct-1, GATA-1, GATA-2, GATA-3, HSF-2, XFD, CRE-BP, IRF-1, 2, Sox-5, GR, and others (Nanjidsuren *et al.*, 2011). Among the transcription factors, the Ap-1 site (−281~−274) plays a crucial role in the activation of the monkey 20α-HSD gene in CHO-K1 cells (Nanjidsuren and Min, 2014) (Fig. 4). AKR1C1 is regulated by NF-Y in human ovaries, lungs, and liver carcinoma cells, and the cisplatin-induced transcription in human ovarian carcinoma cells (Pallai *et al.*, 2010). The increase in AKR1C1 mRNA is transcriptionally regulated, at least in part by the transcription factor Sp1 in HT29 human colon cancer cells (Selga *et al.*, 2008).

CONCLUSION

We summarized the molecular function of 20α-HSD on reproductive tissues. 20α-HSD mRNA and protein were expressed in several tissues including those of the ovaries, placenta, testes, etc. The transcription factors Sp-1, Ap-1, NF-Y, and Nur77 play a significant role in the expression of the 20α-HSD gene in luteal cells (Fig. 5). Thus, we suggest that 20α-HSD has a pivotal function

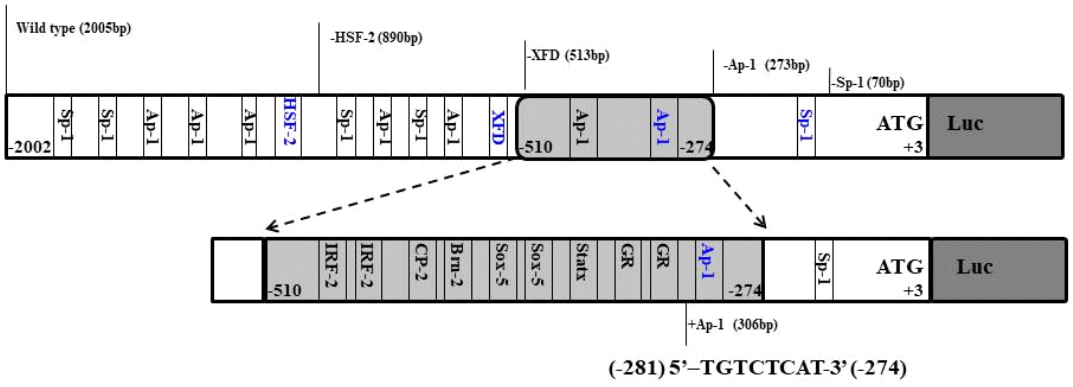

Fig. 4. Putative transcription factor-binding sites in the 5′-flanking region of the monkey 20α-HSD gene. Sequences approximately 2.5 kb upstream from the translational start codon of the monkey 20α-HSD. Putative transcription factor binding sites were identified using the TF search software (Nanjidsuren and Min, 2014).

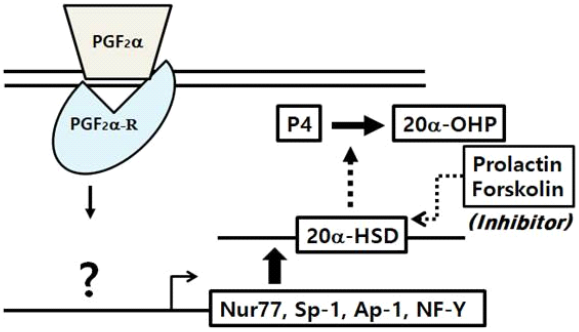

Fig. 5. Mechanism of 20α-HSD inductions in luteal cells. Among the species, 20α-HSD promoter was regulated by several transcriptional factors (Nur77, Sp-1, Ap-1, NF-Y). This Fig was modified (Stocco *et al*., 2002).

in the ovaries during estrous cycle/pregnancy and in the placenta during pregnancy. Furthermore, we suggest that 20α-HSD activity may be important for protecting the fetus from high progesterone levels during parturition in several mammalian species including primates. However, little is known about the specific functions of the 20α-HSD protein in the ovaries and placenta during the estrous cycle and pregnancy. We suggest that the roles of the 20α-HSD protein in tissues except those of the ovaries and placenta need to be clearly elucidated.

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REFERENCES

1. Albarracin CT, Parmer TG, Duan WR, Nelson SE, Gibori G (1994): Identification of a major prolactin-regulated protein as 20α-HSD: coordinate regulation of its activity, protein content, and messenger ribonucleic acid expression. Endocrinology 134:2453-2460.
2. Bao L, Tessier C, Prigent-Tessier A, Li F, Buzzio OL, Callegari EA, Horseman ND, Gibori G (2007): Decidual prolactin silences the expression of genes detrimental to pregnancy. Endocrinology 148:2326-2334.
3. El-Kabbani O, Dhagat U, Hara A (2011): Inhibitors of human 20α-hydroxysteroid dehydrogenase (AKR1C1). J Steroid Biochem and Mol Bio 125:105-111.
4. Higaki Y, Kamiya T, Usami N, Shintani S, Shiraishi H, Ishikura S, Yamamoto I, Hara A (2002): Molecular characterization of two monkey dihydrodiol dehydrogenases. Drug Metab Pharmacokinet 17:348-356.
5. Hirabayashi K, Ishida M, Suzuki M, Yamanouchi K, Nishihara M (2004): Characterization and functional analysis of the 5'-flanking region of the mouse 20α-hydroxysteroid dehydrogenase gene. Biochem J 382: 975-980.
6. Ishida M, Chang K, Hirabayashi K, Nishirara M, Takahashi M (1999): Cloning of mouse 20α-hydroxysteroid dehydrogenase cDNA and its mRNA localization during pregnancy. J Reprod Dev 45:321-329.
7. Jayasekara WS, Yonezawa T, Ishita M, Yamanouchi K, Nishihara M (2004): Molecular cloning of goat 20 α-hydroxysteroid dehydrogenase cDNA. J Reprod Dev 50:323-331.
8. Jayasekara WS, Yonezawa T, Ishita M, Yamanouchi K, Nishihara M (2005): Expression and possible role of 20α-hydroxysteroid dehydrogenase in the placenta of the goat. J Reprod Dev 51:265-272.
9. Jez JM, Bennett MJ, Schlegel BP, Lewis M, Penning TM (1997): Comparative anatomy of the aldoketo reductase superfamily. Biochem J 326:625-636.
10. Kim SH, Shin YS, Kang MH, Yoon JT, Min KS (2014): Gene expression and localization of 20α-HSD in reproductive tissues during early pregnancy of cattle. Anim Reprod Sci 147:1-9.
11. Liu H, Bellemare V, Labrie F, Luu-The V (2007): Molecular characterization of the cynomolgus monkey Macacafascicularis steroidogenic enzymes belonging to the aldo-keto reductase family. J Steroid Biochem Mol Biol 104:75-80.
12. Meyer HH (1994): Luteal versus placental progesterone: the situation in the cow, pig and bitch. Exp Clin Endocrinol 102:190-192.
13. Miura R, Shiota K, Noda K, Yagi S, Ogawa T, Takahashi M (1994): Molecular cloning of cDNA for rat ovarian 20α-hydroxysteroid dehydrogenase (HSD1). Biochemistry J 15:561-567.
14. Naidansuren P, Park CW, Kim SH, Nanjidsuren T, Park JJ, Yun SJ, Sim BW, Hwang SS, Kang MH, Ryu BY, Hwang SY, Yoon JT, Yamanouchi K, Min KS (2011): Molecular characterization of bovine placental and ovarian 20α-HSD. Reproduction 142:723-731.
15. Naidansuren P, Park CW, Nanjidsuren T, Park JJ, Yun SJ, Kang MH, Yamanouchi K, Min KS (2012): Ovarian and placental of 20α-HSD during pregnancy in deer. Anim Reprod Sci 130:63-73.
16. Nanjidsuren T, Min KS (2014): The transcription factor Ap-1 regulates monkey 20α-hydroxysteroid dehydrogenase promoter activity in CHO cells. BMC Biotechnology 14:71.

17. Nanjidsuren T, Naidansure P, Park CW, Park JJ, Yun SJ, Sim BW, Kang MH, Lee SR, Chang KT, Min KS (2011): Expression and localization of the 20α-hudroxysteroid dehydrogenase enzyme in the reproductive tissues of the cynomolgus monkey Macaca fascicularis. J Steroid Biochem Mol Biol 127:337-344.
18. Nanjidsuren T, Yun SJ, Park CW, Kim MS, Kang MH, Min KS (2014): Expression and localization of 20α-HSD in porcine reproductive tissues during pregnancy. Anim Reprod Sci 148:63-71.
19. Nishizawa M, Nakajima T, Yasuda K, Kanzaki H, Sasaguri Y, Watanabe K, Ito S (2000): Close kinship of human 20α-hydroxysteroid dehydrogenase gene with three aldo-keto reductase genes. Genes Cells 5:111-125.
20. Pallai R, Simpkins H, Chen J, Parekh HK (2010): The CCAT box binding transcription factor, nuclear factor-Y (NF-Y) regulates, transcription of human aldo-keto reductase 1C1 gene. Gene 549:11-23.
21. Park CW, Jeong SK, Nanjidsuren T, Byambaragchaa M, Kang MH, Sim BW, Min KS (2018): Characterization of transgenic mice expressing EGFP under the control of the monkey 20α-hydroxysteroid dehydrogenase promoter. Sci China Life Sci DOI https://doi.org/10.1007/s11427-017-9111-9.
22. PenningTM (2011): Human hydroxysteroid dehydrogenases and pre-receptor regulaltion: Insights into inhibitors design and evaluation. J Steroid Biochem Mole Biol 125:46-56.
23. Rinzer TL, Smuc T, Rupreht R, Sinkovec J, Penning TM (2006): AKR1C1 and AKR1C3 may determine progesterone and estrogen ratios in endometrial cancer. Mol Cell Endocrinol 248:126-135.
24. Schuler G, Teichmann U, Kowalewski MP, Hoffmann B, Madore E, Fortier MA, Klisch K (2006): Expression of cyclooxygenase-II (COX-II) and 20alpha-hydroxysteroid dehydrogenase/prostaglandin F-synthesis in bovine placentomes: implications for the initiation of parturition in cattle. Placenta 27:1022-1029.
25. Selga E, Noe V, Ciudad CJ (2008): Transcriptional regulation of aldo-keto reductase 1C1 in HT29 human colon cancer cells resistant to methotrexate: role in the cell cycle and apoptosis. Biochem Pharmacol 75:414-426.
26. Seo KS, Naidansuren P, Kim SH, Yun SJ, Park JJ, Sim BW, Park CW, Nanjidsuren T, Kang MH, Seo HW, Ka HK, Kim NH, Hwang SY, Yoon JT, Yamanouchi K, Min KS (2011): Expression of aldo-keto reductase family 1 member C1 (AKR1C1) gene in porcine ovary and uterine endometrium during the estrous cycle and pregnancy. Reprod Biol Endocrinol 9:e139.
27. Seong HH, Min KS, Kang MH, Yoon JT, Jin HJ, Chung HJ, Chang WK, Yun SG, Shiota K (2002): Change in ovarian and placental 20α-HSD activity during the pregnancy in the rat. Asian-Aust J Anim Sci 16:342-347.
28. Seong HK, Seo KS, Kim JS, Her CG, Kang MH, Sim BW, Yoon JT, Min KS (2017): Studies on steroid hormone concentration during the estrous cycle in the medikinetics micropig. Reprod Dev Bio 41:1-6.
29. Shiota K, Seong HH, Noda K, Hattori N, Ikeda A, Ogura A, Itagaki S, Takahashi M, Ogawa T (1993): 20α-HSD activity in rat placenta. Endocrine J 40:673-681.
30. Stocco CO, Lau LF, Gibori G (2002): A calcium/calmodulin-dependent activation of ERK1/2 mediates Jun D phosphorylation and induction of nur77 and 20α-HSD genes by prostaglandin $F_2\alpha$ in ovarian cells. J Biol Chem 277:3293-3302.
31. Stocco CO, Zhong L, Sugimoto Y, Ichikawa A, Lau LF, Gibori G (2000): Prostaglandin $F_2\alpha$-induced expression of 20α-hydroxysteroid dehydrogenase involves the transcription factor NUR77. J Biol Chem 275:37202-37211.
32. Tsumagari S, Kamata J, Takagi K, Tanemura K, Yoshi A, Takeishi M (1994): 3 beta-hydroxysteroid dehydrogenase activity and gestagen concentrations in bovine cotyledons and caruncles during gestation and parturition. J Reprod Fetil 102:35-39.
33. Zhang Y, Dufort I, Rheault P, Luu-The V (2000): Characterization of a human 20α-HSD. J Mol Endocrinol 25:221-228.
34. Zhong L, Ou J, Barkai U, Mao J, Frasor J, Gibori G. (1998): Molecular cloning and characterization of the rat ovarian 20alpha-hydroxysteroid dehydrogenase gene. BBRC 249:797-803.

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