Changes in Ovarian and Placental 20α-hydroxysteroid Dehydrogenase Activity during the Pregnancy in the Rat

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ABSTRACT : The enzyme 20α-hydroxysteroid dehydrogenase (20α-HSD) catabolizes progesterone to 20α-dihydroprogesterone (20α-OHP), and is appeared in rat corpora luteal and placenta. A polled samples of 10-15 placental and ovarian tissues collected from each individual rat were subjected to measurement of 20α-HSD activity. A 20α-HSD activity in the cytosol fraction was based on the generation of NADPH. In this study, it is designed to study cytosolic 20α-HSD activity in rat ovarian and placenta during pregnancy, and its relationship to embryonic mortality. It was found that from days 5 to 18 of pregnancy the 20α-HSD activities steady by decreased but at parturition time rapidly increased in ovary. On the other hand, placental cytosolic 20α-HSD activities were high detected from days 8 to 10 of pregnancy, not detectable from days 11 to 20 of pregnancy, but again very high at the time of parturition. Analysis of DEAE column chromatography revealed that two different types of 20α-HSD (HSD-1 and HSD-2) were found with similar activity in the placental cytosol on day 10 of pregnancy. The number of fetuses on day 10 of pregnancy was 15.4 and decreased significantly to 12.9 on day 12. The results suggested that expression of 20α-HSD in the placental tissues seems to be related the number of fetal survived in the specific time (days 11 and 12) which spontaneous fetal loss occurs. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 342-347)***

Key Words : 20α-HSD Activity, Progesterone, 20α-dihydroprogesterone, Rat Ovary

INTRODUCTION

The enzyme 20α-hydroxysteroid dehydrogenase (20α-HSD) catabolizes progesterone to 20α-dihydroprogesterone (20α-OHP), a biologically inactive steroid and plays a key role in the regulation of ovarian function in the rodent (Noda et al., 1991; Seong et al., 1992). In a variety of mammalian tissues and cell, 20α-HSD activity has also been demonstrated as testis (Sato et al., 1971; Pineda et al., 1985), ovary (Nakajin et al., 1989; Seong et al., 1998; Yoshida et al., 1999; Zetser et al., 2001), placenta (Fukuda et al., 1986; Shiota et al., 1993), erythrocytes (Nancarrow et al., 1981), macrophages (Matsuyama et al., 1990) and lymphocytes (Chaouat et al., 1990) and also in microorganisms (Happel et al., 1985). The main source of progesterone in the rat is the ovary throughout pregnancy, and an increase in ovarian 20α-HSD activity results in a reduction in progesterone secretion (Noda et al., 1991; Telleria et al., 1999).

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The activity of 20α-HSD is associated with luteal regression and is known to increase at the termination of pseudopregannncy and pregnancy (Kuhn and Briley, 1970). The activity of 20α-HSD in the functional corpus luteal (CL) of the early and middle phase of pseudopregnancy is suppressed under the control of prolactin and at the end of pseudopregnancy (Seong et al., 1998). However, 20α-HSD activities appear mainly in the functional CL without suppressive action of prolactin (Matsuda et al., 1990). Histochemical evidences illustrated that 20α-HSD activities in the theca cells was strongly showed in a few large follicle but not in the granulosa cells (Seong et al., 1998).

Cloning (Miura et al., 1994) of 20α-HSD cDNA and genome DNA (Zhong et al., 1998) revealed that its mRNA expressed the ovaries from normal adult rats contained in a 1.2 kb. The sequence showed a high similarity with those of rat liver 3α-HSD, bovine lung prostaglandin F synthesis (PGFS), human liver chlordecone reductase (CDR), frog lens rho-crystallin and aldoreductase, and hence, indicating that 20α-HSD belongs to the aldo-keto reductase family. Matsuda et al. (1990) reported that ovarian cytosolic 20α-HSD activities are derived 3-5 times higher in the luteal ovarian tissue than in the non-luteal ovarian tissue at the end of pseudopregnancy. In addition, it was reported that two types of 20α-HSD isozymes (HSD-1 and HSD-2) with very similar molecular structures are present in the rat ovary (Noda et al., 1991, Seong et al., 1992). The increase in total enzyme activity and appearance of HSD-2 activity observed at late pseudopregnancy was accompanied by an increase in

the number of 20α-HSD-positive luteal cells (Seong et al., 1992).

In the present study, we investigated the changes in 20α-HSD activity and their physiological roles of 20α-HSD in rat placental and ovarian tissues during pregnancy.

MATERIALS AND METHODS

Animals

The animals (n=10~15) used were 10 to 12 weeks old Wistar-Imamichi rats (body weight 200-250 g) under controlled lighting conditions of 14 h light: 10 h dark (lights on 05:00-19:00 h) and at a temperature of 22°C. Food and water were available *ad libitum*. Only animal that had shown regular 4 or 5 day oestrous cycles for more than two cycles were used. In the evening of the proestrous day, each female was housed with a male, and the day sperm was observed in the vaginal smear was designated day 0 of pregnancy.

Ovary and placenta tissue sample and collection of blood : The rats were sacrificed by decapitation between 09:00 and 12:00 h. The antimesometrial wall of the uterus was cut and the conceptus was shelled out of the uterus with forceps. After removal of the embryo, the placental tissues and ovaries were collected from pregnant rats on days 5 to 21 at 1- or 2- day intervals. The tissue samples after freed from adherent tissues were kept at -70°C until preparation of the cytosol. Pooled samples of 4-5 placental tissues collected from each individual rat were subjected to measurement of 20α-HSD activities. Blood samples were allowed to clot at room temperature for 2 h and centrifuged at 1,500×g for 20 min. The sera thus obtained were stored at -20°C until the hormone assay.

Preparation of ovarian and placental cytosol : Each frozen ovarian and placental tissues were prepared as reported previously (Noda et al., 1992; Seong et al., 1992). Briefly, the frozen tissue was weighed, minced and homogenized in 5 volumes (v/w) of 5 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM dithiothreitol , 5 ug/ml leupeptin and 10% glycerol for placenta and in 20 volumes for ovary. The homogenate was centrifuged at 105,000×g for 90 min. The supernatant after centrifugation was used as the cytosol fraction. All procedures were performed at 4°C.

Measurement of 20α-Hydroxysteroid dehydrogenase activity

Activity of 20α-HSD was measured by the method of Wiest et al. (1968) by a few modifications. The assay was carried out in 0.1 M Tris-HCl buffer (pH 8.0) containing 60 uM 20α-dihydroprogesterone, 300 uM NADP, 1 mM EDTA, 1 mM dithiothreitol and 3% ethanol for steroid solubilization. NADP and dithiothreitol were added to the buffer immediately prior to use, and 500 μl of assay mixture medium was preincubated at 37°C in special microcuvette (0.7 ml). The enzyme reaction was initiated by introducing a 25 μl sample into the cuvette with rapid mixing. The temperature during the assay was maintained at 37°C. Initial velocities at an absorbance of 340 nm were followed spectrophotometrically for 3 min with a Hitachi U-2000 spectrophotometer. A molar absorbance of 6220 at 340 nm was used to calculate NADPH concentrations. One unit of enzyme activity was defined as the amount that could reduce 1 μM NADP at 37°C.

Separation of placental 20α-HSD activity and protein assay : The placental cytosol in day 10 of pregnancy was applied to a DEAE-Toyopearl (0.7×6.7 cm, 650S) anion-exchange column on a high-performance liquid chromatography system (Water's model 650: Waters, Bedford, MA). Chromatography was performed at 1 ml per min using 5 mM potassium phosphate buffer solution (pH 7.0) containing 1 mM EDTA, 1mM dithiothreitol and 10% glycerol (buffer A) and eluted with a linear gradient of increasing KCl concentration in buffer A. Elutes were fractionated into 500 μl per tube and the enzyme activity was measured as already described. Protein was quantified by the method of Bradford (1976) using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA, USA) with bovine serum albumin as a standard.

Steroid hormone assay : Concentrations of serum progesterone and 20α-dihydroprogesterone were determined by radioimmunoassay using specific antibodies raised as described by Matsuyama et al.(1990).

Statistics

Duncan's multiple-range test was used for statistical evaluation of the results. Differences at $p<0.05$ were considered to be statistically significant.

RESULTS

Changes of progesterone and 20α-OHP levels during pregnancy

The progesterone concentration was 100 μg/ml at 5 days and gradually increased to 140 at 12 days, then slightly declined to 130. It was further depressed to 30 at 21 days. A 20α-OHP concentration was 85 μg/ml at 5 days and sharply dropped to 50 at 9 days. It increased to 12 days and dramatically increased to 150 μg/ml at 20-21 days. The serum progesterone and 20α-dihydroprogesterone concentrations were positively correlated in pregnant rat (Figure 1).

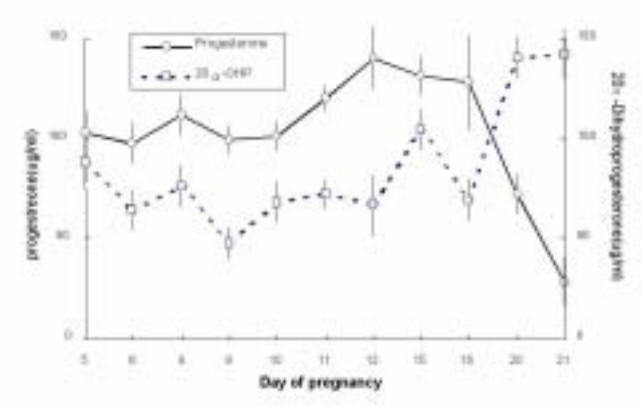

Figure 1. Concentration of progesterone and 20α-dihydroprogesterone in serum of pregnant rats. The day sperm was observed in the vaginal smear was designated day 0 of pregnancy. Each column and vertical bar represents the mean±SEM for 10 15 animals.

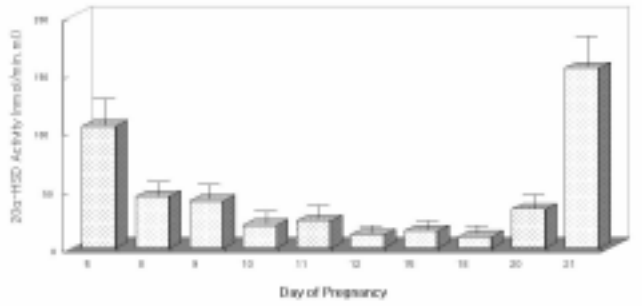

Figure 2. Changes in activities of ovarian cytosolic 20α-hydroxysteroid dehydrogenase (20α-HSD) in pregnant rats. Each column and vertical bar represents the mean±SEM for 3 5 animals. 20α-HSD activities were measured as mM NADPH/min with 1 unit of activity defined as the amount that can induce 1 mM NADPH /min at 37°C.

The enzyme (20α-HSD) converting progesterone to a 20α-OHP, a biologically inactive steroid, is play a pivotal roles for pregnant maintenance, ovary and placenta functions during pregnancy in rat.

Cytosolic 20α-HSD activity in ovary and placenta during pregnancy

In order to identify the cytosolic 20α-HSD activities, we investigated in rat placenta and ovary tissues. Its relationship to embryonic mortality was considered the mesometrial endometrium (including the ectoplacenta on days 8-11 of pregnancy). Cytosolic 20α-HSD activities in ovary were detected during pregnancy. It was linear decreased from days 5 to 18 of pregnancy and rapidly increased at parturition time. Its activity was almost undetected from day 12 to 18 (Figure 2).

Placental cytosolic 20α-HSD activities were detected as high level from days 8 to 10 of pregnancy. The activity of

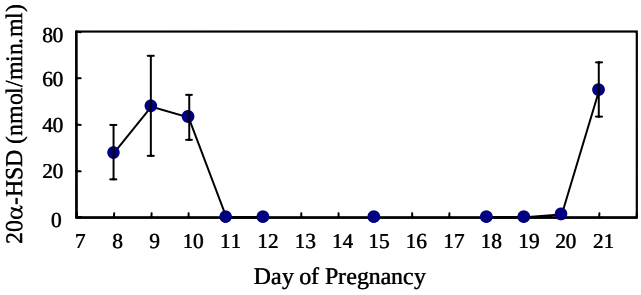

Figure 3. Changes in activities of placental cytosolic 20α-hydroxysteroid dehydrogenase (20α-HSD) in pregnant rats. Each column and vertical bar represents the mean±SEM for 3 5 animals. 20α-HSD activities were measured as mM NADPH/min with 1 unit of activity defined as the amount that can induce 1 mM NADPH /min at 37°C.

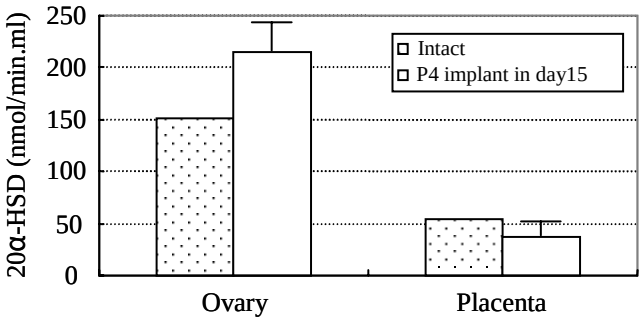

Figure 4. Ovarian and placental cytosolic 20α-hydroxysteroid dehydrogenase (20α-HSD) activities at day 20 in pregnant rats (intact) and after progesterone tube implant in day 15 of pregnancy. 20α-HSD activities were measured as mM NADPH/min with 1 unit of activity defined as the amount that can induce 1 mM NADPH /min at 37°C.

20α-HSD in the placenta was highest on day 9 and then rapidly decreased to a minimum value. The activity was not detected from days 11 to 20 of pregnancy. On day 20, the 20α-HSD activities began to increase and a sudden burst activity was detected at the time of parturition (Figure 3). The activity of ovary was 2 times than placenta in early pregnancy and increased 3 times at parturition time. To determine the role of progesterone affecting 20α-HSD activity, we transplanted into subcutaneous the silicon tube which contained progesterone hormone on day 15 of pregnancy. The activity of 20α-HSD was measured in ovary and placenta obtained on day 21 of pregnancy. The 20α-HSD activity of ovary implanted progesterone tube was higher (133%) than control ovary. However, the activity was a similar level between intact and progesterone implanted group in the placenta (Figure 4).

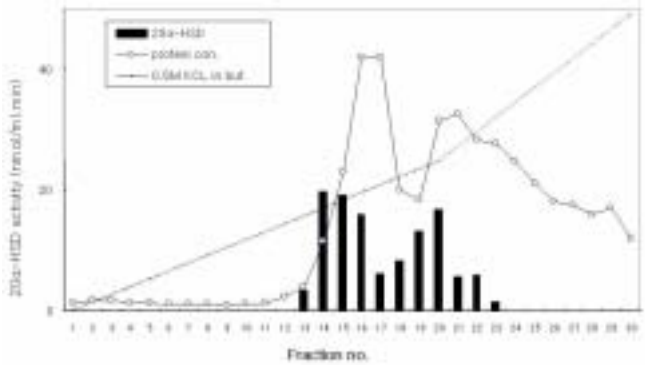

Figure 5. DEAE-Toyopearl chromatography of cytosol fraction from rat placenta at day 10 of pregnancy. The cytosol fraction from rat placenta at day 10 of pregnancy was applied to a DEAE-Toyopearl 650S column on a medium-pressure inert liquid chromatography system. No enzyme activity was detected in the effluent. The combined substances were eluted with a KCl gradient at a flow rate of 1 ml/min. and collected in 1 ml fractions.

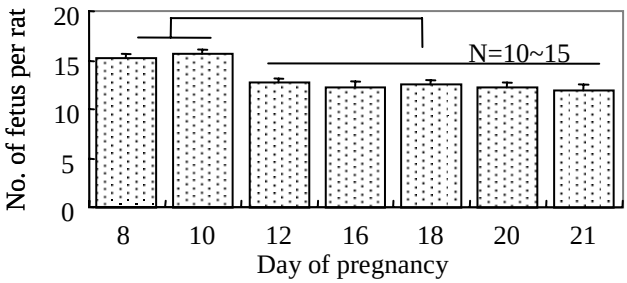

Figure 6. Number of fetuses during pregnancy in the rat.
* Significant at $p<0.05$ (day 8 and 10)

DEAE chromatography of cytosol fraction from rat placenta at day 10 of pregnancy and number of fetuses

We also analyzed by DEAE chromatography to determine the molecular cytosol fractions prepared in placenta on day 10 of pregnancy. Analysis of DEAE column chromatography revealed that these tissues contain two different types of 20α-HSD activities (HSD-1 and HSD-2). The HSD-1 eluted between fraction 13 and 17, and HSD-2 between fractions 18 and 23. The activity of the two types was almost the same in the placental cytosol at day 10 of pregnancy (Figure 5).

Correlation between 20α-HSD activities in each placenta and number of fetuses during pregnancy

To identify the correlation between 20α-HSD activity and number of fetuses, we checked the number of fetuses throughout pregnancy. The number of fetuses on day 10 of pregnancy was 15.4 and decreased significantly to 12.9 on day 12 (Figure 6). And we also analyzed 20α-HSD activities in each individual placenta. The 20α-HSD activities were shown very different variation on day 10 of pregnancy (Table 1).

DISCUSSION

Results of this investigation have been revealed that the cytosolic 20α-HSD activities was changed in rat ovarian and placenta during pregnancy, and its relationship to embryonic mortality were considered. The 20α-HSD activity has been demonstrated in a variety of mammalian tissues and cell and has a pivotal role during pregnancy (Sato et al., 1971; Pineda et al., 1985), ovary (Nakajin et al., 1989; Seong et al., 1998; Yoshida et al., 1999; Zetser et al., 2001), placenta (Fukuda et al., 1986; Shiota et al., 1993), erythrocytes (Nancarrow et al., 1981), macrophages (Matsuyama et al., 1990) and lymphocytes (Chaouat et al., 1990) and thymus (Hirabayashi et al., 2001) and also in microorganisms (Happel et al., 1985).

The 20α-HSD in the corpus luteum is detrimental to the normal progress of pregnancy, this enzyme is totally silenced throughout gestation but becomes highly expressed at the end of pregnancy (Albarracin et al., 1994). At this stage it plays a key role in the initiation of labor, which

Table 1. 20α-HSD activity in each individual placenta on10 day of pregnancy

Placenta	20α-HSD activity (NADPH nmol/min.ml)			
	A	B	C	D
1	-	-	-	15.1
2	171.8	-	-	-
3	-	-	-	-
4	-	-	66.7	29.8
5	47	133.9	11.3	146.9
6	-	-	-	23.8
7	81.9	-	93.9	82.1
8	-	-	-	-
9	28.3	-	-	-
10	102.7	-	-	56.7
11	-	-	17	169.3
12	52.7	32.9	52.5	142.8
13	159.3	33.8	18.3	63.1
14	49.9	24.7	-	6.8
15	147.5	-	109.6	
16	106.9		-	
17	-		-	
18			27.1	
	(10/16)	(4/15)	(8/18)	(10/14)

depends on a drop in circulating progesterone. Thus, the interrelationships between 20α-HSD and progesterone have an important bearing on pregnancy maintenance in the ovary and placenta of rat.

The molecular cytosol fractions were prepared in placenta on day 10 of pregnancy. Results revealed that these tissues contain two different types of 20α-HSD activity (HSD-1 and HSD-2), the activity of the two types was almost the same in the placental cytosol on day 10 of pregnancy. There are several distinctive proteins that have 20α-HSD activity. The rat ovarian 20α-HSD differs from testicular and placental enzymes in that progesterone is its principal substrate rather than 20α-OHP and that its role is clearly defined in the corpus luteum (Zhong et al., 1998). But the other groups reported that the same molecular species as ovarian 20α-HSD is expressed in thymic lymphocytes. Therefore, 20α-HSD may play a role of T-lymphocyte proliferation and differentiation processes. We also think that the same molecular express in ovary and placenta of rat. We reported previously that there are two molecular types of 20α-HSD in the ovary and that HSD-1 is dominant in the corpus luteum at the end of the luteal phase (Seong et al., 1992; Noda et al., 1991). Thus, HSD-1 is responsible for the regulation of progesterone secretion and its expression is under the suppressive control of prolactin. Expression of 20α-HSD activity in the placental tissues seems to lower the concentration of progesterone in the fetal environment. The ovarian 20α-HSD activities were physiologically under inhibitory control. The decrease time for fetuse number was correlated with 20α-HSD activities in each placenta during pregnancy. The fetuses that lowered 20α-HSD activities could be absorpt into the uterus and does not reach until parturition term. It is very important time for fetuses to develop on 10 day of pregnancy.

The rat ovarian 20α-HSD plays a key physiological role and its timely regulation is crucial for the progress of a normal pregnancy. The abrupt expression of 20α-HSD at the end of pregnancy may be due, at least in part, to $PGF2_{\alpha}$ which rises at the end of pregnancy (Zester et al., 2001). A $PGF2_{\alpha}$ markedly stimulates the activity of this enzyme (Strauss et al., 1974). In recently, the molecular mechanism by which PGF2α stimulates 20α-HSD activity was a little elucidate (Stocco et al., 2000, 2002).

The results showed that the 20α-HSD activity in the placental tissues seems to control progesterone concentration and the number of fetal. Thus, the detection of 20α-HSD activity in the placenta as well as ovary at term may contribute to the mechanism of reproductive physiology through the control of peripheral progesterone concentrations during pregnancy.

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