Distribution and Chronological Changes of Hydroxysteroid Dehydrogenase (HSD) in the Cat Placenta.

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ABSTRACT. The distribution of hydroxysteroid dehydrogenase (HSD) was examined histochemically in the placenta and ovary of the cat at 14 gestational stages to discuss the possibility of steroidogenesis in the cat placenta. The enzymes investigated in this study were Δ^5 -3 β -HSD, 17 β -HSD, 20 α -HSD, 20 β -HSD and G-6-PDH. The activity of Δ^5 -3 β -HSD was detected in the trophoblast of placental labyrinth and junctional zone. The activity of Δ^5 -3 β -HSD was weak at the early stage of gestation, but being gradually increased with the progress of gestation. The activity was strongest at the stage of an 88-mm fetus in CRL (day 45), then decreased slightly toward term. No activity of 17 β -HSD was detected in the trophoblast, but a weak activity was detected in the glandular epithelium, persisting throughout pregnancy. In the corpus luteum, the activity of Δ^5 -3 β -HSD was strong or moderate until the stage of an 80-mm fetus (day 43), then abruptly decreased toward term. These results suggested that the conversion of pregnenolone into progesterone may proceed in the trophoblast of the cat placenta and support the fact that ovariectomy after day 45 or 49 of gestation did not interrupt pregnancy.—**KEY WORDS:** cat placenta, hydroxysteroid dehydrogenase, trophoblast.

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Steroid hormones, particularly estrogen and progesterone are essential for the maintenance of pregnancy. The placenta has been thought to be able to synthesize steroid hormones to an extent which varies according to several mammals. Ovariectomy after a certain stage of gestation does not terminate pregnancy in man, monkey, ewe, mare and guinea pig but does in sow, goat, rabbit, mouse, hamster and dog [1]. In the cat, ovariectomy after day 45 [20] or 49 [5] of gestation does not interrupt pregnancy. This suggests that after these stages in the cat, the placenta plays an important role in steroidogenesis instead of the ovary. In the cat placenta, Δ^5 -3 β -hydroxysteroid dehydrogenase (HSD) was demonstrated biochemically and hence, the possibility of steroid hormone synthesis was suggested [4, 17].

According to some histochemical studies [12, 18], Δ^5 -3 β -HSD is detected in the trophoblast of the placental labyrinth, suggesting the possibility of steroid hormone synthesis therein.

In this study, histochemical distribution of HSD was surveyed in the placentae and ovaries obtained at various stages of gestation in the cat with reference to the chronological changes of its intensity, and the results were discussed by comparing with those by Ferguson & Christie [12] and Malassiné & Juillard [18].

MATERIALS AND METHODS

Fourteen pregnant cats were used in this study. Crown-Rump-Length (CRL) of fetuses was ranged from 12-mm (approximately day 21 of gestation) to 120-mm (term) [10]. The transverse slice of the placental girdle was obtained from the anti-mesometrial zone and was immediately

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Table 1. Kinds of enzymes, substrates and co-factors used in this study

Enzymes	Substrates	Co-factors
Δ^5 -3 β -HSD	Dehydroepiandrosterone (DHEA)	NAD
	Pregnenolone	NAD
	17 α -hydroxypregnenolone	NAD
17 β -HSD	Estradiol-17 β	NAD
	Testosterone	NAD
20 α -HSD	20 α -hydroxyprogesterone	NADP
20 β -HSD	20 β -hydroxyprogesterone	NAD

Fig. 1. Transverse section of the cat placenta. It is divided into four parts; placental labyrinth (PL), junctional zone (JZ), glandular layer (GL) and marginal hematoma (MH). HE. $\times 12$.

frozen within cold acetone. The tissue blocks of placenta and ovary were sectioned at $10\mu\text{m}$ in a cryostat maintained at about -20°C and the sections were mounted on glass slides and dried in the air.

Activities of the following HSD were investigated in the placenta; Δ^5 -3 β -HSD, 17 β -HSD, 20 α -HSD, 20 β -HSD and G-6-PDH. The kinds of HSD and the corresponding substrates and co-factors used in this study are listed in Table 1. In the ovary, only Δ^5 -3 β -HSD was investigated.

The sections were incubated for 2 hr at 37°C in a medium containing 9 ml 0.1 M phosphate buffer (pH 7.5), 2.0 mg Nitro-BT, 4.0 mg co-factor and 1 ml substrate (1.8 mg/ml) dissolved in dimethylformamide [7]. After incubation, the sections were fixed in 10% formalin and were mounted with

glycerol-gelatin. The control sections were incubated in a similar medium without substrate. The degree of the activities was graded as negative (−), weak (+), moderate (++) and strong (+++) according to the intensity of the deposited tetrazolium salts. G-6-PDH, which is an important source of NADPH required for hydroxylations during steroidogenesis, was investigated by the method of Rudolph & Klein [19]. The sections were incubated for 1 hr at 37°C in a medium containing 2 mg G-6-P, 2.5 mg NADPH, 2 ml Tris buffer (0.1M, pH 7.2), 1 ml Nitro-BT (1 mg/ml) and 2 ml distilled water and were treated in the same way as for HSD. For histological observation, the placental tissues were embedded in paraffin and the sections were stained with hematoxylin and eosin (HE).

Fig. 2. Placental labyrinth. Maternal capillaries (MC) and giant cells (GC) are sandwiched in between adjacent layers of the trophoblast (ST and CT). HE. $\times 400$.

Fig. 3. Junctional zone. Trophoblast cells (arrowed) which have invaded into the glandular lumen appear vacuolated. HE. $\times 200$.

RESULT

The placenta of the cat was divided into four parts; placental labyrinth, junctional zone, glandular layer and marginal hematoma (Fig. 1), based on the description by Amoroso [2]. In the placental labyrinth, maternal capillaries and giant cells were sandwiched in between adjacent layers of the trophoblast. These maternal elements and the trophoblast formed the darkly-stained lamellae which were clearly separated by lightly stained strands of mesenchyme in which the fetal capillaries were distributed. Trophoblasts were composed of the syncytiotrophoblast (ST) situated on the maternal side of the trophoblast and the cytotrophoblast (CT) situated on the fetal side (Fig. 2). In the late gestational period, the CT lost its continuity and the trophoblast was composed only of ST.

The tip of the trophoblast which invaded into the endometrium formed the junctional zone which contained the uterine gland, its

Fig. 4. Marginal hematoma. Extravasated erythrocytes (Er) are stored between the trophoblast (Tr) and the endometrium (En). HE. $\times 150$.

secretions, cell debris and giant cells. In this zone, the trophoblast partly projected into the glandular lumen where trophoblast cells appeared to be vacuolated and showed enlarged columnar shapes (Fig. 3).

Table 2. Distribution of Δ^5 -3 β -HSD in the trophoblast of the cat placenta

Stages in CRL (mm)	Substrates					
	DHEA ^{a)}	Preg ^{b)}	17 α -OH-P ^{c)}	DHEA	Preg	17 α -OH-P
	Placental labyrinth			Junctional zone		
12	+	+	—	—	+	—
27	+	+	—	+	+	—
48	+	+	—	+	+	—
58	+	+	—	++	++	—
61	++	+	—	++	+	—
68	++	+	—	++	—	—
80	+	—	—	++	—	—
88	++	+	—	+++	++	—
94	++	+	—	+++	++	—
105	+	+	—	+++	+	—
110	+	+	—	+++	+	—
113	+	+	—	+++	+	—
115	+	+	—	+++	+	—
120	+	—	—	++	+	—

a) DHEA=Dehydroepiandrosterone.

b) Preg=Pregnenolone.

c) 17 α -OH-P=17 α -hydroxypregnenolone.Table 3. Distribution of 17 β -HSD in the cat placenta

Stages in CRL (mm)	Epithelium of uterine gland		Uterine epithelium of paraplacenta	
	Estr ^{a)}	Test ^{b)}	Estr ^{a)}	Test ^{b)}
12	+	—	+	—
27	+	+	—	—
48	+	+	+	+
58	+	+	+	+
61	+	+	+	+
68	+	+	+	—
80	—	—	+	+
88	—	—	—	—
94	+	+	—	+
105	+	+	—	—
110	+	+	—	—
113	+	—	—	—
115	+	+	—	+
120	—	—	—	—

a) Using Estradiol-17 β as substrate.

b) Using Testosterone as substrate.

The marginal hematoma was formed at both ends of the placental labyrinth. Between the trophoblast and the uterine epithelium, the extravasation of the mater-

nal blood and the phagocytosis of extravasated erythrocytes by the trophoblast were observed (Fig. 4).

Histochemical distribution of HSD in the cat placenta is summarized in Tables 2 and 3.

Δ^5 -3 β -HSD: The activity of Δ^5 -3 β -HSD was detected when DHEA or pregnenolone was used as a substrate but was not detected in all trials when 17 α -Hydroxypregnenolone was used. The activity when DHEA was used as a substrate was stronger than that when pregnenolone was used, at all stages of gestation. The intensity of activities tended to be increased in the mid-pregnancy, followed by a subsequent decline. Such a chronological change was particularly obvious when DHEA was used as a substrate. In the placental labyrinth, the activity was localized in the trophoblast exclusively, and was detected neither in the other fetal elements nor in all maternal elements. The deposits of the reaction products were scattered within the cytoplasm of the trophoblast. Chronologically, a

Fig. 5. Δ^5 - 3β -HSD activity in the placental labyrinth with DHEA as a substrate. $\times 80$. a: Weak activity is detected in the trophoblast. CRL 12mm. (day 21). b: Strong activity is detected in the trophoblast. Maximum activity is observed at this stage. CRL 88mm. (day 45). c: The activity is decreased toward term. CRL 120mm. (term).

weak activity was detected in the trophoblast at the stage of a 12-mm fetus (day 21) (Fig. 5a), and thereafter the activity was gradually increased with the progress of gestation. Then the activity attained its maximum level at the period of 88- to 94-mm fetuses (day 45 and 46) (Fig 5b), followed by a slight decrease toward term (Fig. 5c). In the junctional zone, the activity was detected in the trophoblast and the deposits were evenly scattered within the cytoplasm. The activity was not detected in the maternal elements (Fig. 6). The chronological change of the enzyme activity which attained the maximum at the period of 88- to 94-mm fetuses (day 45 and 46) was similar to that in the placental labyrinth. At all stages of gestation the activity in this zone was stronger than in the labyrinth.

In the glandular layer and the marginal hematoma, the activity was not detected in all elements during pregnancy.

17 β -HSD: No activity of 17 β -HSD was detected in both trophoblasts of the placen-

tal labyrinth and the junctional zone throughout gestation. In the epithelia of uterine gland and endometrium of the marginal hematoma, a weak activity was detected which persisted throughout gestation (Fig. 7). No difference in activities of the enzyme between the two substrates (estradiol-17 β and testosterone) was observed.

20 α -HSD and 20 β -HSD: These enzyme activities were not detected in any elements throughout gestation.

G-6-PDH: In the placental labyrinth, the activity was detected in the trophoblast (Fig. 8). Both CT and ST showed the activity, and the formazan deposits were scattered in all areas of the cytoplasm. The trophoblast in the junctional zone and the glandular epithelium also showed the activity which was graded as moderate or strong. Such a degree of activity of this enzyme persisted throughout gestation.

Histochemical distribution of Δ^5 - 3β -HSD in the ovary is summarized in Table 4. The activity of Δ^5 - 3β -HSD (substrates; DHEA

Fig. 6. Δ^5 -3 β -HSD activity in the junctional zone with DHEA as a substrate. The activity is detected in the trophoblast of the placental labyrinth (PL) and the junctional zone (JZ). No activity is detected in the glandular layer (GL). CRL 113mm. (day 52). $\times 80$.

Fig. 7. 17 β -HSD activity in the glandular layer with testosterone as a substrate. The activity is limited in the epithelia of the uterine gland (G). No activity is detected in the trophoblast of the placental labyrinth (PL) and the junctional zone. CRL 94mm. (day 46). $\times 80$.

Fig. 8. G-6-PDH activity in the placental labyrinth. The activity is detected in the trophoblast. CRL 27mm. (day 29). $\times 80$.

Table 4. Distribution of Δ^5 -3 β -HSD in the cat ovary

Stages in CRL (mm)	Theca interna and Interstitial tissue		Corpus luteum	
	DHEA ^{a)}	Preg ^{b)}	DHEA ^{a)}	Preg ^{b)}
12	+++	+++	+++	+++
27	+++	++	+++	++
48	++	++	++	++
58	++	++	++	++
61	++	++	++	++
68	+++	++	++	++
80	++	++	++	++
88	++	++	+	+
94	++	+	+	+
105	++	+	+	+
110	++	+	+	+
113	++	+	+	+
115	++	+	+	+
120	++	+	+	+

a) Using Dehydroepiandrosterone as substrate.

b) Using Pregnenolone as substrate.

Fig. 9. Δ^5 - 3β -HSD activity in the ovary with DHEA as a substrate. $\times 80$. a: Strong activity is detected in the corpus luteum. CRL 27mm. (day 29). b: Weak activity is detected in the corpus luteum. CRL 88mm. (day 45).

and pregnenolone) was detected in the corpus luteum, granulosa cells, theca interna and interstitial gland. When pregnenolone was used as a substrate, the chronological change of the intensity of this enzyme activity was remarkable in such a way that the activity was graded as strong or moderate during the stages of 12- to 80-mm fetuses (day 21 to 43), followed by a gradual decrease with the progress of gestation (Fig. 9).

DISCUSSION

Δ^5 - 3β -HSD is important for the biosynthesis of various steroid hormones and is known as "key enzyme" [9]. The existence of this enzyme signifies the steroidogenesis. Therefore, the possibility of steroid hormone synthesis in the placenta can be pursued by examination of the localization of Δ^5 - 3β -HSD.

In the cat placenta, Bloch & Newman [4] and Malassiné & Ferré [17] proved biochemically the conversion of pregnenolone into progesterone under the existence of Δ^5 - 3β -HSD. Ferguson & Christie [12] and Malassi-

né & Juillard [18] showed histochemically the distribution of Δ^5 - 3β -HSD activity in the trophoblast. Although the above authors dealt with chronological changes of the intensity of this enzyme during the gestational period, they did not note at what stage the activity would attain its maximum and what changes would follow in the activity thereafter.

In this study, the activity of Δ^5 - 3β -HSD was shown to be localized in the trophoblast of the labyrinth and the junctional zone, and was detected in the specimens from the early stage of gestation. The activity was increased with the progress of gestation and was strongest at the stages of 88- and 94-mm fetuses (day 45 and 46), followed by a slight decrease toward term.

The problem as to which trophoblast, ST or CT, shows the enzyme activity has not been clearly solved. However, in this study, as the CT loses its continuity and disappears from the placental labyrinth at a late stage of pregnancy, it is possible that the enzyme activity is distributed in the ST. In the junctional zone, the activity of Δ^5 - 3β -HSD is also considered to be distributed in ST,

because the most of CT cells in this zone were characterized by a vacuolar appearance.

On the other hand, in the dog placenta which is unable to maintain pregnancy after ovariectomy [21], the activity of Δ^5 -3 β -HSD in the trophoblast was trace or weak throughout gestation [15]. Thus, whether pregnancy is interrupted or not after ovariectomy may be concerned with the intensity of Δ^5 -3 β -HSD activity in the placenta.

In the corpus luteum of the cat ovary, the activity of Δ^5 -3 β -HSD was strong at the early stage of gestation and tended to be decreased gradually with the progress of gestation. Although the chronological change of the intensity of this enzyme observed in this study was almost in accord with that observed by Juillard & Malassiné [13], it is important that this activity is abruptly decreased at the stage of an 88-mm fetus (day 45), since this may indicate a reduction in capacity of the steroid hormone synthesis in the ovary. Therefore, such chronological changes in activity of Δ^5 -3 β -HSD in the cat placenta and ovary suggest that the cat placenta has an ability of progesterone synthesis to maintain pregnancy instead of the ovary and that ovariectomy after the stage of an 88-mm fetus (day 45) does not interrupt pregnancy.

The activity of 17 β -HSD was detected in the epithelium of the uterine gland and in the endometrium of the marginal hematoma. The activities in these sites may be considered to take part in the metabolism of estrogen, because the uterine gland is the target of estrogen.

G-6-PDH is an important source of NADPH required for hydroxylation during steroidogenesis. In this study, the activity of G-6-PDH was detected in the trophoblast and in the epithelium of the uterine gland. This enzyme contributes to the steroidogenesis by providing the trophoblast with NADPH in the cat placenta.

Generally, the ultrastructure of the steroid hormone-secreting cells is characterized by a large quantity of smooth endoplasmic reticulum (SER), lipid droplets, Golgi complex, lysosome and mitochondria which have tubular cristae [11]. Ultracytochemically, the Δ^5 -3 β -HSD activity in steroidogenic cells was localized in the cytoplasm, or in SER, or in mitochondria [3, 14]. On the other hand, usually, the trophoblast in the placentae of various animals is not provided with the above characteristics of steroid hormone-secreting cells. In the cat placenta, neither CT nor ST has these characteristics, and especially, CT has poor organelles [6, 16, 22]. Malassiné & Ferré [17] showed the existence of the activity of Δ^5 -3 β -HSD in microsomal and mitochondrial fractions. Therefore, the possibility of steroid hormone synthesis in ST, which contains more abundant organelles than CT, is suggested. The conception of "feto-placental unit" is now established and the placenta is not considered to synthesize steroids *de novo* [8]. Accordingly, the placenta does not show the characteristics of the steroid-secreting cells and utilizes the precursors of steroid materials from either maternal or fetal origin, and synthesizes steroid hormones.

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要 約

ネコ胎盤におけるハイドロキシステロイド脱水素酵素の局在と経時的变化について：伊藤純治，木曾康郎，山内昭二（大阪府立大学農学部家畜解剖学教室）——ネコの胎盤および卵巣についてハイドロキシステロイド脱水素酵素（HSD）を組織化学的に検出し，胎盤におけるステロイドホルモン産生能を検討した。胎盤における Δ^5 - 3β -HSD 活性は迷路部および接合帯の栄養膜のみに局在し，妊娠初期から弱度ながら活性がみとめられ妊娠経過とともに徐々に上昇して，妊娠約45日前後に最高に達し，その後やや減少した。 17β -HSD 活性は妊娠期を通じて弱度の活性が子宮腺の腺上皮にのみ局在した。 20α および 20β -HSD 活性は全時期検出されなかった。 $G-6-PDH$ 活性は栄養膜および腺上皮に局在した。卵巣における Δ^5 - 3β -HSD 活性は，黄体，卵胞膜および間質腺に局在した。黄体における酵素活性は妊娠初期から中期にかけて強度であり，その後減少した。以上の成績から，ネコの胎盤における progesterone 産生は迷路部および接合帯の栄養膜で行なわれ，妊娠45日以後の妊娠維持にきわめて重要であることが示唆された。