論文の内容の要旨

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論文題目　**The Role of 20α-Hydroxysteroid Dehydrogenase in the Maintenance of Pregnancy in Mice**
(マウスの妊娠維持における20α-水酸化ステロイド脱水素酵素の役割)

In all mammalian species, progesterone is prerequisite for the maintenance of pregnancy and is secreted from the ovary or placenta depending on both the animal species and the stage of pregnancy. Therefore, the precise control of both the synthesis and degradation of this steroid hormone during the different reproductive states of the animal is crucial for reproductive success. 20α-hydroxysteroid dehydrogenase (20α-HSD) is a steroid-metabolizing enzyme, which catalyzes the reaction that converts progesterone to its biologically inactive metabolite, 20α-dihydroprogesterone (20α-OHP). 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. 20α-HSD plays a crucial role in promotion of functional luteolysis and the expression of 20α-HSD is elevated at the end of the luteal phase of pseudopregnancy as well as pregnancy. In addition, 20α-HSD is supposed to be involved in normal recurrence of the estrous cycle in rats. Apart from the well-studied function of ovarian 20α-HSD, this enzyme is also expressed in the placenta, pregnant uterus, and fetal tissue. Although the precise role of 20α-HSD expressed in extraovarian tissues is currently unknown, it has been postulated that this enzyme may be involved in protecting developing fetuses from toxicity of

progesterone during pregnancy, since high levels of progesterone exert cytotoxic effects.

Mice deficient in 20α-HSD (20α-HSD$^{-/-}$ mice) have been generated in our laboratory. In 20α-HSD$^{-/-}$ mice, neither 20α-HSD activity in the corpus luteum nor an increase in the serum concentrations of 20α-OHP at the end of luteal phase was detected. Preliminary experiments analyzing their reproductive phenotypes showed that the length of estrous cycle and the duration of pseudopregnancy were prolonged in 20α-HSD$^{-/-}$ mice. In addition, the number of offspring from 20α-HSD$^{-/-}$ females crossed with 20α-HSD$^{-/-}$ males was smaller than that from wild type (20α-HSD$^{+/+}$) females crossed with 20α-HSD$^{+/+}$ males. These results suggested the importance of 20α-HSD for the successful development of fetuses and/or delivery as well as for the maintenance of pregnancy. To examine the roles of 20α-HSD in mice reproduction, the following studies were carried out in this thesis. In chapter 1, the general reproductive phenotype with special interests in luteal function of 20α-HSD$^{-/-}$ females was analyzed in detail. In chapter 2, the roles of extraovarian 20α-HSD for successful achievement of pregnancy and fetal survival were investigated.

Chapter 1: Reproductive Phenotype of Mice Deficient in 20α-Hydroxysteroid Dehydrogenase Gene

In the present experiments, male and female 20α-HSD$^{+/-}$ mice of F9 or later generation backcrossed to C57BL/6J strain mice were crossed, and resulting littermates of each genotype were used (in Chapter 1, only 20α-HSD$^{+/+}$ and 20α-HSD$^{-/-}$ mice were used). To examine the role of 20α-HSD in the luteal function, the length of estrous cycle and pseudopregnancy were compared between 20α-HSD$^{+/+}$ and 20α-HSD$^{-/-}$ mice. In 20α-HSD$^{-/-}$ mice, the duration of continuous diestrus during the estrous cycle was significantly prolonged (2.5 ± 0.2 days in 20α-HSD$^{+/+}$ vs. 4.1 ± 0.5 days in 20α-HSD$^{-/-}$), suggesting that functional luteolysis after ovulation was delayed when 20α-HSD was not expressed. The duration of pseudopregnancy was also prolonged in the 20α-HSD$^{-/-}$ mice (11.5 ± 0.3 days in 20α-HSD$^{+/+}$ vs. 14.0 ± 0.6 days in 20α-HSD$^{-/-}$). These observations suggest that 20α-HSD is indeed involved in functional luteolysis at term of pseudopregnancy as well as during the estrous cycle in rodents. However, the prolongation was only about 1.5 days for the estrous cycle and 2.5 days for pseudopregnancy, and the next ovulation occurred shortly thereafter, suggesting that 20α-HSD plays a relatively minor role in functional luteolysis and that some other mechanism may be responsible for it. This is more evident in the case of pregnancy, in which the prolongation was less than 24 h in the 20α-HSD$^{-/-}$ mice (18.0 ± 0.0 days in 20α-HSD$^{+/+}$ vs. 18.6 ± 0.2 days in 20α-HSD$^{-/-}$). On the day

before parturition, serum progesterone concentrations in 20α-HSD$^{-/-}$ mice decreased to similar levels to those in 20α-HSD$^{+/+}$ mice (19.5 ± 4.3 ng/ml in 20α-HSD$^{+/+}$ vs. 22.6 ± 2.7 ng/ml in 20α-HSD$^{-/-}$). Besides the luteal function, preliminary experiments in our laboratory suggested the importance of 20α-HSD for the successful development of fetuses and/or delivery in mice. To further confirm this result, 20α-HSD$^{-/-}$ females were crossed with 20α-HSD$^{-/-}$ males and the number of offspring was compared with that from 20α-HSD$^{+/+}$ females crossed with 20α-HSD$^{+/+}$ males. The number of offspring from 20α-HSD$^{-/-}$ females was significantly lower than that from 20α-HSD$^{+/+}$ females (9.8 ± 0.4 pup s from 20α-HSD$^{+/+}$ vs. 1.0 ± 0.4 pups from 20α-HSD$^{-/-}$). These findings suggest that, although the slight delay in functional luteolysis has been observed in 20α-HSD$^{-/-}$ mice, there should be some other mechanisms responsible for functional luteolysis in mice. In addition, it was demonstrated that 20α-HSD plays an important role in the survival of fetuses during pregnancy in mice.

Chapter 2: Involvement of 20α-Hydrosysteroid Dehydrogenase in Fetal Survival in Mice

The experiments in Chapter 2 were conducted to elucidate how 20α-HSD is involved in fetal survival in mice. First, in order to know whether the decrease in the number of offspring is due to the lack of 20α-HSD in fetuses or in dams, 20α-HSD$^{+/-}$ females were crossed with 20α-HSD$^{+/-}$ males, and the genotype ratio of delivered pups was calculated. The total number of pups of each genotype from 22 dams was 50 for 20α-HSD$^{+/+}$, 63 for 20α-HSD$^{+/-}$, and 18 for 20α-HSD$^{-/-}$, respectively. The ratio of each genotype (20α-HSD$^{+/+}$: 20α-HSD$^{+/-}$: 20α-HSD$^{-/-}$) was 1: 1.3: 0.4, which did not match with the expected ratio based on Mendel's laws of inheritance. This indicates that the loss of 20α-HSD$^{+/-}$ and 20α-HSD$^{-/-}$ fetuses occurred during pregnancy and suggests that the fetal 20α-HSD plays a role in their own development. To investigate when the loss of 20α-HSD$^{+/-}$ and 20α-HSD$^{-/-}$ fetuses occurs during pregnancy, the ratios of each genotype of fetuses were examineded on days 13, 15, and 18 of pregnancy (day 0 was defined as the day when vaginal plug was observed). On day 13, the ratio of 20α-HSD$^{+/+}$: 20α-HSD$^{+/-}$: 20α-HSD$^{-/-}$ was not significantly different from the expected ratio, but on days 15 and 18, the ratios did not match with Mendel's laws. This result suggests that the loss of 20α-HSD$^{+/-}$ and 20α-HSD$^{-/-}$ fetuses occurred after day 13 of pregnancy. The role of maternal 20α-HSD in maintaining pregnancy was also investigated by embryo transfer experiment, i.e., 20α-HSD$^{+/+}$ embryos were transferred into the oviduct of pseudopregnant 20α-HSD$^{+/+}$ and 20α-HSD$^{-/-}$ mice. The number of pups from 20α-HSD$^{-/-}$ females was significantly smaller

than that from 20α-HSD$^{+/+}$ females, and the loss of transferred embryos took place before day 17 of pregnancy. These results suggest that maternal 20α-HSD is important for fetal development as well as fetal 20α-HSD.

In accordance with the above results, the expression of both 20α-HSD mRNA and protein, which was examined by RT-PCR and immunohistochemistry, respectively, in extraovarian tissues of 20α-HSD$^{+/+}$ mice such as fetal skin, placenta, and pregnant uterus was confirmed. Expression of 20α-HSD mRNA in these tissues was found to progressively increase from day 11 to 18 of pregnancy. To know the role of extraovarian 20α-HSD in regulating progesterone concentrations in fetuses, progesterone and 20α-OHP concentrations in fetal homogenates on day 17 of pregnancy were measured. Progesterone concentrations were significantly higher in 20α-HSD$^{-/-}$ fetuses (26.3 ± 2.4 ng/g in 20α-HSD$^{+/+}$ vs. 44.5 ± 5.7 ng/g in 20α-HSD$^{-/-}$), while 20α-OHP levels were low in 20α-HSD$^{-/-}$ fetuses (15.3 ± 5.2 ng/g in 20α-HSD$^{+/+}$ vs. 7.1 ± 2.8 ng/g in 20α-HSD$^{-/-}$), and thus the progesterone/20α-OHP ratio was much higher in 20α-HSD$^{-/-}$ than 20α-HSD$^{+/+}$ fetuses. Finally, to ascertain if high levels of progesterone indeed exert detrimental effects on fetuses, progesterone was directly injected into the amniotic cavity on day 15 of pregnancy and its effect on fetal survival was examined. It was found that all the fetuses treated by progesterone died before parturition.

Taken together, the findings obtained in the present study using 20α-HSD$^{-/-}$ mice suggest that the role of 20α-HSD in functional luteolysis is relatively minor in mice, and that both maternal and fetal 20α-HSD plays a pivotal role in fetal survival during the late pregnancy. The 20α-HSD expressed in the fetal skin, placenta, and pregnant uterus may contribute to local withdrawal of progesterone concentrations in and around the fetuses. Given that high levels of progesterone exert cytotoxic effects, it is suggested that 20α-HSD is involved in protecting developing fetuses from high levels of progesterone, which is needed to suppress uterine contraction during pregnancy.