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Reproductive Phenotypes in Mice with Targeted Disruption of the 20 α -Hydroxysteroid Dehydrogenase Gene

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Abstract. In the corpus luteum of rats and mice, 20 α -hydroxysteroid dehydrogenase (20 α -HSD) catalyzes the conversion of progesterone to a biologically inactive metabolite, 20 α -dihydroprogesterone (20 α -OHP). The reduction of progesterone by 20 α -HSD is believed to be important for functional luteolysis in these rodent species. In addition to the corpus luteum, expression of 20 α -HSD has been demonstrated in tissues such as the placenta, endometrial epithelia, and fetal skin, although the roles it plays in the latter tissues remain to be determined. To determine the contribution of 20 α -HSD to functional luteolysis and to the rodent reproductive system more generally, we generated a strain of mice with targeted disruption of the 20 α -HSD gene. In the 20 α -HSD^{-/-} mice we obtained, which lacked the genomic region essential for catalytic reaction, neither 20 α -HSD activity in the corpus luteum nor an increase in the serum concentrations of 20 α -OHP during pseudopregnancy or pregnancy was detected. The durations of the estrous cycle, pseudopregnancy, and pregnancy were significantly prolonged in the 20 α -HSD^{-/-} mice, although the serum progesterone levels decreased to levels low enough for delivery of pups at term of pregnancy. In addition, the number of pups, especially live pups, was markedly decreased in the 20 α -HSD^{-/-} mice. These findings suggest that the role of 20 α -HSD in functional luteolysis is relatively minor but that it is involved in the survival of newborn mice.

Key words: 20 α -Hydroxysteroid dehydrogenase (20 α -HSD), Luteolysis, Mouse, Ovary, Reproduction (J. Reprod. Dev. 53: 499–508, 2007)

The enzyme 20 α -hydroxysteroid dehydrogenase (20 α -HSD) is expressed in the rodent corpus luteum, and it catalyzes the conversion of

progesterone to its inactive form, 20 α -dihydroprogesterone (20 α -OHP). In the estrous cycle of rats, 20 α -HSD is thought to play a crucial role in recurrence of unique short cycles through promotion of functional luteolysis [1, 2]. The finding that the transcription factors Sp1 and Sp3 stimulate the promoter activity of mouse 20 α -HSD

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5'-flanking region [3] suggests that 20 α -HSD is constitutively expressed with the establishment of luteinization. The cervical stimulation associated with copulation causes a prolactin (PRL) surge, which induces the corpus luteum to secrete progesterone through inhibition of the expression of 20 α -HSD [4], thereby leading to the luteal phase. Expression of 20 α -HSD is also inhibited by progesterone through the glucocorticoid receptor, which is constitutively expressed in the corpus luteum during pregnancy [5]. Expression of 20 α -HSD is then elevated at the end of the luteal phase of pseudopregnancy and pregnancy [6, 7]. The effects of prostaglandin F_{2 α} (PGF_{2 α}), a well-known luteolytic factor, include promotion of the expression of 20 α -HSD through the transcription factor NUR77 [8] in addition to inhibition of the synthesis of progesterone. PGF_{2 α} -receptor knockout (FP^{-/-}) mice failed to deliver pups due to lack of decline of serum progesterone levels at parturition [9]; at that time, no elevation of 20 α -HSD activity was observed [8]. Furthermore, it has recently been reported that 20 α -HSD^{-/-} mice exhibited a phenotype similar to FP^{-/-} mice at parturition; notably, the fetuses of the latter mice all died in utero [10]. Thus, expression of 20 α -HSD appears to be closely related to functional luteolysis in rodent reproduction. It is possible, however, that the contributions of luteolytic factors, including 20 α -HSD, to functional luteolysis differ in the corpus luteum at different reproductive stages. In fact, the estrous cycle of FP^{-/-} mice is normal [9] despite the luteal expression of PGF_{2 α} receptor in estrous cycle of wild-type mice [11].

In rodents, activity or expression of 20 α -HSD is detected not only in the ovaries but also in other tissues, such as the thymus [12–14], placenta [15–17], uterus [16, 17], and fetal skin [16]. The 20 α -HSD activity in the thymus is thought to play a role in protecting lymphocytes from the growth-inhibitory effect of progesterone [12]. In rats [15], mice [16], and goats [17], the 20 α -HSD expressed in the placenta, pregnant uterus, and fetal tissue may be involved in protecting developing fetuses from progesterone, since high levels of progesterone exert cytotoxic effects [18]. Thus, although 20 α -HSD appears to play roles in mitigating the effects of high levels of progesterone in several non-ovarian tissues during pregnancy, the precise biological significance of its expression remains to be clarified.

The enzyme 20 α -HSD belongs to the aldo-keto reductase superfamily [19]. Among the members of this superfamily, the four amino acids (Asp⁵⁰, Tyr⁵⁵, Lys⁸⁴ and His¹¹⁷) that constitute the cofactor [NADP(H)] binding site are conserved [20], with mutations of Tyr⁵⁵ and Lys⁸⁴ in particular causing loss of catalytic activity [21]. We generated 20 α -HSD^{-/-} mice that lack the genomic region encoding the cofactor binding site located in exons 2–4. In this study, using these mice, we investigated several reproductive phenotypes to determine the significance of 20 α -HSD in luteal regression and fetal development in mice.

Materials and Methods

Construction of 20 α -HSD targeting vector

The λ 2431 genomic DNA of 20 α -HSD was isolated from the 129 SvJ mouse genomic library (Stratagene, La Jolla, CA, USA) [22]. The targeting vector was constructed by replacing the genomic region from exons 2–4 (approximately 3-kb, *Bam*HI-*Hinc*II fragment) with a foreign fragment containing exon 2 with the stop codon (TGA) at the 55th bp of exon 2 followed by enhanced green fluorescent protein (EGFP; Clontech, Palo Alto, CA, USA) with an internal ribosomal entry site (IRES) [23] and PGK-neo-bpA cassette [24], and by ligating the diphtheria toxin A (DT-A) fragment [25] at the 5' end of the vector (Fig. 1A). A neomycin resistance gene under the control of the phosphoglycerate kinase (PGK) 1 promoter was inserted as a positive selection marker, and DT-A fragment DNA under the control of the polyoma enhancer/herpes simplex virus thymidine kinase promoter (MC-1) was used for negative selection for screening of recombinant embryonic stem (ES) cells. Homologous regions at the 5' and 3' ends of the targeting vector were about 5- and 1.5-kb, respectively.

Generation of 20 α -HSD^{-/-} mice

The targeting vector linearized by *Sac*II (20 μ g) was electroporated (250 V, 500 μ F) into 10⁷ E14.1 ES cells [26] and selected with 250 μ g (active form)/ml G418 (Gibco BRL, Grand Island, NY, USA) for 7 days, as described previously [27]. Homologous recombination was confirmed by Southern blot analysis as described below. For the generation of chimeric mice, ES cells were aggregated with two

Fig. 1. Generation of 20 α -HSD^{-/-} mice by gene targeting. (A) The strategy for targeting of the mouse genomic 20 α -HSD locus. Probes used for Southern blot analysis are indicated as the 5' and 3' probes. Restriction enzyme sites used for Southern blot analysis are also indicated in the maps. Arrows: expected sizes of DNA fragments obtained by restriction enzyme digestions. Open arrowheads: primers (P1-P3) used for genotyping PCR. (B) Southern blot analysis of DNA from the livers of mice bred by heterozygous intercrossing. Hybridization analyses of *Bgl*I-digested DNA with the 5' probe and *Pst*I-digested DNA with the 3' probe are shown on the left and right, respectively.

(C57BL/6 $\times$ BDF1) F1 8-cell stage embryos using a method described previously [28]. Offspring (129/Ola $\times$ C57BL/6J) were genotyped with PCR analysis. The primers used for genotyping were 5'-ATG CAG TCA TGG TCT CTC ACT AGG-3' (P1), 5'-CAG GAT CAT CTC CAG TTG TCT ACG-3' (P2), and 5'-ATC TGC ACG AGA TCA GTG AGA CGT-3' (P3; Fig. 1A). PCR mixtures containing 1 $\times$ Ex Taq buffer, 0.5 mM of each primer, 0.2 mM of dNTPs, and 2.5 $\times 10^{-3}$ U/ μ l of Ex Taq polymerase (TaKaRa, Ohtsu, Japan) were first incubated at 95 C for 2 min, and this was followed by 40 cycles of 94 C for 1 min, 64 C for 30 sec, and 72 C for 1 min.

Southern blot analysis

Southern blot analysis was performed as previously reported [28]. Genomic DNA extracted

from ES cell clones or mouse livers were digested by *Bgl*I or *Pst*I for 5' or 3' probes flanking the targeting vector, respectively. For analysis of the targeted 20 α -HSD gene, the *Kpn*I-*Bam*HI fragment and *Bal*I-*Pst*I fragment corresponding to the 5' upstream and 3' downstream regions of the targeting vector were used as the 5' and 3' probes, respectively (Fig. 1A, B).

Northern blot analysis

Total RNA was isolated from ovaries using TRIzol Reagent (Gibco BRL). A 5-microgram portion of total RNA was hybridized to the full length of the mouse 20 α -HSD cDNA [16] released by *Bam*HI from the cloned plasmid. Labeling of the probe and the conditions of hybridization were the same as for Southern hybridization described

above. After hybridization, membranes were washed in $2 \times$ saline-sodium citrate (SSC) containing 1% SDS at 42 C for 30 min and $0.5 \times$ SSC containing 1% SDS at 55 C for 30 min. Signals were visualized by exposure to X-ray film (Kodak, Rochester, NY, USA) overnight at -80 C.

Western blot analysis

Ovaries were homogenized on ice in homogenization buffer [20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide, 25 mM NaF, 50 mM β -glycerophosphate, 2 mM PMSF, and protease inhibitor cocktail (Santa Cruz Biotechnology, Santa Cruz, CA, USA)]. The homogenized solution was placed on ice for 30 min and then centrifuged at 12,000 rpm for 15 min at 4 C. An aliquot of the supernatant was subjected to protein measurement using the Bradford method with BSA as the standard. A 20-microgram portion of protein was boiled for 5 min in sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% glycerol, 2.5% β -mercaptoethanol and 0.1% bromophenol blue) and separated on 12.5% SDS-PAGE gels in electrophoresis buffer (25 mM Tris, 192 mM glycine and 0.1% SDS). This was followed by transfer to a polyvinylidene difluoride (PVDF) membrane in transfer buffer (25 mM Tris and 5% methanol) at 80 mA for 1 h. The blots were placed in blocking solution [20 mM Tris-HCl, pH 7.6, 137 mM NaCl and 0.1% Tween 20 (TBST) with 5% non-fat dry milk] for 2 h at room temperature. They were then incubated with the polyclonal antibody against rat 20 α -HSD (kindly provided by Dr. G. Gibori, University of Illinois at Chicago, IL, USA; 1:4,000 dilution with TBST) overnight at 4 C and then washed and incubated with a secondary antibody, peroxidase-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:6,000 dilution with TBST), for 2 h at room temperature. The blots were also incubated with monoclonal anti- β -actin (Sigma-Aldrich, St. Louis, MO, USA; 1:10,000 dilution with TBST) for 2 h at room temperature and then washed and incubated with a secondary antibody conjugated to horseradish peroxidase (1:10,000 dilution with TBST) for 2 h at room temperature. Signals were detected using an ECL plus Western Blotting Detection System (Amersham Biosciences, Buckinghamshire, UK) and the LAS-1000 Plus software (Fuji Film, Tokyo, Japan).

Measurement of 20 α -HSD activity

Ovaries were homogenized in 5 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM dithiothreitol (DTT), and 10% glycerol. The cytosolic fraction was separated by centrifugation at 47,000 rpm for 90 min at 4 C, and the supernatant was stored at -80 C until use. Reaction with the substrate (60 μ M 20 α -OHP) was catalyzed by addition of the cytosolic fraction to the assay medium [0.1 M Tris-HCl buffer (pH 7.0) containing 300 μ M β -NADP, 1 mM EDTA, 1 mM DTT and 3% ethanol (as a solvent of steroids)] and incubation at 37 C for 3 min. The amount of β -NADPH generated was measured spectrophotometrically as the increase in absorbance at 340 nm. The activity of 20 α -HSD in the ovarian cytosol was expressed as the initial velocity of generation of β -NADPH in nmol/min/mg protein as described previously [29]. The protein concentrations of the samples were measured using the Bradford method with BSA as the standard.

Detection of the fluorescence of enhanced green fluorescent protein (EGFP)

Images from frozen sections (25 μ m) of ovaries were captured with a fluorescence microscope (BX 50-FLA, Olympus, Tokyo, Japan), and digital imaging was performed with a DP50 CCD camera using the Viewfinder Lite software (Olympus).

Analysis of reproductive phenotypes

Mice were housed under controlled conditions with a 12 h light/12 h dark cycle (lights on, 0700–1900 h). The experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals, Graduate School of Agricultural and Life Sciences, The University of Tokyo. For analysis of the estrous cycle, 10 female wild-type and 20 α -HSD $^{-/-}$ mice (6–8 weeks old) were housed together. Vaginal smears were collected daily for 2 weeks. Stages of the estrous cycle were determined by vaginal smear cytology. Pregnancy and pseudopregnancy were induced by crossing with fertile and infertile male mice of the same genetic background, respectively. The day on which vaginal plug formation was noted was considered day 0. Total numbers and numbers of live pups were counted on the day of parturition.

Steroid hormone assay

Blood samples were collected by puncture of the

Fig. 2. Expression of ovarian 20 α -HSD and EGFP on the day of parturition. (A) Northern blot analysis of 20 α -HSD mRNA expression. Full-length 20 α -HSD cDNA was used as a probe. (B) Detection of 20 α -HSD protein by western blotting analysis. The arrowhead indicates the protein immunoreactive with polyclonal antibody to rat 20 α -HSD. The result is representative of three animals. (C) 20 α -HSD activity in ovaries. Each column and vertical bar represent the mean and SE for three animals. (D) Detection of EGFP fluorescence in the ovary of a 20 α -HSD^{-/-} mouse. The same section is shown below without a fluorescence filter.

orbital vein with heparinized capillaries under light ether anesthesia. Blood samples were centrifuged at 3,000 rpm for 10 min at 4 C, and the sera were kept at -20 C until use. The serum (50 μ l) was diluted to 1 ml with distilled water and extracted with 4 ml of diethyl ether for measurement of progesterone and 20 α -OHP. The extracts were subjected to radioimmunoassay using specific antibodies as described previously [30].

Statistical analysis

Mann-Whitney's U test was used for statistical evaluation of results, with findings of $P < 0.05$ considered significant.

Results

Generation of 20 α -HSD^{-/-} mice

The 20 α -HSD gene isolated from the 129 SvJ mouse genomic library was about 18-kb in length and was composed of nine exons [22]. Because the

four amino acids required for the catalytic activity of 20 α -HSD are encoded within exons 2–4 of the 20 α -HSD gene, the 20 α -HSD gene was inactivated by designing exons encoding the catalytic domain as the target for homologous recombination (Fig. 1A). Four independent ES cell clones were obtained out of 1,050 recombinant clones by screening with Southern blot analysis. Aneuploids were excluded, and two clones were used for production of chimeric mice. Germline transmission of the target gene was observed in both of the clones. Offspring from one clone backcrossed to C57BL/6J strain mice were used for subsequent examinations.

Homologous recombination of the 20 α -HSD genomic DNA was verified by Southern blot analysis. Genotyping of the mice revealed shifts in the size of a restriction fragment detected by 5' and 3' probes from 20- to 10-kb and 4- to 2.2-kb, respectively (Fig. 1B). Expression of 20 α -HSD was examined using ovaries on the day of parturition, which is when expression of 20 α -HSD is prominent

Table 1. Duration of continuous diestrus during the estrous cycle, pseudopregnancy and pregnancy

Genotype	Diestrus during estrous cycle		Pseudopregnancy		Pregnancy	
	Days	n	Days	n	Days	n
+/+	2.5 ± 0.2	10	11.5 ± 0.3	10	18.0 ± 0.0	8
-/-	4.1 ± 0.5*	10	14.0 ± 0.6*	12	18.6 ± 0.2*	11

*P<0.05 vs. -/-.

in wild-type mice [8]. Northern blot analysis using full-length 20 α -HSD cDNA as a probe revealed the existence of a truncated form of mRNA in both the heterozygous and homozygous mice (Fig. 2A). Sequencing analysis revealed that the shorter mRNA lacked exons 2–4 (data not shown). Despite the existence of the shorter mRNA, there was no translated product immunoreactive with the antibody to rat 20 α -HSD (Fig. 2B). No 20 α -HSD activity was detected in the ovarian cytosol of the homozygous mice (Fig. 2C). The fluorescence of EGFP, the gene for which was inserted in the targeting vector, was detected in the corpora lutea of ovaries from 20 α -HSD^{-/-} mice (Fig. 2D).

Duration of diestrus during the estrous cycle, pseudopregnancy, and pregnancy

As determined by vaginal smear cytology, the estrous cycle was irregular in many of the wild-type and 20 α -HSD^{-/-} mice, but the duration of continuous diestrus was prolonged by about 1.5 days in the 20 α -HSD^{-/-} mice compared with the wild-type mice (Table 1). Pseudopregnancy, which was defined as beginning with formation of vaginal plugs in females mated with infertile males, was extended by 2.5 days in the 20 α -HSD^{-/-} mice (Table 1). Although pregnancy was prolonged by 2 days in 2 of the 11 20 α -HSD^{-/-} mice, one of which delivered live pups, 6 animals delivered within the normal term of pregnancy (day 18) and 3 animals delivered one day later (day 19). The mean duration of pregnancy was significantly prolonged in the 20 α -HSD^{-/-} mice, although the prolongation was less than 24 h (Table 1).

Changes in progesterin concentrations during pseudopregnancy and pregnancy

In contrast to the changes in 20 α -OHP levels in the wild-type mice, the levels of 20 α -OHP in the 20 α -HSD^{-/-} mice remained low throughout pseudopregnancy (Fig. 3A) and at term of pregnancy (Fig. 3B). In the wild-type mice, the

mean serum 20 α -OHP levels were elevated on day 12, and the mean serum progesterone levels decreased to about 16 ng/ml on day 10 of pseudopregnancy. In the 20 α -HSD^{-/-} mice, the mean serum progesterone levels decreased gradually in late pseudopregnancy, but were still about 24 ng/ml on day 10. The serum 20 α -OHP levels were elevated on the day before parturition (day -1) at term of pregnancy in the wild-type mice and progesterone levels fell to 15 ng/ml. In the 20 α -HSD^{-/-} mice, the mean serum progesterone levels on day -1 fell to 19 ng/ml without any increase in the serum 20 α -OHP levels.

Number of pups

Although the total number of pups for the 20 α -HSD^{-/-} mice was significantly less than for the wild-type mice, all of the 20 α -HSD^{-/-} mice used delivered (Table 2). The number of live pups counted on the day of parturition was even more significantly decreased for the 20 α -HSD^{-/-} mice than for the wild-type mice. Live pups were obtained from 76.5% of the 20 α -HSD^{-/-} dams, but whether the pups that died did so before or after parturition could not be determined in the present study. However, since the placenta and fetal membranes were not present, even around the dead pups, and since the dead pups did not drink milk based on their stomach contents, the pups probably died around the time of parturition or immediately after parturition.

Discussion

In the present study, we successfully generated mice with targeted disruption of the 20 α -HSD gene by elimination of the genomic region essential for catalytic reaction. From the homologously recombined mutant gene of the 20 α -HSD^{-/-} mice, we detected two transcriptional products. One was from the imported EGFP gene, and EGFP

Fig. 3. Changes in the serum progesterone (dotted line) and 20 α -OHP (solid line) concentrations throughout pseudopregnancy (A) and at term of pregnancy (B). Day 0 in (A) and (B) was defined as the day when a vaginal plug was found and the day of parturition, respectively. Each point and vertical bar represents the mean and SE, respectively, of 5 (A) and 6–8 (B) animals of each genotype.

Table 2. Number of total and live pups one day after parturition

Genotype	Total pups		Live pups	
	Number	n	Number	n
+/+	7.3 $\pm$ 0.6	12	7.1 $\pm$ 0.3	12
-/-	5.2 $\pm$ 0.7*	17	3.6 $\pm$ 0.8*	17

*P<0.05 vs. -/-.

fluorescence was observed in functionally regressing corpora lutea on the day after parturition, as expected. The other was a truncated form of 20 α -HSD lacking exons 2–4. Despite the existence of the mRNA of the truncated form of 20 α -HSD, there was neither protein

immunoreactivity for anti-rat 20 α -HSD antibody nor 20 α -HSD activity in the ovarian cytosol of the 20 α -HSD^{-/-} mice.

Since the discovery of its presence in rat ovarian tissue in the 1950s [31], the roles of 20 α -HSD in functional luteolysis in rodents have been

intensively studied [1, 2, 4, 5, 7, 32], and this enzyme has long been believed to be required for recurrence of the unique short estrous cycle of rodents. In the present study, continuous diestrus during the estrous cycle was significantly prolonged in the 20α -HSD^{-/-} mice, 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. 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 is 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.

Piekorz *et al.* [10] recently reported that the parturition of mice lacking the 20α -HSD gene is delayed by 2–3 days and that the pups of these mice die in utero. In their study, the progesterone levels at term of pregnancy were 3–4 times higher than those in wild-type mice. In the present study, the serum progesterone level declined before parturition to only about 1.3 times higher than that in the wild-type mice, a level low enough for delivery of pups. Although the reason for the difference in findings related to 20α -HSD^{-/-} mice between the two studies is unknown, our findings suggest that there are some other mechanisms responsible for functional luteolysis in 20α -HSD^{-/-} mice. For example, PGF₂ α exerts its luteolytic effect through elevation of the luteal expression of endothelin-1 [33], suppression of the expression of sterol carrier protein-2 [34] and steroidogenic acute regulatory protein [35], and promotion of the expression of 20α -HSD. In addition, using the cDNA subtraction technique, we identified 26-cholesterol hydroxylase as an enzyme with increased expression in the corpus luteum toward the end of pseudopregnancy [36]. This enzyme catalyzes the conversion of cholesterol to 26-hydroxycholesterol and thereby blocks utilization of cholesterol in cells. One of the mechanisms leading to decrease in progesterone levels at the end of pregnancy in rodents may thus be

suppression of progesterone synthesis rather than progesterone inactivation. The findings of this study support the notion that a pre-partum fall in progesterone secretion preceded any rise in 20α -OHP and that 20α -HSD only accounts for part of functional luteolysis [32, 37, 38].

In the present study, the number of pups decreased and the number of live pups was further decreased in the 20α -HSD^{-/-} mice. Other than luteal tissue, we have also previously demonstrated the presence of 20α -HSD mRNA, protein, and/or enzyme activity in the placental and fetal tissues of rats including the mesometrial endometrium, chorioallantoic placenta and visceral yolk sac [15]; in the endometrial epithelium, maternal placental endothelial cells, and fetal epidermal cells of mice [16]; and in the endometrial epithelium on the caruncle side of the placenta of goats [17]. In addition, it has been shown that progesterone causes fetal death if injected in large quantities into the amniotic sac [39], suggesting the presence of a mechanism through which fetuses can avoid the cytotoxic effects of progesterone during pregnancy. The findings obtained in the goat show that the 20α -OHP concentration in fetal serum is always higher than that in maternal serum and that the progesterone levels in fetal serum and amniotic fluid remain extremely low throughout pregnancy [17], and this suggests that 20α -HSD is important for fetal development. Furthermore, in the baboon, the activity of 20α -HSD in the placenta appears to be important for fetal development and/or parturition based on the finding of an increase in 20α -OHP concentrations in the fetal compartment in late pregnancy [40]. It is thus likely that the decrease in number of pups of the 20α -HSD^{-/-} mice observed in the present study was due to the detrimental effects of progesterone on fetal development caused by lack of placental and/or fetal 20α -HSD expression.

There may also be another way to interpret the role of 20α -HSD during pregnancy. We have previously reported expression of 20α -HSD in the endometrial epithelia at mid- and late-pregnancy in mice [16] and goats [17] and have suggested that the endometrial expression of 20α -HSD might play a role in maintenance of pregnancy. It appears paradoxical that the enzyme that inactivates progesterone is expressed in the pregnant uterus, which is under the control of high levels of progesterone during pregnancy. *In vivo* findings

regarding estrogen and progesterone control of endometrial epithelial cells in ovariectomized rats indicate that progesterone inhibits estrogen-induced cell proliferation through effects on cell cycle regulation [41, 42]. If proliferation of endometrial epithelial cells at mid- and late-pregnancy is required for physical extension of the uterine lumen of the pregnant uterus, lack of 20 α -HSD expression in endometrial epithelial cells may result in exposure of these cells to high levels of progesterone, resulting in a decrease in their proliferation. Although further studies are needed to clarify the reasons for the decrease in the number of live pups from the 20 α -HSD^{-/-} mice, the present study has demonstrated that 20 α -HSD plays an

important role in the survival of fetuses and/or newborn pups.

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