Changes in the Activities of Hydroxysteroid Dehydrogenases in Rabbit and Hamster Oocytes during Meiotic Maturation

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Abstract: The activities of hydroxysteroid dehydrogenases (HSDs) were histochemically demonstrated in rabbit and hamster oocytes in the process of maturation, and the changes in steroid metabolism during meiotic maturation were examined. In rabbits, the percentages of oocytes showing the activities of Δ^5-3β-HSD (with DHEA as the substrate), 17β-HSD (testosterone) and 20β-HSD (20β-hydroxyprogesterone) were always high and did not change during maturation, whereas those showing the activities of Δ^5-3β-HSD (pregnenolone and 17α-hydroxypregnenolone), 17β-HSD (estradiol-17β), 20α-HSD (20α-hydroxyprogesterone) and 20β-HSD (17α-hydroxyprogesterone) decreased as the time after the hCG injection was prolonged. On the other hand, the activities of Δ^5-3β-HSD, 17β-HSD, 20α-HSD and 20β-HSD with eight substrates were almost always observed in hamster oocytes from 0 to 13 hrs after the hCG injection. The present findings suggested that the metabolic abilities of 20β-hydroxyprogesterone and androgen are constantly present in rabbit oocytes in the process of maturation, whereas those of progesterone, 17α-hydroxyprogesterone, 17α, 20β-dihydroxyprogesterone, 20α-hydroxyprogesterone and estrogen decrease during maturation. And it was also confirmed in hamster oocytes that the metabolic abilities of progesterone, 17α-hydroxyprogesterone, 17α, 20β-dihydroxyprogesterone, 20α-hydroxyprogesterone, 20β-hydroxyprogesterone, estrogen and androgen are always present and do not vary with maturation.

Key words: Rabbit oocyte, Hamster oocyte, Meiotic maturation, Hydroxysteroid dehydrogenase,

Received: July 4, 2003
Accepted: August 29, 2003
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Histochemistry

It was reported that steroid metabolism in the cytoplasm alters with nuclear maturation of oocytes. Namely, it has been biochemically confirmed that the activity of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) with pregnenolone as the substrate, which is involved in progesterone metabolism, increases in rat oocytes after germinal vesicle breakdown, and that this activity reaches a peak at the time of ovulation, indicating that nuclear maturation in rat oocytes is associated with progesterone metabolism [1]. We also reported that the activities of Δ^5-3β-HSD (with 17α-hydroxypregnenolone as the substrate) and 20β-HSD (17α-hydroxyprogesterone) were observed in almost all oocytes collected from antral follicles of mice, rabbits, pigs and cattle, and that they disappeared from the oocytes of these animals as nuclear maturation progressed, except for the mouse [2]. From these results, it was inferred that the nuclear maturation of rabbit, porcine and bovine oocytes is closely related to 17α-hydroxyprogesterone metabolism, and 17α, 20β-dihydroxyprogesterone metabolized from 17α-hydroxyprogesterone may possibly serve as a substance inducing the maturation of oocytes of these animals [2].

Recently, Takano and Niimura [3] have reported that the rates of porcine oocytes with the activities of Δ^5-3β-HSD (DHEA), 17β-HSD (testosterone) and 20β-HSD (20β-hydroxyprogesterone) did not change during maturation culture, whereas those with the activities of Δ^5-3β-HSD (pregnenolone and 17α-hydroxypregnenolone), 17β-HSD (estradiol-17β), 20α-HSD (20α-hydroxyprogesterone) and 20β-HSD (17α-

Table 1. HSDs investigated, and substrates, solvents and cofactors used for their histochemical analyses

HSDs	Substrates	Solvents	Cofactors
Δ^5-3β-HSD	DHEA	Acetone	NAD
	Pregnenolone	Acetone	NAD
	17α-Hydroxypregnenolone	Dimethylformamide	NAD
17β-HSD	Estradiol-17β	Acetone	NAD
	Testosterone	Acetone	NAD
20α-HSD	20α-Hydroxyprogesterone	Acetone	NADP
20β-HSD	17α-Hydroxyprogesterone	Acetone	NAD
	20β-Hydroxyprogesterone	Acetone	NAD

NAD: Nicotinamide adenine dinucleotide, NADP: nicotinamide adenine dinucleotide phosphate.

hydroxyprogesterone) decreased as the time of culture was prolonged. They have also reported that the resumption of nuclear maturation was completely inhibited in porcine oocytes treated with olomoucine, an inhibitor of resumption of nuclear maturation, and the decrease in number of oocytes with the activities of such HSDs was also inhibited. These results suggested that the nuclear maturation in porcine oocytes was determined to be closely associated with the metabolic abilities of progesterone, 17α-hydroxyprogesterone, 20α-hydroxyprogesterone, 17α, 20β-dihydroxyprogesterone and estradiol-17β, and the disappearance of these metabolic abilities could be a characteristic of the resumption of meiotic maturation in porcine oocytes [3].

Changes in the metabolic abilities of steroids in mammalian oocytes with maturation have been investigated in rats [1], mice [2], rabbits [2], pigs [2, 3] and cattle [2], as mentioned above, though sufficient numbers of different kinds of HSDs were demonstrated only in pigs [3].

In the present study, the activities of various kinds of HSDs were histochemically demonstrated in rabbit and hamster oocytes in the process of maturation *in vivo* by using as many substrates as possible, in order to investigate the changes in the metabolic abilities of steroids with oocyte maturation.

Materials and Methods

Animals used and collection of oocytes

Fifty-five female mature Japanese white rabbits and 70 female mature golden hamsters were used in the present study. These animals were housed in autoclaved metal cages and were given a standard chow (RC-4 or MF, Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum* in an air-conditioned room (24°C), under controlled-lighting conditions (14L/10D).

In order to observe oocytes during meiotic maturation *in vivo*, rabbits were intravenously injected with 60 i.u. of hCG (Gonatropin®, Teikoku Hormone Manufacturing Co. Ltd., Tokyo, Japan), and the oocytes were collected from antral follicles 0 and 7 hrs after the hCG injection and from oviducts 14 hrs after the hCG injection. As for hamsters, oocytes were obtained by superovulation treatment; the animals were subjected to subcutaneous injections with 25 i.u. of eCG (Serotropin®, Teikoku Hormone Manufacturing Co. Ltd.) at 10:00 am on Day 1 (the day when post-oestrous discharge [4] existed in the vagina) and with 25 i.u. of hCG (Gonatropin®, Teikoku Hormone Manufacturing Co. Ltd.) at 2:00 pm on Day 4. Hamster oocytes were collected from antral follicles 0, 3 and 8 hrs after the hCG injection and from oviducts 13 hrs after the hCG injection.

Observation of HSD activities

Cumulus-oocyte complexes (COCs) collected from antral follicles and oviducts were immersed in phosphate buffered saline (PBS, pH 7.4) [5] containing 0.1% hyaluronidase (Sigma-Aldrich, MO, USA) to disperse their cumulus cells. In order to detect the activities of HSDs shown in Table 1, the method used by Niimura and Ishida [6] was employed: denuded oocytes were placed at 37°C in a solution containing 1.8 mg substrate (Sigma-Aldrich) which had been dissolved in 0.5 ml acetone or dimethylformamide, 4.0 mg cofactor (Sigma-Aldrich), 2.0 mg nitroblue tetrazolium salt (Sigma-Aldrich), and 10.0 ml 0.1 M phosphate buffer solution (pH 7.5). Oocytes incubated in a solution containing the substrate solvent, acetone or dimethylformamide, but devoid of the substrates were observed as negative controls. The incubation time was

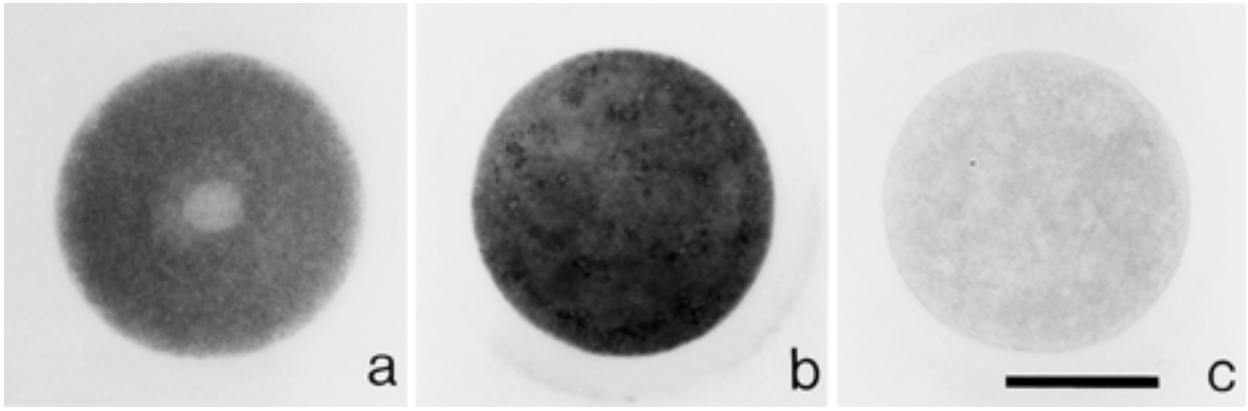

Fig. 1. Diformazan granules showing the presence of the activity of 17β-HSD with estradiol-17β as substrate (a) and 20β-HSD with 20β-hydroxyprogesterone as substrate (b) are deposited in the cytoplasm of rabbit oocytes, but not in the cytoplasm of a negative control oocyte, which was incubated in a substrate-free solution (c). Scale indicates 50 μm. (a) and (c) oocytes 0 hr after the hCG injection, (b) an oocyte 14 hrs after the hCG injection.

60 to 120 min, because this was the period within which unspecified or endogenous dehydrogenase reactions never appeared in the control oocytes incubated in a substrate-free solution. The same procedures for the demonstration of HSDs were applied 3 times to oocytes from each period after the hCG injection. Atretic oocytes were eliminated from the observation. As the histochemical reaction of HSDs depends on the reaction of $NADH_2$ dehydrogenase ($NADH_2$-DH) or $NADPH_2$ dehydrogenase ($NADPH_2$-DH) [7], the demonstration of these enzymes was carried out according to the Barka and Anderson method [8]. As negative controls, some oocytes were incubated in a substrate-free solution for 60 min at 37°C. After incubation in the substrate solution, the oocytes were washed in PBS, and were placed on glass slides to be photographed under a light microscope (OPTIPHOT-2, Nikon, Tokyo, Japan).

Observation of nuclear maturation

In order to investigate nuclei, oocytes collected from antral follicles and oviducts at various periods after the hCG injection were fixed in 25% (v/v) acetic acid in ethanol for 24 hrs at 4°C. The fixed oocytes were stained with 1.0% aceto-orcein and examined for evidence of nuclear maturation under a light microscope.

Results

Nuclear maturation

At 0 hr after the hCG injection, the nuclei of rabbit and hamster oocytes were all in the germinal vesicle (GV) stage (30/30 and 30/30). The nuclei of rabbit oocytes 7 hrs after the hCG injection were in the GV (19%, 5/26), diakinesis (42%, 11/26) and metaphase I (39%, 10/26) stages, and those 14 hrs after the hCG injection were all in the metaphase II stage (25/25). The nuclei of hamster oocytes 3 hrs after the hCG injection were in the diakinesis (52%, 15/29) and metaphase I (48%, 14/29) stages, and those 8 hrs after the hCG injection were in the metaphase I to metaphase II stages, mostly telophase I stage (56%, 23/41). Of the hamster oocytes 13 hrs after the hCG injection, 100% (30/30) were in the metaphase II stage.

Activities of HSDs

When rabbit and hamster oocytes in the process of maturation *in vivo* were immersed in a substrate solution, diformazan granules were found to be deposited in the cytoplasm (Figs. 1a, 1b, 2a and 2b). Since such granules were not observed in the oocytes immersed in a solution containing no substrate (negative control; Figs. 1c and 2c), the granules were considered to represent the activity of HSDs. With the method of Barka and Anderson [8] to demonstrate $NADH_2$-DH and $NADPH_2$-DH, deposited diformazan granules were found in the cytoplasm of every oocyte from each period after the hCG injection. These granules did not appear in the negative control oocytes.

The activities of various HSDs in rabbit oocytes in the process of maturation *in vivo* are shown in Table 2. Of oocytes 0 hr after the hCG injection, 75 to 100% showed the activities of Δ^5-3β-HSD, 17β-HSD, 20α-HSD and 20β-HSD with eight substrates. Although the rates of

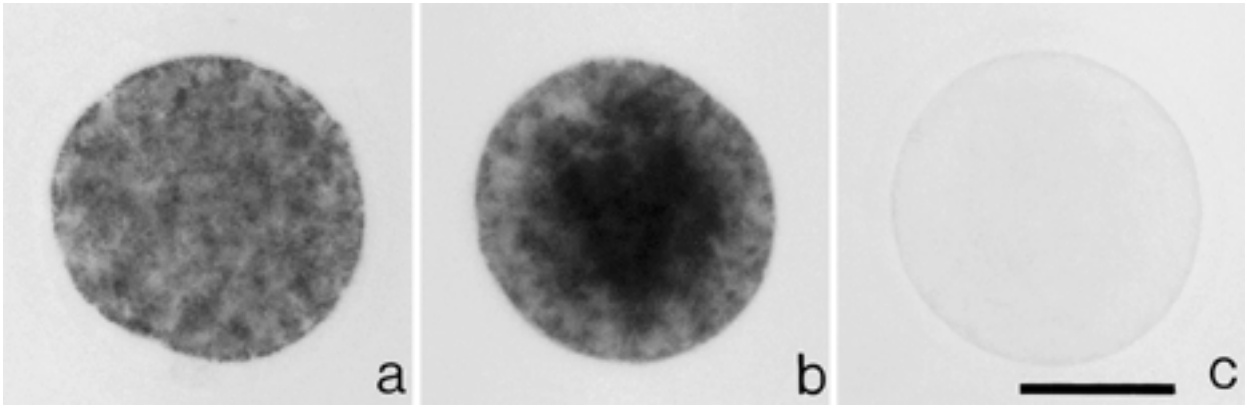

Fig. 2. Diformazan granules showing the presence of the activity of 20α-HSD with 20α-hydroxyprogesterone as substrate (a) and Δ^5-3β-HSD with pregnenolone as substrate (b) are deposited in the cytoplasm of hamster oocytes, but not in the cytoplasm of a negative control oocyte, which was incubated in a substrate-free solution (c). Scale indicates 50 μm. (a) and (c) oocytes 8 hrs after the hCG injection, (b) an oocyte 3 hrs after the hCG injection.

Table 2. Activities of HSDs in rabbit oocytes during meiotic maturation

Hours after hCG injection	Δ^5-3β-HSD						17β-HSD			
	DHEA[1)]		Pregnenolone[1)]		17α-Hydroxy-pregnenolone[1)]		Estradiol-17β[1)]		Testosterone[1)]	
	+[2)]	–[2)]	+	–	+	–	+	–	+	–
0	30 (100)*	0 (0)	12 (75)	4 (25)	36 (82)	8 (18)	14 (88)	2 (12)	20 (80)	5 (20)
7	21 (84)	4 (16)	7 (41)	10 (59)	14 (47)	16 (53)	11 (37)	19 (63)	13 (81)	3 (19)
14	26 (100)	0 (0)	2 (11)	16 (89)	0 (0)	28 (100)	2 (14)	12 (86)	18 (86)	3 (14)

Hours after hCG injection	20α-HSD		20β-HSD			
	20α-Hydroxy-progesterone[1)]		17α-Hydroxy-progesterone[1)]		20β-Hydroxy-progesterone[1)]	
	+	–	+	–	+	–
0	16 (84)	3 (16)	38 (84)	7 (16)	13 (93)	1 (7)
7	13 (54)	11 (46)	11 (34)	21 (66)	18 (86)	3 (14)
14	2 (8)	22 (92)	0 (0)	34 (100)	13 (87)	2 (13)

[1)] Substrates for enzyme-histochemistry. [2)] + Positive, – negative.
*The number of oocytes with percentages in parentheses.

oocytes with the activities of Δ^5-3β-HSD (DHEA), 17β-HSD (testosterone) and 20β-HSD (20β-hydroxyprogesterone) did not change during maturation, those with the activity of Δ^5-3β-HSD (pregnenolone), Δ^5-3β-HSD (17α-hydroxypregnenolone), 17β-HSD (estradiol-17β), 20α-HSD (20α-hydroxyprogesterone) and 20β-HSD (17α-hydroxyprogesterone) decreased with time after the hCG injection and reached 11, 0, 14, 8 and 0%, respectively, 14 hrs after the hCG injection.

On the other hand, the percentages of hamster oocytes showing the activities of Δ^5-3β-HSD, 17β-HSD, 20α-HSD and 20β-HSD with eight substrates did not vary during meiotic maturation *in vivo* (Table 3).

Discussion

Changes in the metabolic abilities of steroids in mammalian oocytes with maturation have been investigated in rats [1], mice [2], rabbits [2], pigs [2, 3] and cattle [2], though sufficient numbers of different kinds of HSDs were demonstrated only in pigs [3].

In the present investigation, the activities of Δ^5-3β-HSD (DHEA), 17β-HSD (testosterone) and 20β-HSD (20β-hydroxyprogesterone) were almost always demonstrated in rabbit oocytes during meiotic maturation *in vivo*, whereas oocytes showing the activities of Δ^5-3β-HSD (pregnenolone and 17α-

Table 3. Activities of HSDs in hamster oocytes during meiotic maturation

Hours after hCG injection	Δ^5-3β-HSD						17β-HSD			
	DHEA[1)]		Pregnenolone[1)]		17α-Hydroxy-pregnenolone[1)]		Estradiol-17β[1)]		Testosterone[1)]	
	+[2)]	–[2)]	+	–	+	–	+	–	+	–
0	26 (93)*	2 (7)	29 (83)	6 (17)	40 (93)	3 (7)	24 (86)	4 (14)	30 (100)	0 (0)
3	24 (100)	0 (0)	28 (80)	7 (20)	32 (91)	3 (9)	26 (84)	5 (16)	23 (100)	0 (0)
8	29 (100)	0 (0)	30 (100)	0 (0)	26 (84)	5 (16)	26 (90)	3 (10)	29 (100)	0 (0)
13	29 (100)	0 (0)	30 (86)	5 (14)	37 (86)	6 (14)	31 (91)	3 (9)	34 (100)	0 (0)

Hours after hCG injection	20α-HSD		20β-HSD			
	20α-Hydroxy-progesterone[1)]		17α-Hydroxy-progesterone[1)]		20β-Hydroxy-progesterone[1)]	
	+	–	+	–	+	–
0	30 (100)	0 (0)	35 (80)	9 (20)	28 (100)	0 (0)
3	21 (88)	3 (13)	27 (93)	2 (7)	29 (100)	0 (0)
8	29 (100)	0 (0)	28 (97)	1 (3)	28 (100)	0 (0)
13	26 (90)	3 (10)	28 (90)	3 (10)	29 (100)	0 (0)

[1)] Substrates for enzyme-histochemistry. [2)] + Positive, – negative.
*The number of oocytes with percentages in parentheses.

hydroxypregnenolone), 17β-HSD (estradiol-17β), 20α-HSD (20α-hydroxyprogesterone) and 20β-HSD (17α-hydroxyprogesterone) decreased in number with maturation and had almost disappeared 14 hrs after the hCG injection. On the other hand, the activities of Δ^5-3β-HSD (DHEA, pregnenolone and 17α-hydroxypregnenolone), 17β-HSD (estradiol-17β and testosterone), 20α-HSD (20α-hydroxyprogesterone) and 20β-HSD (17α-hydroxyprogesterone and 20β-hydroxyprogesterone) were almost always observed in hamster oocytes during meiotic maturation *in vivo*. Because nuclear maturation was found to progress in the rabbit and hamster oocytes observed in the present study, as in previous reports [9–11], nuclear maturation was considered to progress normally. These results suggested that in rabbit oocytes the metabolic abilities of 20β-hydroxyprogesterone and androgen remain unchanged, whereas those of progesterone, 17α-hydroxyprogesterone, 17α, 20β-dihydroxyprogesterone, 20α-hydroxyprogesterone and estrogen decrease during maturation *in vivo*. And it was also suggested that the metabolic abilities of progesterone, 17α-hydroxyprogesterone, 17α, 20β-dihydroxyprogesterone, 20α-hydroxyprogesterone, 20β-hydroxyprogesterone, estrogen and androgen in hamster oocytes do not vary with maturation.

In porcine oocytes, it appeared that nuclear maturation was closely associated with the metabolic abilities of progesterone, 17α-hydroxyprogesterone, 17α, 20β-dihydroxyprogesterone, 20α-hydroxyprogesterone and estrogen, and the disappearance of these metabolic abilities could be a characteristic of the resumption of meiotic maturation [3]. From the results of the recent investigation and of Takano and Niimura [3], it is confirmed that changes in the metabolic abilities of steroids in oocytes with maturation are different among species, and that the metabolic abilities of steroids in rabbit oocytes are similar to those in porcine oocytes. Nevertheless, the results of the present study could not provide the information needed to clarify the relationship between nuclear maturation and changes in the metabolic abilities of steroids in rabbit oocytes. This issue should be further studied in the future.

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