TEMPORAL EXPRESSION OF FUNCTIONAL LOW DENSITY LIPOPROTEIN RECEPTORS AND CATALYTIC ENZYMES IN STEROID HORMONE SYNTHESIS OF MOUSE PREIMPLANTATION EMBRYOS

Naoki Sato and Yukihiro Terada

(received 13 March 2012, accepted 23 March 2012)

Department of Obstetrics and Gynecology, Akita University Graduate School of Medicine, Akita 010-8543, Japan

Grant support : This work was supported by Grant-in-Aid for Scientific Reserch from the Japanese Ministry of Education, Science, Sports, and Culture, and Uehara Memorial Foundation to Y.T

Key words : Development, LDL, LDL receptor, Preimplantation embryo, Steroidogenesis

Abstract

Mammalian preimplantation embryos, which lack a storage organ for cholesterol which plays an essential role in the composition of cell membranes may exhaust the cholesterol without uptake from lipoprotein during development. LDL is the major lipoprotein carrier in mammals. The aim of this study was to investigate the possibility of de novo steroidsynthesis in mouse preimplantation embryos. Using collected murine oocytes and embryos, the temporal expressions of either LDL receptor (LDLR) or enzymes to synthesize steroid hormones were analyzed at the mRNA level using RT-PCR techniques. The temporal expressions of LDLR and P450scc protein were analyzed by immunostaining. To monitor the uptake of LDL in embryos, an LDL labeled with a probe was used. LDLR mRNA was detected at the oocyte, 8-cell, morula, blastocyst, and hatched blastocyst stages. P450scc mRNA was detected at the oocyte, 1-cell, 2-cell, 4-cell, blastocyst and hatched blastocyst stages. β-HSD VI mRNA was detected at the oocyte, blastocyst and hatched blastocyst stages. P450arom mRNA was detected only at oocyte. The expression of LDLR protein was detected in trophectoderm cells of blastocysts, whereas P450scc was detected in cytoplasm of oocytes and all embryos. Labeled LDL was only taken in by blastocysts, but not oocytes or 4-cell stage embryos. These results suggest that murine blastocysts could utilize LDL as a source of cholesterol for de novo progesterone synthesis.

Introduction

The transport of cholesterol through the circulatory system is facilitated by its packaging into lipoprotein carriers. These spherical particles comprised of proteins and phospholipid shells surround a core of neutral lipid, including unesterified or esterified cholesterol and triglycerides. The liver synthesizes a precursor lipoprotein, very low-density lipoprotein, which is converted during circulation to low-density lipoprotein (LDL). While the majority of the LDL receptors (LDLR) are expressed in liver, all other tissues also express the LDL

Correspondence : Yukihiro Terada, M.D., PhD.
Department of Obstetrics and Gynecology, Akita University Graduate School of Medicine,
1-1-1 Hondoh, Akita 010-8543, Japan
Tel : 81-18-884-6163
Fax : 81-18-884-6447
E-mail : teraday@obgyn.med.akita-u.ac.jp

receptors to supply cells with cholesterol[1].

Cholesterol is indispensable for embryonic development, as sources of nutrients[2-8]. Avian embryos take in lipoproteins with the specific receptors, and accumulate them into the yolk for future development[3-8]. A mutation of chicken oocytic LDLR gene causes atherosclerosis and hen sterility[9]. During development, the chicken embryo utilizes the cholesterol supplied from yolk as one of the major sources of nutrients[5]. The mammalian embryo also requires large quantities of nutrients, including cholesterol, for its growth[2]. In preimplantation embryos, cholesterol is utilized as a precursor of steroid hormone synthesis, while the porcine blastocyst has the potential to produce estrogen at the preimplantation period[10-15]. In addition, cholesterol is also an important constituent of cell membranes in the development of preimplantation embryos. The mouse preimplantation embryos are capable of synthesizing membrane sterols from the 8-cell stage onwards and the sterol composition of membranes is important for normal cytokinesis and compaction of the embryos[20].

In the rodent fetus, cholesterol is synthesized two different ways, inside the cells (endogenous) and supplied from lipoproteins taken into the cells through specific receptors (exogenous)[21]. Recently, the mechanisms of maternal-fetal transportation of cholesterol have been elucidated. In the hamster, uptake of maternal lipoproteins in the placenta, yolk sac and decidua are mediated by receptor-dependent as well as receptor independent processes[22]. A significant amount of cholesterol is mainly taken up as maternal-delivered LDL and HDL[23]. In humans, the LDL binding sites of placenta are present as early as the 6th week of pregnancy, and display the same high affinity and specificity for LDL as those of the term trophoblast[24]. During murine fetal development after implantation, the HDL receptor, SR-BI is considered to play a role in the maternal-fetal lipoprotein cholesterol transport system, supplying HDL cholesterol for either membrane construction or steroid hormone synthesis[25]. Furthermore, a difference of HDL cholesterol concentration and/or composition in maternal serum can affect the size of the fetus and sterol metabolism in the yolk sac and placenta in the mouse[26].

Preimplantation embryos start to secrete progesterone from the blastocyst stage[12,27,28]. Thus, cholesterol would be an important precursor in steroid hormone synthesis. During development of mammalian preimplantation embryos, because of the lack of a cholesterol storage organ for cholesterol such as a yolk in chicken embryos, the cholesterol may be exhausted in embryos without uptake from lipoproteins. However, to our knowledge, there have been no reports concerning the role of LDL in the steroidgenesis in mouse preimplantation embryos.

We reported the temporal expression of LDLR and uptake of LDL in mouse preimplantation embryos[29]. The aim of this study was to investigate the possibility of de novo steroid synthesis in early mouse embryos through the LDL/LDLR pathway. We sought to determine the expression of catalytic enzymes in the synthesis steroid hormones.

Materials and Methods

Collection of Mouse oocytes and embryos

Female IVCS mice aged 9 week (Institute for Animal Reproduction, Ibaragi, Japan) were superovulated with intraperitoneal injections 5 IU of pregnant mare serum gonadotropin (Sigma, St. Louis, MO) followed 48 h later by 5 IU of human chorionic gonadotropin (hCG) (Sigma). Oocytes and 1-cell embryos were collected from the oviducts of the non-mated or mated female mice 12-14 h after hCG injection, and incubated in human tubal fluid (HTF) medium[30] containing 1 mg/ml hyaluronidase (Sigma) for 5 min at 37°C to remove cumulus cells. Two-cell embryos were collected from the oviducts of the mated female mice 46-47 h after hCG injection. After washing three times with M2 medium (Sigma), oocytes, 1-cell and 2-cell embryos were used for study without incubation. Groups of 15 randomly selected embryos were placed in 30 μl drops of HTF medium up to the stages of purpose (4-cell, 8-cell, morula : 32 cells, blastocyst : 100 cells, and hatched blastocyst) at 37°C in 5% CO_2 in air. All of eggs free of fragmentation or denaturing were used for this study. All procedures involving the care and use of the animals were approved by the Animal Research Committee, Akita University School of Medicine.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Nested PCR

RT-PCR and nested PCR for oocytes and preimplantation embryos were performed according to previously described methods[29,31,32]. Briefly, the poly (A)+ mRNA was isolated from 30 mouse oocytes or preimplantation embryos of each stage (1-cell, 2-cell, 4-cell, 8-cell, morula, blastocyst and hatched blastocyst stage), and each mRNA was reverse transcribed into cDNA. Exogenous rabbit α-globin mRNA (Life Technologies, Inc., Rockville, MD) was added to each sample before mRNA extraction in order to evaluate the efficiency of mRNA extraction and RT accuracy. The amount of cDNA subjected to each PCR reaction was equivalent to the number of genomes, so that each PCR product was derived from the same number of transcribing genomes.

Primers of LDLR, 3β-hydroxysteroid dehydrogenase (3b-HSD) and aromatase P450 (P450arom) were made based on published sequences[33,34,35]. Primers of cholesterol side-chain cleavage cytochrome P450 (P450scc) were made referring to the unpublished sequence [DBGET; AF195119]. Primers and programs of PCR are shown in Table 1. For positive controls, mouse placenta cDNA was amplified simultaneously. For negative controls, the specimen in which water was substituted for mRNA was amplified. Due to small amounts mRNA in oocytes and preimplantation embryos, nested PCR was needed to obtain optimal result against LDLR, P450scc, 3β-HSD VI, and P450arom mRNAs.

The PCR products were separated by 2% agarose gel electrophoresis (AgaroseLE, Nacarai Tesque, Inc., Kyoto, Japan) in the presence of ethidium bromide solution (Sigma), and visualized with an UV transilluminator (Funako-

Table 1. Primers used for RT-PCR and nested PCR, product sizes, PCR cycles and temperatures for amplification of the different eDNA

Transcript	PCR round		Primer sequence (5′-3′)	Size of product (bp)
LDLR	1st	sense	GACCAACGAGTGTTTGGACA	351
		Antisense	GCCACCACATTCTTCAGGTT	
	2nd	sense	CACATCTGCAAGGACCTCAA	314
		antisense	GCCACCACATTCTTCAGGTT	
P450scc	1st	sense	TTACACAGACGCATCAAGCA	345
		antisense	CTGGCTGAAGTCTCGCTTCT	
	2nd	sense	TTACACAGACGCATCAAGCA	257
		antisense	GCATGGTCCTTCCAGGTCT	
3β-HSD VI	1st	sense	GCCTGTATCCAAGCCAGTGT	576
		Antisense	GCTCACAGTTTCCAGCATGA	
	2nd	sense	GTGCATTAAGGCCCATGTTT	359
		antisense	GCTCACAGTTTCCAGCATGA	
P450arom	lst	sense	CACCGTAAGCAACTGGGTTT	1146
		antisense	AGCAGCAATCCTGAAGGAGA	
	2nd	sense	GACAGGCACCTTGTGGAAAT	358
		antisense	AGCAGGAGACCAGACGTGTT	
β-actin		sense	GGACCTCACTGACTACCTCATGAA	524
		antisense	GGTGGAAGGTGGTCAACACCTAG	
α-globin		sense	GCAGCCACGGTGGCGAGTAT	257
		antisense	GTGGGACAGGAGCTTGAAAT	

PCR cycles : denaturation at 94°C for 30 sec, anealing at 55°C for 30 sec, extension at 72°C for 30 sec, total 35 cycle were performed.

shi, Tokyo, Japan). Each PCR product was cycle sequenced, and analyzed using the ABI 100 DNA sequencer (PE Applied Biosystems, Tokyo, Japan).

Immunostaining

Oocytes and embryos of each stage were fixed with 4% paraformaldehyde in PBS for 1 h at 4°C and blocked with 10% normal goat serum (Dako, Kyoto, Japan) for 30 min at room temperature. Each sample was incubated overnight at 4°C with 1 : 10 dilution of rabbit anti-LDLR antibody (Research Diagnostic Inc., NJ) and 1 : 1,000 dilution of rabbit anti-cytochrome P450scc antibody (Research Diagnostic Inc.) in 1% PBS-BSA/0.1% TritonX-100 (Sigma). After three washes in cold PBS, each sample was incubated with 1.0 μg/ml of goat anti-rabbit Cy3 fluorescein antibody (Chemicon, Temecula, CA) in 1% PBS-BSA for 1 h at room temperature in the dark. After three washes in cold PBS, samples were applied into the drop of anti-fade mounting medium (Dako) on the slide. Each sample was analyzed under an epifluorescence microscope (Olympus). For negative controls, primary antibodies were replaced by the same concentrations of rabbit IgG (Dako)

Uptake of DiO-LDL in preimplantation embryos

DiO-LDL (Biomedical Technologies Inc. MA) was used to monitor the uptake of LDL in embyos. The DiO-LDL is the purified human LDL labeled with the fluorescent probe DiO with an excitation wavelength of 484 nm. To avoid the effect on the affinity and specificity to the LDLR, the labeling of LDL was given by replacing phospholipids or cholesterols with DiO. Ten oocytes, 4-cell embryos or blastocysts were incubated at 37°C in 2 ml HTF medium containing 10 μg/ml of DiO-LDL. After five washes in PBS, oocytes and embryos were mounted on the slide and analyzed under an epifluorescence microscope (Olympus). For controls, oocytes and embryos were incubated in 2 ml HTF medium containing 10 μg/ml of DiO-LDL and 100 μg/ml of unlabeled-LDL.

Results

Temporal expression of LDLR and catalytic enzymes in the synthesis of steroid hormone mRNAs in mouse oocytes and preimplantation embryos

RT-PCR followed by nested PCR was performed to detect mRNAs for LDLR and catalytic enzymes in the synthesis of steroid hormones in the mouse oocytes and early embryos at different stages (1-cell, 2-cell, 4-cell, 8-cell, morula, blastocyst and hatched blastocyst). As shown in Fig. 1 and summarized in Table 2, LDLR mRNA was detected as 314 bp bands at oocytes, 8-cell, morula, blastocyst, and hatched blastocyst stage (Fig. 1). P450scc mRNA was detected as 257 bp bands at the oocyte, 1-cell, 2-cell, 4-cell, blastocyst and hatched blastocyst stage. 3β-HSD VI mRNA was detected as 359 bp bands at the oocyte, blastocyst and hatched blastocyst stage. P450arom mRNA was detected as 358 bp bands at the oocyte. As loading controls, no significant differences were observed in the intensities of α-globin and β-actin amplification products among oocytes and preimplantation embryos at different stages.

Expression of LDLR and P450scc proteins in mouse oocytes and preimplantation embryos

Immunostaining was performed to determine the expression of LDLR and P450scc proteins in mouse oocytes

Table 2. Expression of steroid honnones catalytic enzymes in mouse preimplantation embryos

		oocyte	1-cell	2-cell	4-cell	8-cell	morula	blastocyst	hatched blastocyst
P450 sec	mRNA	+	+	+	+	−	−	+	+
	protein	+	N/A	N/A	+	N/A	N/A	+	N/A
3β-HSD VI	mRNA	+	+	+	+	−	−	+	+
P450arom	mRNA	+	−	−	−	−	−	−	−

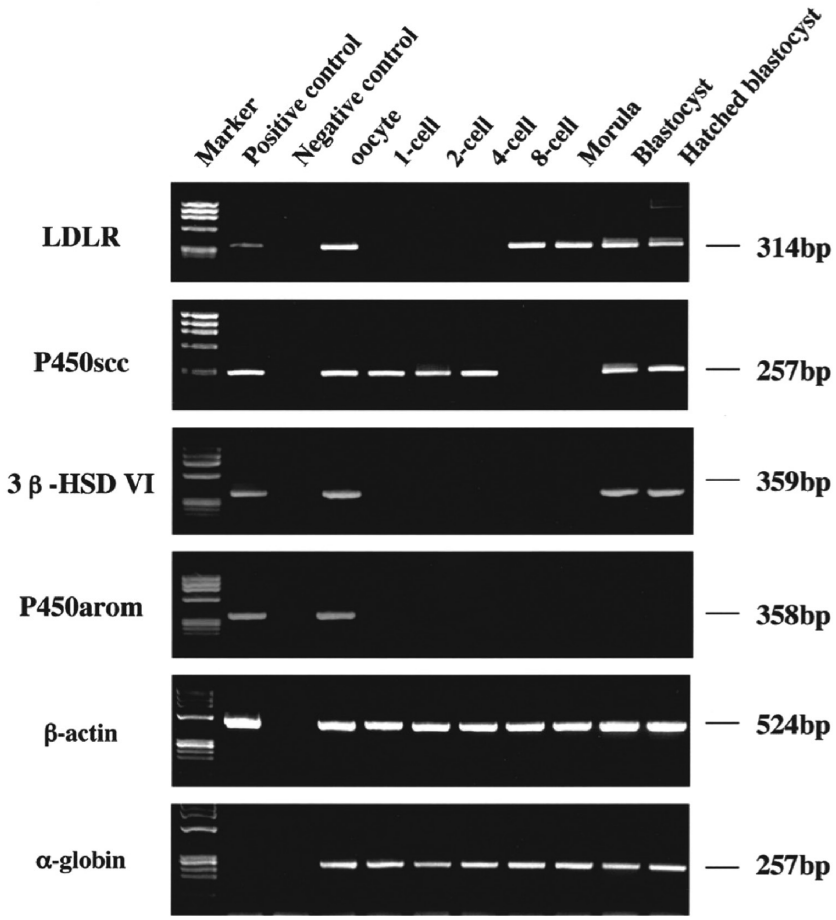

Fig. 1. Reverse transcription-polymerase chain reaction (RT-PCR) detection of LDLR, P450scc, 3β-HSD VI and P450arom mRNAs from mouse oocytes and preimplantation embryos. Fifteen oocytes and embryos at different stages were used for mRNA extraction. In order to compare amounts of the PCR product from the same number of actively transcribing genomes, the amount of cDNA for each PCR reaction was corrected using the genome copies. Exogenous α-globin mRNA was added to each sample before mRNA extraction to evaluate the efficiencies of mRNA extraction and RT. For internal control, β-actin was amplified simultaneously in each PCR reaction. Due to the low number of oocytes and preimplantation embryos under study, nested PCR was needed to obtain optimal results. No significant difference is observed in the band intensities of α-globin and β-actin amplification products among oocytes and embryos at different stages. Experiments in the present study were performed three times with reproducible results. Marker, φx 174-Hae III digest ; posi, positive control for LDLR, P450scc, 3β-HSD VI, P450arom, α-globin and β-actin=mouse adipose tissue cDNA ; nega, negative control=distilled water.

and embryos at 4-cell and blastocyst stage. The expression of LDLR was detected in trophectoderm cells of blastocysts (Fig. 2E), whereas P450scc was detected in oocytes and all embryos examined (4-cells, blastocysts) (Fig. 3A, C, E). The localization of P450scc protein was identified in cytoplasm but not on plasma membrane. The specificities of these immunoreactivities were demonstrated by the absence of staining in specimens incubated with non-immunized rabbit IgG (LDLR, Fig. 2B, D, F ; P450scc, Fig. 3B, D, F).

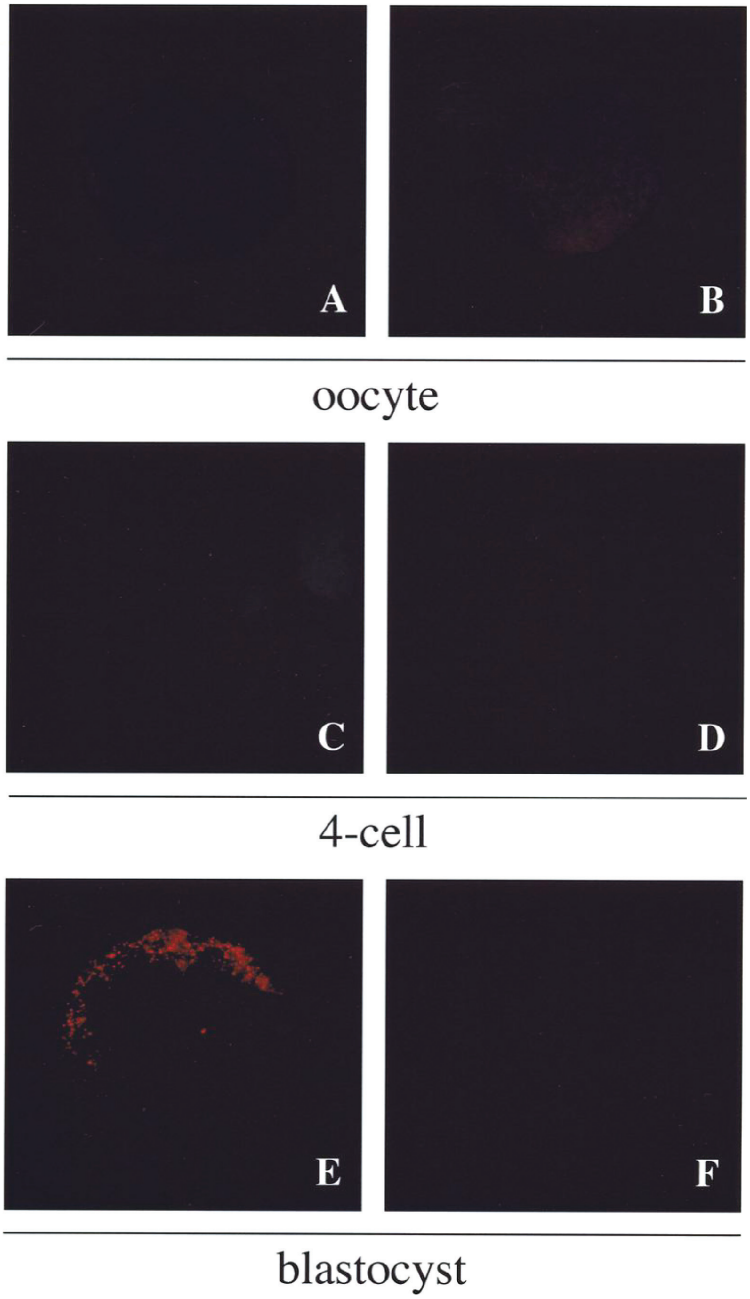

Fig. 2. Immunofluorescence staining of LDLR in mouse oocytes, 4-cells and blastocysts. Shown are epifluorescence images of the following : A and B, oocytes ; C and D, 4-cell stage embryos ; E and F, blastocyst stage embryos. Samples were fixed in 4% paraformaldehyde and stained using 1 : 10 dilution of rabbit anti-LDLR antibody as primary antibodies and 1.0 μg/ml of goat anti-rabbit IgG Cy3 fluorescein antibody as secondary antibodies. Immunoreactivities are detected in the trophectoderm cells of blastocysts (E). For negative controls, primary antibodies were replaced by the same concentration of non-immunized rabbit IgG (B, D, F). Representative results are shown and similar results were obtained in the other samples.

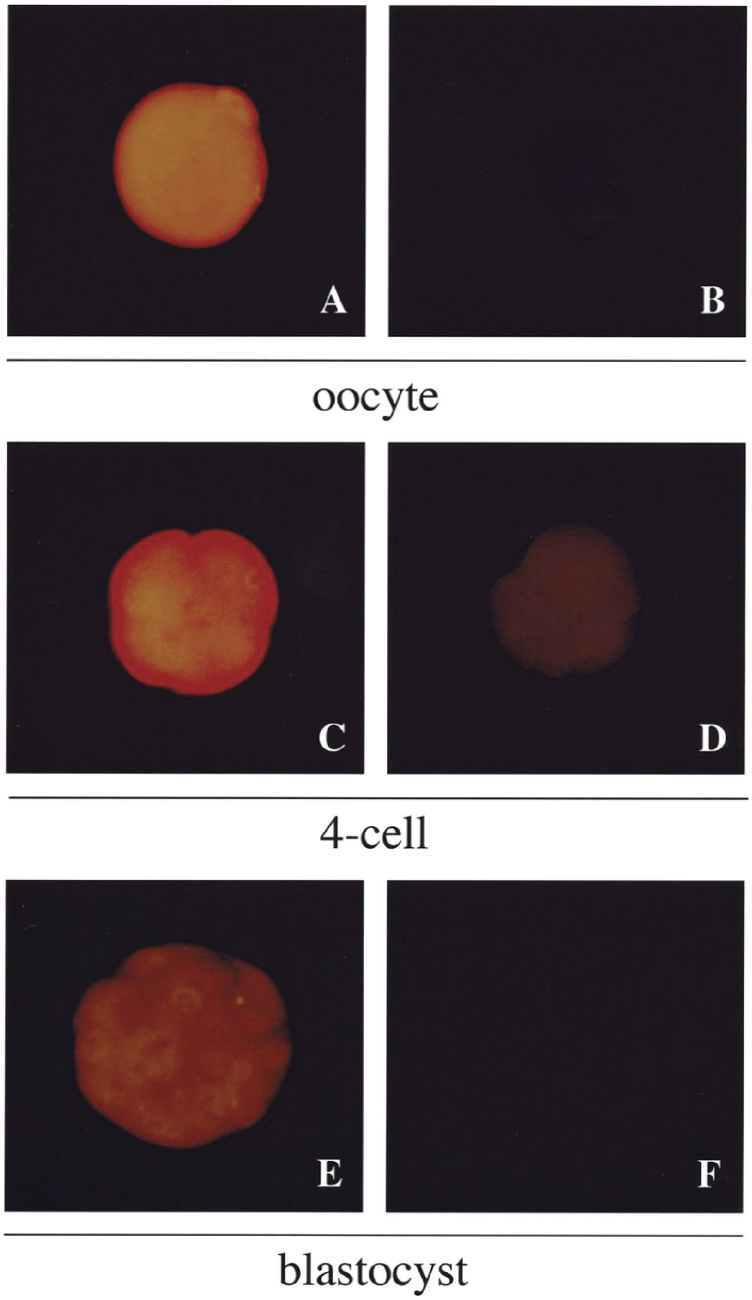

Fig. 3. Immunofluorescence stainings of P450scc in mouse oocytes, 4-cells and blastocysts. Shown are epifluorescence images of the following : A and B, oocytes ; C and D, 4-cell stage embryos ; E and F, blastocyst stage embryos. Samples were fixed in 4% paraformaldehyde and stained using 1 : 1,000 dilution of rabbit anti-P450scc antibody as primary antibodies and 1.0 μg/ml of goat anti-rabbit IgG Cy3 fluorescein antibody as secondary antibodies. Immunoreactivities are detected in the cytoplasm of oocytes (A), 4-cells (C) and blastocysts (E). For negative controls, primary antibodies were replaced by the same concentration of non-immunized rabbit IgG (B, D, F). Representative results are shown and similar results were obtained in the other samples.

Uptake of DiO-LDL in mouse oocytes and preimplantation embryos

To confirm the expression of functional LDLR in mouse preimplantation embryos, binding studies were performed using DiO-LDL. Only blastocytes took in DiO-LDL (Fig. 4C), while neither oocytes (Fig. 4A) nor 4-cell stage embryos (Fig. 4B) did. The clustered fluorescent signals were detected homogenously in trophectoderm cells (TE) at the blastocyst stage (Fig. 4C). For controls, signals almost fully disappeared when embryos were incubated with the DiO-LDL in the presence of a 10-fold excess of unlabeled LDL (Fig. 4D).

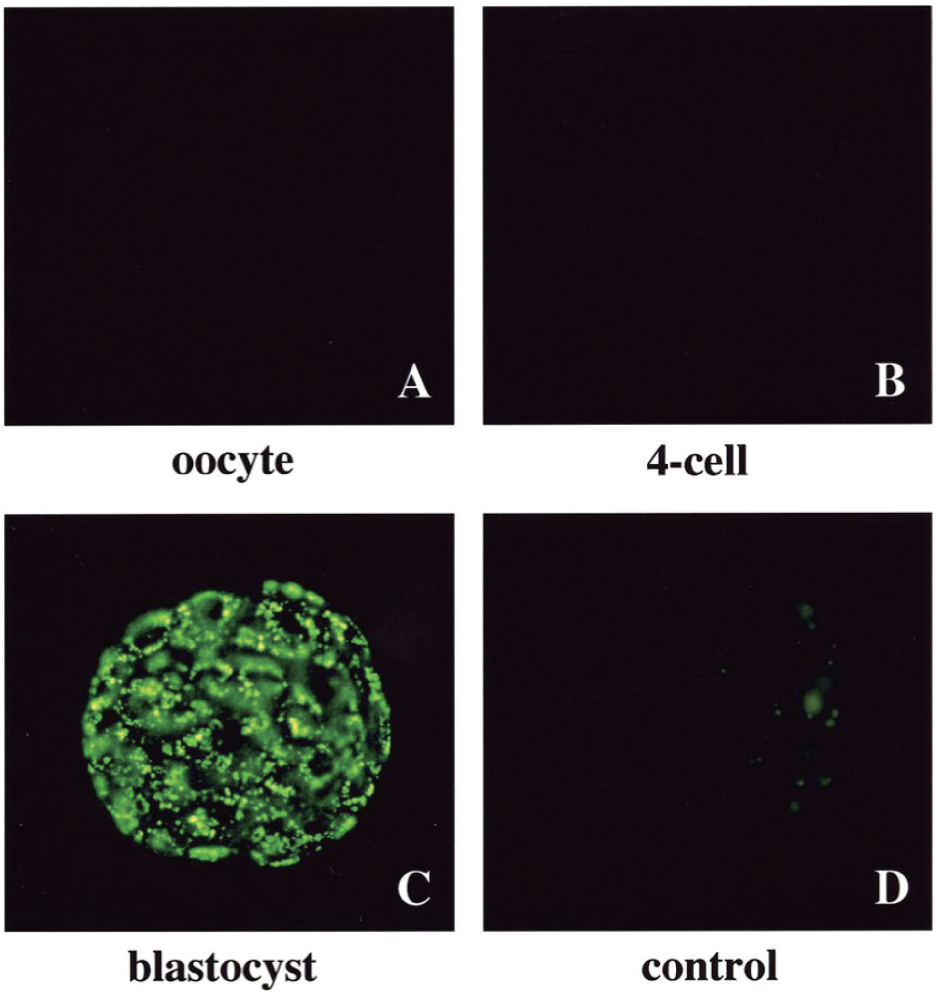

Fig. 4. Uptakes of DiO-LDL in mouse preimplantation embryos. Shown are epifluorescence images of optical sections of the following : oocytes (A), 4-cells (B) and blastocysts (C, D). Oocytes and embryos were incubated with 10 μg/ml of DiO-LDL, the purified Human LDL labeled with the fluorescent probe DiO, in HTF medium for 5 h at 37°C in 5% CO_2 in air. The clustered fluorescent signals of DiO-LDL are found homogenously in trophectoderm cells (C). Oocytes (A) and 4-cell stage embryos (B) were lacked fluorescent signals. For controls of nonspecific bindings, blastocysts were incubated with a 10-fold excess of unlabeled LDL (D). The fluorescent signals in controls are much weaker than specific signals in C. Consistent signals were observed in at least three experiments.

Discussion

In the present study, we demonstrate the temporal expression of LDLR and steroid hormone catalytic enzyme mRNAs and proteins in mouse oocytes and preimplantation embryos. Using binding assays, fluorescent labeled LDL could bind to TE cells at the blastocyst stage in clustering patterns, suggesting the existence of receptor-ligand complexes[36,37]. In contrast, oocyte and 4-cell stage embryos lacked specific fluorescent signals. The results of both RT-PCR and binding assay also suggest that oocyte and 4-cell stage embryos do not express functional receptors. Thus, through the receptor-mediated process, exogenously supplemented LDL could be taken into blastocysts expressing the LDLR.

The temporal expression pattern of LDLR was different between mRNA and protein levels. LDLR mRNA was detected at oocytes and embryos at the 8-cell, morula, blastocyst, and hatched blastocyst stage, whereas only the blastocyst stage expressed LDLR at the protein level. Furthermore, from the binding studies, the functional receptor was found in blastocysts, not in oocytes and 4-cell stage embryos. During murine embryonic development, embryonic genome activation, which is an event of transition from maternally inherited RNA and proteins to newly translated embryonic ones, is known to take place between the early and late 2-cell stages[38,39], and the de novo translations from embryonic mRNAs initiate at the 8-cell stage[39]. Therefore, in the oocyte, it is possible that the LDLR mRNA is maternally inherited and not translated into a functional protein. Furthermore, de novo synthesis of LDLR mRNA would be started at the 8-cell stage and subsequently translated into a functional receptor at the blastocyst stage.

To examine whether mouse preimplantation embryos have the potential to execute steroidgenesis, we examined the expression of steroid hormone catalytic enzymes in embryos. P450scc is the enzyme catalyzing the first and key regulatory reaction controlling the production of steroid hormones. The presence of 3β-HSD, a key enzyme in the metabolism of steroid hormones, is strong evidence for steroidogenesis because 3β-HSD catalyzes the conversion of Δ^5-3β-hydroxysteroids to Δ^4-3β-ketosteroids, a reaction that is essential for the biosynthesis of active steroid hormones, including progesterone and estrogen. 3β-HSD VI is one of the isoforms of 3β-HSD expressed during early embryogenesis in embryonic cells and in uterine tissue undergoing implantation, as well it is involved in the production of progesterone[34]. For progesterone synthesis, P450scc catalyzes the conversion of cholesterol to pregnenolone, followed by the conversion of pregnenolone to progesterone by 3β-HSD. Estrogen is also synthesized from cholesterol, and its synthesis is dependent on P450arom.

In mammalian preimplantation embryos, previous reports have demonstrated the steroidgenesis of estrogen and progesterone including porcine, rabbit, bovine, hamster and rat [porcine : 13, 40, 41] [rabbit : 11, 28] [bovine : 10, 12] [rat : 42] [Hamster : 43]. In the present study, as summarized in Table 2, P450scc mRNA was detected at the oocyte, 1-cell, 2-cell, 4-cell, blastocyst and hatched blastocyst stage. 3β-HSD VI mRNA was detected at the oocyte, blastocyst and hatched blastocyst stage. P450arom mRNA was detected only in the oocyte. In the protein level, the expression of P450scc was detected in oocytes and all embryos examined. Therefore, our results strongly suggest that mouse preimplantation embryos have the potential to synthesize progesterone, but have no ability to synthesize estrogen. This result is consistent with our previous results obtained in rabbit blastocysts[27]. Because the active uptake of LDL was only observed at the blastocyst stage embryos, cholesterol supplied from LDL might be the only available source of progesterone in the blastocyst. In contrast to our results, one group failed to detect P450scc in mouse blastocysts[14], but others have shown evidence of sex steroid production, including progesterone in rodent preimplantation embryos[11,42,44].

Synchronous development of both the embryo and endometrium is a prerequisite for blastocyst implantation. Uterine "receptivity" is a functional term to suggest a state of the uterus where it can receive embryonic inputs and allow the blastocyst to attach, penetrate, and induce localized changes in the stroma leading to decidual support and placentation. Progesterone secreted from luteal cells in the ovary acts on an estrogen-primed uterus to induce glandular secretory differentiation and vascular maturations [reviewed in 45)]. On the basis of

previous reports and our results, preimplantation mammalian embryos, at least at the blastocyst stage, possess the necessary machinery to metabolize progesterone. Thus, it is highly speculated that blastocysts can secrete progesterone into the surrounding milieu. Although the functions of embryo-secreted progesterone in signaling endometrial responses at implantation remain to be elucidated, it is now well established that levels of hormones in systemic circulation do not necessarily reflect the local milieu of a target tissue. Therefore, at the implantation site, the blastocyst maintains the high concentration of progesterone, potentially allowing for the completion of a receptive endometrium.

In conclusion, we demonstrated that the temporal expression of LDLR and catalytic enzymes are present on the synthesis of steroid hormones in mouse preimplantation embryos. The functional LDLR was expressed in TE cells of blastocyst stage embryos. In the blastocysts, the key enzymes for progesterone synthesis such as P450scc and 3b-HSD IV were detected, while P450arom was not. These observations strongly suggest that the blastocyst could utilize LDL as a source of cholesterol for de novo progesterone synthesis.

References

1) Krieger, M. (1998) The "best" of cholesterols, the "worst" of cholesterols : a tale of two receptors. *Proc. Natl. Acad. Sci. U S A*, **95**, 4077-4080.
2) Young, S.G. (1996) Using genetically modified mice to study apolipoprotein B. *J. Atheroscler Thromb*., **3**, 62-74.
3) Bujo, H., Hermann, M., Lindstedt, K.A., Nimpf, J. and Schneider, W.J. (1997) Low density lipoprotein receptor gene family members mediate yolk deposition. *J. Nutr*., **127**, 801-804.
4) Schneider, W.J., Osanger, A., Waclawek, M. and Nimpf, J. (1998) Oocyte growth in the chicken : receptors and more. *Biol. Chem*., **379**, 965-971.
5) Schneider, W.J. (1992) Lipoprotein receptors in oocyte growth. *Clin. Investig*., **70**, 385-390.
6) Elkin, R.G. and Schneider, W.J. (1994) Visualization of the chicken oocyte lipoprotein receptor by ligand blotting with biotinylated plasma and yolk very low density lipoproteins. *Poult. Sci*., **73**, 1127-1136.
7) Barder, D.L., Sanders, E.J., Aebersold, R. and Schneider, W.J. (1991) The receptor for yolk lipoprotein deposition in the chicken oocyte. *J. Biol. Chem*., **266**, 18761-18770.
8) Hermann, M., Mahon, M.G., Lindstedt, K.A., Nimpf, J. and Schneider, W.J. (2000) Lipoprotein receptors in extraembryonic tissues of the chicken. *J. Biol. Chem.,* **275**, 16837-16844.
9) Bujo, H., Yamamoto, T., Hayashi, K., Hermann, M., Nimpf, J. and Schneider, W.J. (1995) Mutant oocytic low density lipoprotein receptor gene family member causes atherosclerosis and female sterility. *Proc. Natl. Acad. Sci. U S A*., **92**, 9905-9909.
10) Chiappe, M.E., Lattanzi, M.L., Colman-Lerner, A.A., Baranao, J.L. and Saragueta, P. (2002) Expression of 3 beta-hydroxysteroid dehydrogenase in early bovine embryo development. *Mol. Reprod. Dev.,* **61**, 135-141.
11) Dickmann, Z., Dey, S.K. and Gupta, J.S. (1975) Steroidogenesis in rabbit preimplantation embryos. *Proc. Natl. Acad. Sci. U S A*, **72**, 298-300.
12) Shemesh, M., Milaguir, F., Ayalon, N. and Hansel, W. (1979) Steroidogenesis and prostaglandin synthesis by cultured bovine blastocysts. *J. Reprod. Fertil.,* **56**, 181-185.
13) Chu, X., Corbin, C.J., Kaminski, M.A. and Conley, A.J. (1999) Unique regulation of CYP17 expression in the trophectoderm of the preattachment porcine blastocyst. *Endocrinology,* **140**, 632-640.
14) Stromstedt, M., Keeney, D.S., Waterman, M.R., Paria, B.C., Conley, A.J. and Dey, S.K. (1996) Preimplantation mouse blastocysts fail to express CYP genes required for estrogen biosynthesis. *Mol. Reprod. Dev.,* **43**, 428-436.
15) Mondschein, J.S., Hersey, R.M., Dey, S.K., Davis, D.L. and Weisz, J. (1985) Catechol estrogen formation by pig blastocysts during the preimplantation period : biochemical characterization of estrogen-2/4-hydroxylase and correlation with aromatase activity. *Endocrinology*, **117**, 2339-2346.
16) Chakraborty, C., Davis, D.L. and Dey, S.K. (1990) Estradiol-15 alpha-hydroxylation : a new avenue of estrogen metabolism in peri-implantation pig blastocysts. *J. Steroid. Biochem.,* **35**, 209-218.
17) Conley, A.J., Christenson, L.K., Ford, S.P. and Christenson, R.K. (1994) Immunocytochemical localiza-

tion of cytochromes P450 17 alpha-hydroxylase and aromatase in embryonic cell layers of elongating porcine blastocysts. *Endocrinology,* **135**, 2248-2254.

18) Hoversland, R.C., Dey, S.K. and Johnson, D.C. (1982) Aromatase activity in the rabbit blastocyst. *J. Reprod. Fertil.*, **66**, 259-263.

19) Ko, Y., Choi, I., Green, M.L., Simmen, F.A. and Simmen, R.C. (1994) Transient expression of the cytochrome P450 aromatase gene in elongating porcine blastocysts is correlated with uterine insulin-like growth factor levels during peri-implantation development. *Mol. Reprod. Dev.*, **37**, 1-11.

20) Pratt, H.P., Keith, J. and Chakraborty, J. (1980) Membrane sterols and the development of the preimplantation mouse embryo. *J. Embryol. Exp. Morphol.,* **60**, 303-319.

21) Woollett, L.A. (2001) Fetal lipid metabolism. *Front. Biosci.,* **6**, D536-545.

22) Wyne, K.L. and Woollett, L.A. (1998) Transport of maternal LDL to the fetal membranes and placenta of the Golden Syrian hamster is mediated by receptor-dependent and receptor-independent processes. *J. Lipid. Res.,* **39**, 518-530.

23) Woollett, L.A. (1996) Origin of cholesterol in the fetal Golden Syrian hamster : contribution of de novo sterol synthesis and maternal-derived lipoprotein cholesterol. *J. Lipid. Res.,* **37**, 1426-1457.

24) Alsat, E., Bouali, Y., Goldstein, S., Malassine, A., Berthelier, M., Mondon, F. and Cedard, L. (1984) Low-density lipoprotein binding sites in the microvillous membranes of human placenta at different stages of gestation. *Mol. Cell Endocrinol.,* **38**, 197-203.

25) Hatzopoulos, A.K., Rigotti, A., Rosenberg, R.D. and Krieger, M. (1998) Temporal and spatial pattern of expression of the HDL receptor SR-BI during murine embryogenesis. *J. Lipid. Res*., **39**, 495-508.

26) McConihay, J.A., Honkomp, A.M., Granholm, N.A. and Woollett, L.A. (2000) Maternal high density lipoproteins affect fetal mass and extra-embryonic fetal tissue sterol metabolism in the mouse. *J. Lipid. Res.,* **41**, 424-432.

27) Tanaka, T., Fujimoto, S., Ichinoe, K., Ono, T. and Imai, Y. (1980) Activity of cholesterol side-chain cleavage enzyme system of rabbit preimplantation blastocysts and corpora lutea. *Acta. Obst. Gynaec. Jpn.,* **31**, 243-247.

28) Angle, M.J. and Mead, R.A. (1979) The source of progesterone in preimplantation rabbit blastocysts. *Steroids*, **33**, 625-637.

29) Sato, N., Kawamura, K., Fukuda, J., Honda, Y., Sato, T., Tanikawa, H., Kodama, H. and Tanaka, T. (2003) Expression of LDL receptor and uptake of LDL in mouse preimplantation embryos. *Mol. Cell. Endocrinol.,* **202**, 191-194.

30) Quinn, P., Kerin, J.F. and Warnes, G.M. (1985) Improved pregnancy rate in human in vitro fertilization with the use of a medium on the composition of human tubal fluid. *Fertil. Steril.,* **44**, 493-498.

31) Kawamura, K., Fukuda, J., Ito, K., Kodama, H., Kumagai, J., Kumagai, A. and Tanaka, T. (2000) Chaperonin 10 in the rat oocytes and early embryos : its expression and activity for early pregnancy factor. *Am. J. Reprod. Immunol.,* **44**, 242-248.

32) Kawamura, K., Fukuda, J., Kodama, H., Kumagai, J., Kumagai, A. and Tanaka, T. (2001) Expression of Fas and Fas ligand mRNA in rat and human preimplantation embryos. *Mol. Hum Reprod*., **7**, 431-436.

33) Polvono, W.J., Dichek, D.A., Mason, J. and Anderson, W.F. (1992) Molecular cloning and nucleotide sequence of cDNA encoding a functional murine low-density-lipoprotein receptor. *Somat. Cell. Mol. Genet.,* **18**, 443-450.

34) Abbaszade, I.G., Arensburg, J., Park, C.H., Kasa-Vubu, J.Z., Orly, J. and Payne, A.H. (1997) Isolation of a new mouse 3beta-hydroxysteroid dehydrogenase isoform, 3beta-HSD VI, expressed during early pregnancy. *Endocrinology*, **138**, 1392-1399.

35) Terashima, M., Toda, K., Kawamoto, T., Kuribayashi, I., Ogawa, Y., Maeda, T. and Shizuta, Y. (1991) Isolation of a full-length cDNA encoding mouse aromatase P450. *Arch. Biochem. Biophys*., **285**, 231-237.

36) Beaudet, A., Nouel, D., Stroh, T., Vandenbulcke, F., Dal-Farra, C. and Vincent, J.P. (1998) Fluorescent ligands for studying neuropeptide receptors by confocal microscopy. *Braz. J. Med. Biol. Res.*, **31**, 1479-1489.

37) Fabry, M., Langer, M., Rothen-Rutishauser, B., Wunderli-Allenspach, H., Hocker, H. and Beck-Sickinger, A.G. (2000) Monitoring of the internalization of neuropeptide Y on neuroblastoma cell line SK-N-MC. *Eur. J. Biochem*., **267**, 5631-5637.

38) Flach, G., Johnson, M.H., Braude, P.R., Taylor, R.A.

and Bolton, V.N. (1982) The transition from maternal to embryonic control in the 2-cell mouse embryo. *EMBO. J.,* **1**, 681-686.

39) Nothias, J.Y., Majumder, S., Kaneko, K.J. and De Pamphilis, M.L. (1995) Regulation of gene expression at the beginning of mammalian development. *J. Biol. Chem.,* **270**, 22077-22080.

40) Niemann, H. and Elsaesser, F. (1987) Steroid hormones in early pig embryo development. In Bavister, B.D. (ed.) *The Mammalian Preimplantation Embryo.* Plenun publishing corporation, pp. 117-132.

41) Choi, I., Simmen, R.C. and Simmen, F.A. (1996) Molecular cloning of cytochrome P450 aromatase complementary deoxyribonucleic acid from periimplantation porcine and equine blastocysts identifies multiple novel 5′-untranslated exons expressed in embryos, endometrium, and placenta. *Endocrinology,* **137**, 1457-1467.

42) Dickmann, Z. and Dey, S.K. (1974) Steroidogenesis in the preimplantation rat embryo and its possible influence on morula-blastocyst transformation and implantation. *J. Reprod. Fertil*., **37**, 91-93.

43) Dickmann, Z. and Sen Gupta, J. (1974) Delta5-3beta-hydroxysteroid dehydrogenase and estradiol-17beta-hydroxysteroid dehydrogenase activity in preimplantation hamster embryos. *Dev. Biol.,* **40**, 196-198.

44) Wu, J.T. and Liu, Z.H. (1990) Conversion of pregnenolone to progesterone by mouse morulae and blastocysts. *J. Reprod. Fertil.,* **88**, 93-98.

45) Norwitz, E.R., Schust, D.J. and Fisher, S.J. (2001) Implantation and the survival of early pregnancy. *N. Engl. J. Med.,* **345**, 1400-1408.