Expression of Scavenger Receptor-BI and Low-Density Lipoprotein Receptor and Differential Use of Lipoproteins to Support Early Steroidogenesis in Luteinizing Macaque Granulosa Cells

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An ovulatory hCG stimulus to rhesus macaques undergoing controlled ovarian stimulation protocols results in a rapid and sustained increase in progesterone synthesis. The use of lipoproteins as a substrate for progesterone synthesis remains unclear, and the expression of lipoprotein receptors [very-low-density lipoprotein receptor (VLDLR), low-density lipoprotein receptor (LDLR), and scavenger receptor-BI (SR-BI)] soon after human chorionic gonadotropin (hCG) ($<$12 h) has not been characterized. This study investigated lipoprotein receptor expression and lipoprotein (VLDL, LDL, and HDL) support of steroidogenesis during luteinization of macaque granulosa cells. Granulosa cells were aspirated from rhesus monkeys undergoing controlled ovarian stimulation before or up to 24 h after an ovulatory hCG stimulus. The expression of VLDLR decreased within 3 h of hCG, whereas LDLR and SR-BI increased at 3 and 12 h, respectively. Granulosa cells isolated before hCG were cultured for 24 h in the presence of FSH or FSH plus hCG with or without VLDL, LDL, or HDL. Progesterone levels increased in the presence of hCG regardless of lipoprotein addition, although LDL, but not HDL, further augmented hCG-induced progesterone. Other cells were cultured with FSH or FSH plus hCG without an exogenous source of lipoprotein for 24 h, followed by an additional 24 h culture with or without lipoproteins. Cells treated with hCG in the absence of any lipoprotein were unable to maintain progesterone levels through 48 h, whereas LDL (but not HDL) sustained progesterone synthesis. These data suggest that an ovulatory stimulus rapidly mobilizes stored cholesterol esters for use as a progesterone substrate and that as these are depleted, new cholesterol esters are obtained through an LDLR- and/or SR-BI-mediated mechanism. **(*Endocrinology* 150: 957–965, 2009)**

The midcycle surge of LH in primates signals the initiation of the 36- to 42-h periovulatory interval, during which time granulosa cells of the preovulatory follicle luteinize, the oocyte resumes meiosis, and the follicle wall ultimately ruptures to extrude a fertilizable oocyte (1–3). Part of the luteinization process is a rapid and dramatic increase in progesterone biosynthetic capacity; within 3 h of an ovulatory stimulus, intrafollicular concentrations of progesterone increase more than 40-fold relative to levels before human chorionic gonadotropin (hCG) (4).

Cholesterol necessary to initiate and sustain steroid synthesis can be obtained from *de novo* synthesis, from hydrolysis of stored cholesterol ester (CE) droplets, and/or from circulating or follicular fluid lipoproteins (5–8). The rapidity with which progesterone increases after hCG suggests that hydrolysis of stored CE (4, 9) supports the earliest increase in progesterone synthesis after hCG, whereas lipoprotein-derived CE forms the source for late periovulatory and luteal steroidogenesis.

It is currently unclear which lipoproteins and lipoprotein re-

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Abbreviations: CE, Cholesterol ester; hCG, human chorionic gonadotropin; HDL, high-density lipoprotein; IVF, *in vitro* fertilization; LDL, low-density lipoprotein; LDLR, LDL receptor; NLGC, nonluteinized granulosa cells; PVA, polyvinyl alcohol; r-hFSH, recombinant human FSH; SR-BI, scavenger receptor-BI; TL, Tyrode's lactate; VLDLR, very-low-density lipoprotein receptor.

ceptors mediate periovulatory progesterone synthesis. Conflicting data have been published using granulosa cells from women in *in vitro* fertilization (IVF) protocols and rhesus macaques undergoing controlled ovarian stimulation cycles to suggest that high-density lipoprotein (HDL) (women) or low-density lipoprotein (LDL) (women and macaques) is the preferred steroidogenic substrate (10–13). The issue of lipoprotein use is further confused by the fact that the scavenger receptor-BI (SR-BI) binds the apolipoprotein moiety of HDL and LDL to internalize CE, whereas the LDL receptor (LDLR) is specific to LDL particles (14, 15). Although no data exist in human or primate detailing changes in the expression of SR-BI during the periovulatory interval, SR-BI mRNA in human IVF-derived granulosa cells associates positively with estrogen levels at the time of oocyte retrieval (16). In contrast, the LDLR has been shown to be induced by an ovulatory stimulus in several species and the very-low-density lipoprotein receptor (VLDLR) is present in human granulosa cells (17–22), although the temporal relationship of either receptor to the onset of periovulatory progesterone has not been definitively determined. These data collectively indicate the potential importance and complexity of lipoprotein use during the periovulatory interval.

The goals of the current study are to determine the expression pattern of VLDLR, LDLR, and SR-BI in luteinizing granulosa cells and to establish the preferred type of lipoprotein (HDL, LDL, or VLDL) leading to progesterone synthesis by luteinizing macaque granulosa cells.

Materials and Methods

Animals

Adult female rhesus macaques (*Macaca mulatta*) were housed at the California National Primate Research Center as described previously (23). After the onset of menstruation, adult female rhesus monkeys were treated with recombinant human FSH (r-hFSH; Ares-Serono, Randolph, MA, or Organon, West Orange, NJ; 37.5 IU, im, twice daily) for 7 d. Antide (Ares-Serono; 5 mg/kg body weight, sc, single injection daily) was administered daily to prevent endogenous gonadotropin secretion. Follicles were aspirated the morning after the last dose of r-hFSH by an ultrasound-guided procedure as described previously (23), and the characteristics of the follicular cohort in this model have been described (24). The resulting cells are referred to as nonluteinized granulosa cells (NLGC). A subset of animals received an ovulatory bolus of r-hCG (1000 IU, sc, Ares-Serono) on the morning of d 8, and follicles were aspirated before (0 h) or 3, 6, 12, and 24 h after hCG (n ≥3 per time point). Aspirates representing the pooled contents of multiple follicles from each animal were maintained at approximately 35 C within a temperature-controlled isolette at all times. Oocytes were removed by transferring the aspirate to a 24-mm diameter, 70-μm pore size filter (Netwell Inserts 3479; Corning, Inc., Acton, MA), and the tube was rinsed with fresh Tyrode's lactate (TL)-HEPES/0.1 mg/ml polyvinyl alcohol (PVA) that was also poured onto the filter. This rinse was repeated until blood cells were removed from the filter. Granulosa cells were recovered by centrifugation of the cell suspension for 5 min at 300 × *g* to pellet the red cells and then increased to 500 × *g* for an additional 5 min, resulting in a thin layer of granulosa cells over the red cell pellet. The layer of granulosa cells was transferred to a 40% Percoll gradient in medium 199 (Sigma-Aldrich Corp., St. Louis, MO) and centrifuged for 30 min at 500 × *g*. The granulosa cells were recovered from the surface of the Percoll with a Pasteur pipette, washed twice with TL-HEPES-PVA, and centrifuged at 500 × *g* for 10 min. The cell pellet was resuspended in 1 ml TL-HEPES-PVA in a 15-ml centrifuge tube, and total cell number was determined on a hemocytometer. An additional 14 ml TL-HEPES-PVA was added to the cell suspension, and tubes were capped and sealed with Parafilm. The cells were shipped in a biohazard shipping container by overnight delivery at ambient temperature from September to June. All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of California Davis and the University of Maryland at Baltimore animal care and use committees.

Cell culture and treatments

Macaque granulosa cells were plated at 37 C with an initial seeding density of 2×10^5 viable cells per well in 24-well fibronectin-coated plates (Biocoat; Roche, Indianapolis, IN) in 400 μl DMEM/Ham's F-12 medium supplemented with 0.1× insulin-transferrin-selenium (Sigma), penicillin/streptomycin (50 U/ml), and 25 ng/ml hFSH (Sigma) with or without 20 IU/ml hCG (Sigma) to induce luteinization. Treatment groups included FSH or FSH+hCG in the presence or absence of VLDL, LDL, or HDL (Calbiochem, La Jolla, CA) for 24 h from the start of culture (0–24 h). Another group was cultured with FSH or FSH+hCG for 24 h in the absence of lipoproteins and then treated as above for the subsequent 24 h (24–48 h). Lipoprotein concentrations were 5, 25, and 50 nM or 1, 5, and 10 μg/ml based on cholesterol content. These concentrations are in the range reported for bovine and human follicular fluid (25, 26). The cholesterol content was provided by the manufacturer (HDL, 5.83 mg/ml; LDL, 7.56 mg/ml; VLDL, 4.81 mg/ml).

RIA

Progesterone and 17β-estradiol levels were determined using commercially available RIA kits (Siemens Healthcare Diagnostics Inc., Deerfield, IL). All samples were determined in a single assay. The within-assay variability was 0.50% and 0.51% for progesterone and 17β-estradiol, respectively.

RT-PCR

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) and reverse transcribed with Maloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time RT-PCR (Applied Biosystems, Inc., Foster City, CA) was performed for SR-BI, LDLR, and VLDLR. Primers and 6-carboxy fluorescein-labeled probes for the target gene of interest and carboxy-(VIC)-labeled probe for the endogenous control ribosomal protein L19 were synthesized by Applied Biosystems and used in the same reaction (Table 1). For relative quantification of mRNA levels, a standard curve was generated using a pool of H295R (human adrenocortical cell line) cDNA. The target gene was normalized to ribosomal protein L19.

Western blot

Total cell lysates from granulosa cells obtained before (0 h) or up 24 h after hCG were prepared using 5% SDS, 50 mM Tris-Cl (pH 7.6) buffer

TABLE 1. Real-time probe and primer sequences

Target	Primers/probe (5′–3′)
SRB1	
Forward	TTCCTCGAGTACCGCACCTT
Reverse	ATGTTGGGCATGACGATGTAGTC
6Fam-	CCTCCAAGTCCCACGGCTCGG
LDLR	
Forward	GGCAGTGTGACCGGGAATAT
Reverse	AGTCTCTAGCCATGTTGCAGACTTT
6Fam-	ACACTCTGCGAGGGACCCAACAAGTTC
VLDLR	
Forward	GAACCAAGAGGAAGTTCCTGTTTAA
Reverse	TTCACCCCAGTCTGACCAGTAA
6Fam-	TGACTTGCGAGAGCCTGCCTCCA

in the presence of protease inhibitor cocktail (1:100) and phenylmethylsulfonyl fluoride (1 mg/ml) (both from Sigma). Aliquots of the lysates (8 μg protein/lane) were subjected to SDS-PAGE in 4–10% gradient gels (Bio-Rad Laboratories, Hercules, CA) and then transferred onto polyvinylidene fluoride membranes overnight at 4 C. Blots were blocked with 5% milk for 1 h, incubated with polyclonal anti-SR-BI (Novus Biologicals) (1:3000), anti-LDLR (Abcam, Cambridge, MA) (1:200), or anti-VLDLR (Novus Biologicals, Littleton, CO) (1:600) at 37 C for 1 h, rinsed three times with TBS-0.1% Tween plus 5% milk, reacted with antirabbit horseradish peroxidase-labeled IgG (Cell Signaling, Danvers, MA; 1:3000) at room temperature for an additional hour and then rinsed three more times with TBS-0.1% Tween. Bands were visualized using an Amersham ECL chemiluminescence kit (GE Healthcare, Piscataway, NJ). Western analysis was performed on lysates from two animals per time point.

Statistical analysis

All data were tested for heterogeneity of variance with Bartlett's χ^2 test. Data not normally distributed were transformed to log+2 before analysis. Levels of mRNA were analyzed by one-way ANOVA. Data for steroid measurements are presented as the percentage of gonadotropin (FSH or FSH+hCG) controls to normalize the typical variability that exists between nonhuman primates and analyzed by repeated-measures two-way ANOVA followed by Bonferroni post-tests. Differences were considered significant when $P < 0.05$. All data are presented as mean ± SEM from three to four samples unless otherwise stated.

Results

Lipoprotein receptor levels in response to hCG *in vivo* and *in vitro*

Total RNA was isolated from granulosa cells aspirated before (0 h) and 3, 6, 12, and 24 h after an ovulatory hCG bolus to macaques undergoing controlled ovarian stimulation cycles. Levels of SR-BI, LDLR, and VLDLR mRNA were analyzed by real-time RT-PCR (Fig. 1). SR-BI mRNA was present in all samples before hCG but increased 23- and 30-fold by 12 and 24 h after hCG ($P < 0.05$). LDLR mRNA was increased significantly (7-fold, $P < 0.05$) 3–12 h after hCG, declining to pre-hCG levels by 24 h (Fig. 1). In contrast, VLDLR mRNA decreased by 140-fold within 3 h of hCG injection ($P < 0.05$) and remained at that level throughout 24 h (Fig. 1).

To verify changes in lipoprotein receptor expression during *in vitro* luteinization, NLGC were cultured in the presence of FSH or FSH+hCG for 1, 3, 6, 12, 24, and 48 h. Treatment of cells with FSH+hCG increased SR-BI mRNA 9-fold ($P < 0.05$) at 6 h compared with time-matched FSH and remained elevated throughout the experiment (Fig. 1). LDLR mRNA increased 3–12 and 48 h after FSH+hCG treatment compared with FSH (3-, 5-, 2.4-, and 6-fold at 3, 6, 12, and 48 h, respectively; $P < 0.05$; Fig. 1). VLDLR mRNA did not change after FSH+hCG treatment with the exception of a 2-fold decrease ($P < 0.05$) at 12 h compared with FSH only (Fig. 1).

Protein levels of SR-BI measured by Western blot increased qualitatively 6 h after hCG administration *in vivo* and appeared to peak at 12 h with appreciable protein present at 24 h. LDLR increased 3 h after hCG and remained elevated throughout the study interval (Fig. 2A). Because VLDLR mRNA expression is rapidly and dramatically decreased after hCG (Fig. 1) and, importantly, to conserve limited and difficult to obtain 3-, 6-, and 12-h samples, VLDLR protein was assessed only before (0 h) and 24 h after hCG *in vivo*. Levels of VLDLR protein were markedly reduced 24 h after the ovulatory hCG bolus (Fig. 2B).

Lipoprotein support of steroid synthesis in vitro: progesterone

Progesterone and estrogen concentrations in culture media after FSH and FSH+hCG are listed in Table 2. After the initial 24 h culture (0–24 h), progesterone levels increased 25-fold ($P < 0.05$) in FSH+hCG- *vs.* FSH-treated samples, whereas during the second 24-h interval (24–48 h), levels of progesterone were not different between FSH and FSH+hCG. Progesterone synthesis did not change in FSH-treated samples between 0–24 and 24–48 h, whereas media progesterone levels decreased 3.4-fold in FSH+hCG samples at 0–24 and 24–48 h.

To understand lipoprotein support of steroidogenesis during luteinization, monkey granulosa cells were cultured in serum-free media and treated with HDL, LDL, and VLDL based on molar concentration during the initial 24 h culture (0–24 h) in the presence of FSH or FSH+hCG. In the absence of lipoprotein support, progesterone levels increased 25-fold ($P < 0.05$) after hCG treatment relative to FSH only (Fig. 3A and Table 2). In the presence of FSH alone, lipoproteins did not significantly alter media levels of progesterone. In the presence of FSH+hCG, the 25- and 50-nM doses of LDL and VLDL increased progesterone relative to FSH+hCG control, whereas HDL did not increase progesterone synthesis (Fig. 3A).

To deplete stored CE, cells were treated with FSH or FSH+hCG for 24 h before the addition of lipoproteins for an additional 24 h (24–48 h). Basal levels of progesterone were not different between FSH and FSH+hCG during the 24- to 48-h interval (Fig. 3B and Table 2). All doses of LDL and VLDL increased progesterone relative to FSH+hCG controls, and HDL did not result in changes to media progesterone.

Because HDL, LDL, and VLDL carry different amounts of CE, the above study was repeated with doses based on mass of cholesterol for each lipoprotein. During the initial 24-h interval (0–24 h), lipoproteins did not support progesterone synthesis, although there was a tendency for progesterone to increase with 10 μg/ml LDL and VLDL relative to FSH+hCG alone ($P = 0.06$ and 0.09, respectively; Fig. 3C). During the 24- to 48-h culture interval, 1, 5, and 10 μg/ml LDL and 5 and 10 μg/ml VLDL increased progesterone relative to controls ($P < 0.05$), although there was a trend for HDL to increase progesterone at the 10 μg/ml dose ($P = 0.06$; Fig. 3D).

Lipoprotein support of steroid synthesis *in vitro*: estrogen

Estrogen levels were not different between FSH and FSH+hCG during the first 24-h interval and declined 15-fold ($P < 0.05$) as a result of hCG treatment during the 24- to 48-h culture period. Estrogen did not change 0–24 h compared with 24–48 h during FSH treatment but was reduced 17-fold ($P < 0.05$) 24–48 h compared with 0–24 h in the FSH+hCG group (Table 2).

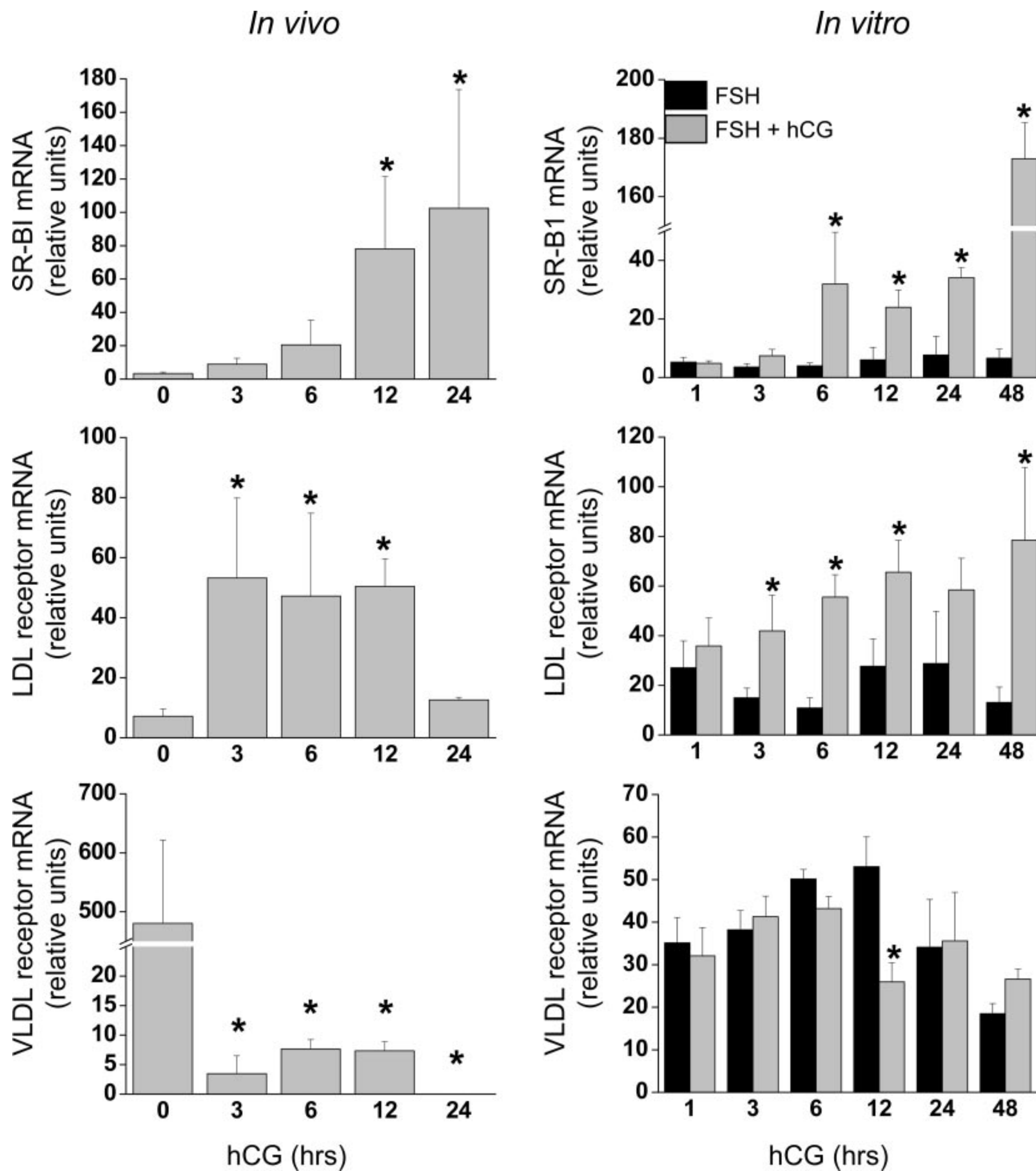

FIG. 1. Expression of SR-BI, LDLR, and VLDLR mRNA during luteinization. *In vivo* (*left panels*), granulosa cells were isolated from rhesus monkeys undergoing controlled ovarian stimulation protocols before (0 h) and 3, 6, 12, or 24 h after an ovulatory hCG stimulus (n = 4, 4, 3, 3, 4). A model of *in vitro* (*right panels*) luteinization was used in which cells aspirated before an ovulatory stimulus were cultured in the presence of FSH to maintain a nonluteinized phenotype or FSH+hCG to induce luteinization (n = 3). Total RNA was isolated for use in real-time RT-PCR. *, Significantly different from 0 h (*in vivo*) or time-matched FSH controls (*in vitro*).

During the initial 24-h interval (0–24 h), media concentrations of estrogen in the presence of FSH and FSH+hCG tended to increase with the highest dose of HDL and LDL (Fig. 4, A and C; note that the samples in Fig. 3C reflect n = 2). In the subsequent 24-h period (24–48 h), 50 nM HDL and 25 and 50 nM LDL increased ($P < 0.05$) estrogen levels in the presence of FSH alone (Fig. 4B), and 5, 25, and 50 nM LDL significantly ($P < 0.05$) increased estrogen after FSH+hCG treatments (Fig. 4B). Balancing treatments based on cholesterol content during the 24- to 48-h interval resulted in a significant increase in estrogen with FSH treatment in the presence of 5 and 10 μg/ml LDL and 10 μg/ml VLDL, whereas only the highest dose of LDL elicited an increase in estrogen during FSH+hCG exposure (Fig. 4D).

Discussion

In rhesus monkeys undergoing controlled ovarian stimulation cycles, serum progesterone doubles within 15 min in response to the ovulatory hCG stimulus and remains at high levels until the initiation of luteolysis (4, 9, 27). The remarkable levels of progesterone produced during the luteal phase of the menstrual cycle suggest a profound adaptation of the corpus luteum to use circulating lipoproteins as a source of cholesterol (28, 29). Data from the current study support the hypothesis that steroidogenesis during luteal development occurs in a two-step process in which hCG promotes the use of stored CE leading to the initial rapid increase in progesterone and also increases the expression of SR-BI and LDLR as a means to ensure cholesterol substrate for

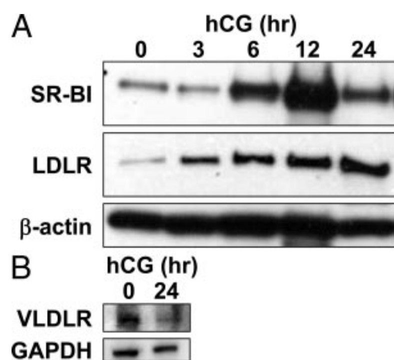

FIG. 2. Changes in SR-BI, LDLR, and VLDLR protein during luteinization. Granulosa cells were isolated from rhesus monkeys undergoing controlled ovarian stimulation protocols before or up to 24 h after an ovulatory hCG stimulus (n = 2). Western blot analysis was used to detect SR-BI, LDLR (A), or VLDLR (B), and blots were reprobed with β-actin (SR-BI and LDLR) or GAPDH (VLDLR) as a loading control.

luteal progesterone production. Interestingly, LDL (and to a lesser extent VLDL), but not HDL, is the preferred substrate for progesterone synthesis during luteinization *in vitro*.

All three of the major lipoprotein receptors (VLDLR, LDLR, and SR-BI) are expressed by macaque granulosa cells. However, an ovulatory stimulus rapidly and profoundly reduces levels of VLDLR mRNA and protein (21). The expression of VLDLR has been shown to be suppressed by cAMP in trophoblast cells in a sterol-independent manner (30); this is consistent with the rapid reduction of granulosa cell VLDLR mRNA levels *in vivo*. In contrast, the expression of VLDLR mRNA *in vitro* was largely unchanged with hCG treatment; given the relative levels of VLDLR mRNA before hCG *in vivo* (~500) *vs. in vitro* (~35), it is hypothesized that expression *in vitro* was reduced in response to culture rather than hormonal treatment. The expression of VLDLR mRNA in nonluteinized granulosa cells *in vivo* appears to be promoted by an endocrine/paracrine factor in the follicular fluid that is missing from culture, possibly IGFs or member(s) of the TGFβ family. The expression of LDLR mRNA increased within 3 h of hCG, both *in vivo* and *in vitro*. This is consistent with a number of other models, including macaque, cultured human, and porcine granulosa cells (17–19, 22, 31). Interestingly, Miranda-Jimenez and Murphy (32) recently showed a decrease in LDLR protein in porcine granulosa cells during the transition from large, nonluteinized follicles to early corpora lutea. The basis for this difference is not clear, although given the limited follicular LDL in both porcine and human (33–35), the data would argue against a major role for the LDLR in periovulatory events.

SR-BI expression also increases rapidly after an ovulatory hCG bolus. There is evidence in rats and humans that SR-BI mediates the selective uptake of both HDL-CE and LDL-CE (36), potentially making SR-BI a key component of periovulatory and luteal steroidogenesis. Consistent with a role for SR-BI in periovulatory steroidogenesis, Velasco *et al.* (16) reported that women with lower SR-BI expression in granulosa cells have reduced levels of estrogen at the time of follicle aspiration during IVF cycles. SR-BI mRNA is expressed at high levels in theca cells of preovulatory rat follicles and is only apparent in granulosa cells a full 24 h after an ovulatory hCG injection (37, 38). A similar 24-h latency from hCG to increased SR-BI expression has been reported in bovine granulosa cells, whereas SR-BI protein is first apparent in porcine granulosa cells around 30 h after hCG (32, 39). It is noteworthy that SR-BI protein apparently increases before a significant increase in mRNA (6 *vs.* 3 h, respectively), suggesting that an ovulatory stimulus increases the rate of translation or enhances the stability of SR-BI as a means to rapidly increase protein levels. Further studies are clearly warranted in support of this observation. In primates undergoing controlled ovarian stimulation protocols, follicular fluid progesterone is increased markedly within 3 h of an ovulatory hCG bolus (4) and clearly precedes the increase in SR-BI mRNA and protein, suggesting that this receptor does not mediate the initial uptake of CE after hCG leading to the initial increase in steroidogenesis but rather supports later periovulatory and luteal function.

The short latency between an ovulatory hCG bolus to measurable changes in circulating progesterone suggests strongly the activation of latent factors leading to the mobilization of cholesterol such as lipoprotein or hormone-sensitive lipase (9, 40, 41). Nonluteinized granulosa cells have the capacity to synthesize substantial amounts of progesterone in response to hCG *in vitro* without the provision of an exogenous source of cholesterol for 24 h. Although there are limited data quantifying stored CE in nonluteinized granulosa cells, Seachord *et al.* (42) recently showed the presence of a limited number of small lipid droplets in granulosa cells before hCG. These stored CE are hypothesized to be used up rapidly under hCG stimulation, and in order for subsequent periovulatory and luteal steroidogenesis to occur, a source of CE must be available. These data suggest that hCG/LH-induced progesterone synthesis occurs in two steps: lipase-mediated mobilization of CE and the subsequent recovery of CE via lipoproteins.

Because fully luteinized granulosa cells are laden with lipid droplets (42), the use of these cells to determine the preferential use of lipoprotein as an early source of cholesterol for steroidogenesis may not be ideal. The present *in vitro* studies used 1) nonluteinized granulosa cells from macaques to determine lipoprotein usage at early time points during luteinization and 2) luteinizing granulosa cells with depleted CE stores. Progesterone synthesis during FSH treatment is low regardless of the presence or absence of lipoproteins, consistent with the concept that hCG has multiple effects on steroidogenesis. During the first 24 h of hCG treatment, progesterone was modestly augmented by LDL and VLDL. Presumably, progesterone synthesis is largely sup-

TABLE 2. Steroid concentrations after FSH or FSH+hCG for 24 h

	FSH	FSH+hCG
Progesterone (ng/ml)		
0–24 h	41.4 ± 6.7[a]	1038.3 ± 95.2[b]
24–48 h	111.3 ± 20.4[a]	304.0 ± 76.5[a]
Estrogen (pg/ml)		
0–24 h	40.8 ± 35.1[a]	127.5 ± 85.0[a]
24–48 h	114.8 ± 82.4[a]	7.5 ± 7.5[b]

[a] and [b] Significant differences between treatments.

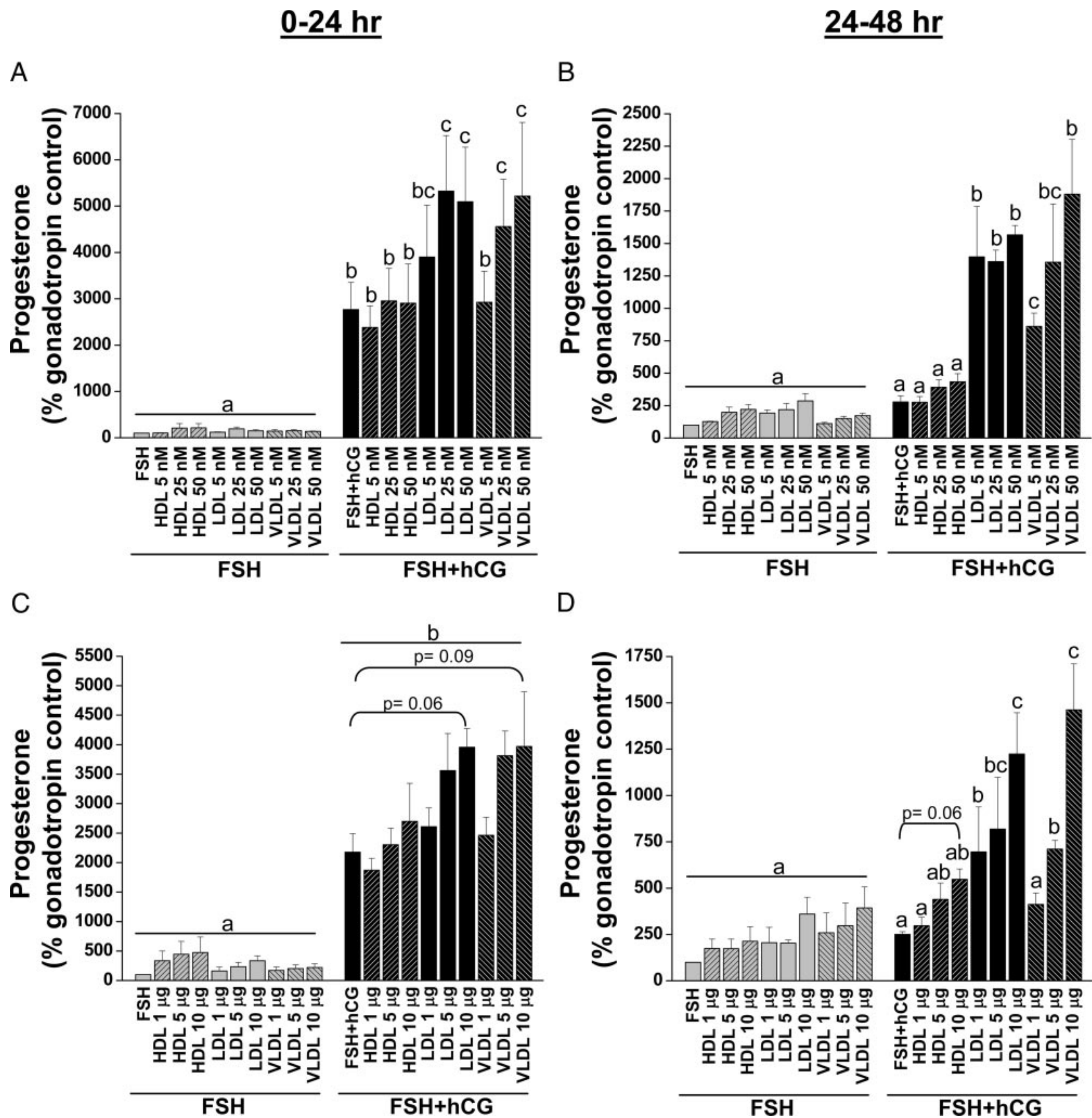

FIG. 3. The effects of exogenous lipoproteins on progesterone synthesis during luteinization *in vitro*. Nonluteinized granulosa cells were isolated from rhesus monkeys undergoing controlled ovarian stimulation before an ovulatory stimulus. Cells were held in culture for 24 h in the presence of FSH±hCG with or without lipoproteins HDL, LDL, and VLDL for 24 h from the start of culture (0–24 h) (A and C). Another group of cells was cultured in FSH±hCG for 24 h and then treated as above for the next 24 h (24–48 h) (B and D). Lipoprotein concentrations were based on molarity (5, 25, and 50 nM) (A and B) or based on mass of cholesterol/volume (1, 5, and 10 μg/ml) (C and D). Progesterone concentrations were determined by RIA and all data expressed as the percentage of the FSH-only treatment. *Different superscript letters* indicate significant differences ($P < 0.05$) between treatments as determined by two-way ANOVA (one repeated measure) ($n = 3$).

ported by CE stores at this time, and thus cholesterol substrate is not the limiting resource. During the subsequent 24 h, *i.e.* after stored CE is exhausted, LDL and VLDL support progesterone synthesis, whereas HDL is not effective. It is not clear why HDL is less effective than molar equivalents of LDL, although Brannian and Stouffer (11) reported a similar finding in fully luteinized macaque granulosa cells. The use of human granulosa cells derived from IVF settings has proven equivocal on this topic. Tureck and Strauss (43) suggested that LDL, but not HDL, promoted progesterone production, whereas other studies have shown that VLDL, LDL, and HDL are effective (10, 21). One intriguing possibility is that the use of specific lipoprotein species as steroidogenic substrates evolved to favor the less abundant (in follicular fluid) but cholesterol-rich LDL over the more abundant but cholesterol-poor HDL. It is noteworthy that follicular fluid HDL is particularly deficient in cholesterol content compared with serum HDL (26) and thus likely represents a poor steroidogenic substrate.

Estrogen levels tend to be increased by lipoproteins in the presence of either FSH or FSH+hCG during the initial 24 h. During the second 24-h interval, principally LDL supported es-

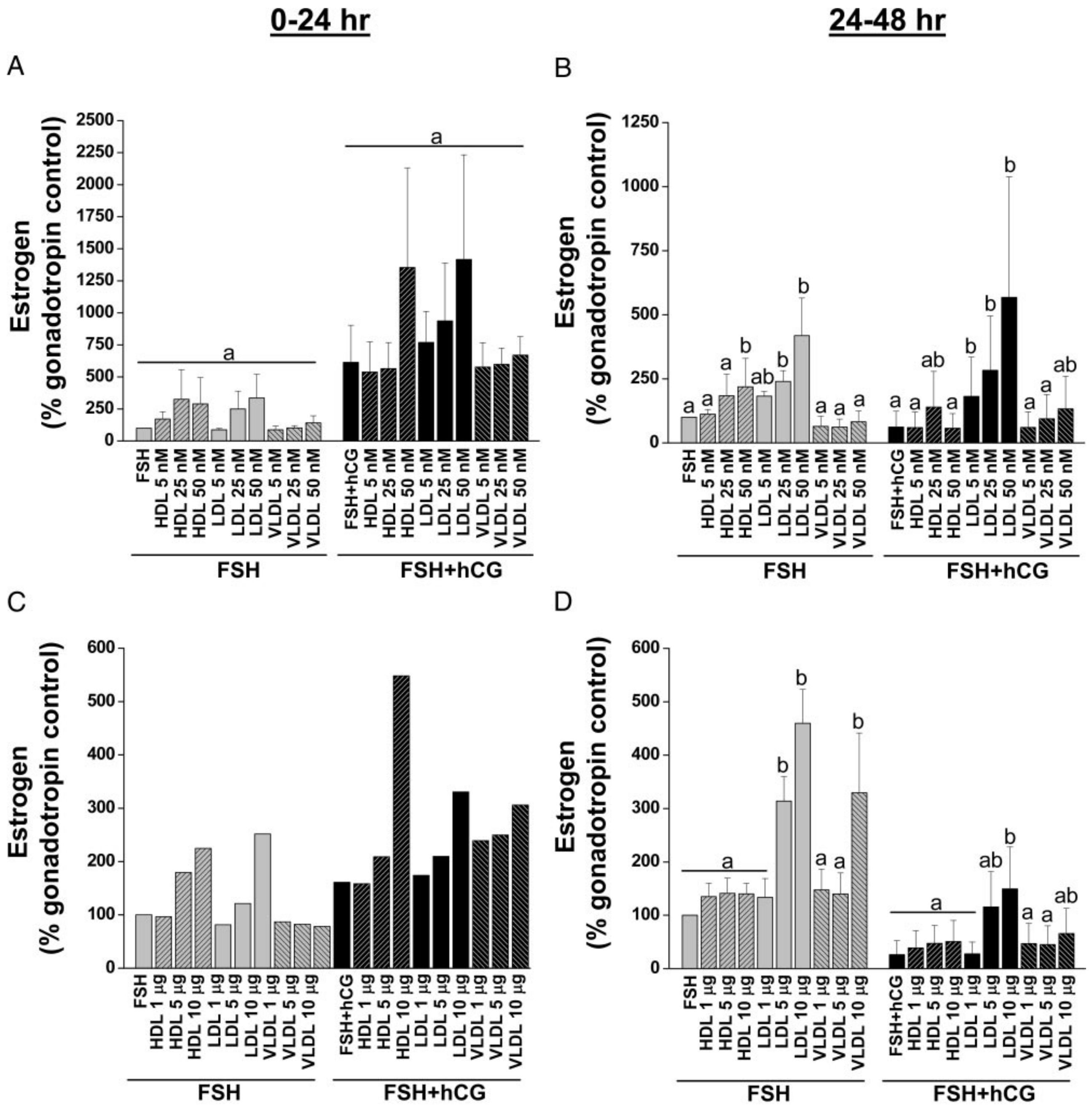

FIG. 4. The effects of exogenous lipoproteins on estrogen synthesis during luteinization *in vitro*. Nonluteinized granulosa cells were isolated from rhesus monkeys undergoing controlled ovarian stimulation before an ovulatory stimulus. Cells were held in culture for 24 h in the presence of FSH±hCG with or without lipoproteins HDL, LDL, and VLDL for 24 h from the start of culture (0–24 h) (A and C). Another group of cells was cultured in FSH±hCG for 24 h and then treated as above for next 24 h (24–48 h) (B and D). Lipoprotein concentrations were based on molarity (5, 25, and 50 nM) (A and B) or based on mass of cholesterol/volume (1, 5, and 10 μg/ml) (C and D). Estrogen concentrations were determined by RIA and all data expressed as the percentage of the FSH-only treatment. Different *superscript letters* indicate significant differences ($P < 0.05$) between treatments as determined by two-way ANOVA (one repeated measure) (n = 3, except C in which n = 2).

trogen synthesis in the presence of both FSH and hCG. It is not entirely clear by what mechanism LDL was taken up in the presence of FSH, although SR-BI protein is clearly detectable in nonluteinized granulosa cells; this may be the mechanism whereby LDL, and to a lesser extent VLDL, support estrogen synthesis before hCG. It is possible that sufficient levels of combined LDLR and SR-BI protein are present to mediate uptake of CE from exogenous LDL, although this remains to be determined. Importantly, for both estrogen and progesterone, balancing lipoproteins by concentration of CE (nanomolar) and lipoprotein mass yielded qualitatively similar results. These data indicate that macaque granulosa cells in the early phases of luteinization (<36 h after hCG) preferentially use LDL more than VLDL more than HDL to support hCG-mediated progesterone production.

Before an ovulatory stimulus, levels of HDL in follicular fluid are much higher than LDL (33–35); given that macaque granulosa cells do not appear to efficiently use HDL as a steroidogenic

precursor, this could suggest alternative functions for HDL particles, for example, apolipoprotein A1-mediated signaling (*cf.* Ref. 44). After LH/hCG, the breakdown of the basement membrane ensues with attendant vascular permeability (45, 46), potentially providing a source of serum-derived LDL to luteinizing granulosa cells. Although nonluteinized granulosa cells appear to possess enough stored CE to promote the initial increase in progesterone that occurs within the first several hours of the ovulatory stimulus, the expression of LDLR and SR-BI mRNA increases soon thereafter, and late periovulatory and luteal steroidogenesis likely relies on serum-borne lipoproteins. Because a mix of LDL and HDL is present in serum and presumably available to steroidogenic luteal cells of the mature corpus luteum, SR-BI is hypothesized to play a critical role in luteal CE uptake. Overall, these data support the hypothesis of a two-step process leading to periovulatory progesterone synthesis in the macaque in which rapid CE hydrolysis is the initial response to LH/hCG, and lipoprotein-CE uptake is the sustaining mechanism.

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