Low expression of COX-2, reduced cumulus expansion, and impaired ovulation in SULT1E1-deficient mice

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ABSTRACT **The SULT1E1-encoded estrogen sulfotransferase (EST) catalyzes sulfation of estrogen, resulting in its inactivation. Reduced fertility observed in SULT1E1 knockout (KO) female mice has previously been attributed to the deleterious effect of chronic exposure to high levels of circulating estrogen on placental function. We herein suggest that, in addition to placental dysfunction, this phenotype demonstrates that an excess of estrogen impairs ovulation. The role of SULT1E1 in ovulation is suggested by the substantially low ovulatory response in hCG-treated SULT1E1 KO mice; a similar effect was observed when 17β-estradiol was administered to wild-type (WT) females. The normal rate of ovulation in SULT1E1 KO females may be restored by PGE2. Along this line, ovaries of human Chorionic Gonadotropin (hCG)-treated SULT1E1 KO mice expressed low levels of cyclooxygenase-2 (COX-2) and its downstream TSG6; moreover, their ovaries contained a reduced number of expanded cumuli. Our results demonstrate, for the first time, that estrogen inactivation may allow the expression of COX-2 and subsequent cumulus expansion, enabling normal ovulation. Our findings may be applied to novel treatments of human ovulatory failure.—Gershon, E., Hourvitz, A., Reikhav, S., Maman, E., Dekel, N. Low expression of COX-2, reduced cumulus expansion, and impaired ovulation in SULT1E1-deficient mice. *FASEB J.* 21, 1893–1901 (2007)**

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The involvement of estrogen in ovulation was proposed by studies of different knockout (KO) mouse models. Specifically, the ovaries of ERβ null mutants contain fewer corpora lutea, a phenotype that apparently represents less frequent ovulated oocytes and is further manifested by subfertility (1). Similarly, null mice lacking aromatase [aromatase knockout mice (ARKO)], the enzyme that converts androgen to estrogen, fail to ovulate (2). In view of its major role in ovulation, it is widely believed that estrogen is subjected to strict regulation.

It is not only the production of estrogen but also the availability of its active form that is regulated. One of the controls on estrogen activity is elicited by the sulfotransferase family members. Steroid sulfotransferases are cytosolic enzymes that catalyze the sulfonation of hydroxyl groups, using 3′-phosphoadenosine-5′-phosphosulfate as a sulfate donor (3). The resulting sulfated steroids become hydrophilic, are unable to cross the membrane to reach their receptors, and are thus rendered inactive. Two types of steroid sulfotransferases can be distinguished biochemically: hydroxysteroid sulfotransferases (HSST) that bind primarily to dehydroepiandrosterone (DHEA) and to pregnenolone and estrogen-specific sulfotransferases (4). HSSTs are expressed primarily in the liver (5, 6, 7), whereas the tissue distribution of estrogen-specific sulfotransferases (EST) includes various reproductive organs (8, 9, 10).

Recently, a new EST gene, SULT1E1, was cloned and characterized in mice testicular Leydig cells (11). This enzyme, initially defined as testis-specific, was later found in the liver of humans and in male mice and rats (12). Male mice lacking the SULT1E1 gene exhibit an age-related phenotype: specifically, testes from mice more than 12 mo old display abnormal structures, including hyperplasia and hypertrophy, and significantly lower sperm motility is also observed. In connection with that, treating young knockout males with estradiol accelerated the appearance of this phenotype (13). Furthermore, Leydig cells from mice lacking SULT1E1 produced only 30–50% of the amount of testosterone made by wild-type cells, due to a reduction in 17α-hydroxylase-17,20-lyase activity (14). The impaired sperm motility in older males, coupled with the fact that in these mice the seminiferous tubule was also damaged (13), led to the notion that this enzyme is expressed in other parts of the male reproductive tract as well. A more recent study (15) indeed demonstrated expression of SULT1E1 in the epididymis and in the vas deferens.

In previous studies, SULT1E1 was not detected in the ovaries of female mice but rather in the late-stage placenta and pregnant uterus (13, 16, 17). Therefore,

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subfertility observed in SULT1E1 knockout (SULT1E1 KO) females was attributed to placental thrombosis and, in fact, placental degeneration was demonstrated in these females. Similar placental defects were induced in wild-type (WT) animals exposed to high doses of estrogen (18). Ovarian expression of SULT1E1 was later detected in rat (19) and equine (20) that had been administrated by human Chorionic Gonadotropin (hCG).

In the present study, we employed an ovulation-selective cDNA library constructed by the suppression subtractive hybridization (21) and found that SULT1E1 is expressed in mouse ovaries; furthermore, this expression is stimulated by an ovulatory dose of hCG. We characterized the temporal and spatial patterns of expression of both SULT1E1 mRNA and its protein product, EST, in ovarian follicles and investigated its role in ovulation. Based on our results, we conclude that down-regulation of estrogen activity by EST enables the Luteining Hormone (LH)/hCG-induced expression of cyclooxygenase-2 (COX-2) and its downstream TSG6, which in turn stimulates the cumulus expansion that is essential for normal ovulation.

MATERIALS AND METHODS

In vivo protocols

Sexually immature, 24-day-old female C57BL/6 mice and WISTAR rats were purchased from Harlan (Haralan Laboratories, Rehovot, Israel). SULT1E1 KO females were kindly provided by Dr. W. Song (University of Pennsylvania School of Medicine). The animals were injected with 5 IU of pregnant mare serum gonadotropin (PMSG; Chrono-gest Intervest), followed by hCG, administrated 48 h later. Ovulation was examined in females killed 20 h after treatment with hCG, as described previously (22, 23). Ovaries isolated at various time points either before or after administration of hCG were subjected to the indicated analysis described below.

To examine SULT1E1 expression during the estrous cycle, ovaries from sexually mature, cycling female Wistar rats (7–9 wk old) were used. The specific stages of the estrous cycle were monitored by microscopic analysis of the vaginal cell population. Only rats showing at least three consecutive 4 day cycles were studied.

All experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council, National Academy of Sciences, Bethesda, MD, USA).

Indomethacin and RU486 treatments

Two subgroups of mice (n=5 each) treated with PMSG and hCG were injected with either indomethacin (ICN, Irvine, CA, USA) or RU486 (Sigma, Rehovot, Israel), prepared as described previously (22, 25). Indomethacin (0.7 mg/animal) and RU486 (0.5 mg/animal) were injected subcutaneously 3 h after treatment with hCG. Ovaries were extracted 6 h after hCG administration.

17β-Estradiol and prostaglandin E2 administration

A group of C57bl female mice (n=5) treated with PMSG and hCG was injected with or without 17β-estradiol (5 μg/animal, kindly provided by Dr. Fourtona Kohen) 3 h after hCG administration. Ovulation rate was determined as described above.

PGE_2 first dissolved in ethanol and then diluted in saline was injected subcutaneously into SULT1E1 KO mice (40 μg/mouse), concurrent with hCG administration, followed by a second injection of PGE_2, 3 h later. Ovulation rate was determined as described above.

RNA isolation

Total RNA was isolated from the ovaries of immature 25-day-old C57BL/6 female mice undergoing the ovulation induction protocol described above. Total RNA was isolated from the following nonovarian tissues of these mice: brain, heart, kidney, liver, spleen, stomach, small intestine, large intestine, adrenal glands, uterus, muscle, and lung. Total RNA from the testis was extracted from mature C57BL/6 male mice. Total RNA was isolated using Tri-Reagent (Sigma), according to the manufacturer's instructions.

Northern blot analysis

Northern blot analysis was preformed as described previously (21). Briefly, total RNA (20 μg) was separated on denaturing 1% agarose-formaldehyde gels and transferred to nylon membranes (Magna Graph, MSI, Westboro, MA, USA) that had been prehybridized for 2 h, followed by hybridization overnight with SULT1E1 probe at 42°C. Membranes were then sequentially washed and exposed to a phosphor screen (Molecular Images System; Bio-Rad, Hercules, CA, USA). The resulting digitized data were analyzed with Molecular Analyst software (Bio-Rad).

Semiquantitative reverse transcriptase-polymerase chain reaction

First-strand cDNA was synthesized by mixing 7.5 μg total RNA and 1 μl of oligo $(dT)_{12–18}$ (Promega, Madison, WI, USA) in water to a final volume of 12 μl. The mixture was then heated to 70°C for 2 min, and the reaction finally quenched on ice for 2 min. Reverse transcription reactions were carried out by adding 4 μl of MMLV-RT 5× buffer (Promega), 10 mM deoxynucleotide triphosphates (dNTP), 40 U of RNasin, an RNase inhibitor (Promega), and 200 U of Moloney murine leukemia virus reverse transcriptase (MMLV reverse transcriptase, Promega). This mixture was incubated at 37°C for 2 h.

cDNAs corresponding to various experimental time points or tissue types were used for polymerase chain reaction (PCR) amplification. A primer set was included for β-actin (0.5 μM each; forward primer, 5′-CCCCATTGAACATGGCATTGTTAC-3′; reverse primer, 5′-TTGATGTCACGCACGATTTCC-3′) or SULT1E1-primer sequences (0.5 μM each; forward primer, 5′-CTTCCAGTATCATTTTGGGAAAAG-3′; reverse primer, 5′- TGGATTGTTCTTCATCTC-3′) in a 25 μl reaction volume with 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1%Triton X-100 (Promega), 2.5 mM $MgCl_2$, 400 μM each D-NTP and 0.625 U of *Taq* DNA polymerase (Promega). PCR was performed for 29 cycles (initial denaturation at 94°C for 3 min, then 29 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and a final incubation at 72°C for 7 min). The number of cycles used ensured that the reaction could be quantified within the log phase of the amplification reaction. The reaction mix (24 μl) was run on a 1.5% agarose gel, and the PCR products were stained with ethidium bromide using ultraviolet imaging (Gel Doc 1000, Bio-Rad).

In situ hybridization

In situ hybridization was performed as described previously (21). Briefly, mouse ovaries obtained from immature gonadotropin-primed animals at the indicated time points were immediately fixed in 4% paraformaldehyde. Paraffin-embedded tissues were sectioned (5 μm). Sections were hybridized in 100 μl of a hybridization solution [1 ml of 10× salt solution (22.8 g NaCl, 2.8 g Tris-HCl, 0.68 g Tris base, 1.38 g $NaH_2PO_4H_2O$, 1.42 g NaH_2PO_4, and 20 ml 0.5 M EDTA), 5 ml formamide, 2 ml 50% dextran sulfate, 1 ml yeast tRNA (10 mg/ml), and 200 μl 50× Denhardt's, containing 1 μg/ml of DIG-labeled SULT1E1 mouse antisense probe]. Sections were stained using anti-DIG antibody (1:1000; Roche, Basel, Switzerland) conjugated to alkaline phosphatase, washed, and incubated at 37°C with chromogen (Zymed, San Francisco, CA, USA) until the color appeared. The sections were then examined under an E-800 microscope (Nikon, Melville, NY, USA).

Antibodies and Western blot analysis

Anti-SULT1E1 and COX-2 antibodies were purchased from Biomeda (Foster City, CA, USA) and Upstate (Lake Placid, NY, USA), respectively. Anti-PR was kindly provided by Dr. Fortuna Kohen (Weizmann Institute of Science). Total ovarian proteins were extracted, and Laemmli buffer (125 mM Tris, pH 6.8, 4% SDS, 10% glycerol, 0.006% bromphenol blue, and 2% β-mercaptoethanol) was added. The samples were then separated on a 12% acrylamide gel, followed by their transfer to a nitrocellulose membrane. After blocking with 10% skimmed milk, the membranes were incubated with the primary antibodies overnight at 4°C, then with the secondary antibodies for 1 h at room temperature. The immunoreactive bands were detected by ECL (Amersham, Buckinghamshire, England). Anti-total-MAPK antibodies (Sigma) or anti-β-tubulin antibodies (Sigma) were used as a marker for equal protein loading. The intensity of the signal was quantified by computerized densitometry (quantity one software).

Immunofluorescence

Immunofluorescence assays were performed according to the manufacturer's instructions. The sections were deparaffinized, washed in tap water and TBST (0.1 m Tris, pH=7.2, 0.15 M NaCl, and 0.1% Tween-20), and blocked in 3% fetal calf serum in TBST for 30 min. The sections were then incubated with anti-SULT1E1 antibody (2 μg/ml, Biomeda) overnight at 4°C. Sections were washed with TBST and immonureacted with the Alexa 488-conjugated secondary antibody for 1 h at room temperature, washed again with TBST, and visualized using fluorescence microscope (Eclipse, Nikon).

Real-Time PCR

Real-time PCR analysis was performed as described previously (24). Briefly, the following reaction protocol was employed: 15 min at 95°C for enzyme activation, followed by 45 cycles of: 15 s at 95°C, 30 s at 55°C, and 15 s at 72°C, at the end of which fluorescence was measured with a Rotor-Gene (Corbett Research, Sydney, Australia). All reactions contained the same amount of cDNA, 10 μl Absolute QPCR Master Mix, primers, and UltraPure PCR-grade water (Fisher Biotec, Subiaco, Australia) to a final volume of 20 μl.

Statistical analysis

Each experiment was carried out at least three times, using at least three mice at each time point. Data points are mean ± SE. Statistical significance (Fisher's protected least significance difference) was determined by ANOVA to assess differences between mean densities, adjusted for the average background density. All analyses were performed using Statview for Macintosh (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Identification of SULT1E1 in the SSH ovulation-selective cDNA library

In our ovulation-selective cDNA library, 13 different clones were identified as SULT1E1 mRNA (21). This cDNA fragment is highly homologous (E-value=4e-86) to a segment of the mouse SULT1E1 gene (accession numbers S78182, BC034891, NM023135, and AK015750) that was originally cloned in male Mus Musculus testes and epididymis and later in the uterus of pregnant females and liver of obese and diabetic animals (11, 12, 13).

Ovulatory expression of mouse and rat SULT1E1 mRNA and its protein product

In line with previous results (11, 13), we were unable to detect SULT1E1 in ovaries of mice that had not been treated by gonadotropins. However, we did demonstrate a significant increase ($P<0.05$) in SULT1E1 mRNA expression in the ovaries of PMSG-primed mice that were further administered with hCG. Specifically, we demonstrated that SULT1E1 mRNA expression peaked 6 h after hCG administration and substantially decreased 2 h later. (**Fig. 1*A***). The presence of SULT1E1 mRNA could not be detected during the luteal phase (24 and 48 h after hCG injection; data not shown).

Ovulatory-specific expression of the rat homologue of SULT1E1 (designated STE) was recently reported in the literature (19). We corroborated and extended these findings, showing that, similar to findings in the mouse, SULT1E1 mRNA expression in the rat is undetectable 48 h after PMSG but is expressed 6 h after hCG injection (Supplemental Fig. S1). In addition, real-time PCR analysis of rat ovaries at the various stages of the estrous cycle revealed that SULT1E1 mRNA expression, undetectable during diestrus, sharply increased on the evening of proestrus, soon after the preovulatory surge of LH. This increase is followed by a mild reduction on the night between proestrus and estrus, the time when ovulation occurs. Rat SULT1E1 mRNA expression could not be detected on the day of estrous (**Fig. 2*A***).

The expression of the SULT1E1 protein product EST was also ovulatory dependent: although absent in PMSG-primed mice and rat ovaries, it could be initially detected 4 h after hCG administration (Figs. 1*B*, 2*B*). This protein reached its maximal levels at 6 h and remained high for at least an additional 2 h (Figs. 1*B*,

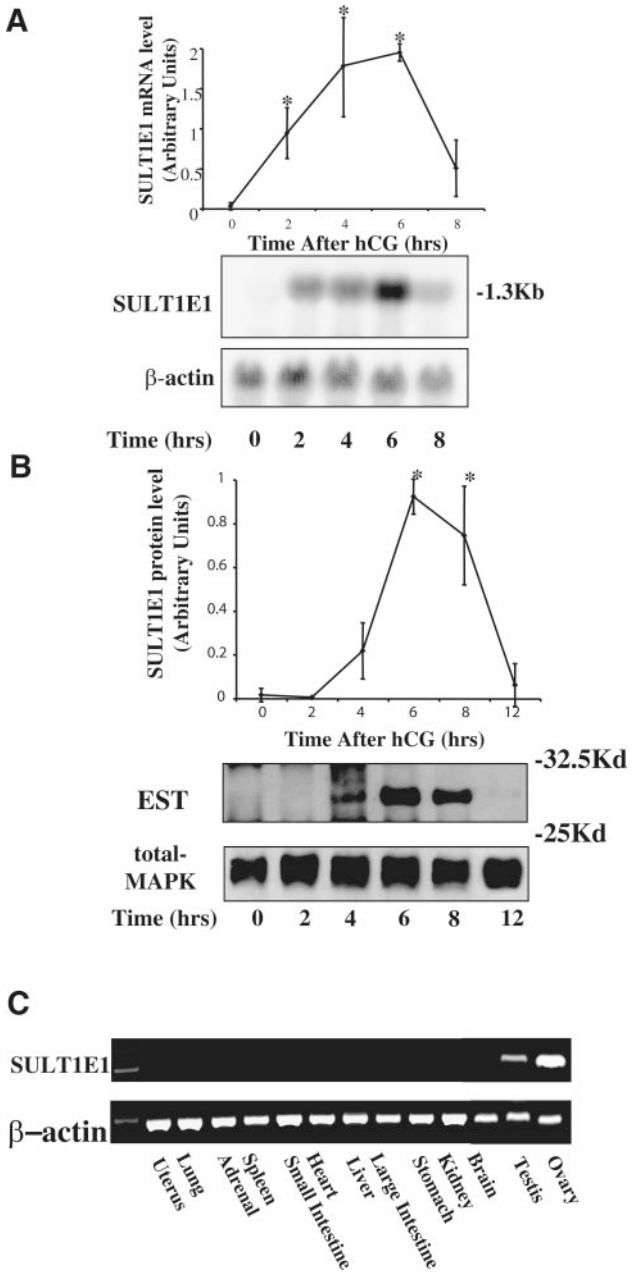

Figure 1. Characterization of the expression SULT1E1 and its protein product in mouse ovaries. *A)* Ovulatory expression of SULT1E1 mRNA. Ratio of SULT1E1/β-actin was calculated at various time points after hCG administration to PMSG-primed mice. Mean ± SE of 4 independent experiments is presented. *Statistically significant difference ($P<0.05$), as compared to time 0 (48 h after PMSG administration). *B)* Expression of EST: ratio of EST/total-MAPK was calculated at various time points after hCG administration to PMSG-primed mice. Mean ± SE of 3 independent experiments is presented. *Statistically significant difference ($P<0.05$), as compared to time 0 (48 h after PMSG administration). *C)* Ovarian-specific expression of SULT1E1 mRNA. Semiquantitative RT-PCR was performed on RNA extracted from indicated mouse tissues, using specific primers for SULT1E1. Results of 1 representative experiment out of a total of 3 independent experiments with similar findings are presented.

2*B*). However, unlike the mouse, in which EST levels substantially decreased 12 h after exposure to hCG, high levels of the protein were still present in the rat at this time point (Figs. 1*B*, 2*B*).

Tissue-specific expression of SULT1E1 was studied by semiquantitative RT-PCR analysis of RNA extracted from 14 different tissues. We found that SULT1E1 expression in mouse females was restricted to hCG-stimulated ovaries, with no signal detected in the other tissues examined (Fig. 1*C*). Males expressed the SULT1E1 gene in their testes.

SULT1E1 mRNA and protein product localization in the ovary

In situ hybridization revealed that the message encoding SULT1E1 is expressed exclusively in granulosa and cumulus cells of the large antral follicles (**Fig. 3*D***), with no detectable signal in either the oocyte or the theca cells (Fig. 3*D*). Time course analysis confirmed that SULT1E1 mRNA expression increases from undetectable levels 48 h after PMSG, to maximal levels 6 h after hCG administration (Fig. 3, *A–E*).

Immunofluorescence analysis revealed that, similar to the SULT1E1 mRNA, its protein product, EST, is expressed in the granulosa and cumulus cells of the large antral follicles 6 h after hCG injection and is absent in both the theca cells and oocytes (Fig. 3, *F–G*).

COX-2, but not progesterone, mediates the effect of hCG on SULT1E1 expression

The indispensable role of prostaglandins in ovulation is indicated by the LH-stimulated increase in the follicular expression of COX-2 (27, 28), a key enzyme in the biosynthesis of prostaglandins; ovulation failure is seen in mice lacking this enzyme (29). To examine whether prostaglandins mediate the effect of LH on SULT1E1 expression, female mice that hCG had been administered to were concomitantly treated with indomethacin, a COX-2 inhibitor. After confirming the inhibitory effect of indomethacin on ovulation (Supplemental Fig. S2*A*), we showed that the expression levels of both ovarian SULT1E1 mRNA and its protein product were significantly lowered in animals treated with this drug (**Fig. 4**, *A–B*).

Knockout female mice lacking the progesterone receptor (PR) express COX-2 but do not ovulate (30). These findings suggest that progesterone and prostaglandin use independent mechanisms to influence ovulation. To examine whether the progesterone pathway mediates the effect of LH on SULT1E1 expression, female mice that hCG had been administered to were treated with the progesterone antagonist RU486. The effect of RU486 on ovulation was verified (Supplemental Fig. S2*B*) however, the same dose of RU486 did not affect SULT1E1 mRNA expression levels in the ovary (Fig. 4, *C–D*).

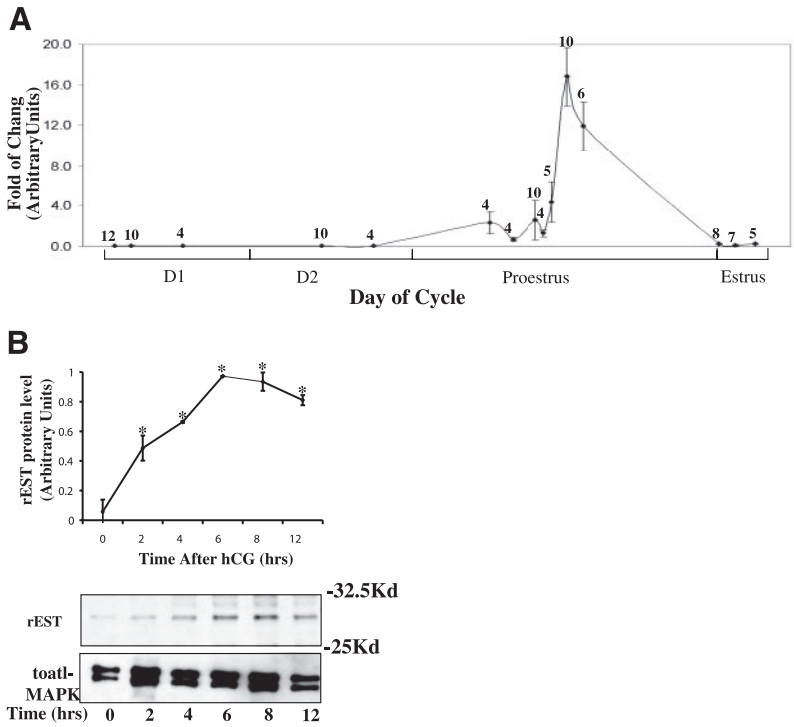

Figure 2. Characterization of SULT1E1 expression in rat ovaries. *A)* SULT1E1 mRNA expression during estrous cycle of sexually mature females. Numbers of animals examined at each time point are indicated. *B)* Ovulatory expression of rat EST (rEST) protein. Ratio of rEST/total-MAPK was calculated at various time points after hCG administration to PMSG-primed mice. Mean ± SE of 3 independent experiments is presented. *Statistically significant difference ($P<0.05$) as compared to time 0 (48 h after PMSG administration).

The role of SULT1E1 in ovulation

After verification that the hCG-treated SULT1E1 KO mice do not express this protein (Supplemental Fig. S3), we examined their ovulatory response. This experiment revealed a reduced ovulation rate in SULT1E1 KO mice. Specifically, the ovulation rate in hCG-treated SULT1E1 KO females reached <50% of that exhibited by wild-type animals receiving similar treatment (25.9±1.9 ova/mouse in SULT1E1 KO females and 57.6±4.75 ova/mouse in WT animals, **Fig. 5*A***). Assuming that the lower rate of ovulation may represent the effect of the relatively high levels of active estrogen maintained in the absence of SULT1E1, we injected WT animals with 17β-estradiol 3 h after hCG administration. We found a 40% reduction in the ovulation rate of the estrogen-treated animals as compared to controls (35.5±5.5 ova/mouse in 17β-estradiol-treated females, and 57.6±4.75 ova/mouse in control animals, Fig. 5*A*).

In view of a recent report regarding down-regulation of COX-2 and up-regulation of PR in rats exposed to high doses of estrogen (31), we examined their levels of expression in the ovaries of hCG-treated SULT1E1 KO mice. Western blot analysis performed 5 h after hCG administration revealed substantially lower expression levels of COX-2 in the SULT1E1 KO females, as compared to similarly treated WT animals (Fig. 5*B*). This difference in COX-2 expression levels could not be detected at earlier time points (Fig. 5*C*). Similar levels of PR expression were obtained in KO and WT ovaries (Fig. 5*B*).

To bypass the lack of SULT1E1 and compensate for the low levels of COX-2 expression, SULT1E1 KO females were treated with PGE_2 concomitantly with hCG administration. We found that ovulation in seven out of eight SULT1E1 KO animals treated with PGE_2 was restored, reaching a number of ovulated eggs that was similar to that obtained in WT animals (50±1.6 in PGE_2-treated KO females and 57.6±4.75 in WT animals; Fig. 5*A*).

Cumulus expansion and TSG6 gene expression in SULT1E1 KO mice

COX-2 catalyzes prostaglandin synthesis that, in turn, stimulates cumulus expansion, a response that is mediated by TSG6 (32). Cumulus-oocyte complexes (COCs) recovered from the ovarian follicles of WT and SULT1E1 KO mice 6 h after hCG administration were examined microscopically for their expansion (**Fig. 6*A***). We found that whereas 90% of the COCs in the WT mice were expanded, only 50% of the COCs in the SULT1E1 KO animals showed this phenotype (Fig. 6*B*). COCs were collected and further subjected to real-time PCR analysis, with specific primers for TSG6. This analysis revealed a significant decrease in TSG6 gene expression in the COCs of SULT1E1 KO mice, as compared to WT animals (Fig. 6*C*).

DISCUSSION

The significant role of estrogen availability in ovulation has been previously demonstrated in a number of KO mouse models (1, 2). On the other hand, the critical decrease in estrogen levels necessary to enable normal ovulation is presented herein for the first time. Moreover, our findings specifically suggest that it is the expression of COX-2 and its downstream TSG6, as well

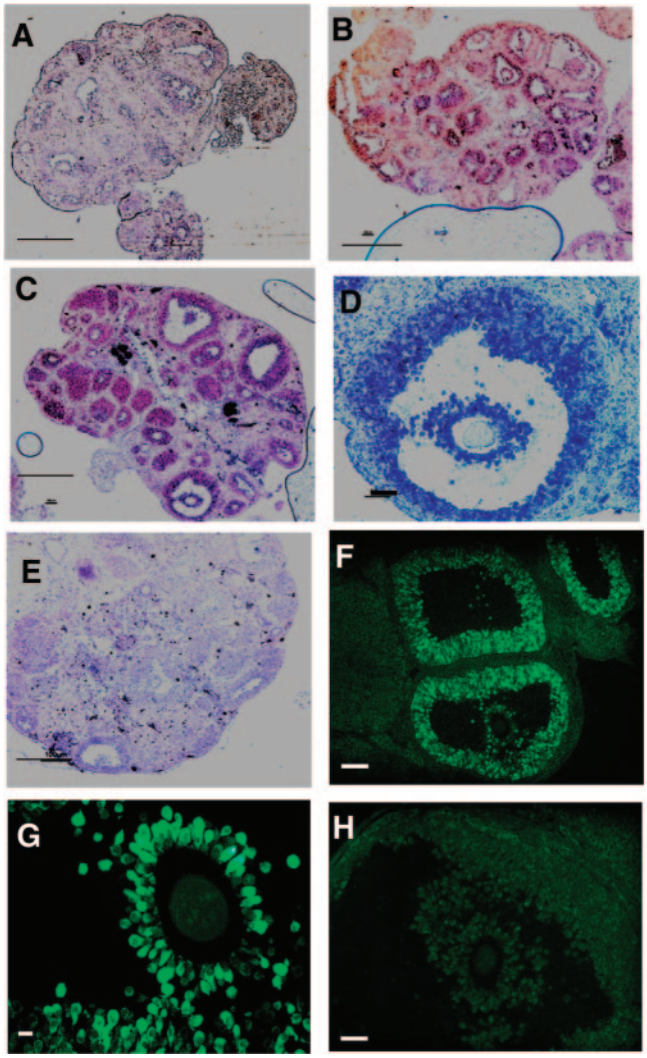

Figure 3. Localization of SULT1E1 mRNA and its protein product, EST, in ovary. *A)* Lack of SULT1E1 mRNA signal in an ovary not previously exposed to hCG. Scale bar: 500 μm. *B)* Positive SULT1E1 mRNA signal in some granulosa cells of a few follicles in a PMSG-primed female mouse, 4 h after hCG administration. Scale bar: 500 μm. *C)* Strong SULT1E1 mRNA signal in granulosa cells of antral follicles, observed 6 h after hCG administration. Scale bar: 500 μm. *D)* Detailed view of 1 representative follicle, 6 h after hCG administration. SULT1E1 mRNA is expressed in cumulus and granolusa cells and is absent in both theca cells and oocyte. Scale bar: 50 μm. *E)* No SULT1E1 mRNA signal is detected 8 h after hCG administration. Scale bar: 500 μm. Localization of EST in ovary. *F)* EST is localized in granolusa and cumulus cells and is absent in both oocyte and theca cells of an ovarian section, 6 h after hCG treatment. Scale bar: 100 μm. *G)* Detailed view of 1 representative COC. EST is expressed by cumulus cells and not by oocyte. Scale bar: 50 μm. *H)* No EST staining was observed when sections were incubated only with Alexa-488, the secondary antibody. Scale bar: 100 μm.

as the subsequent cumulus expansion, that are negatively regulated by high estrogen levels. Moreover, the decrease in the availability of active estrogen is absolutely required to enable ovulation.

The detrimental effects of an excess of circulating estrogen is apparently not only manifested in impaired ovulation. A previous study demonstrated that ablation of the SULT1E1 gene caused placental thrombosis and, subsequently, spontaneous fetal loss (18). This phenotype was associated with elevated systemic estrogen, the effects of which could be reversed by administration of anti-estrogens. In fact, the reduced fertility seen in the SULT1E1 null females could possibly represent the effect of estrogen excess, demonstrated herein for the ovary and reported previously for the placenta (18).

The notion that systemic estrogen concentrations decrease soon after the preovulatory surge of LH was established in the early seventies (33, 34). Later studies (35, 36) suggested that these reduced levels of circulating estrogen may be attributed to the inhibitory effect of the LH surge on the expression of aromatase mRNA. By studying mature cycling rats on different days of the estrus cycle, we demonstrate that the sharp reduction in aromatase mRNA after the LH surge is apparently accompanied by a rapid elevation of SULT1E1 levels. Taken together, these findings suggest that several complementary mechanisms, including but perhaps not limited to a decrease in estrogen availability and its subsequent inactivation, act to ensure highly efficient, negative control of estrogen levels after the surge of LH. Furthermore, as granolusa cells constitute the main producers of estrogen in the ovary and also express estrogen receptors (37, 38), localization of SULT1E1 in these ovarian cells can serve as an excellent means to increase the efficacy of estrogen regulation in its target tissue.

It was previously demonstrated that exposure to high doses of estrogen leads to down-regulation of COX-2 expression (31). It was further shown that COX2 is necessary for TSG6 expression and subsequent cumulus expansion (32). In agreement with these findings, we demonstrate herein that in SULT1E1 KO mice, COX-2 protein expression levels fail to rise in response

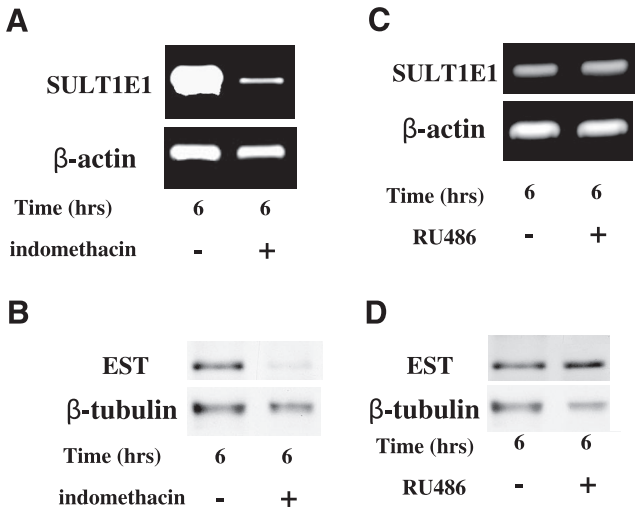

Figure 4. Effect of indomethacin on hCG-induced SULT1E1 expression. *A, C)* Expression levels of SULT1E1 mRNA. *B, D)* Expression levels of EST, its protein product. Results of 1 representative experiment out of at least 3 independent experiments with similar findings are presented.

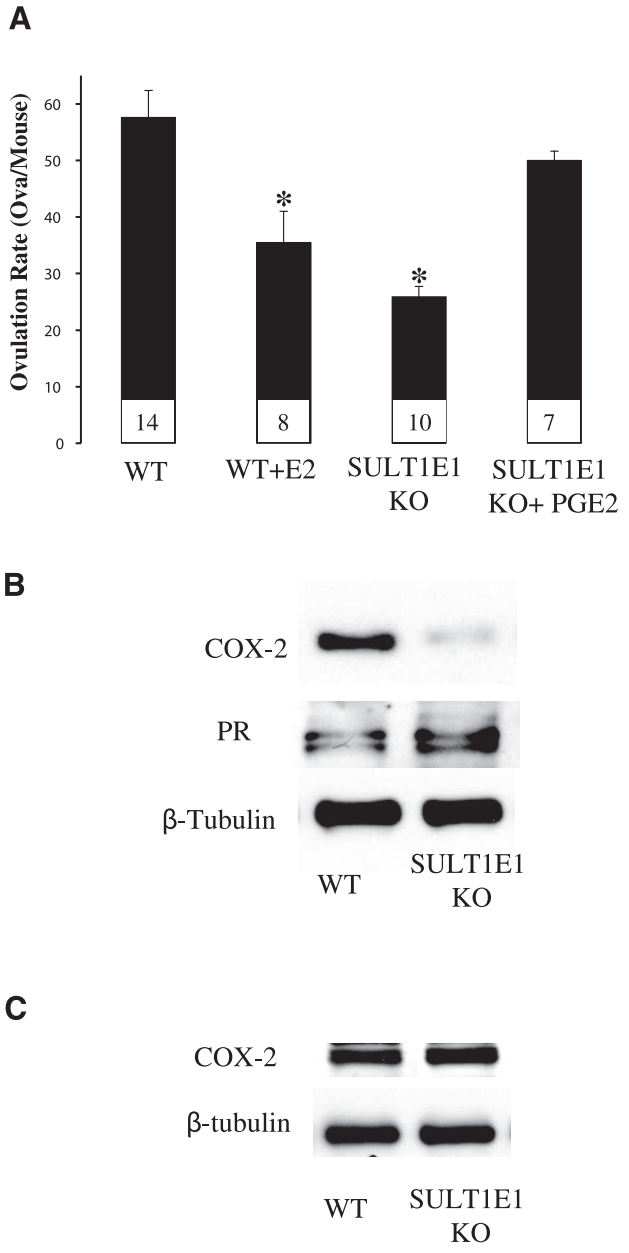

Figure 5. Role of SULT1E1 in ovulation. *A)* Ovulation rates in PMSG/hCG-treated WT animals administrated with or without 17β-estradiol and in PMSG/hCG-treated SULT1E1 KO female administrated with and without PGE_2. Total number of animals examined is indicated. Mean ± SE of 4 independent experiments is presented. *Statistical significance of $P < 0.05$. *B)* COX-2 and PR expression in ovaries of PMSG-primed SULT1E1 KO females 5 h after hCG administration; 1 representative experiment out a total of 3 independent experiments with similar results is presented. *C)* COX-2 expression in ovaries of PMSG-primed SULT1E1 KO females 2 h after hCG administration. Results of 1 representative experiment, out a total of at least 3 independent experiments with similar findings, are presented.

to hCG. Coupled with previous findings, our results suggest that the EST-catalyzed inactivation of estrogen enables hCG-induced COX-2 up-regulation, prostaglandin production, and TSG6 expression. The latter enzyme acts as an essential mediator of LH-induced cumulus expansion. Localization of SULT1E1 in the cumulus cells, demonstrated in our studies as well as others (39), supports this conclusion.

SULT1E1 was initially identified in male mice as a testis-specific enzyme with no detectable expression in the female gonad (11, 16). Previous failures to demonstrate expression of SULT1E1 in the ovary can appar-

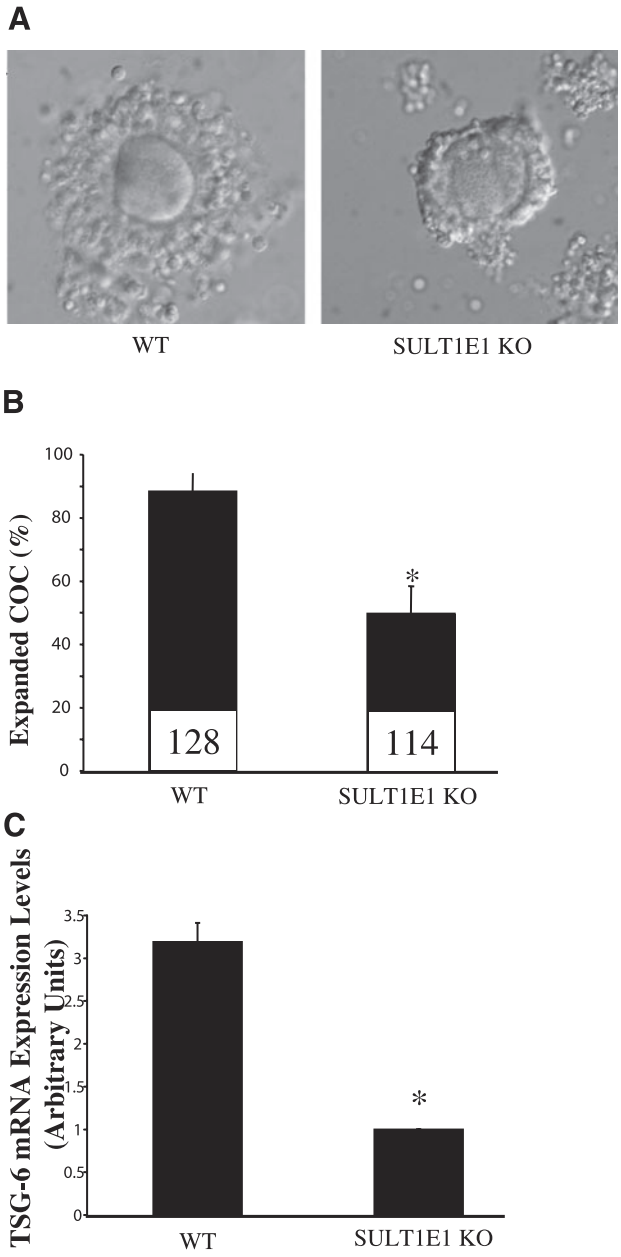

Figure 6. Cumulus expansion and TSG6 gene expression in SULT1E1 KO mice. *A)* Representative COC displaying an expanded appearance recovered from PMSG/hCG-treated WT females, as compared to compact organization of a COC recovered from SULT1E1 KO mice treated in a similar manner. *B)* Fraction of expended COCs in the 2 mouse models described in *A*. Mean ± SE of 3 independent experiments is presented. *Statistical significance of $P < 0.05$. *C)* TSG-6 mRNA expression in COCs of SULT1E1 KO mice 5 h after hCG administration, as compared to that in WT animals. Mean ± SE of 3 independent experiments is presented. *Statistical significance of $P < 0.05$.

ently be attributed to the fact that analyses were performed on animals that were not exposed to LH/hCG. In fact, the present study also revealed the absence of SULT1E1 expression in ovaries recovered from mice primed by PMSG, with no further hCG administration. Moreover, the time window of SULT1E1 expression, 2 h after hCG injection, is quite limited: when SULT1E1 mRNA is initially detected, it peaks at 6–8 h but is totally undetectable 2 h later. In a similar manner, SULT1E1 mRNA expression in sexually mature female rats is limited to the evening of proestrus, soon after the preovulatory surge of LH, with no detectable levels at any other stage of the estrous cycle. As in the ovary, SULT1E1 expression in Leydig cells is also subjected to regulation by LH (12, 40).

In humans, the sulfotransferase family consists of at least 10 functional members (41), including SULT1E1. Thus far, human SULT1E1 has been identified in the endometrium but not in the ovary (42, 43). The narrow window of expression of ovarian SULT1E1, demonstrated herein for rodents, suggests that reexamination of human ovaries at various specific stages throughout the menstrual cycle may yet reveal its expression. In that case, SULT1E1-catalyzed down-regulation of estrogen activity, seen immediately before ovulation in humans, could be of great significance and needs to be carefully considered.

Hydroxylated polychlorinated biphenyls (PCB) are ubiquitous environmental contaminants (44). Studies of wildlife and laboratory animals show that exposure to PCB can adversely affect reproductive and endocrine function (45, 46). Recently, it has been shown that PCBs are inhibitors of SULT1E1 in humans (47). Our knowledge of the effects of PCBs on human reproduction is very limited and has thus far been demonstrated only in males. Studies have shown that exposure to high levels of PCB reduces sperm motility (48) and also has a negative impact on the integrity of sperm chromatin (49). Our findings emphasize the dangers of PCBs, and the seriousness of their deleterious effects on the fertility of both animals and humans.

In summary, our results demonstrate that SULT1E1 is expressed in the ovarian follicle in response to LH/hCG. We also show that the lack of SULT1E1 in the ovary, which apparently manifests itself in high levels of active estrogen, prevents TSG6 expression and cumulus expansion, and leads to impaired ovulation. Taken together, these results imply that ovarian SULT1E1 participates in the regulation of estrogen activity locally in the follicle. As to its protein product, the EST-catalyzed down-regulation of estrogen activity enables normal ovulation by a mechanism involving prostaglandins, TSG6, and cumulus expansion. These findings broaden our understanding of estrogen regulation on ovulation and may lead to the establishment of novel treatment protocols in specific cases of reproduction failures. Fj

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