Expression and localization of transcription factor Ets-1 in the rat ovary during the estrous cycle and pregnancy

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Objective: To examine the expression and localization of Ets-1 in the rat ovary during the estrous cycle and pregnancy, and to investigate its effects on ovarian function.
Design: Prospective, randomized study.
Setting: Department of Physiology at Harbin Medical University.
Animal(s): Pubertal female Wistar rats.
Intervention(s): Vaginal smears were taken daily from female rats to determine the stage of the estrous cycle. Pregnancies were achieved by caging female and male rats together overnight. Ovaries were collected from both cycling and pregnant rats for tissue sectioning and RNA and protein extractions.
Main Outcome Measure(s): Real-time quantitative polymerase chain reaction, Western blot, in situ hybridization, and immunohistochemistry were performed to investigate the expression and localization of Ets-1 messenger RNA (mRNA) and protein in the rat ovary during the estrous cycle and pregnancy.
Result(s): During the estrous cycle, the levels of Ets-1 mRNA and protein expression increased during the follicular phase, achieving their highest measurements at proestrus and lowest at metestrus. The expression of Ets-1 mRNA and protein fluctuated during pregnancy, increasing during early pregnancy, then decreasing during mid-pregnancy, and again increasing until parturition. Ets-1 mRNA and protein were present throughout the estrous cycle and pregnancy, principally localized in follicles of various sizes and in the corpus luteum.
Conclusion(s): Ets-1 may participate and play an important role in the regulation of follicular development, corpus luteum formation, maintenance, and regression. (Fertil Steril® 2009;91:1998–2005. ©2009 by American Society for Reproductive Medicine.)

Key Words: Ets-1, follicle, corpus luteum, estrous cycle, pregnancy, rat

The Ets genes are a family of genes comprising more than 30 members, all functioning as transcription factors (1). All Ets factors share an evolutionally conserved DNA-binding domain; the Ets domain, comprising a sequence of 85 amino acids, binds to a consensus DNA sequence centered on the core GGAA/T motif, aptly named the Ets-binding site or PEA3. Ets-1, the first member of the Ets transcription factor family, was first identified as the cellular progenitor of the viral oncogene v-ets in the genome of the avian leukemia retrovirus E26 (2). Originally detected in lymphoid cells of adult tissues (3), the expression of Ets-1 is also observed in a variety of cells, including endothelial cells, vascular smooth muscle cells, lymphocytes, and epithelial cancer cells (4, 5). A large number of studies reported that Ets-1 played a pivotal role in diverse physiologic and pathologic processes, such as cell growth, migration, differentiation, apoptosis, hematogenesis, angiogenesis, osteogenesis, embryogenesis, and tumor invasion (6–10). Another group of closely related studies found that Ets-1 interacted with the promoter regions of these genes, such as urokinase-type plasminogen activator, matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinase-1, and vascular endothelial growth factor (VEGF) and its receptors, suggesting that it may play a fundamental role in the regulation of the activities of these genes (11–17).

During the estrous cycle and pregnancy, a series of morphologic and functional changes occurs in the rat ovary, coupled with the fluctuation of hormone levels, such as gonadotropin-releasing hormone (GnRH), gonadotrophins, and sex steroids. Numerous cytokines and growth factors, regulated by GnRH and sex steroids, contribute to cell proliferation, apoptosis, angiogenesis, and extracellular matrix degradation. It has been proposed that these factors may be involved in ovarian oocyte maturation, follicular development, ovulation, and corpus luteum (CL) formation and

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regression, such as VEGF, transforming growth factor (TGF)-β, MMPs, and plasminogen activator system (18–22).

Recent data have suggested that Ets-1 is critically involved in female reproduction. Ets-1 was expressed in theca and interstitial cells in adult mouse ovary, in addition to the mammalian and human glandular tubular epithelial cells of the endometrium, during the estrous or menstrual cycle (23). Ets-1 expression was also detected in reproductive system tumors, such as ovarian carcinoma and endometrial cancer (24). Despite numerous studies having suggested the relationship between Ets-1 and female reproduction, there is no clear evidence to suggest the effects of Ets-1 on ovarian function during the estrous cycle and pregnancy. In the present study, we have determined the expression and localization of Ets-1 messenger RNA (mRNA) and protein in rat ovary at various stages during the estrous cycle and pregnancy, so as to investigate the biological function of Ets-1 involved in follicular development, ovulation, corpus luteum formation, maintenance, and regression.

MATERIALS AND METHODS

Animals and Tissue Collection

Pubertal female Wistar rats (aged 2 to 3 months), weighing approximately 200 g, were randomly bred at room temperature (approximately 25°C) in a constant photoperiod (light/dark cycle, 12 hours/12 hours) with free access to water and food. These rats were allowed 7 days to acclimatize to the new surroundings. All animals were handled in accordance with the guidelines for care and use of experimental animals for scientific purposes, and this research was approved by the Harbin Medical University institutional review board.

To determine the stage of the estrous cycle, vaginal smears were taken daily from these animals for at least two consecutive 4-day cycles. Pregnancies were achieved naturally by caging female and male rats together, overnight. The morning of the day on which sperm in female vaginal smears were detected was designated as day 1 of pregnancy, and the first day after parturition was designated P1.

Cycling and pregnant rats were killed at proestrus, estrus, metestrus, and diestrus, on days 1, 3, 5, 7, 9, 18, and 22 of pregnancy, and on P1 (n = 6 per stage). Ovaries were collected and divided into two parts. One part was snap-frozen in liquid nitrogen to be stored at −80°C for RNA and protein extraction and frozen tissue sections. The other parts were immediately fixed and dehydrated for paraffin-embedded sections.

RNA Extraction and Real-Time Quantitative PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, Ca), in accordance with the manufacturer's instructions, and demi-quantified with an ultraviolet spectrophotometer (Ultrospec 1100 Pro; Amersham, Buckinghamshire, United Kingdom) at an absorbance of 260 nm.

Single-stranded complementary DNA (cDNA) was synthesized using avian myeloblastosis virus (AMV) reverse transcriptase (TaKaRa, Otsu, Japan). A reverse transcription reaction system (with a final volume of 20 μL) containing 5 U AMV reverse transcriptase, 4 μL 5× AMV buffer, 2 μL 10 mmol/L deoxyribonucleoside triphosphate mixture, 50 pmol oligo $(dT)_{18}$ primer, 20 U ribonuclease inhibitor, and 1 μg RNA was maintained for 10 minutes at room temperature, followed by incubation at 42°C for 60 minutes and then 95°C for 5 minutes. The cDNA was stored immediately at −20°C until the polymerase chain reaction (PCR).

Real-time quantitative PCR was performed in a 20-μL final reaction volume using an SYBR Premix Ex Taq kit (TaKaRa) according to the manufacturer's protocol. The sequence-specific primers for rat Ets-1 were 5′-CTACCCTTCTGTCATTCTCC-3′ (forward) and 5′-GAGGCGGTC ACAACTATC-3′ (reverse), and the size of the product was 181 base pairs (bp). Rat β-actin was used as internal control (primers were 5′-GTTTGAGACCTT CAACACCCC-3′ and 5′-GTGGCCATCTCTCTTGCTCGA AGTC-3′) with the expected size of 320 bp. The amplification reaction consisted of 10 μmol/L of each primer, 10 μL of 2× SYBR Premix Ex Taq, and 2 μL cDNA template. Real-time PCR was carried out in duplicate for each sample in the LightCycler real-time PCR thermal cycler (Roche, Mannheim, Germany) with capillary system (TaKaRa) using the following parameters: 1 cycle at 95°C for 10 seconds, followed by 40 cycles at 95°C for 5 seconds (denaturation), 60°C for 15 seconds (annealing), and 72°C for 10 seconds (extension). A PCR system devoid of template cDNA was included as negative control. Fluorescence values in each tube were measured at the end of each cycle using the single acquisition mode. Melting curve analysis was performed after the end of the last cycle. The standard curves were generated using average fluorescence values of duplicate standards. Average fluorescence values of samples were then used to calculate the concentration of Ets-1 mRNA in each sample, with LightCycler Data Analysis software. All data were normalized by dividing the amount of Ets-1 by the amount of β-actin used as the control.

In Situ Hybridization

Digoxigenin (DIG)-labeled RNA probes for in situ hybridization analysis were prepared using T7 and SP6 polymerases according to the manufacturer's instructions supplied with a kit (DIG RNA Labeling Kit; Roche). Both sense and antisense probes were routinely used.

The localization of Ets-1 mRNA in frozen tissue sections was detected by in situ hybridization. The process of in situ hybridization was performed as described previously (25). Sections of rat ovaries (5 μm) were hybridized with DIG-labeled antisense or sense rat Ets-1 complementary RNA (cRNA) probes. The frozen sections on poly-L-lysine–coated slides were quickly thawed and fixed in 4% paraformaldehyde (pH 7.4) at 4°C for 15 minutes. The slides were washed twice in phosphate-buffered solution (PBS) containing 0.1% active diethyl pyrocarbonate for 15 minutes each time, and then in 5× saline sodium citrate (SSC; 1× SSC

contained 0.15 mol/L NaCl and 0.015 mol/L sodium citrate) for 15 minutes. Prehybridization was carried out at 56°C for 2 hours in prehybridization solution (50% deionized formamide, 5× SSC, and 10 mg/mL salmon sperm DNA). Slides were then hybridized with 2 μg/mL DIG-labeled antisense cRNA probes for Ets-1 diluted in prehybridization solution at 55°C for 18 hours in a moist chamber. After hybridization, the slides were serially washed in 2× SSC for 30 minutes at room temperature, then in 2× SSC and in 0.1× SSC at 65°C for 1 hour, respectively. After incubation with anti-DIG-alkaline phosphatase (diluted 1:3,000) for 2 hours at room temperature, the slides were rinsed twice for 15 minutes in buffer A (100 mmol/L Tris, 150 mmol/L NaCl, pH 7.5) and 5 minutes in buffer C (100 mmol/L Tris, 100 mmol/L NaCl, 50 mmol/L $MgCl_2$, pH 9.5). Color development was performed with the NBT/BCIP kit (Zhongshan, Beijing, China), in accordance with the manufacture's instructions. The sense probes were used as negative controls for the hybridization specificity and background levels.

Western Blot

Ovary proteins were extracted using radio immunoprecipitation assay (RIPA) reagent (Beyotime, Beijing, China) containing a protease inhibitor cocktail tablet (Roche). A total of 50 mg of ovary was homogenized in 1 mL RIPA reagent on ice with a homogenizer. After homogenization, samples were centrifuged at 4°C at 12,000 rpm for 15 minutes; the supernatant was then collected and stored at −20°C until use. The protein concentration of the supernatant was measured with the BCA Protein Assay Kit (Beyotime). Equal amounts of protein were loaded in each lane (approximate 50 μg/mL), then separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel and electro-transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA) using a wet immunoblot apparatus (Tanon, Shanghai, China). Then, after blocking with 3% bovine serum albumin at 37°C for 1 hour, the membrane was incubated at 37°C for 1 hour with rabbit anti-Ets-1 polyclonal antibody (200 μg/mL, 1:800 dilution) (C-20; Santa Cruz Biotechnology, Santa Cruz, CA) followed by three washes with TBST (1× Tris-buffered saline containing 0.05% Tween 20) for 10 minutes each. The membrane was incubated at 37°C for 30 minutes with alkaline phosphatase goat anti-rabbit IgG (1:800 dilution) (Zhongshan), again followed by washing with TBST three times for 10 minutes each. Visualization of Ets-1 protein was detected by the NBT/BCIP kit (Zhongshan). Membranes were re-incubated with anti-actin (rabbit polyclonal antibody against rat; Santa Cruz Biotechnology), which was used as a loading control. Optical density values for Ets-1 bands were evaluated by an image analyzer (Tanon GIS 2020), and results were represented as densitometric ratios normalized to actin.

Immunohistochemistry

Immunohistochemistry was performed by using a streptavidin/peroxidase kit (Zhongshan). Briefly, paraffin-embedded sections (4 μm) were deparaffinized with xylene and washed. After treatment with 3% H_2O_2 at room temperature for 10 minutes to quench endogenous peroxidase, sections were blocked for 30 minutes with normal goat serum at room temperature and incubated for 2 hours at 37°C with the primary antibody against Ets-1 (200 μg/mL rabbit polyclonal antibody diluted at 1:150; Santa Cruz Biotechnology). After rinsing with PBS, sections were processed further using the streptavidin/peroxidase kit; reaction products were developed with a diaminobenzidine kit (Zhongshan). Negative controls were incubated with PBS instead of the primary antibody. All sections were counterstained with hematoxylin and protected with coverslips. Immunolocalization of Ets-1 protein was then examined and photographed under microscopy (Olympus, Tokyo, Japan).

Statistical Analysis

All data are presented in figures as mean ± SEM. Differences among groups were analyzed by one-way analysis of variance and Student Newman–Keuls q tests to determine whether statistical significance existed, using SPSS 11.5 (SPSS, Chicago, IL). A P value of $< .05$ was considered statistically significant.

RESULTS

Expression of Ets-1 mRNA in the Rat Ovary During the Estrous Cycle and Pregnancy

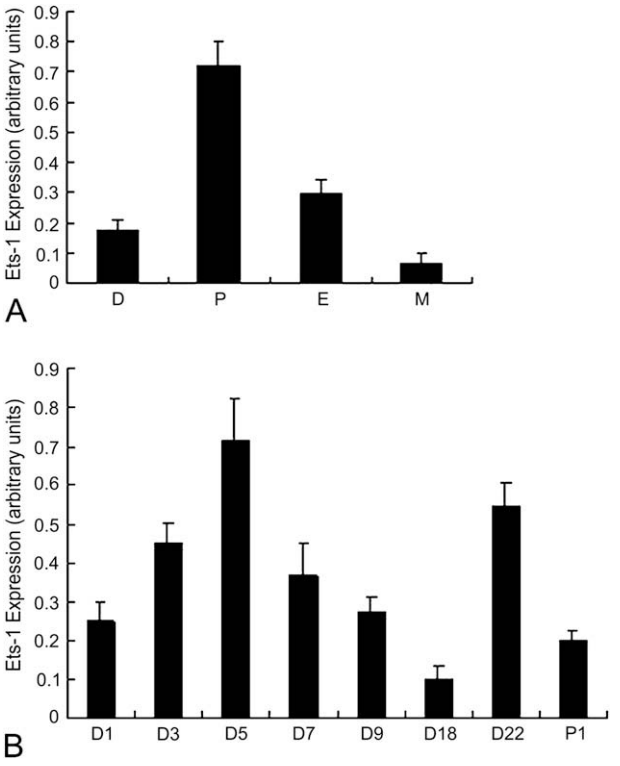
Real-time quantitative PCR was performed to quantify the relative amounts of Ets-1 mRNA expression in the rat ovary. As shown in Figure 1, the most striking feature of these data is that the levels of Ets-1 mRNA expression were significantly different during the estrous cycle and pregnancy ($P<.05$). Figure 1A shows the representative Ets-1 mRNA expression pattern observed in cycling rats. The level of Ets-1 mRNA expression was higher at diestrus and estrus, as compared with its expression at metestrus, coming to a peak at proestrus ($P<.05$). As shown in Figure 1B, expression of Ets-1 mRNA increases during early pregnancy and decreases during mid-pregnancy, initially peaking at day 5 of pregnancy; after this, Ets-1 mRNA expression intensity elevates again during late pregnancy and declines markedly after parturition (P1), coming to a second peak just before parturition at day 22 of pregnancy ($P<.05$).

Localization of Ets-1 mRNA in the Rat Ovary

In situ hybridization analysis revealed the localization of Ets-1 mRNA in ovaries of cycling and pregnant rats. As shown in Figure 2, hybridization signals of Ets-1 mRNA were consecutively detected in follicles of various sizes and corpus luteum throughout the estrous cycle and pregnancy. During the estrous cycle, Ets-1 mRNA expression was primarily localized in oocytes, granulosa, and luteal cells (Fig. 2A–D). In pregnant rat (Fig. 2E–M), Ets-1 mRNA was principally localized in the functional corpus luteum; weak

FIGURE 1

Real-time quantitative PCR analysis of Ets-1 mRNA expression in the rat ovary. (**A**) Level of Ets-1 mRNA expression during the estrous cycle. (**B**) Expression intensity of Ets-1 mRNA during pregnancy. The expression of Ets-1 mRNA was normalized on the basis of β-actin mRNA expression. All data were expressed as mean ± SEM (n = 6 per stage) ($P<.05$). D = diestrus; P = proestrus; E = estrus; M = metestrus; D1, D3, D5, D7, D9, D18, D22 and P1 = days 1, 3, 5, 7, 9, 18, 22 of pregnancy and day 1 after parturition, respectively.



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signals of Ets-1 mRNA can also be observed in ovarian interstitial cell.

Expression of Ets-1 Protein in the Rat Ovary During the Estrous Cycle and Pregnancy

Western blot analysis revealed the presence of a single 55-kd band for Ets-1 protein in the rat ovary throughout the estrous cycle and pregnancy (Fig. 3A, C). Actin (43-kd band) was used as a loading control. The densitometric ratios of Ets-1/actin represented the concentrations of corresponding Ets-1 protein at various stages during the estrous cycle and pregnancy (Fig. 3B, D). As shown in Figure 3B, Ets-1 expression was apparent at diestrus and estrus, faint at metestrus, and highest at proestrus ($P<.05$). Figure 3D shows that the levels of Ets-1 protein expression fluctuated during pregnancy ($P<.05$). During early pregnancy there appeared an increase in the level of Ets-1 that came to a peak on day 5 of pregnancy. After day 5, Ets-1 expression decreased until day 9, then increasing to the second peak on day 22, which was followed by a dramatic decline after parturition (P1).

Spatial and Temporal Localization of Ets-1 Protein Expression in the Rat Ovary

Immunohistochemical results showed that the spatial and temporal localization of Ets-1 protein was similar to that of its mRNA. As shown in Figure 4, Ets-1 protein was constantly detected in follicles of various sizes as well as corpus luteum throughout the estrous cycle and pregnancy, primarily restricted to oocytes, granulosa, and luteal cells. Ets-1 was expressed in primordial follicles, small follicles with theca interna (exhibiting the beginning of antrum formation), atretic follicles, and even some old corpus luteum from previous cycles (Fig. 4A). Expression in the cumulus–oocyte complex and granulosa cells in preovulatory follicles during follicular development and ovulation was also detected (Fig. 4B). Expression of Ets-1 protein was detected in granulosa cells and luteal cells in luteinized follicles and corpus luteum (Fig. 4C, D). In the pregnant ovary, Ets-1 protein expression was persistent in the functional corpus luteum during the entire pregnancy (Fig. 4E–L). Slight immunostaining of Ets-1 was also observed in theca cells and ovarian interstitial cells.

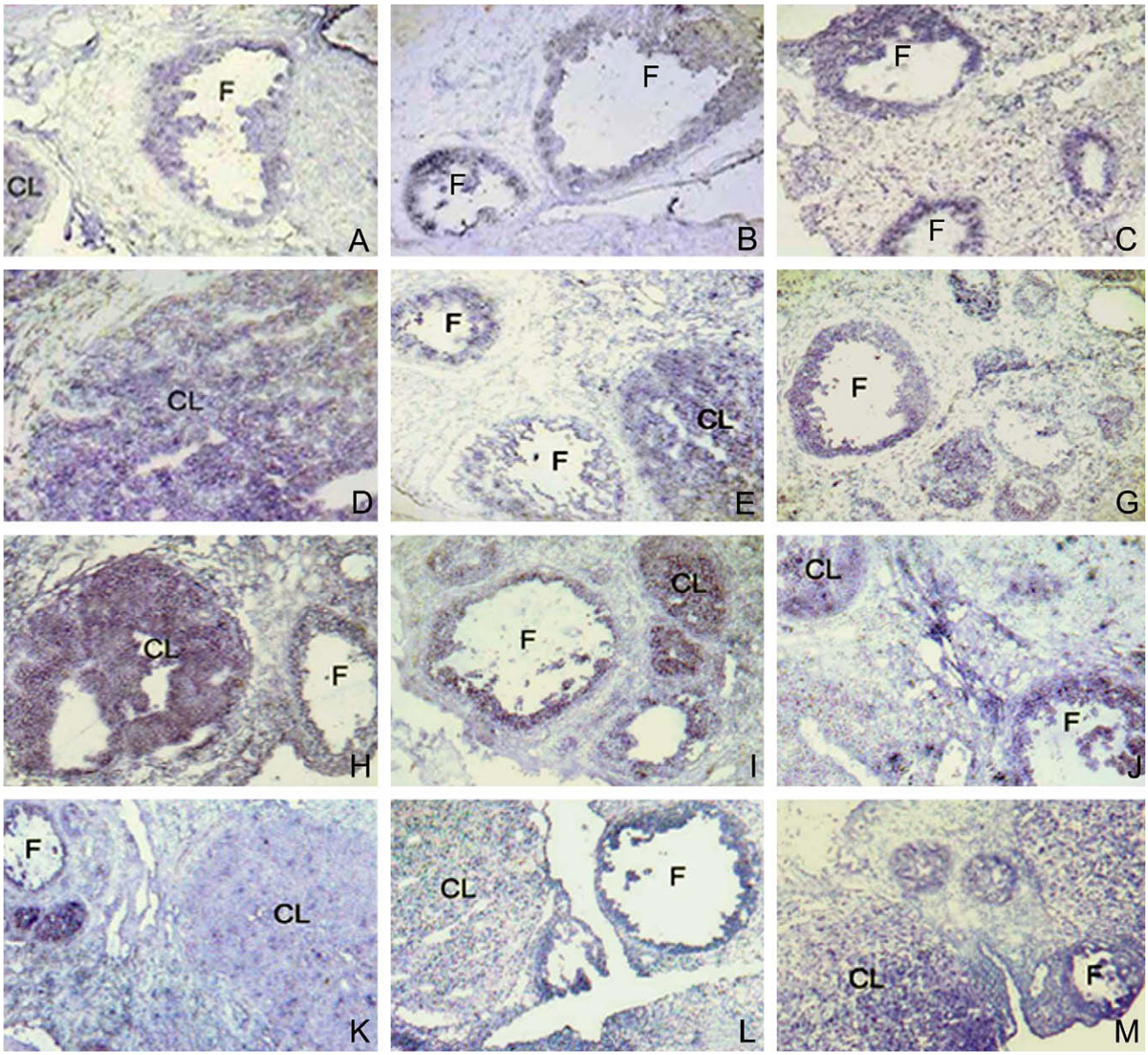
DISCUSSION

The proto-oncogene Ets-1 is a transcription factor, originally identified by sequence homology with the viral v-ets oncogene of the E26 avian leukemia retrovirus. Ets-1 expression has been detected in various cells, with the role of Ets-1 gene expression in mesodermal lineage cells (such as fibroblasts and endothelial cells) drawing wide attention in the fields of embryogenesis and angiogenesis (26, 27). A large body of studies have also demonstrated that Ets-1 exhibits multiple activities in the transcriptional regulation of numerous genes involved in many physiologic and pathologic processes, such as cell proliferation, angiogenesis, extracellular matrix remodeling, self-tolerance, and cancer invasion (28–32). Ets-1 expression has been reported in the glandular epithelial cells of mammalian endometrium and in various reproductive system tumors (33, 34). However, little is known about the potential effects of Ets-1 on the regulation of ovarian function. In this study the involvement of Ets-1 in the ovarian cycle was demonstrated and examined with expression and localization of Ets-1 mRNA and protein in the rat ovary during the estrous cycle and pregnancy.

During the estrous/menstrual cycle and pregnancy, mammalian ovaries undergo structural and functional changes, including the dynamic processes of cell proliferation, differentiation, angiogenesis, and tissue remodeling. The most significant and closely regulated events in mammalian reproduction are the formation, maintenance, regression, and steroidogenesis of the follicles and corpus luteum (35, 36).

FIGURE 2

In situ hybridization analysis of Ets-1 mRNA expression in the rat ovary during the estrous cycle and pregnancy. Ets-1 mRNA expression was persistently detected in the follicles of various sizes in addition to the corpus luteum during the estrous cycle and pregnancy, primarily in granulosa and luteal cells. (**A**) Diestrus; (**B**) proestrus; (**C**) estrus; (**D**) metestrus; (**E–M**) days 1, 3, 5, 7, 9, 18, 22 of pregnancy, and day 1 after parturition, respectively. CL = corpus luteum; F = follicle. Original magnification, ×100.



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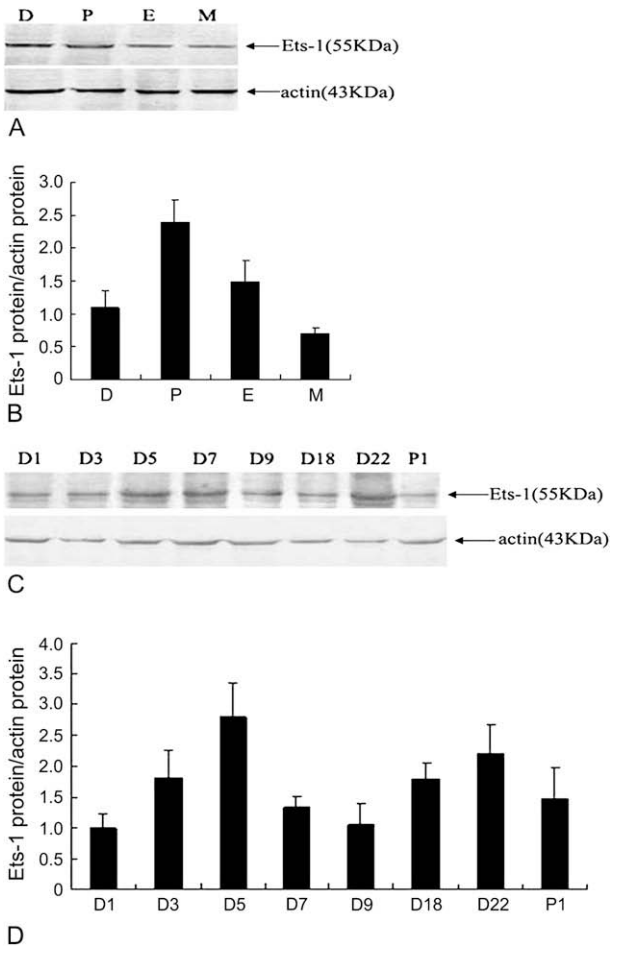
Numerous hormones play essential roles in the control of ovarian cycle and pregnancy, such as GnRH, FSH, LH, estrogen (E), and progesterone (P). Gonadotropin-releasing hormone and FSH are well recognized as the physiologic stimulants of follicular survival, growth, and E production in the ovary during the follicular phase. High concentrations of E promote granulosa cell proliferation and oocyte maturation. Luteinizing hormone surge seems to be rapidly induced by an E peak, followed by a rapid decline after ovulation. During the luteal phase, P secretions increase during corpus luteum maturation and reduce as corpus luteum regresses. These hormones (gonadotrophins, sex steroids) exert their function on cell proliferation and angiogenesis during the estrous cycle and pregnancy, participating as critical signals in the cross-talk of cell death and cell survival pathways (37).

The data presented here demonstrate the spatiotemporal features of Ets-1 expression in rat ovary during the estrous

FIGURE 3

Western Blot analysis of Ets-1 protein expression in the rat ovary during the estrous cycle and pregnancy. (**A**) The single 55-kd band of Ets-1 protein was observed in rat ovary across all stages of the estrous cycle. Actin (43-kd band) was used as a loading control. (**B**) Densitometric ratios of Ets-1/actin showing the level of Ets-1 protein expression in rat ovary during the estrous cycle. Ets-1 expression was apparent at diestrus and estrus, faint at metestrus, and highest at proestrus (n = 6 per stage) ($P < .05$). (**C**) Expression of Ets-1 protein (55-kd band) and actin (43-kd band) in rat ovaries during pregnancy. (**D**) Ets-1/actin densitometric ratios representing the expression intensity of corresponding Ets-1 protein. The expression intensity of Ets-1 protein fluctuated at various stages during pregnancy (n = 6 per stage) ($P < .05$). D = diestrus; P = proestrus; E = estrus; M = metestrus; D1, D3, D5, D7, D9, D18, D22 and P1 = days 1, 3, 5, 7, 9, 18, 22 of pregnancy and day 1 after parturition, respectively.



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cycle and pregnancy, suggesting its specific roles in both the follicular and luteal stages. Ets-1 expression in ovaries throughout the estrous cycle was abundant in follicles at various developmental stages, increasing alongside follicular maturation; this proved to be significantly highest at proestrus and lowest at metestrus. During pregnancy, Ets-1 expression is maximal on D5 and D22 and relatively low through mid-pregnancy. The selective localization of Ets-1 expression in oocytes, granulosa, and luteal cells suggests that it may play potentially critical roles in follicular development, luteolysis, and the establishment of pregnancy.

The diffused expression of Ets-1 in stromal cells supports its role in the vascular system. The altered expression of Ets-1 in rat ovary coincided with the fluctuation in levels of several gonadotrophins and steroid hormones, such as GnRH, E, and P. This change in Ets-1 expression may be regulated by the presence of E, which could potentially play an important role in regulating vascular development in rat ovary. These findings suggest that Ets-1 contributes to the regulation of follicular and luteal function, implying that Ets-1 may be an essential upstream regulator of these hormones.

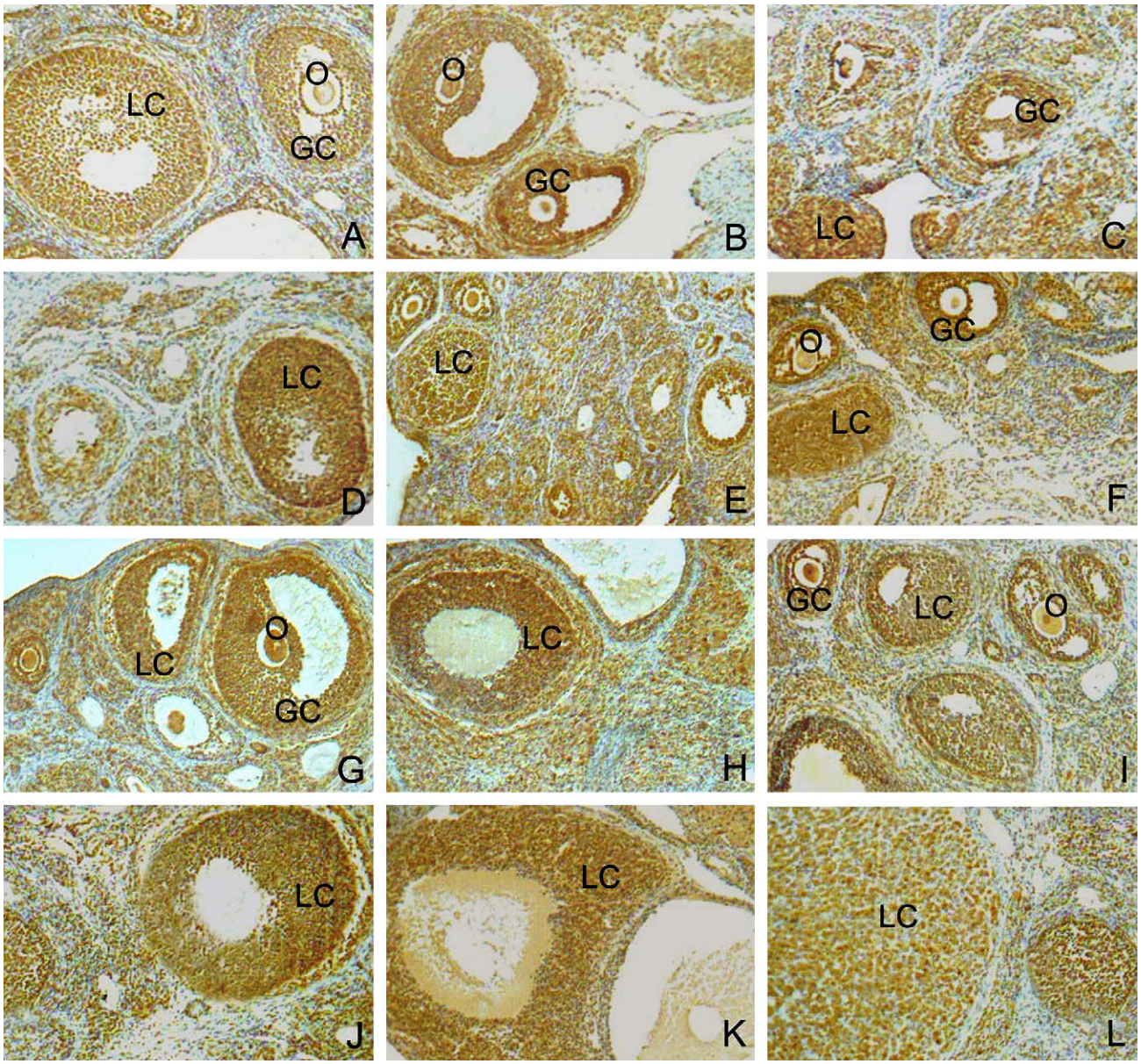
Numerous cytokines and growth factors, including VEGF, TGF-β, growth differentiation factor-9, MMPs and plasminogen activator system, and insulin-like growth factor-1, have been proposed to be involved in oocyte maturation, follicular development, ovulation, corpus luteum formation, and regression (18–22, 38). Vascular endothelial growth factor, a crucial angiogenic factor, plays significant roles in cell proliferation and sex steroid–dependent angiogenesis in the ovary during both the estrous cycle and pregnancy; synthesis takes place in the developing follicle and corpus luteum (39–41). Recent studies have shown that the Ets-1 protein links to the promoter region of these cytokines and growth factors, suggesting that it may play an important role in the regulation of these genes.

Ets-1 is a pivotal regulator in TGF-β signaling, controlling TGF-β receptor II expression. Activity of Ets-1 may also be regulated by angiogenic factors and phosphorylated by growth factor–induced kinases (42, 43). Recent data have reported that during the menstrual cycle, Ets-1 expression in endothelial cells increases after changes in the VEGF level (23). Transcription factor Pit-1 cooperates with pituitary Ets-1 to stimulate prolactin synthesis, suggesting that Ets-1 also plays a role in pituitary hormone secretion (44). Our results demonstrate that the large increase of Ets-1 expression during the estrous cycle and pregnancy might facilitate ovulation, corpus luteum formation, and regression, by interacting with these cytokines and growth factors.

In summary, the expression pattern and spatiotemporal distribution of Ets-1 during the estrous cycle and the process of pregnancy illustrate the involvement of Ets-1 in ovarian follicular development and luteal function. It also provides the first evidence suggesting Ets-1 contributions to the regulation of dominant follicular development, ovulation, corpus

FIGURE 4

Localization of Ets-1 protein in the rat ovary during the estrous cycle and pregnancy. The immunostaining of Ets-1 protein was detected in follicles of various sizes and corpus luteum throughout the estrous cycle and pregnancy, and primarily in oocytes, granulosa, and luteal cells. (**A**) Diestrus; (**B**) proestrus; (**C**) estrus; (**D**) metestrus; (**E–L**) days 1, 3, 5, 7, 9, 18, 22 of pregnancy, and day 1 after parturition, respectively. LC = luteal cells; GC = granulosa cells; O = oocyte. Original magnification, ×100.



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luteum formation, and regression. It is hypothesized that there may be novel pathways existing among the Ets transcription factors, hormones, numerous cytokines, and growth factors. Further research on the regulatory factors involved in the expression of Ets-1 is needed to give more information on the roles of Ets-1 and these novel pathways modulating the mammalian reproductive processes. More information about the roles of Ets-1 is likely to suggest some new therapeutic clues to the management of fertility.

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