Expression of Sperm Protein 22 (SP22) in the Rat Ovary During Different Reproductive States

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Sperm protein 22 (SP22) is correlated with fertility in rats. It has been identified in testis and implicated in sperm-egg interaction, protection against oxidative stress, and androgen receptor function. SP22 is widespread in rat and human tissues but has not yet been reported in the ovary. Using reverse transcription polymerase chain reaction, we identified the presence of SP22 transcripts in the rat ovary. We assessed the cellular distribution of the SP22 protein by collecting ovaries from rats in each of the following groups: 30, 60, and 90 days old; Days 9.5, 14.5, 16.5, 18.5, and 20.5 of pregnancy; and Days 1, 2, 8, and 19 of lactation. Tissue sections were stained immunohistochemically for SP22, and some serial sections were stained for relaxin or cytochrome P450 cholesterol side-chain cleavage enzyme (SCC). Weak staining for SP22 was evident in some corpora lutea (CL) and some interstitial gland cells in nonpregnant adult rats. At Day 9.5 of pregnancy, SP22 was detected in all CL, but staining intensity was weak. Staining intensity for SP22 in CL increased from Day 9.5 to 20.5 of pregnancy but was low on postpartum Day 1 and thereafter. A similar temporal pattern of staining intensity in CL was observed for relaxin. Strong immunoreactivity for SCC was present in the CL throughout pregnancy, and the spatial distribution of staining for SP22 in CL and in some areas of ovarian stroma was similar to that for SCC. There was weak staining of some theca cells in some antral follicles of pregnant and early postpartum rats when heat-induced antigen retrieval was used. There was inconsistent staining of oocytes for SP22, particularly in 30-day-old rats. In summary, the expression of SP22 was most prevalent in the CL and increased during pregnancy. Exp Biol Med 232:910–920, 2007

Key words: CAP1; corpus luteum; cytochrome P450 cholesterol side-chain cleavage enzyme; DJ-1; ovary; pregnancy; relaxin; sperm protein SP22

Introduction

Sperm protein 22 (SP22; also called DJ-1 in humans and contraception-associated protein 1 [CAP1] in rats and hamsters) was originally classified as an oncogene (1). A precise role of SP22 in tumorigenesis has not been defined, but its expression is elevated in lung (2), prostate (3), and breast (4) cancers. SP22 has been shown to be widely expressed; however, of the organs examined, it is particularly abundant in the testis (5, 6).

SP22 has been localized to specific areas in rat (6) and human (7) sperm. It is highly correlated to fertility in male rats, and concentrations decrease in association with loss of fertility that occurs subsequent to exposure of male rats and hamsters to toxicants (6, 8–11). Furthermore, studies have demonstrated that exposure of sperm to anti-SP22 results in decreased fertilization rates both *in vivo* and *in vitro* (12, 13), and SP22 also may be necessary for binding of sperm to the zona pellucida of the egg (13). The mechanism by which SP22 affects fertility has not been elucidated. However, SP22 has both protease and antioxidant characteristics (14). Proteases are needed for penetration of the zona pellucida (15, 16), and it is well known that spermatozoa are particularly vulnerable to oxidative stresses (17–19). SP22 is induced by oxidative stress (20, 21) and has been shown to decrease the effects of oxidative stress in cells by oxidizing and thus removing reactive oxygen species (ROS). Evidence has shown that an appropriate ratio of ROS and antioxidants is required for fertilization with low levels of ROS being beneficial for capacitation and the acrosome reaction, whereas excess ROS result in reduced sperm motility, alter the sperm plasma membrane and DNA, and decrease fertility (19, 22). In addition, SP22 is a positive regulator of androgen receptor (AR) function (23), and androgens are essential for spermatogenesis.

This work was supported by a grant from the National Institutes of Health (MD00233).

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Received June 6, 2006.
Accepted March 3, 2007.

1535-3702/07/2327-0910$15.00

Despite accumulating evidence that SP22 plays a significant role in gonadal function and fertility in the male, it has not yet been identified in the ovary. Ovarian ROS are thought to play a role in oocyte quality and regression of the corpus luteum (CL; Refs. 24–26). Like the testis, the ovary contains ARs (27–30) and produces androgens that are thought to participate in oocyte development, follicular development, and follicular atresia (31). Since SP22 is involved in the differentiation and proliferation of cells, protection from ROS, and regulation of AR in the male gonad, it is important to determine whether SP22 is also present in the ovary, where it may play a role in ovarian function.

Therefore, we used reverse transcription polymerase chain reaction (RT-PCR) to determine if SP22 transcripts were present in the ovaries of adult cyclic, pregnant, and lactating rats. In addition, ovaries were collected from prepubertal, adult cyclic, pregnant, and lactating rats for immunohistochemical localization of SP22 protein in ovarian cells in different functional states. We report the presence of SP22 transcripts in the rat ovary and the presence of SP22 in specific ovarian cells, the expression of which differs with reproductive status of the rat.

Materials and Methods

Reagents. General laboratory supplies and reagents were purchased from Fisher Scientific (Fairlawn, NJ), VWR International (Suwanee, GA), and Sigma-Aldrich (St. Louis, MO). RT-PCR reagents were obtained from Applied Biosystems (Branchburg, NJ). TRIzol Reagent, TOPO TA Cloning Kit, and oligonucleotide primers were obtained from Invitrogen (Carlsbad, CA). QIAprep Spin Miniprep Kits were obtained from Qiagen, Inc. (Valencia, CA). Horseradish peroxidase (HRP)–conjugated donkey anti-goat secondary antibody, goat anti-SP22 and rabbit anti-relaxin primary antibodies, normal rabbit and goat IgG, and SP22 blocking peptide were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Diaminonobenzidine (DAB) Plus Kits, aminoethyl carbazole (AEC) Single Solution, and HRP-conjugated goat anti-rabbit secondary antibody (65–6120) were purchased from Zymed Laboratories (South San Francisco, CA). Rabbit anti–cytochrome P450 cholesterol side-chain cleavage enzyme (SCC; RDI-P450SCCabr) was purchased from Research Diagnostics, Inc. (Flanders, NJ). Anti-SP22 was a peptide affinity-purified goat polyclonal antibody raised against 16 amino acids by peptide mapping within the 50 amino acids at the carboxy terminus of DJ-1 of human origin (sc-27006). Santa Cruz Biotechnology recommended it for detection of DJ-1 of mouse, rat, and human origin by Western blotting and immunohistochemistry. We found it to recognize a single protein product of the appropriate size in rat tissues by Western blotting (32). Anti-relaxin was produced against a recombinant protein representing full-length human relaxin (sc-20652). Anti-SCC was produced against a synthetic peptide corresponding to amino acids 421–441 of rat SCC.

Animals and Collection of Tissue. Female rats (CD) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed in rooms with controlled lighting (lights on 0700–1900 hrs daily) and temperature (20°–22°C) and were given Teklad Rodent Diet 8604 (Harlan, Madison, WI) and tap water *ad libitum*. Animals were maintained and used in accordance with the standards of the Institutional Animal Care and Use Committee of the University of South Carolina. All animals were killed by decapitation between 1100 and 1400 hrs.

Four to nine rats were used at each of the following times: 30, 60, and 90 days of age; Days 9.5, 14.5, 16.5, 18.5, and 20.5 of pregnancy; and Days 1, 2, 8, and 19 postpartum. The morning after mating was defined as Day 0.5 of pregnancy. Sixty-day-old rats were used on proestrus (one rat), estrus (two rats), and metestrus (one rat) as determined by examination of vaginal cells collected daily by lavage (33). Ninety-day-old rats were used (two per group) on proestrus, estrus, and metestrus. The uteri were examined grossly at the time of use in pregnant rats to visualize embryos. One ovary from each rat was fixed in 4% paraformaldehyde for 24 hrs and then processed for immunohistochemistry. In some of the rats, the contralateral ovary was fixed in Bouin solution for comparison. In addition, one ovary from each of three adult rats (90-day-old proestrous rat, Day 9.5 of pregnancy, and Day 8 of lactation) was snap-frozen on dry ice and stored at −80°C until processed for recovery of total RNA.

Amplification of Rat Ovarian SP22. Individual ovaries were placed into sterile RNase- and DNase-free microfuge tubes containing 1 ml of TRIzol. Tissue was homogenized using minipestles, processed according to the manufacturer's protocol, quantified using a UV spectrophotometer, and stored at −80°C until used for RT-PCR as described previously (32).

In brief, total ovarian RNA (1 μg) was reverse transcribed using MuLV reverse transcriptase and amplified using AmpliTaq DNA polymerase according to manufacturer's instructions using a GeneAmp PCR Kit (Applied Biosystems). Conditions for PCR were 95°C for 2 mins, 35 cycles of 95°C for 1 min and 64°C for 1 min, and 72°C for 7 mins. Forward (5′-ATGGCATCCAAAAGAGCTCTG-GTC-3′) and reverse (5′-CTAGTCTTTGAGAACAAGCG-GTGC-3′) primers were made using known homologous sequences for rat and hamster SP22 available in GenBank (accession numbers AF157511 and AJ431372, respectively). The primers were designed to amplify the full-length cDNA for the coding region, and the amplicon spans several of the predicted intron-exon boundaries, making it distinguishable from genomic DNA. Amplified cDNAs were determined to be of the correct size (570 base pairs) on agarose gels and were ligated into the pCRII-TOPO vector (Invitrogen) for subsequent DNA sequencing (University of Maine Sequencing Service, Orono, ME).

Immunohistochemistry. Whole ovaries were placed in 4% paraformaldehyde for 24 hrs or Bouin solution for 48–72 hrs and then embedded in paraffin. Serial sections (6 μm) were mounted on Superfrost Plus coated slides (48311-703; VWR International) and stained for SP22, relaxin, or SCC with modifications of procedures previously described (32, 34) using HRP-labeled secondary antibody conjugates. Tissue sections stained for SCC were subjected to heat-induced antigen retrieval prior to incubation with primary antibody to expose antigenic sites within the mitochondria, as previously reported (35, 36). Serial sections stained for SP22 on the same slides as tissue sections stained for SCC also were subjected to heat-induced antigen retrieval. Slides from one rat from each group were included in each run to control for run to run variation. Antibody dilutions were as follows: SP22, 1.33 μg/ml; relaxin, 0.4 μg/ml; and SCC, 1:2000. Specificity of all primary antibodies was confirmed by an absence of staining in serial sections incubated with the same concentration of normal IgG of the same species or with the same dilution of normal rabbit serum. Further specificity tests for SP22 were conducted by preincubating the SP22 antiserum (1.33 μg/ml) with five times the concentration of blocking peptide (6.65 μg/ml; sc-27006 P) for either 2 hrs at room temperature or overnight at 4°C prior to use.

Sections were deparaffinized with xylene, rehydrated with decreasing concentrations of ethanol followed by H_2O and phosphate-buffered saline (PBS). Endogenous HRP activity was blocked by 30 mins' incubation with 0.3% H_2O_2 in methanol. After thorough rinses, slides were incubated with blocking agent (10% donkey or goat serum in PBS) for 1 hr at room temperature. Blocked sections were incubated with primary antibody overnight at 4°C. Slides were then washed with PBS and incubated for 1 hr at room temperature with a 1:600 dilution of HRP-conjugated secondary antibody (goat anti-rabbit for relaxin and SCC; donkey anti-goat for SP22). Sections were incubated with DAB to visualize HRP activity and then counterstained with hematoxylin. For sections stained for SP22 and then incubated with DAB, adjacent sections from all ovaries were stained for SP22 and then incubated with AEC for comparison. Sections were examined and images captured using an Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY) and SPOT camera and software (Diagnostic Instruments, Inc., Amsterdam, The Netherlands). Captured images were then processed using Adobe Photoshop 6.0 software (Adobe Systems, San Jose, CA).

CL Staining Intensity. We quantified the intensity of staining for SP22 using DAB in CL on the different days of pregnancy and Day 1 postpartum. Slides from one rat from Days 9.5, 14.5, 16.5, 18.5, and 20.5 of pregnancy and Day 1 postpartum were included in each of four runs to control for run to run variation. SP22 staining intensity was assessed visually on a scale of 0.5 to 4.0 in increments of 0.5 for all CL in each individual tissue section (1, lightly stained; 2, moderately stained; 3, markedly stained; 4, heavily stained), and these values were averaged. These averages were used to conduct statistics with an n of 4. Analyses were carried out with a one-way ANOVA followed by post hoc Newman-Keuls tests.

Results

Agarose gels of PCR products from ovarian total RNA from all three rats revealed the presence of a transcript that was 570 base pairs. Sequencing of amplicons confirmed that SP22 mRNA is synthesized by cells within the ovary. The cDNA produced had greater than 99% homology to reported sequences for rat (GenBank accession number AF157511). The inferred amino acid sequence was identical to published sequences (37).

Sections shown in all figures are from paraformaldehyde-fixed tissues incubated with DAB (Figs. 1–6) or AEC (Fig. 4). Those shown in Figure 6 were subjected to heat-induced antigen retrieval. The cells stained and the patterns of expression were similar in the additional ovaries fixed in Bouin solution (data not shown).

In 30-day-old prepubertal rats, SP22 immunoreactivity in ovaries incubated with DAB was detected inconsistently in oocytes and was not observed elsewhere in the ovary (Fig. 1A and B). When oocytes were stained for SP22 immunoreactivity, staining intensity varied from barely detectable to the intensity observed in Figure 1B, regardless of follicle size class (primordial, unilaminar and multilaminar preantral, or antral). By contrast, SP22 immunoreactivity was not observed in ovarian tissues including oocytes from 30-day-old rats incubated with AEC.

In 60- and 90-day-old cyclic rats, weak SP22 immunoreactivity was observed in CL (Fig. 1C and D) in tissues incubated with DAB or AEC in 4 of 10 rats (60 days old on proestrus, 90 days old on proestrus, 90 days old on estrus, and 90 days old on metestrus). The intensity of SP22 staining in the CL of these four rats was equivalent to the rank of 0.5 or 1 in Table 1. Weak SP22 immunoreactivity was occasionally observed in interstitial glands in tissues incubated with DAB or AEC. By contrast, weak SP22 immunoreactivity was infrequently observed in oocytes ($<$10% of oocytes) in tissues of 60- and 90-day-old cyclic rats incubated with DAB and not observed in oocytes in tissues of 60- and 90-day-old cyclic rats incubated with AEC.

At Day 9.5 of pregnancy, SP22 was clearly detected in all CL in tissues incubated with DAB (Table 1) (Fig. 2) or AEC, but staining intensity was weak. As pregnancy progressed from Day 9.5 to Day 20.5, staining intensity for SP22 in CL increased but was low on postpartum Day 1 (Table 1; Fig. 2) and thereafter. Similar results were observed when tissues from pregnant and postpartum rats were incubated with AEC. Within the CL, both nuclear and cytoplasmic staining for SP22 was detected (Fig. 3). No staining was observed in control sections in response to incubation with normal IgG prior to incubation with DAB

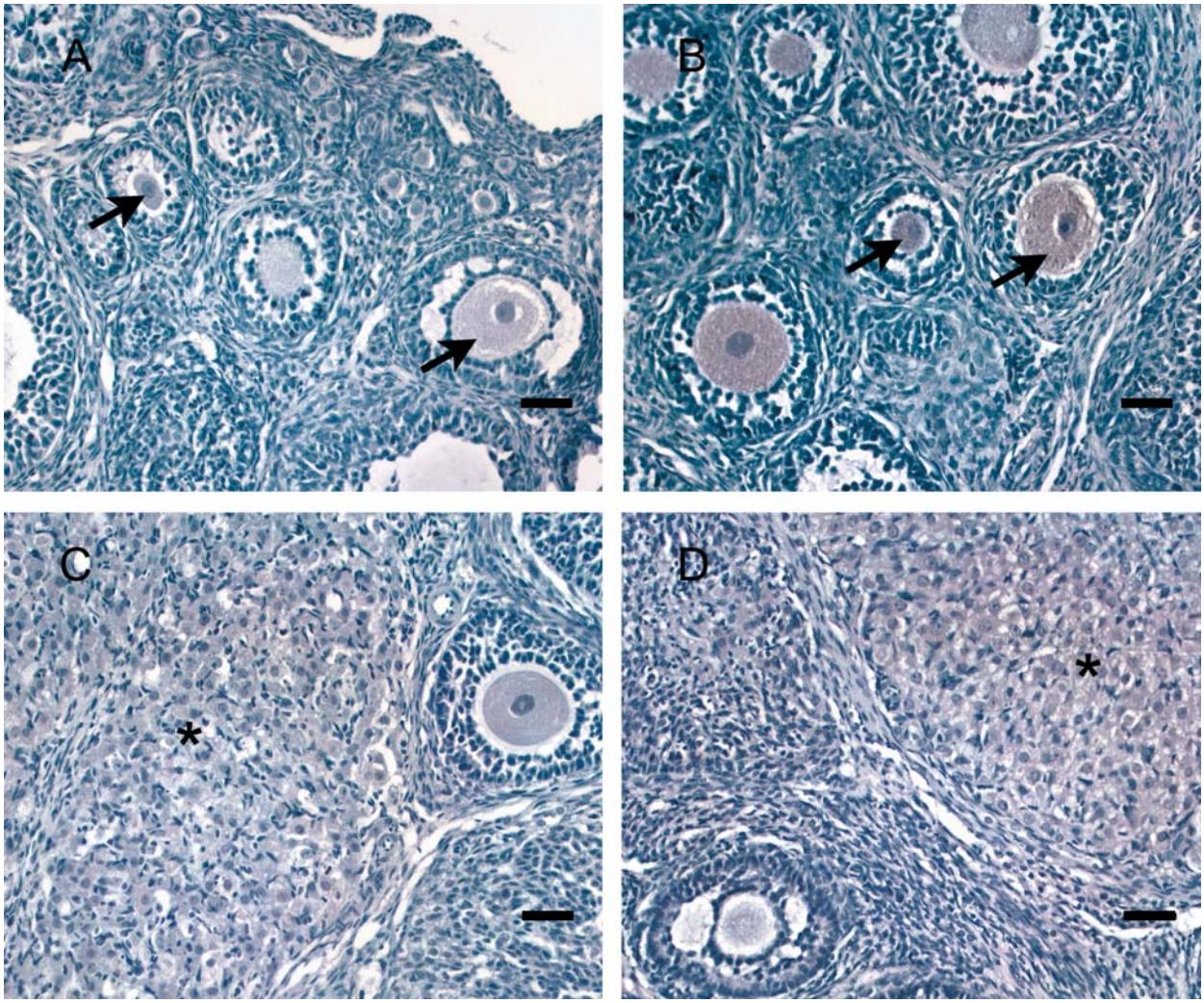

Figure 1. Sections of ovary stained with SP22 antiserum from rats at 30 (Panels A and B), 60 (Panel C, estrus) and 90 (Panel D, metestrus) days. Positive staining for SP22 is shown in brown. At 30 days, SP22 immunoreactivity was detected inconsistently in oocytes and was not observed elsewhere in the ovary. Some oocytes did not stain for SP22 (Arrows in panel A). Other oocytes stained weakly or strongly (Arrows in panel B) for SP22. At 60 (Panel C) and 90 (Panel D) days, some CL (*) stained weakly for SP22. Bar, 50 μm.

(Fig. 2) or AEC (Fig. 4B). Similarly, no staining for SP22 was observed when the SP22 antiserum was preincubated with SP22 blocking peptide prior to incubation with DAB (Fig. 4D) or AEC.

The temporal pattern of increased staining intensity for SP22 in CL during pregnancy was similar to that observed for relaxin (Fig. 5). When heat-induced antigen retrieval was used to stain for SP22 and SCC on the same slides, the temporal pattern of increased staining intensity for SP22 in CL during pregnancy was similar to that observed when heat-induced antigen retrieval was not used; however, staining intensity was increased at all time periods (Fig. 6 vs. Fig. 2). The CL exhibited similar and strong expression of staining for SCC throughout the days of pregnancy evaluated (Fig. 6). Cytoplasmic staining but not nuclear staining was observed for relaxin and SCC in CL. Weak SP22 immunoreactivity was observed infrequently in oocytes (<10% of oocytes) in tissues of pregnant or postpartum rats incubated with DAB (with or without heat-induced antigen retrieval), but no staining was observed in oocytes after incubation with AEC. As expected, relaxin and SCC immunoreactivity was not observed in oocytes (Figs. 5 and 6; Refs. 36, 38).

By Day 1 postpartum, staining for SP22 was dramatically decreased and coincided with the decreased expression of relaxin (Fig. 5) and SCC (Fig. 6). As lactation progressed through Day 19 postpartum, some CL stained intensely for SCC but did not stain or only weakly stained for SP22 (not shown).

When low power was used, there was variable staining intensity for SP22 in interstitial glands in tissues incubated with DAB (Figs. 5 and 6) or AEC during pregnancy that was less obvious postpartum. The spatial distribution of staining for SP22 in ovarian stroma was similar to that for SCC (Fig. 6).

SP22 immunoreactivity was not observed in granulosa cells of healthy follicles, with (Figs. 1–5) or without heat-

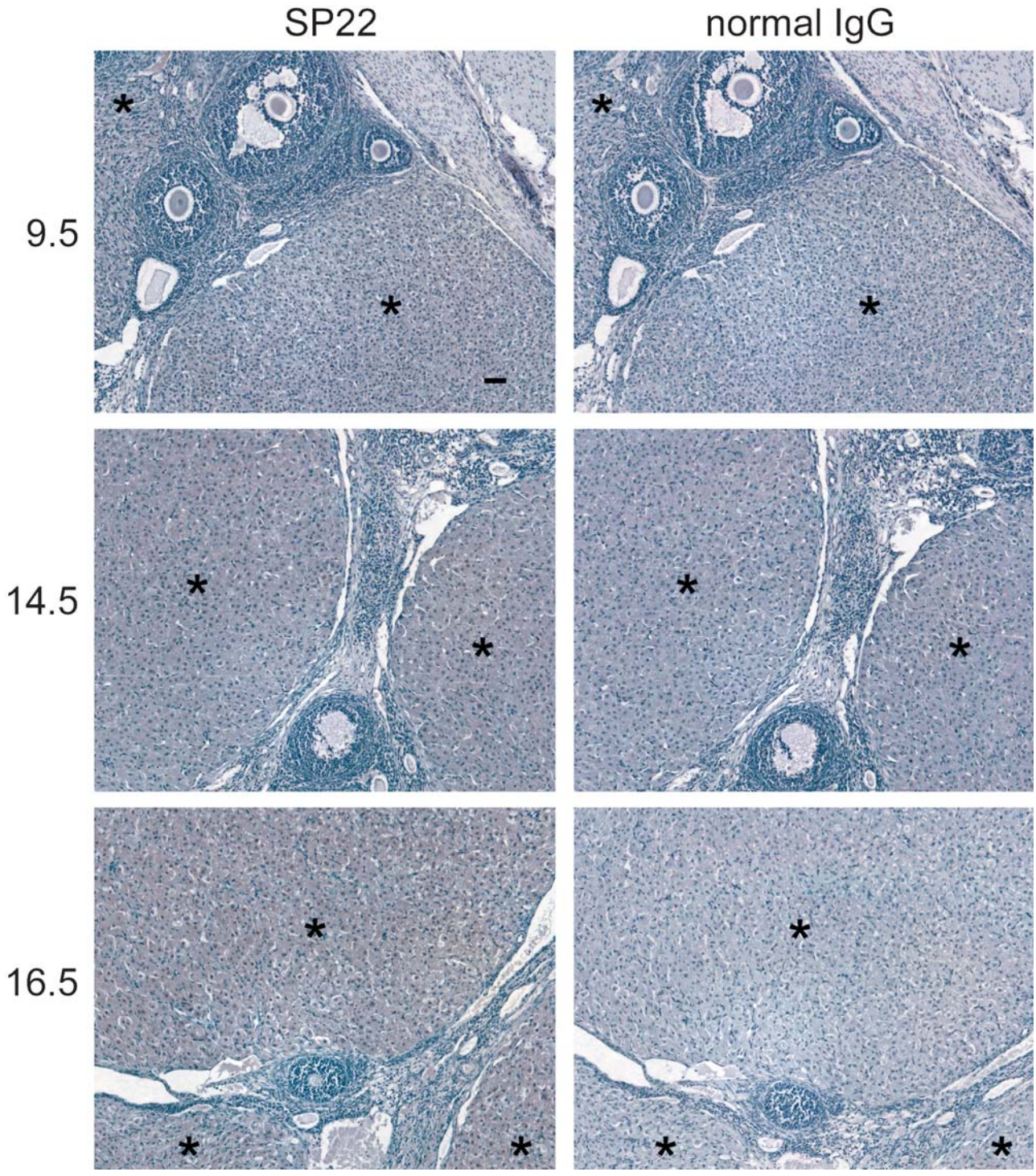

Figure 2. Sections of ovaries from rats at Days 9.5, 14.5, and 16.5 of pregnancy and Days 18.5 and 20.5 of pregnancy and Day 1 postpartum that were stained immunohistochemically in the same run. Positive staining for SP22 is shown in brown. Serial sections were stained for SP22 (Left column) or normal IgG (Right column). SP22 was detected in CL (*) at all time periods. Intensity of signal for SP22 in CL increased dramatically from Day 9.5 to 20.5 and then decreased by Day 1 postpartum (PP1). Staining intensity was quantified (Table 1). Intensity of SP22 staining in CL in the examples shown here are pregnant Day 9.5 = 1.0, Day 14.5 = 1.5, Day 16.5 = 2.0, Day 18.5 = 2.5, and Day 20.5 = 3.5, and PP1 = 1.0 on a scale of 0.5–4.0 for which 4 is the most heavily stained. See Table 1 for definitions. No staining was observed in control sections in response to incubation with normal IgG. Bar, 50 μm. All images are shown at the same magnification.

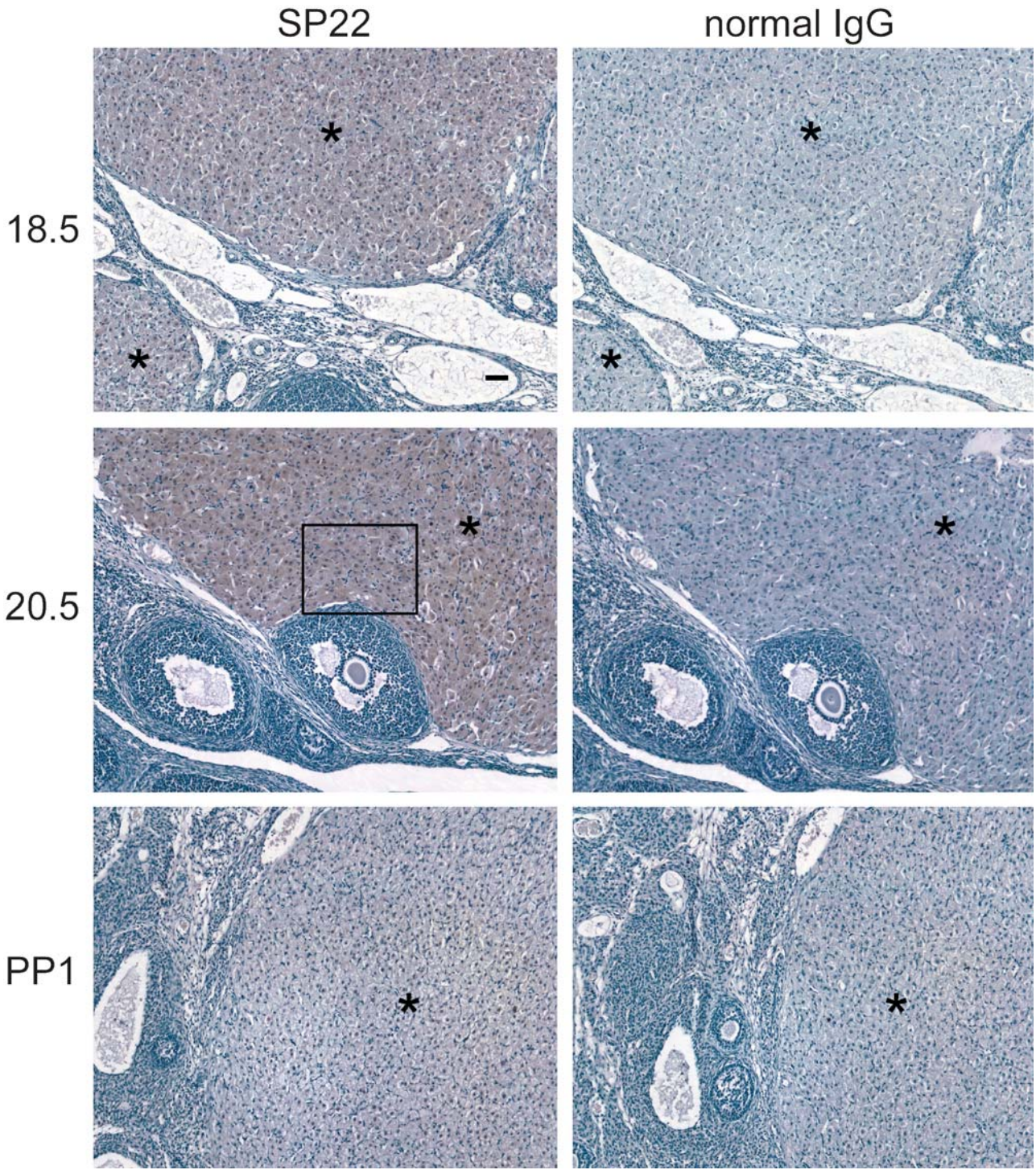

Figure 2. Continued.

induced antigen retrieval (Fig. 6). Weak SP22 immunoreactivity was observed in some theca cells of some healthy antral follicles in pregnant and early postpartum rats but only when heat-induced antigen retrieval was employed. During late pregnancy (on or later than Day 16.5) and early postpartum (Days 1 and 2), a few antral follicles undergoing atresia had some staining for SP22 (<10%) in their granulosa and/or theca cells as observed with or without heat-induced antigen retrieval (not shown).

Discussion

It is well established that SP22 is a sperm protein involved in fertilization (5, 13, 14, 37). The present study demonstrated that SP22 also is synthesized in the ovary,

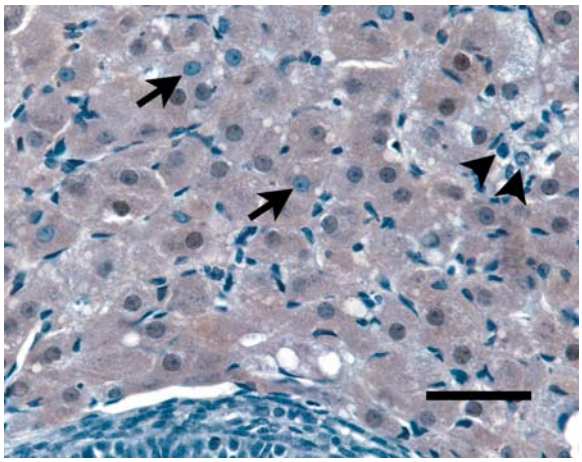

Figure 3. High-power view of the boxed area of rat ovary on Day 20.5 of pregnancy shown in Figure 2. Positive staining for SP22 is shown in brown. In the majority of luteal cells, SP22 was detected in both cytoplasm and nucleus. Other luteal cells were observed in which immunoreactivity for SP22 was in cytoplasm only (arrows) or was not detected (arrowheads). Bar, 50 μm.

indicating that it may play a role in gonadal function in both sexes. We amplified SP22 cDNA from ovarian tissue and localized SP22 protein to specific cell types of the rat ovary. SP22 transcripts and SP22 protein were present in the ovaries of adult cyclic, pregnant, and lactating rats, indicating that SP22 is made in ovaries of adult rats in different reproductive states.

SP22 immunoreactivity was localized primarily to CL and to lesser extent interstitial glands, and the greatest expression was during pregnancy. These observations and the changes in SP22 staining intensity that were quantified in CL from Day 9.5 of pregnancy to postpartum Day 1 were made in paraformaldehyde-fixed tissues incubated with DAB. In confirmation of these results, similar observations were made in paraformaldehyde-fixed tissues incubated with AEC and in Bouin-fixed tissue incubated with DAB (not shown).

SP22 may be involved in the regulation of steroid secretion by the CL during pregnancy. The time frame for the increase in SP22 immunoreactivity in CL from Day 14.5

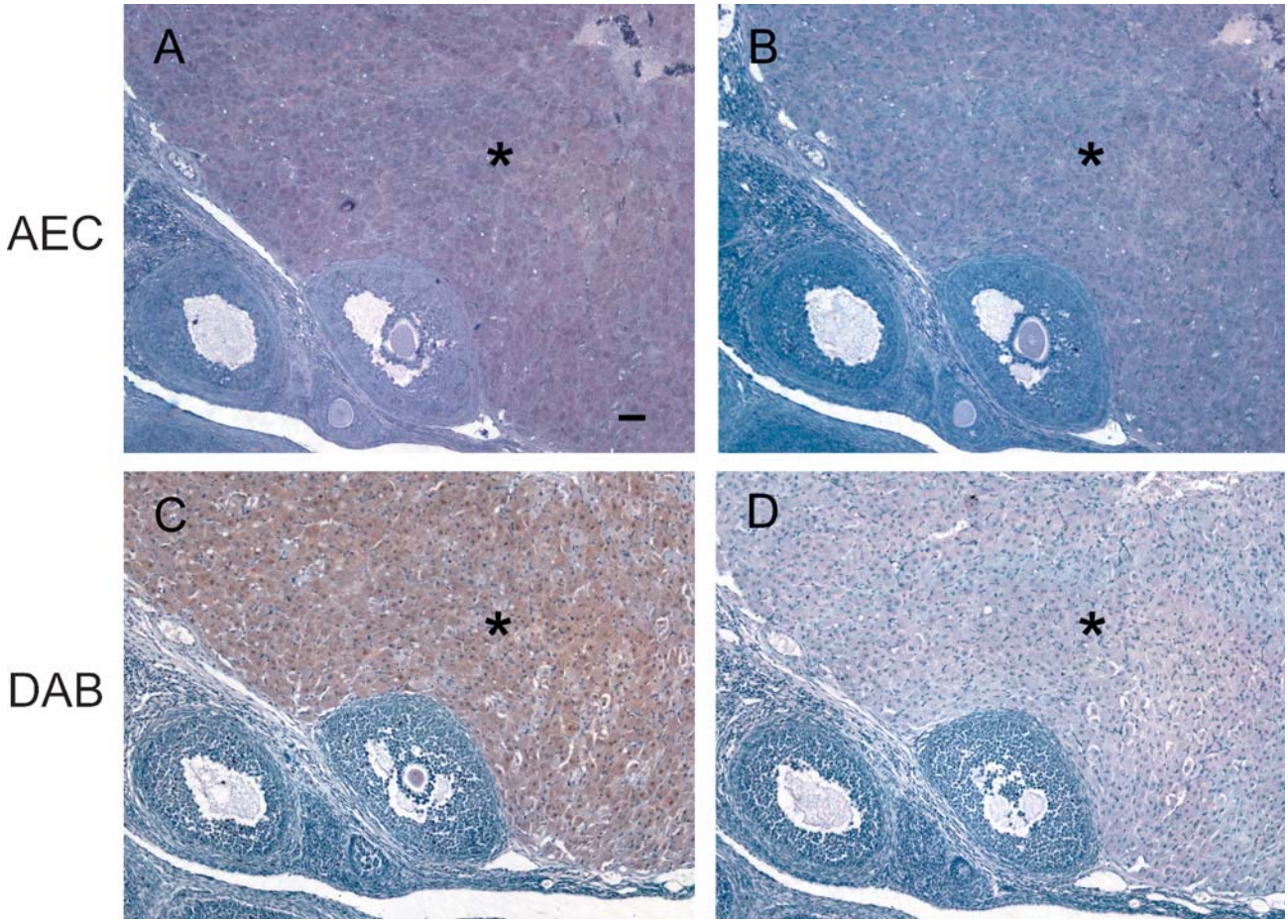

Figure 4. (A–D) Serial sections of a rat ovary on Day 20.5 of pregnancy. Sections were stained for SP22 (Panel A) or IgG (Panel B) and then incubated with AEC. Positive staining for SP22 is shown in reddish-brown. Other sections were incubated with SP22 antiserum (Panel C) or SP22 antiserum preincubated with SP22 blocking peptide (Panel D) prior to incubation with DAB. Positive staining for SP22 is shown in brown. SP22 was detected in CL (*) after incubation with AEC (Panel A) or DAB (Panel B). CL did not stain after incubation with IgG (Panel B) or SP22 antiserum preincubated with SP22 blocking peptide (Panel D). Bar, 50 μm. All images are shown at the same magnification.

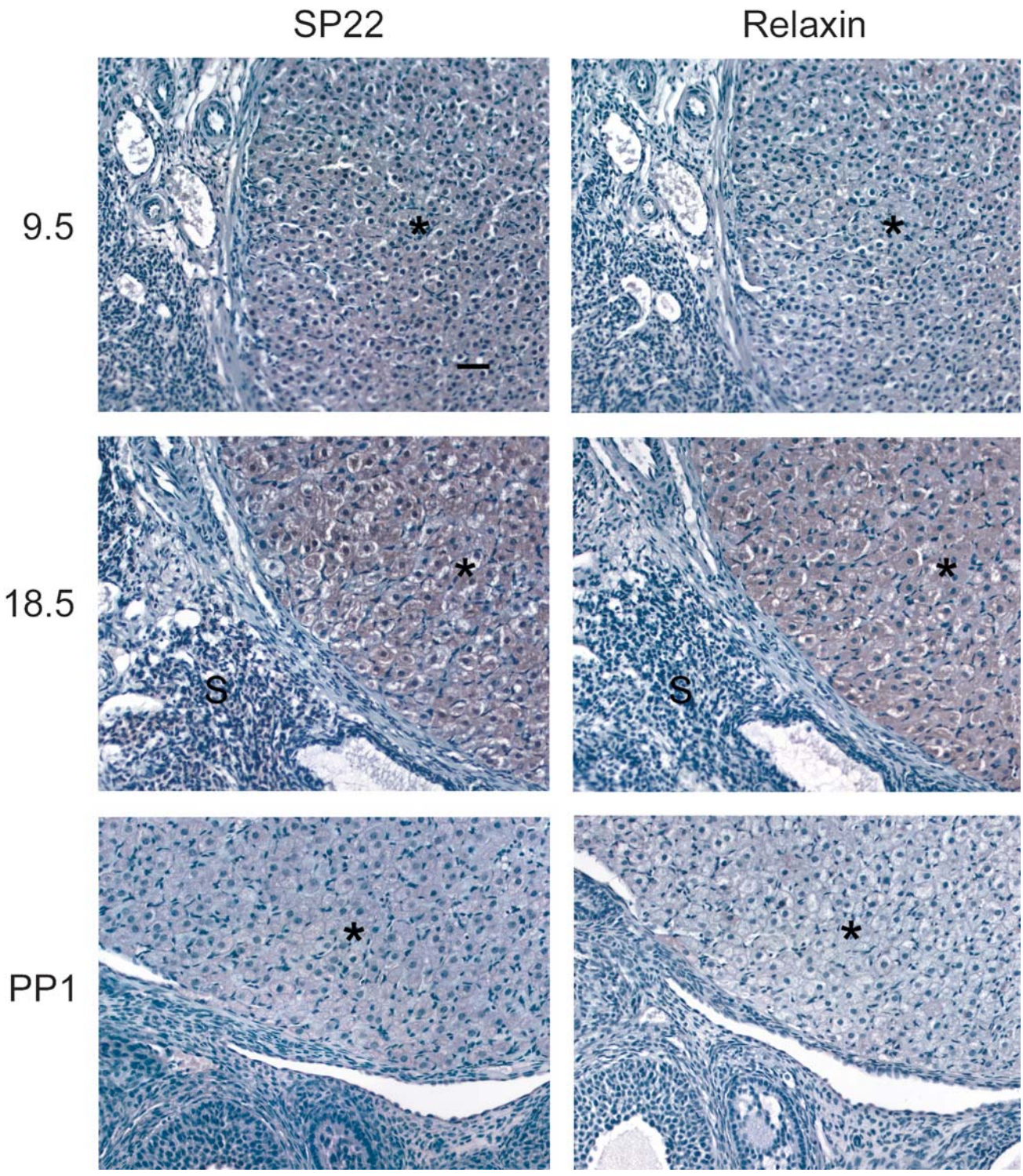

Figure 5. Sections of ovaries from rats at Days 9.5 and 18.5 of pregnancy and on PP1. Serial sections were stained for SP22 (Left column) and relaxin (Right column). Positive staining for SP22 and relaxin is shown in brown. SP22 was detected in CL (*) and within some interstitial glands in the stroma (S) at all time periods. Intensity of signal for SP22 and relaxin in CL increased dramatically during pregnancy and then decreased by PP1. The stroma did not stain for relaxin. Bar, 50 μm. All images are shown at the same magnification.

to 20.5 of pregnancy is similar to the timeframe for a marked increase in mean CL weight, increase in CL estradiol-17β content, and decrease in luteal progesterone content from its peak (39). The peak in SP22 immunoreactivity in the CL at Day 20.5 of pregnancy coincides with the increase in expression of 20α-hydroxysteroid dehydrogenase (40) and the corresponding increase in luteal content of 20α-dihydroprogesterone (39).

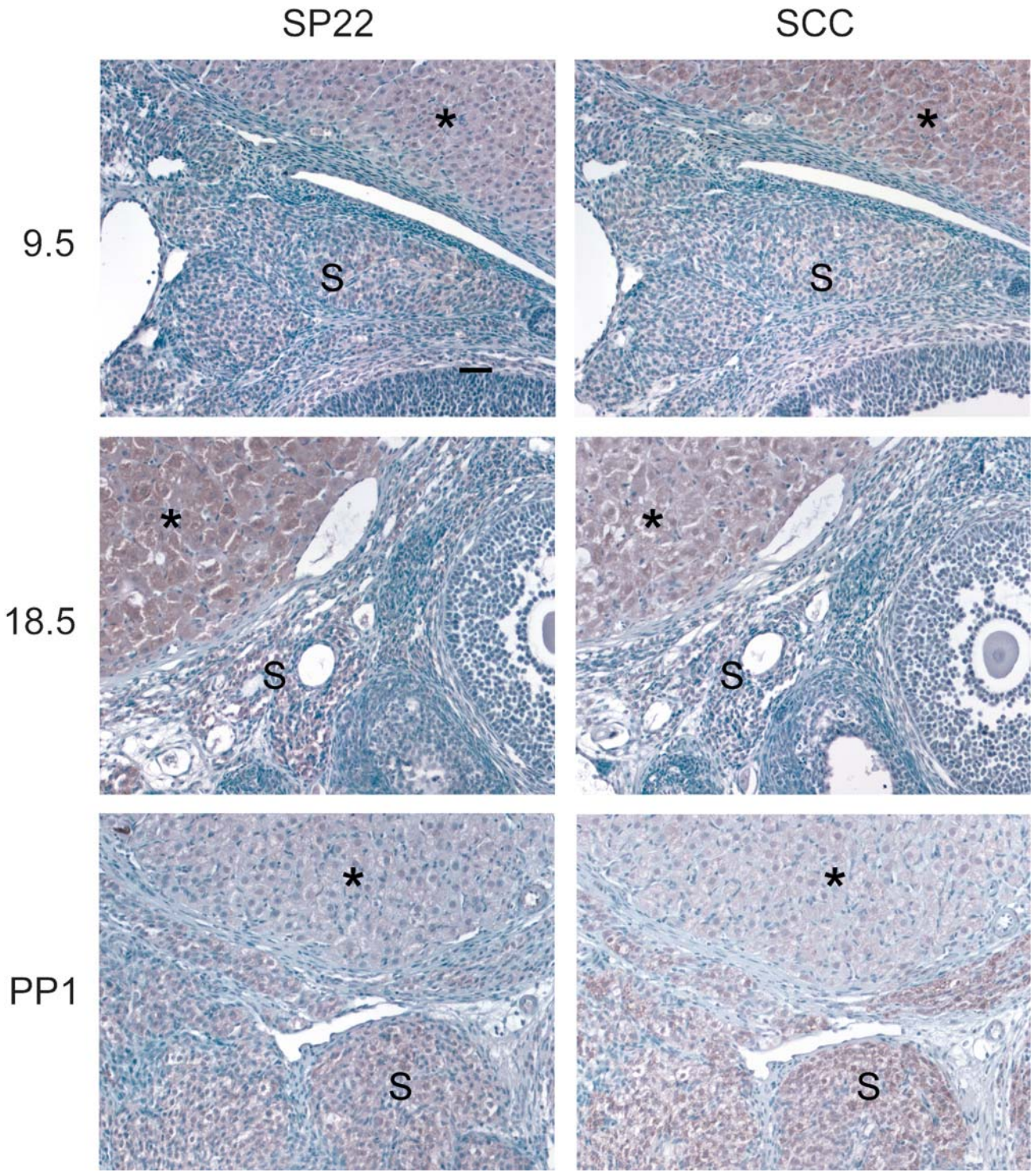

Figure 6. Sections of ovaries from rats at Days 9.5 and 18.5 of pregnancy and on PP1. Serial sections were stained for SP22 (Left column) and SCC (Right column). Positive staining for SP22 and SCC is shown in brown. Following heat-induced antigen retrieval SP22 and SCC were detected in CL (*) and within some interstitial glands in the stroma (S) at all time periods. Intensity of signal in CL for SP22 and SCC increased during pregnancy and decreased by PP1. The spatial distribution of SP22 staining of stroma was similar to that of SCC. Bar, 50 μm. All images are shown at the same magnification.

The presence of SP22 in the nucleus of luteal cells supports the view that those cells may be proliferating or converting to a new hormone secretion profile. Both nuclear and cytoplasmic SP22 have been reported in HeLa cells, and it has been suggested that SP22 may be translocated to the nucleus depending on stage of the cell cycle (1). Nuclear localization of SP22 may be indicative of proliferation or differentiation of cells (1, 6, 37).

Table 1. SP22 Staining Intensity in CL in Pregnant (P) and Postpartum (PP) Rats[a]

Day	P9.5	P14.5	P16.5	P18.5	P20.5	PP1
Staining intensity[b]	1.1 ± 0.1*	1.3 ± 0.1*	2.3 ± 0.3**	2.9 ± 0.4**†	3.5 ± 0.2†	0.6 ± 0.1*

[a] One section through an ovary of one rat in each group was analyzed in each of four runs. SP22 staining intensity was assessed on a scale of 0.5 to 4.0 in increments of 0.5 for all CL in each individual tissue section, and these values were averaged (n = 4). [b] 1, lightly stained; 2, moderately stained; 3, markedly stained; 4, heavily stained (see Fig. 2 for examples).
*,**,† Means ± SE were significantly different than each other ($P < 0.01$).

In humans, SP22 transcription is activated by Sp1 (41). In turn, Sp1 has been implicated in the expression of 20α-hydroxysteroid dehydrogenase in rodents (42). Sp1 also has been implicated in the regulation of 17β-hydroxysteroid dehydrogenase type 7 gene promoter, and it is expressed in rat CL during the same time frame as SP22 (43). Thus, Sp1 may be important in coordinating SP22 transcription with its alteration of genes involved in steroid metabolism.

The ovary is a metabolically active tissue susceptible to production of ROS, and the regression of CL has been linked to the production of ROS (24, 26). SP22 has been shown to modulate cell responses to oxidative stress and as a result is able to modify cell death and apoptosis (20, 21). Thus, the increase in luteal SP22 during pregnancy may function to help maintain the CL.

The predominance of SP22-positive CL late in pregnancy also raises the possibility that SP22 may play a role in relaxin secretion. The time frame for changes in the intensity of SP22 immunoreactivity in CL was similar to that observed for luteal relaxin (38, present study). These changes also are in agreement with ovarian relaxin concentrations in the rat (44, 45). The decreased staining for relaxin in CL on postpartum Day 1 is associated with the release of relaxin into the circulation prior to and during parturition (45). It is unclear what is responsible for the decreased staining of SP22 after parturition.

SP22 has been shown to enhance nuclear AR function, and nuclei of CL in pregnant rats contain SP22 (present study) and ARs (29, 46). Taira *et al.* (47) reported that colocalization of SP22 and AR was important to AR activity. Thus, it is possible that SP22 regulates AR function in CL.

Detection of SP22 in luteal cells and cells of interstitial glands with and without the use of heat-induced antigen retrieval and in some theca cells of antral follicles after employing heat-induced antigen retrieval but not in granulosa cells of healthy follicles suggests that SP22 is localized only in cells that are capable of synthesizing steroids *de novo*. The significance, if any, of SP22 staining of a few granulosa and theca cells in some preantral follicles undergoing atresia during late pregnancy or early lactation is unclear.

In tissues incubated with DAB, inconsistency in the staining of oocytes for SP22 in follicles of all stages raises the possibility that such staining may be nonspecific. The observation that oocytes did not stain for SP22 in tissues incubated with AEC supports this view.

The results of this study demonstrate that the ovary synthesizes SP22 and that its expression increases markedly in CL during pregnancy. Although specific role(s) for SP22 in the ovary have not been defined, its expression coincides with an increase in size of CL, maintenance of the CL during the latter part of pregnancy, and changes in steroid and relaxin secretion by the CL during pregnancy. Further experimentation is warranted to elucidate the potential importance of SP22 in these processes.

The authors thank Neda Osterman and William McAmis for technical assistance.

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