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Gonadotropin-Induced Superovulation Drives Ovarian Surface Epithelia Proliferation in CD1 Mice

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The ovarian surface epithelium (OSE) is a monolayer of cells that surround the ovary and accommodate repeated tear and repair in response to ovulation. OSE cells are thought to be the progenitors of 90% of ovarian cancers. Currently, the total amount of proliferation of the OSE has not been reported in response to one ovulatory event. In this study, proliferation of the OSE was quantified in response to superovulation induced by ip injection of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) in immature 27-d-old CD1 mice using bromodeoxyuridine (BrdU). BrdU incorporation into the OSE cells was measured from the time of hCG injection for a total cumulative label of 12 h. BrdU incorporation was also measured from the time of PMSG injection for a total label of 60 h to correlate proliferation with specific gonadotropin stimulation. The OSE proliferation was significantly higher in superovulated animals compared with control mice at all time points. Proliferation was also analyzed in discrete anatomical sections and indicated that OSE covering antral follicles and corpora lutea proliferated more rapidly than OSE distal to follicular growth. Finally, apoptosis was assessed in response to ovulation, and virtually no cell death within the OSE was detected. These data demonstrate that the OSE, especially near antral follicles and corpora lutea, proliferates significantly in response to the gonadotropins PMSG and hCG. Therefore, ovarian surface cell division in response to ovulation could contribute to ovarian cancer by proliferation-induced DNA mutations and transformed cell progression. (*Endocrinology* 147: 2338–2345, 2006)

THE OVARIAN SURFACE epithelium (OSE) is a single cell layer of squamous and cuboidal cells that express both epithelial and mesenchymal characteristics (1). These cells do not contain discrete markers when compared with other tissues derived from coelemic epithelia, suggesting that they are less differentiated and more pleuripotent (2). The OSE is important to the integrity of the ovary and serves as the regulated barrier at the time of ovulation. The OSE was originally studied to investigate its contribution to ovarian follicular rupture and the subsequent repair (3, 4). Currently, the OSE receives attention because these cells are considered the progenitors of 90% of ovarian cancers (5). The etiology of the disease and mechanisms by which ovarian cancer cells progress to more dangerous phenotypes are poorly understood.

Ovulation induces a rupture site that is closed through reepithelialization by the OSE proliferating and then migrating to cover the gap (6, 7). First proposed by Fathalla (8) in 1971, the "incessant ovulation" hypothesis suggests that continual ovulation subjects the OSE to transformation events, which can lead to ovarian cancer. Increased occurrence of ovarian cancer is associated with increased ovulatory events in women undergoing infertility treatments (9). Additionally, the risk of ovarian cancer is reduced in women who experience fewer total ovulations either by the use of oral contraceptive pills, pregnancy, late menarche, early menopause, or hysterectomy (10, 11). At the site of ovulation, OSE cells suffer DNA oxidative damage and express the tumor suppressor p53, showing the potential of ovulation as a stress factor on OSE cells to give rise to a transformed progenitor cell capable of causing a malignant tumor (12). Domestic hens, the only other species besides humans to form ovarian cancer, have an increased risk of this disease directly related to their number of ovulations and increased oxidative damage (13–15). In this proposed ovarian cancer pathway, ovulation induces DNA damage that escapes endogenous repair mechanisms. After transformation, the increased rate of OSE proliferation, potentially stimulated from ovulation, may contribute to tumor progression (16). The expression of FSH and LH receptors in the OSE reflects on their ability to directly respond the gonadotropins FSH and LH independent of an ovulatory event (17, 18). Here we address the influence of the gonadotropins pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), as well as ovulation, on proliferation of OSE cells in CD1 mice.

Most of the current studies have focused on the proliferative activity of OSE in discrete anatomical areas in relationship to follicles and the hilus at specific times using a pulse of bromodeoxyuridine (BrdU) to label dividing cells during a short window of time. However, the total proliferative activity of the OSE in response to PMSG and hCG stimulation in the mouse has not been reported directly nor has it been analyzed in relation to follicle growth, epithelialization, and

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Abbreviations: BrdU, Bromodeoxyuridine; CL, corpus luteum; DAB, diaminobenzidine; hCG, human chorionic gonadotropin; OSE, ovarian surface epithelium; PMSG, pregnant mare serum gonadotropin; TBS, Tris-buffered saline; TUNEL, terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling.

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formation of the corpus luteum (CL). Additionally, theories regarding increased proliferation near the mesothelium, surrounding the hilus, or in OSE with a specific squamous morphology has not been substantiated using cumulative labeling strategies. We describe the total proliferation of OSE during one superovulation cycle in immature mice, which directly correlates to one ovulatory event without previous follicular rupture to confound results. These studies illuminate the normal proliferative function of the OSE in response to ovulation and are significant for understanding the stress imposed on OSE cells from ovulation and repair during transformation and progression of ovarian cancer.

Materials and Methods

Animals for proliferation study

Female CD-1 mice, age 25 d, were obtained through in-house breeding lines. Mice were maintained in accordance with the policies of the Northwestern University's Animal Care and Use Committee. Mice were housed and bred in a controlled barrier facility within Northwestern University's Center of Comparative Medicine. Temperature, humidity, and photoperiod (12 h light, 12 h dark) were kept constant. Animals were allowed access to phytoestrogen-free breeding chow no. 2919 (Harlan Teklad, Indianapolis, IN) and water *ad libitum*.

Experimental design of proliferation study

Control mice were injected ip with PBS at 0900 h. Superovulated mice were injected ip with 5 IU of PMSG at 0900 and 5 IU of hCG diluted in PBS 48 h later (Sigma, St. Louis, MO). Injections containing BrdU were given ip at 100 mg/kg either at the time of the first and second hormone injection or solely with the second hormone injection (Sigma). Upon first injection of BrdU, water was changed to contain 0.8 mg/ml BrdU to allow for continuous labeling (Fig. 1A). Each experimental group contained seven animals. Mice were killed using CO_2 asphyxiation and cervical dislocation. Ovaries, including fat pad, bursa, oviduct, and partial uterine tube, were collected at 1800, 12 h after the second hormone injection. Ovaries were fixed in 4% paraformaldehyde for 8–12 h, dehydrated with ethanol, paraffin embedded, and serial sectioned at 4 μm.

Immunohistochemistry

All reagents were obtained from Vector Laboratories, Inc. (Burlingame, CA) unless otherwise indicated. Slides were deparaffinized using xylenes and rehydrated with subsequent ethanol dilutions. Antigen retrieval was performed using 1 mM sodium citrate by microwaving for 2 min on high and 7 min on low and then cooled in solution for 20 min. Slides were washed in Tris-buffered saline (TBS) with Tween [20 mM Tris, 500 mM NaCl, 0.1%Tween 20 (pH 7.4)]. Tissues were blocked for 15 min in 3% hydrogen peroxide (Fisher Scientific, Pittsburgh, PA) followed by avidin and biotin according to manufacturer's instructions. Slides were incubated in 10% serum of the secondary antibody host in 3% BSA in TBS for 1 h at room temperature. After blocking, slides were incubated overnight at 4 C in primary antibody in 3% BSA-TBS-10% serum. Slides were rinsed three times for 5 min in TBS-Tween and then incubated at room temperature for 1 h in secondary antibody in 3% BSA-TBS. After washing slides in TBS-Tween, avidin/biotin complex (ABC) reagent was added and incubated for 30 min at room temperature. Slides were then washed in TBS, and antigen-antibody-horseradish peroxidase complex was visualized using diaminobenzidine (DAB) reagent for 3 min. Slides were counterstained using hematoxylin. Control slides received serum block instead of primary antibody.

Antibodies

The primary antibodies against BrdU (sheep; 1:50 dilution; Abcam, Cambridge, MA) and cytokeratin 8 (CK8 TROMA-1 antibody, rat; 1:50; Developmental Studies Hybridoma Bank, Iowa City, IA) were incubated overnight at 4 C with ovary sections. The following secondary antibodies were incubated with their respective sections for 1 h at room temperature: biotinylated antisheep (1:200) and biotinylated antirat (1:200) antibodies. Terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining was performed according to the manufacturer's protocol (Deadend Fluorometric TUNEL System; Promega, Madison, WI). The slides were mounted with Vectastain Mounting Medium (Vector Laboratories) containing 4′,6′-diamidino-2-phenylindole and coverslipped.

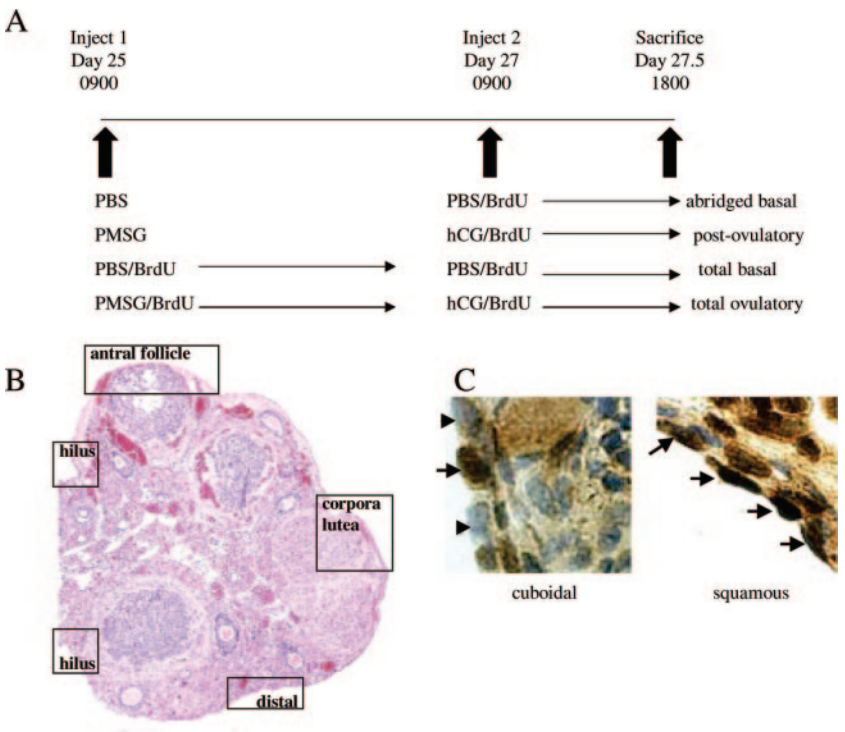

FIG. 1. Schematic of hormone and BrdU injection times, anatomical regions surveyed, and morphologies observed. A, Time intervals are shown for labeling incorporation of BrdU, injections of PMSG and hCG, and time animals were killed. B, Representative superovulated ovary indicating discrete anatomical regions counted. C, Morphological characterization of cuboidal and squamous OSE. Examples of cells stained positive for BrdU are indicated by *arrows* and negative cells are labeled with *arrowheads*.

Imaging and counts

Images were obtained around the perimeter of at least one section per animal using a 20× objective on a Nikon Eclipse E600 microscope (Diagnostic Instruments, Sterling Heights, MI) with a Spot camera and were reconstructed using Adobe Photoshop 7.0. After reconstruction and printing of the image, the perimeter boundaries of the given image were defined. Two separate investigators, blinded to the conditions, independently counted the total number of cells and the total number of positively stained cells (Fig. 1C). Counts for each investigator were averaged and then counts for treatment groups were averaged. Regional counts were completed similarly by partitioning off surface epithelium surrounding follicles with an antrum, the hilus of ovaries when present in the section, distal segment of OSE not overlying any follicles, and in superovulated animals over a CL (Fig. 1B).

Statistical analysis

Total label and regional labels for the distal, follicle, and hilus regions were analyzed using three-factor ANOVA, with genotype, labeling time, and hormone status as the three factors. Regional labels for the CL were analyzed using two-factor ANOVA, with genotype and labeling time as the two factors. In all analyses, tests of main effects were followed by pairwise *t* test comparisons with the *P* value significant at 0.05.

Results

Cumulative proliferation of OSE in response to superovulation

To investigate proliferative changes of OSE cells in response to ovulation, BrdU incorporation of the OSE was quantified in mice that were superovulated (Fig. 1A). In this study, immature mice, with no previous ovulations, were injected with either PBS saline control or a combination of 5 IU of PMSG and hCG to induce superovulation. Once an injection of BrdU was given to an animal, cumulative labeling was achieved by placing BrdU into the drinking water of the animals until the animals were killed. The animals were injected with BrdU to label either background proliferation or that induced from PMSG and hCG. Total basal proliferation was assessed by injecting the animals with PBS and BrdU at 0900 h on d 25 of life and continuing to label all dividing cells until the time the animals were killed on d 27, for a total of 60 h. Abridged basal proliferation was quantified in animals labeled with BrdU at 0900 on d 27 of life until the animals were killed, for a total of 12 h. Total ovulatory proliferation was defined as the mitosis of OSE occurring from 0900 h on d 25 until 2100 h on d 27, for a total of 60 h in animals injected with PMSG and hCG. Proliferation measured in PMSG- and hCG-injected animals from 0900 h on d 27 until the animals were killed is defined as postovulatory proliferation and depicts cell division for 12 h from the time of the hCG injection until the animals were killed. Although the timing of ovulation after hCG varied slightly from animal to animal, the time point when the animals were killed was optimized at 12 h after hCG (data not shown). Ovulation was confirmed in hormone-injected animals based on the presence of CL.

Proliferation of the OSE was detected all around the circumference and in all sections analyzed. The incorporation levels of BrdU in granulosa cells of developing follicles served as an internal positive control to monitor proliferation. OSE cells of both squamous and cuboidal morphologies were found to have proliferated in all of the ovaries analyzed (Fig. 1C). OSE proliferation in the abridged basal animals reached 37.5% after 12 h of labeling and 45.1% in the total basal group after 60 h of labeling (Fig. 2). The basal proliferation rate includes the contribution from endogenous gonadotropins that stimulated the growth of large antral follicles found in every section obtained from the control mice used in the study, suggesting some role for FSH in proliferation of the OSE (19). Stimulation from PMSG and hCG in the total ovulatory animals resulted in a significant increase in the amount of proliferating OSE cells (72.8%) compared with the total basal group (45.1%). Postovulatory animals were injected with BrdU at the time of hCG to label the proliferation that occurred from the time of follicular rupture until repair. The proliferation rate of the OSE in the postovulatory group (56.4%) was significantly higher than in abridged basal mice (37.5%). The rate of proliferation in response to hCG (56.4%) in the postovulatory animals did not differ significantly from the rate of proliferation measured from total ovulation (72.8%). If the contribution from hCG as measured in the postovulatory mice was subtracted from the cumulative labeling index of the total ovulatory animals, the contribution of PMSG to OSE proliferation was 16.4%.

Regional proliferation of the mouse OSE from superovulation

To elucidate whether proliferation of the OSE was occurring in discrete areas in response to follicular development, several anatomical regions of the ovary were selected and counted for the percent of proliferating cells. The schematic in Fig. 1B describes the areas of ovarian surface cells analyzed. The areas selected for evaluation included the OSE directly tandem to the hilus, antral follicles, CL in superovulated animals, and an area distal from follicular development. At least five areas were counted in separate animals to generate the average percent of dividing cells localized around each structure, and the labeling percentage was analyzed between treatment groups. A site distal from follicular growth was chosen to compare the influence of follicle expansion on OSE cells to a region not impacted. The OSE surrounding distal areas did not differ significantly between any of the ovaries, indicating that when follicular maturation is not influencing OSE, the difference in proliferation is not significant (Fig. 3A, i.–v.). These distal areas may be impacted by a variety of factors, making OSE proliferation appear uniform independent of hormone treatment, although the

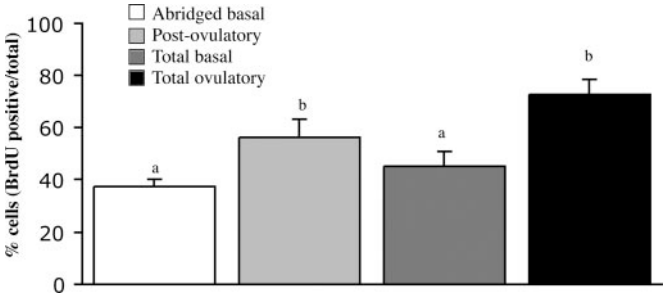

FIG. 2. BrdU incorporation into the OSE over the total surface area of the ovary after gonadotropin stimulation with PMSG and hCG. The data represent the least-square mean (%) of total proliferation and the SE from the mean. The significant differences ($P < 0.05$) are between groups labeled with a *vs.* b.

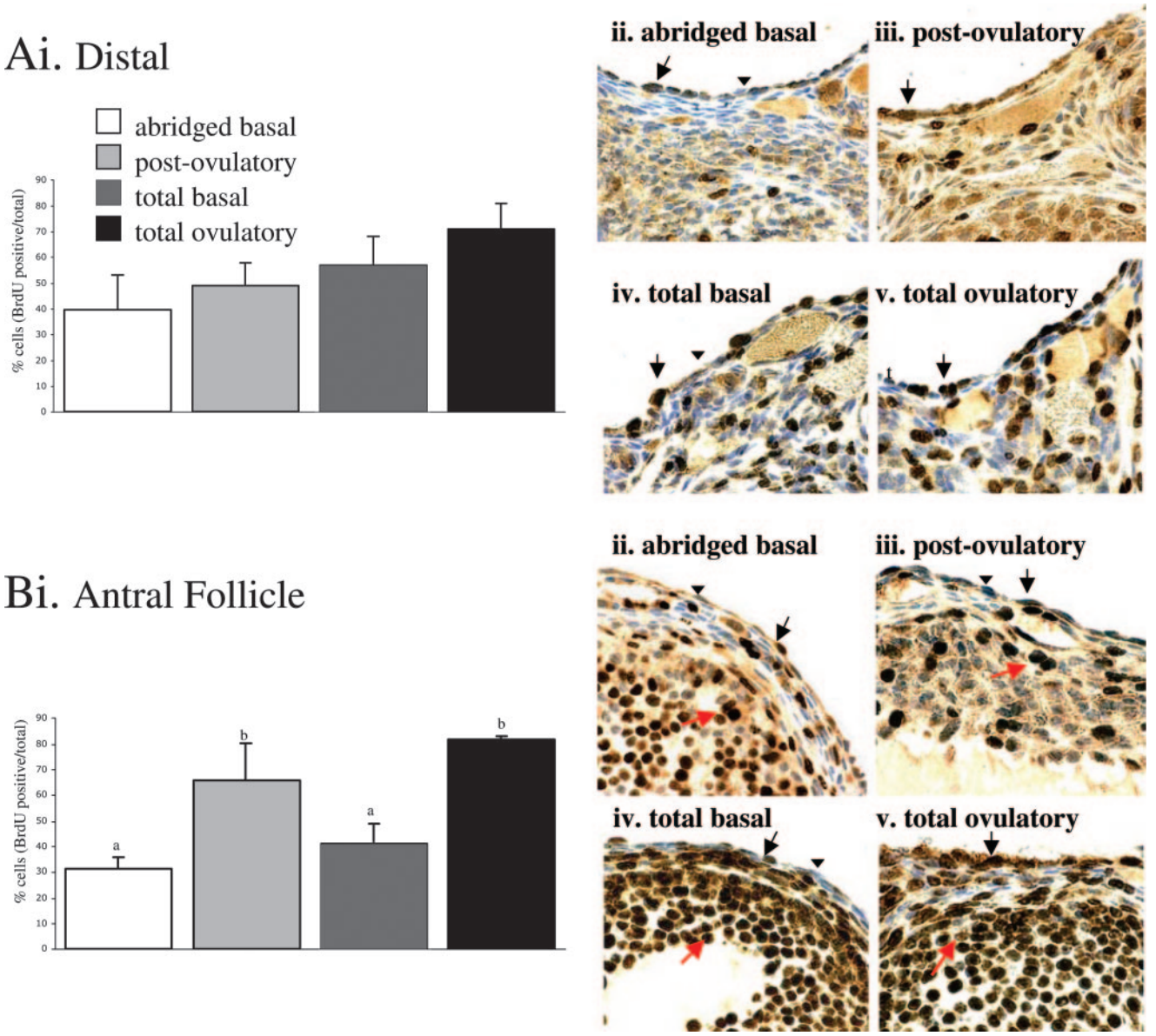

FIG. 3. BrdU incorporation into the OSE covering areas distal from folliculogenesis or tandem to antral follicles. Ai., Data represent the least-square mean (%) of distal proliferation and the SE from the mean. Aii.–iv., BrdU in the OSE of distal areas stained with DAB and counterstained with hematoxylin from abridged basal (ii.), postovulatory (iii.), total basal (iv.), and total ovulatory (v.) treatment groups. Bi., Data represent the least-square mean (%) of proliferation in areas near antral follicles and the SE from the mean. The significant differences ($P < 0.05$) are between groups labeled with a *vs.* b. Bii.–iv., BrdU in the OSE of cells surrounding antral follicles stained with DAB and counterstained with hematoxylin from abridged basal (ii.), postovulatory (iii.), total basal (iv.), and total ovulatory (v.) treatment groups. Cells positive for BrdU are indicated by *arrows*, cells negative for BrdU are labeled with *arrowheads*, and proliferating granulosa cells within antral follicles are labeled with *red arrows*.

label increased according to the amount of time BrdU was being incorporated. The proliferation of OSE adjacent to antral follicles matched the same relative change in proliferation surrounding the entire ovarian surface (Fig. 3B, i.–v.). These data indicate that OSE are partially influenced by the proximity of an expanding follicle to divide. The OSE counts adjacent to follicular development in the total ovulatory group (81.8%) had significantly higher amounts of proliferation than the total basal group (41.2%), and this difference was also significant between the postovulatory and abridged basal groups. The increased OSE cell division stimulated by the gonadotropin injections near antral follicles suggests that OSE respond to systemic hormone administration. Finally, the OSE cells covering the hilus region were not different, with the exception of those cells in the total basal group, which were labeled significantly less than the other three groups (Fig. 4 i.–v.).

Epithelialization of CL after follicular rupture

The OSE proliferation adjacent to CL of animals in the total ovulatory group (83.5%) compared with the postovulatory

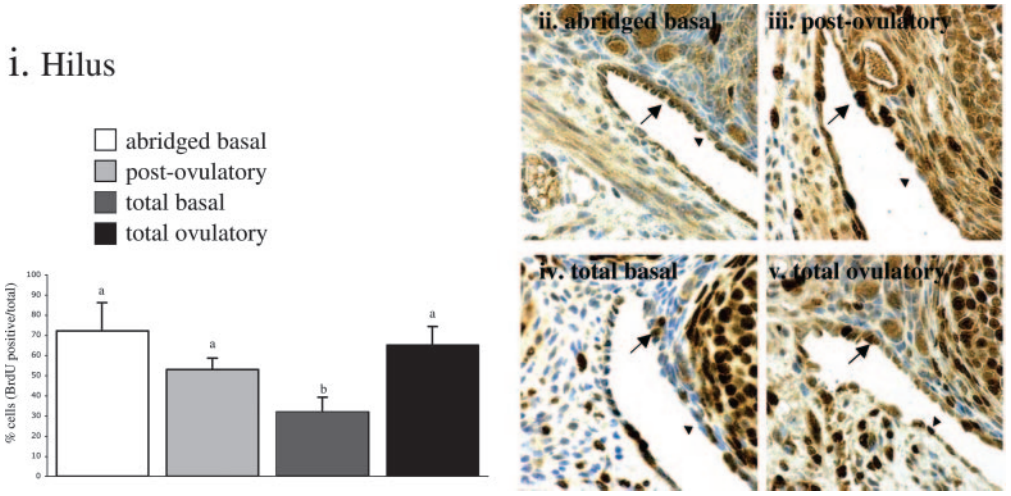

FIG. 4. BrdU incorporation into the OSE adjacent to the hilus. i., Data represent the least-square mean (%) of proliferation adjacent to the hilus and the SE from the mean. The significant differences ($P < 0.05$) are between groups labeled with a *vs.* b. ii.–v., BrdU in the OSE of cells surrounding the hilus stained with DAB and counterstained with hematoxylin from abridged basal (ii.), postovulatory (iii.), total basal (iv.), and total ovulatory (v.) treatment groups. Cells positive for BrdU are indicated by *arrows*, and negative cells are labeled with *arrowheads*.

group (60.0%) was significantly higher, providing evidence that some of the OSE proliferation around a CL occurs in the time before its formation when the follicle is growing (Fig. 5, A and B). Previous investigators have reported that a CL takes several days to fully reepithelialize after follicular rupture (7). To confirm that the measurements obtained in this study reflected the presence of OSE around superovulated ovaries, the known OSE marker cytokeratin 8 was used to distinguish ovarian surface cells. The antigen was detected around the entire ovarian surface and not in any of the follicular compartments (Fig. 5, C and D). The superovulated mouse ovaries were lined with CK8 antigen-positive cells, indicating that OSE had covered over the area of rupture within the 12 h after hCG. These data imply that the covering over of the CL by epithelium occurs rapidly within minutes to hours after the initial formation of the CL from superovulation.

Mouse OSE do not undergo apoptosis in response to superovulation

One possible mechanism monitoring cell levels after proliferation of the ovarian surface may be apoptosis. Apoptosis of the OSE might allow damage incurred during ovulation to be cleared, thereby reducing the risk of developing mutations over time. To evaluate this possibility, ovaries from unstimulated and superovulated animals were stained with TUNEL to quantify the amount of apoptosis in OSE cells. Granulosa cells in atretic follicles served as an internal positive control that the stain was accurately marking apoptotic cells, whereas blood vessels demonstrated autofluorescence. The unstimulated immature ovaries had no apoptotic OSE cells (Fig. 6, A and B). Superovulated ovaries also lacked any signs of apoptosis in the OSE (Fig. 6, C and D).

Discussion

In this study, we have demonstrated three significant findings regarding the proliferation of OSE cells in response to superovulation using cumulative labeling. First, OSE cells in superovulated mice proliferate more than those in unstimulated animals, providing evidence that gonadotropin stimulation influences cell division. Second, the proliferation of the OSE in response to gonadotropins occurs primarily within certain anatomical regions of the ovary, primarily near antral follicles and CL, compared with regions distal from follicular development and occurs both before and during tear and repair. Finally, the amount of apoptosis occurring in OSE cells was investigated and no evidence was found of programed cell death at the time points analyzed either in unstimulated or superovulated ovaries. This study advances the understanding of the normal proliferative function of the OSE in response to each gonadotropin during ovulation, with special attention to the OSE overlying distinct anatomical regions.

Cumulative labeling catalogs all cellular divisions in response to gonadotropins and accurately represents all growth from one ovulatory event. Previous experiments using mice and rats to measure the proliferation of OSE may have underestimated the total amount of proliferation in response to ovulation by using proliferating cell nuclear antigens or short pulses of BrdU (7, 20, 21). However, in this study, between 85 and 90% of all OSE had divided in response to ovulation when measured over a 60-h period. Superovulation has been suggested to overestimate the amount of proliferation occurring in mice due to the increased number of follicles recruited, but this paradigm ensures that only one ovulatory cycle is measured and that each gonadotropin's individual contribution can be assessed. Cumulative labeling also helps to delineate the morphology of dividing cells and reveals that proliferating cells may alternate between a cuboidal type while dividing, when they stain positive for proliferating cell nuclear antigen, but later become either cuboidal or squamous, as depicted in these results (7). This studym by using cumulative labelingm illustrates the proliferative potential of the OSE during one ovulatory event and demonstrates the dynamic change in OSE morphology regardless of cell division.

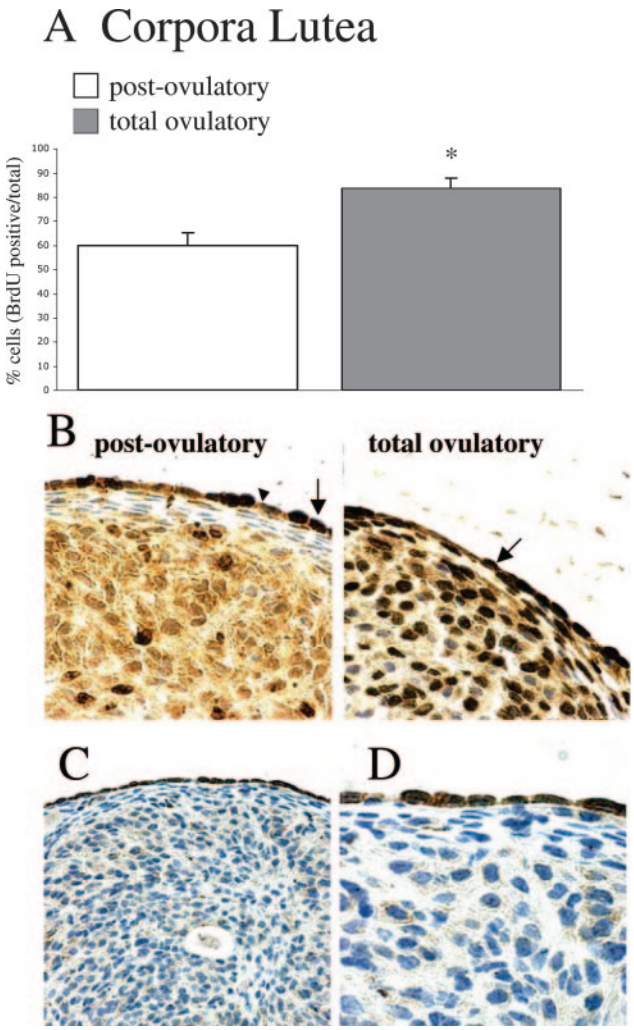

FIG. 5. BrdU incorporation into the OSE tandem to the CL of superovulated mice. A, Data represent the least-square mean (%) of proliferation near CL and the SE from the mean. The significant differences ($P < 0.05$) are between groups labeled with an *asterisk*. B, BrdU in the OSE of cells surrounding the corpus luteum stained with DAB and counterstained with hematoxylin from superovulated animals. Cells positive for BrdU are indicated by *arrows*, and negative cells are labeled with *arrowheads*. C, The CL of superovulated animals stained with the OSE marker cytokeratin 8 demonstrated reepithelialization of the OSE within 12 h after hCG injection, at ×60 (C) and ×100 (D).

The data from this study demonstrate that proliferation of the OSE is related to both follicle proximity as well as systemic gonadotropin stimulation. For example, the rate of proliferation of OSE adjacent to antral follicles and CL exceeded that of areas distal to follicles, consistent with previous findings (7). Also, proliferation of OSE near antral follicles in postovulatory and total ovulatory groups was higher than those surrounding antral follicles in the total basal and abridged basal groups, indicating that the systemic influence of gonadotropins must contribute to the increased proliferation of the OSE. Previously, other investigators concluded that the mesothelium is the local anatomical target of proliferation from ovulation; however, cumulative labeling depicted sporadic staining throughout the OSE, indicating that the entire ovarian surface is capable of cell division (22).

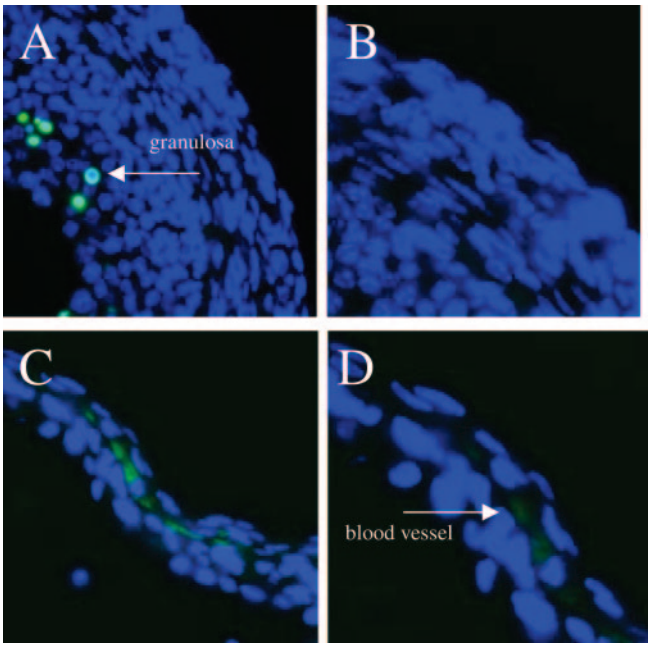

FIG. 6. Mouse OSE does not undergo apoptosis. OSE from ovarian sections were fixed and stained with TUNEL to detect apoptosis. Granulosa cells of atretic follicles serve as an internal positive control. Blood vessels autofluoresce. Nuclei were counterstained with 4′,6′-diamidino-2-phenylindole. Images were taken at ×60 (A) and ×100 (B) for nonsuperovulated animals and ×60 (C) and ×100 (D) for superovulated animals.

Because our study and others have demonstrated a regional increase in proliferation, especially with respect to the position of antral follicles and CL compared with distal areas in the ovary, these data strongly support the idea that both local and systemic factors contribute to OSE proliferation and may offer an explanations why cultured cells do not always proliferate in response to gonadotropins (23–26). Therefore, studying the proliferation of the OSE with relation to anatomical regions and the administration of gonadotropins *in vivo* reveals that both follicular growth as well PMSG and hCG influence OSE cellular division.

Because the ovary must repair the surface after each ovulation, proliferation was primarily thought to occur after rupture in response to the wound, allowing the ovary to heal the exposed area. Our study supports a contribution of PMSG toward proliferation and suggests that proliferation occurs before ovulatory-induced wounds. Consistent with our findings, other reports have provided evidence of proliferation from PMSG, independent of wounding, and in sites distant from follicular rupture (20, 21, 25, 27). In general, the cumulative labeling index of cells covering antral follicles was the same as OSE covering CL when compared against the same relative labeling time. In addition, the proliferation of OSE cells covering CL was higher in animals labeled for 60 instead of 12 h, directly reflecting the prewounding proliferation. Our studies help confirm and extend the concept that proliferation occurs in response to both PMSG and hCG and that follicular growth as well as tear and repair contribute to cell division of the OSE.

The expression of FSH and LH receptors in the OSE reflects on their ability to directly respond to the gonadotropins FSH

and LH suggests that stimulation of these receptors provide the mechanism for proliferation in this study (17, 18). Isolated OSE from rabbits in the estrous phase proliferate in response to hCG and LH but not FSH (25). Human cancer-derived OSE respond to LH, FSH, estradiol, and testosterone *in vitro* by proliferating, whereas progesterone growth-inhibits these cells (24). However, primary OSE scrapes from pre- and postmenopausal women indicate that FSH growth-inhibits the epithelium, and LH had no influence on the proliferation of OSE (26). Although the role of FSH and LH receptors on proliferation of the OSE after ovulation is poorly understood, recent studies concluded that FSH receptors are more highly expressed in cancer compared with normal human OSE and that overexpression of the FSHR activates oncogenic pathways (28, 29). Investigations into the expression of LH and FSH receptors in the mouse would be facilitated by the development of new antibodies. Validating that OSE proliferate in response to gonadotropins because of receptor activation is an important future experimental objective.

Because the ovary exhibits a large amount of proliferation in the surface epithelium in response to ovulation, it may eliminate some cells to compensate for this massive cellular division. To investigate whether cells might be shed through apoptosis, we performed a TUNEL stain on immature and superovulated ovaries. Virtually no apoptosis was demonstrated in either set of ovaries, which is consistent with previous morphological evaluations (21). Although sheep OSE apoptosis is regulated by estrogen and progesterone, in the current study mouse OSE did not appear to undergo apoptosis *in vivo* (12). Future investigations may determine whether the OSE undergo necrosis, are shed during the ovulatory process, or whether the cells undergo an epithelial to mesenchymal transition and become incorporated into the stroma (2). Therefore, most OSE cells are not eliminated through apoptotic mechanisms in superovulated mice.

One hypothesis regarding ovarian cancer is that an increased number of ovulations contribute to the formation of transformed cells. Gonadotropins involved in ovulation were investigated in the current study to determine their role in stimulating OSE proliferation. The gonadotropins responsible for ovulation may stimulate both cancer initiation and progression by stimulating OSE cellular proliferation. Mechanistically, investigators have speculated that transformation events are incurred during ovulation when ROS form DNA adducts in the OSE (12, 30). Also, rat OSE cells continuously proliferated in culture eventually become transformed (31, 32). The current study demonstrates that OSE proliferate significantly in response to follicular growth and administration of the gonadotropins PMSG and hCG. The contribution of ovulation on OSE proliferation as measured in the mouse may help to explain how repeated ovulation in humans might act to transform OSE and progress ovarian cancer.

In conclusion, cumulative BrdU labeling revealed that the ovarian surface epithelium rapidly proliferates in response to superovulation in immature mice. The total amount of proliferation in the OSE appears to most strongly correlate with the proliferation rate of the OSE that are tandem to antral follicles and CL. Both PMSG and hCG contributed to proliferation that could occur before or during ovulation. Apoptotic clearing of damaged OSE cells was not detected in superovulated or unstimulated animals using TUNEL stain. Therefore, ovulation dramatically increases the rate of OSE proliferation without simultaneously increasing programed cell death, potentially creating a system in which damaged cells would be retained and could contribute to the formation of transformed cells.

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