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An Autoradiographic Study of Rabbit Ovarian Surface Epithelium Before and After Ovulation

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ABSTRACT

Morphologic studies suggest that the proliferative activity of the ovarian surface epithelium (OSE) may vary during the reproductive life cycle. To further investigate this phenomenon, rabbit ovaries obtained before and after induction of ovulation with human chorionic gonadotropin (hCG) were incubated in medium containing 3H-methylthymidine and processed for autoradiography. Before ovulation, the labeling index (LI) of OSE cells varied from 0.04% to 0.22%. Twelve hours after hCG, the maximal LI (9.02 ± 0.38%) was seen in OSE cells adjacent to the ovulatory stigma. The LI remained elevated at Days 1 and 5 post-hCG in OSE cells overlying corpora lutea. At Day 12, numerous papillary processes were observed at the apex of each corpus luteum. The maximal LI (16.44 ± 1.31%) had now shifted to the OSE cells covering these processes. Eighteen days after hCG stimulation, the LI of OSE cells near the corpora lutea had returned to prevulatory levels. A slight increase in the LI of OSE cells not associated with ovulatory sites was also observed after ovulation. This study shows that a significant fraction of OSE cells undergoes DNA synthesis throughout most of the postovulatory period.

INTRODUCTION

Changes in the ovarian surface epithelium that overlies the ovulatory follicle have been well described by elegant light and electron microscopy studies (Espey, 1967; Motta et al., 1971; Bjersing and Cajander, 1974b; Van Blerkom and Motta, 1978). Among other findings, these studies have shown that the postovulatory tissue defect is rapidly repaired by surface epithelial cells. Information is lacking of the cell kinetics and mechanism that regulate such repair. Yet this information may be important particularly in view of the controversial relationship between ovulatory frequency, genesis of epithelial inclusion cysts, and common epithelial tumors of the ovary (Fathallah, 1971; Woodruff, 1979). It is significant that the surface epithelium constitutes only a fractional component of the ovary and yet is responsible for over 80% of all ovarian cancers (Blaustein, 1981).

With the exception of recent studies (Adams and Auersperg, 1981; Hamilton et al., 1982), the behavior and regulation of the ovarian surface epithelium have been incompletely investigated. As part of ongoing research on the pathobiology of this tissue (Nicosia and Johnson, 1984a,b; Nicosia et al., 1984, 1985), we now report the results of a study designed to identify the distribution of surface epithelial cells that are engaged in DNA synthesis before and after ovulation.

MATERIALS AND METHODS

Chemicals

Human chorionic gonadotropin (hCG), bovine serum albumin (BSA), Cohn’s fraction V, and Clostridium histolyticum collagenase, type I, were purchased from Sigma Chemical Company (St. Louis, MO). 3H-methylthymidine (sp. act. 6.7 Ci/mmol), Pronase tissue solubilizer, and Econofluor were obtained from New England Nuclear Corporation (Boston, MA) and nuclear track emulsion type NTB-2 was purchased from Eastman Kodak Company (Rochester, NY). Culture medium 199, Hanks’ balanced salt solution (HBSS), and trypan-ethylendiaminetetraacetic acid (EDTA) were purchased from GIBCO (Grand Island, NY).

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Animals

Ovaries were obtained from 20 New Zealand White estrous rabbits (age 4–6 mo). Animals were fed Purina chow ad libitum, individually caged for a minimum of 3 wk, and killed by Nembutal overdose. In test animals (n=16), ovulation was induced by intravenous hCG (50 IU). The animals were then killed at 6, 12, and 24 h and 5, 12, and 18 days following the ovulatory stimulus. These times were chosen to investigate the morphogenetic behavior of the ovarian surface epithelium throughout pseudopregnancy. This luteal phase-like condition lasts in the rabbit approximately 18 days (Everett, 1961). Control animals (n=6) were killed without stimulation.

Morphologic Evaluation

In order to demonstrate the morphology of the ovarian surface, the ovaries of two unstimulated rabbits were fixed in 10% neutral buffered formalin and processed by routine paraffin embedding procedures. Six to 8 mm-thick sections were then stained with hematoxylin-eosin and viewed under brightfield microscopy in a Wild M20 microscope. To study the architecture of the surface epithelium after the postovulatory repair, the ovaries of two rabbits 12 days after stimulation were also processed for scanning electron microscopy. Ovaries were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated in graded alcohols, and critical-point dried in a Denton device. They were then mounted on stubs, sputter-coated on a Polaron device with a 150 mm-thick layer of gold-palladium, and viewed in an AMR 100 microscope operated at 10 kV and with a stage tilt of 0–60°.

Autoradiography

Under aseptic conditions, ovaries were freed from adjacent structures and rinsed three times in HBSS containing penicillin (125 U/ml) and streptomycin (25 μg/ml). Ovaries (n=4/experiment) were then incubated for 2 h in separate flasks containing 10 ml of antibiotic-rich medium 199 and 50 μCi of 3H-methylthymidine. Incubations were carried out at 36.5°C and at 100 rpm under a 5% CO2/95% air atmosphere. At the end of each incubation ovaries were rinsed three times with HBSS to remove unincorporated radiolabel, incubated for 30 min in cold medium 199, and then fixed in 10% neutral buffered formalin for 24 h.

Ovaries were processed for autoradiography using routine paraffin embedding procedures. Each ovary was step-sectioned along a sagittal plane and a minimum of eight 6 μm-thick sections were obtained from each ovary. After deparaffinization, sections were rinsed in tap water, dipped in liquid NTB-2 emulsion, air dried for 1 h in a vertical position, and then placed in light-proof boxes. Autoradiographs were exposed at 4°C for 1 wk and developed using Kodak Dektol developer and F-3 fixer. After development, the sections were stained with hematoxylin and eosin. Autoradiographs were analyzed for the presence and distribution of intranuclear reduced silver grains in surface epithelial cells. A cell was classified as labeled if its nucleus contained more than 20 reduced silver grains. After identifying the corpora lutea, all surface epithelial cells overlying these structures (peritubal surface cells) were counted and scanned for labeled nuclei at 400X magnification. The remainders of the autoradiographs, as well as all ovarian sections from control animals, were divided into four equal quadrants using a rectangular ocular reticle. Surface cells within these quadrants were also scanned for labeled nuclei. A mean of 3200 cells were counted in each ovary and results were expressed as mean labeling index, or LI [(labeled cells/total cells) X100] ± SEM (Baserga and Malamud, 1969).

Measurement of Intracellular Radioactivity

The amount of tritiated thymidine incorporated into surface cell macromolecular material, presumably DNA, was also measured to complement the autoradiographic findings. Ovaries were obtained from 2 unstimulated estrous rabbits and from 2 animals 5 days after induction of ovulation. After incubation of each ovary in medium containing 3H-methylthymidine as outlined above, the surface epithelium of unstimulated animals was obtained by a previously developed isolation procedure that included: exposure of intact ovaries to collagenase (300 U/ml of culture medium) for 1 h, gentle scraping of the ovarian surface with a No. 11 surgical blade under a dissecting microscope at 15X, unit gravity sedimentation of surface epithelial fragments on 5% BSA for 15 min, and cell dissociation in 0.5% trypsin/0.02% EDTA for 20 min (Nicosia et al., 1984). As evaluated by phase-contrast and electron microscopy, a uniform population of surface epithelial cells is routinely obtained by this isolation procedure. Peritubal surface cells and surface cells covering the rest of the ovary were then processed separately. This approach was taken in order to assess the topographical distribution of incorporated radioactivity.

Isolated surface cells were counted, sonicated for 15 min, and treated overnight at 4°C with 2% BSA and 0.4 N perchloric acid (PCA). The precipitated material was washed twice at 4°C with 0.4 N PCA and solubilized in Protosol for 4 h at 55°C in a Dubnoff metabolic shaker. After adding scintillation fluid (Econofluor), the amount of acid-precipitable thymidine counts was then measured in a liquid scintillation counter with a counting efficiency for 3H of 50%. Results were expressed as cpm/10⁴ surface epithelial cells ± SEM.

Statistical Analysis

Autoradiographic and intracellular radioactivity data were comparatively evaluated by the two-tailed Student's t-test.

RESULTS

General Morphology

In the rabbit, the ovarian surface frequently displays papillary or villous processes that emerge rather abruptly from surrounding, flatter intervillous areas (Fig. 1A,C). These processes are covered by a simple or pseudostratified cuboidal to low columnar surface
FIG. 1. Morphology of rabbit ovarian surface epithelium. (A) Ovarian cortex showing numerous villous processes. H & E, X120. (B) Intervillous surface epithelium. Note single layer of cuboidal or low columnar cells separated from the underlying cortex by a distinct basal membrane (arrow). H & E, X400. (C) Villous processes. These projections are lined by surface epithelium and contain a fibrovascular core. H & E, X200.
FIG. 2. Surface morphology of rabbit ovary 12 days after hCG stimulation. (A) Note patches of villous processes radiating from the apical region of two corpora lutea (cl). X30. (B) Variations in size and shape of villous processes. X80. (C) Transition between periluteal villous processes and nonvillus surface epithelium. X190. (D) Higher magnification of transition zone. Note abundant microvilli in cells of both villous processes (vp) and nonvillous epithelium (arrow). X5300.
epithelium that is separated by a basal membrane from a poorly cellular fibrovascular stalk.

In agreement with published data (Everett, 1961), ovulation took place 12 h after administration of hCG and four to six corpora lutea developed in each ovary. Morphologic luteinization was noted in granulosa cells 12 h after stimulation, became prominent between Days 5 and 12, and decreased thereafter. As observed by scanning electron microscopy, the area of the ovulatory stigma was completely covered by a network of large villous processes after 12 days (Fig. 2A, B). The surface cells lining these villous processes appeared more rounded than those of the intervillous areas. Numerous microvilli were present in surface cells of both villous and nonvillous epithelia (Fig. 2C, D).

**Autoradiographic Findings (Fig. 3)**

**Control animals (Day 0).** No corpora lutea were seen in the ovaries of unstimulated rabbits. The labeling index (mean LI ± SEM) of the intervillous surface epithelium was low (0.22 ± 0.05%). A minimal incorporation of tritiated thymidine was also noted in the surface epithelium of villous processes (0.04 ± 0.03%).

**Pseudopregnant animals, Day 1/4.** No obvious corpora lutea were seen 6 h after ovulation.

**Pseudopregnant animals, Day 1/2.** Recently ovulated follicles were present 12 h after stimulation. The LI of the intervillous surface epithelium (0.95 ± 0.17%) and of the epithelium of villous processes (0.77 ± 0.22%) was slightly higher than those observed in control ovaries, suggesting increased DNA synthesis.

**Pseudopregnant animals, Day 1/2.** Recently ovulated follicles were present 12 h after stimulation. The LI of the surface epithelium immediately lateral to the ovulatory stigma was 9.02 ± 0.38% (Figs. 3 and 4). In contrast, the surface epithelium over the rest of the ovary exhibited a LI of only 0.09 ± 0.06%. This difference was statistically significant (P<0.001). A few labeled villous processes (0.02 ± 0.01%)

![Graph](https://academic.oup.com/biolreprod/article-abstract/33/3/729/2764090)

**FIG. 3.** Labeling index of ovarian surface epithelial cells before and at various intervals after ovulation.
were seen, although none near the ovulated follicle.

Pseudopregnant animals, Day 1. Twenty-four hours after stimulation, well-formed corpora lutea were seen and the surface epithelium had already begun to cover the ovulatory stigma. A significant difference (P<0.001) was noted between the LI of periluteal cells (9.07 ± 0.90%) and that of surface cells lining the rest of the ovary (0.25 ± 0.12%). Villous processes were now seen over the corpora lutea and their LI (0.82 ± 0.28%) was higher (P<0.05) than that of other villous processes (0.25 ± 0.12%).

Pseudopregnant animals, Day 5. Five days after stimulation, the ovulatory defect was completely repaired by a monolayer of new surface epithelium (Fig. 5A), yet the LI of nonvillous periluteal epithelium remained high (7.83 ± 0.57%), indicating continued DNA synthesis. This index was significantly higher (P<0.001) than that exhibited by the periluteal villous epithelium (1.92 ± 0.37%) and by the villous (1.13 ± 0.34%) and nonvillous (0.46 ± 0.04%) epithelium covering the rest of the ovary.

Pseudopregnant animals, Day 12. In this group, there was a marked shift in the cell population with active thymidine incorporation. Most of the labeling took place in the surface epithelial cells covering the villous processes that had developed in the apex of corpora lutea (Fig. 5B). These cells displayed a LI of 16.44 ± 1.31%, significantly higher (P<0.001) than that of the periluteal nonvillous surface epithelium (0.69 ± 0.16%) (Fig. 5C) and that of the rest of the ovarian surface (nonvillous epithelium: 0.88 ± 0.35%; villous epithelium: 0.21 ± 0.12).

Pseudopregnant animals, Day 18. Eighteen days after stimulation, DNA synthesis in surface epithelial cells was reduced to levels similar to those observed before stimulation (Fig. 5D). The observed LI were: nonvillous surface epithelium, 0.15 ± 0.05%; villous epithelium, 0.09 ± 0.94%; periluteal nonvillous epithelium, 0.09 ± 0.04%; and periluteal villous epithelium, 0.24 ± 0.11%.

FIG. 4. Autoradiographs of rabbit ovarian surface epithelium 12 h after hCG stimulation. (A) Note apical defect in recently ovulated follicle. Intact surface epithelium is present only at the edges of the defect (rectangle). H & E, X60. (B) Higher magnification of rectangle-enclosed area in A. Note labeled surface epithelial cells (arrow). H & E, X400.
Intracellular Radioactivity (Fig. 6)

Before ovulation, the amount of radiolabel (mean cpm/10^6 cells ± SEM) incorporated into acid-precipitable surface cell macromolecules was 1982 ± 737. Five days after stimulation, the level of radiolabel incorporated by periluteal surface cells increased (P<0.05) to 11,306 ± 1363. Intracellular radioactivity was significantly higher (P<0.05) in these cells than in surface cells covering the rest of the ovary (4836 ± 904).

DISCUSSION

This study clearly shows that the rabbit ovarian surface epithelium is not a static tissue. In fact, a significant fraction of its cell population undergoes DNA synthesis throughout most of the postovulatory period. Extrapolating from the labeling indexes obtained in the present autoradiographic study and from previous estimates of total surface cell number in rabbit ovaries (Nicosia et al., 1984), it can be anticipated that up to 2.4 x 10^5 surface epithelial cells may undergo DNA synthesis in each ovary between the first and the twelfth postovulatory day.

Clearly, postovulatory DNA synthesis in surface epithelial cells is related to the tissue repair that follows ovulation. However, this event continues after the re-epithelialization of the postovulatory defect. This phenomenon may be attributable in part to the propensity of the rabbit ovary to form exuberant villous epithelial processes and intracortical epithelial cords (Cherney et al., 1973; Bjersing and Cajander, 1974a; Motta, 1974). In other animals, such as mice and rats, a high mitotic activity is seen in the surface epithelium immediately surrounding the ovulation site only in the periovulatory period (Bullough, 1942). In the human ovary, epithelial villous or papillary projections have also been observed after...
irritative injury to the ovarian surface, such as with pelvic inflammation and endometriosis (Farhi and Silverberg, 1982). It is possible that the morphogenesis of these structures may also be regulated by hormones, since they have been observed after menopause and in endocrine disorders (Jensen and Norris, 1972; Motta et al., 1980). Follicle-stimulating hormone, luteinizing hormone (LH), and especially, human chorionic gonadotropin (hCG) can stimulate the growth of surface epithelial cells in vitro (Österholzer et al., 1985). In the present study, DNA synthesis also increased significantly after ovulation in surface epithelial cells not overlying corpora lutea. Although not yet reported for normal ovarian surface cells, LH (hCG) receptors have been found in benign and malignant tumors of the human ovary (Rajaneniemi et al., 1981) and growth stimulation by hCG in cell lines derived from human ovarian carcinomas has also been noted (Simon et al., 1983). In a report of epithelial tumors of the ovary in adolescents less than 20 yr of age, 31% of the patients were pregnant or immediately postpartum (Jensen and Norris, 1972). It has also been shown that the surface epithelium of the human fetal ovary undergoes diffuse proliferation, associated with nuclear pleomorphism, during the fourth and fifth months of gestation (Gondos, 1975). Although these changes have been attributed to steroid hormones, a similar influence by pregnancy protein hormones cannot be dismissed. Proliferative changes have also been described in focal areas of the surface epithelium of the adult human ovary at term pregnancy (Forleo, 1961). All these observations indicate that surface epithelial cells may be hormonally modulated. A hormonally mediated or facilitated increase in DNA synthesis may explain why elevated thymidine incorporation is observed locally after cessation of repair of the ovulatory defect and to some extent also in regions of the ovarian surface away from ovulation sites.

The implications of postovulatory DNA synthesis in surface epithelial cells remains to be defined. After re-epithelializing the ovarian surface, these cells may proliferate to form papillary structures (Farhi and Silverberg, 1982). The present study supports this possibility since DNA synthesis is observed almost exclusively in populations of surface epithelial cells covering newly formed villous processes at Day 12 after ovulation. Surface epithelial cells may also invade the ovarian cortex to form cystic inclusions thought to be precursors of ovarian common epithelial tumors (Radisavljevic, 1977; Zajiceck, 1977; Woodruff, 1979). Interestingly, surface epithelial cells that cover papillary processes or line inclusion cysts often develop oncofetal antigens (Blaustein, 1981; Kabawat et al., 1983). Recent reports have shown the protective effect of oral contraceptives in reducing the risk of ovarian common epithelial tumors (Cramer et al., 1982; Centers for Disease Control Cancer and Steroid Hormone Study, 1983). This effect may be direct, by interrupting cyclic DNA replication and entrapment of surface epithelium, or indirect, by suppressing ovarian stimulation by gonadotropins. In addition, gonadotropins can enzymatically activate environmental carcinogens (Bengtsson and Rydstrom, 1983) that may have entered surface epithelial cells (Woodruff, 1979). Experimental verification of these possibilities should be pursuable in appropriate animal models.

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REFERENCES


