# A BRIEF COMMUNICATION

# Morphologic Responses of the Mouse Ovarian Surface Epithelium to Ovulation and Steroid Hormonal Milieu

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**Ovarian cancer of surface epithelial origin is an ovulation- and endocrine-related disease. It appears that a cell transformed by genotoxins generated at follicular rupture is propagated during postovulatory wound repair. A consequent steroid hormonal imbalance favoring the mitogenic estrogens is a prospective predisposing factor in ovarian neoplasia. Protection against epithelial ovarian cancer is conferred by progesterone. The objective of this study was to characterize the acute effects of ovulation and steroid hormonal exposure on morphologic responses of surface epithelial cells of mouse ovaries. Follicular development and ovulation were induced in immature animals with equine and human (=Day 0) choriogonadotropins, respectively. On Day 2 (approximately 36 hrs after ovulation), surface epithelial classifications presented in histologic sections were altered from simple (single-layered) squamous and cuboidal toward stratification; this trend was reversed (i.e., reverted to the control status) on Days 4–8. Shifts in the ovarian epithelium from simple to stratified were accentuated following postovulatory (Days 1–8) treatment with estradiol. Surface epithelia of ovaries obtained after 1 week of progesterone administration were exclusively of a simple phenotype. We conclude that the proliferative/procarcinogenic reaction of the ovarian surface epithelium to ovulation is exacerbated by estrogen and counteracted by progesterone.** Exp Biol Med 232:277–280, 2007

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Mammalian ovaries are encased by a layer of epithelial cells that originate during embryogenesis from the coelomic (peritoneal) mesothelium. Ovarian surface epithelial cells typically vary in type from simple squamous to cuboidal to pseudostratified columnar. Preferential outgrowth of a follicle destined to ovulate brings it into close apposition with the ovarian surface (1).

Most cancers of the ovary are derived from surface epithelial cells (2). The sequences of events that lead to common ovarian cancer are multifactorial. Several aberrant stages are undoubtedly required to yield a phenotype with distinct growth and metastatic advantages. A first step can apparently involve disturbances to the surface epithelium inflicted by genotoxins produced during the mechanics of ovulatory follicular rupture. Depending upon the extent of DNA damage, cells are normally repaired or become relegated to programmed death. Proliferation and migration of surface cells reconciles voids in the ovarian epithelium created by ovulation. It is a unifocal escape from repair/apoptosis, yielding a cell harboring a mutational lesion, which hence undergoes clonal expansion, which could be problematic (3). A stratified ovarian epithelium is suggestive of a proliferative response prejudice toward disease progression (4–6).

Estrogens and progesterone are putative facilitators and suppressors of ovarian carcinogenesis, respectively. Estrogens are thought to act primarily as mitogens (7). Progesterone invokes a cell-cycle arrest, allotting the time

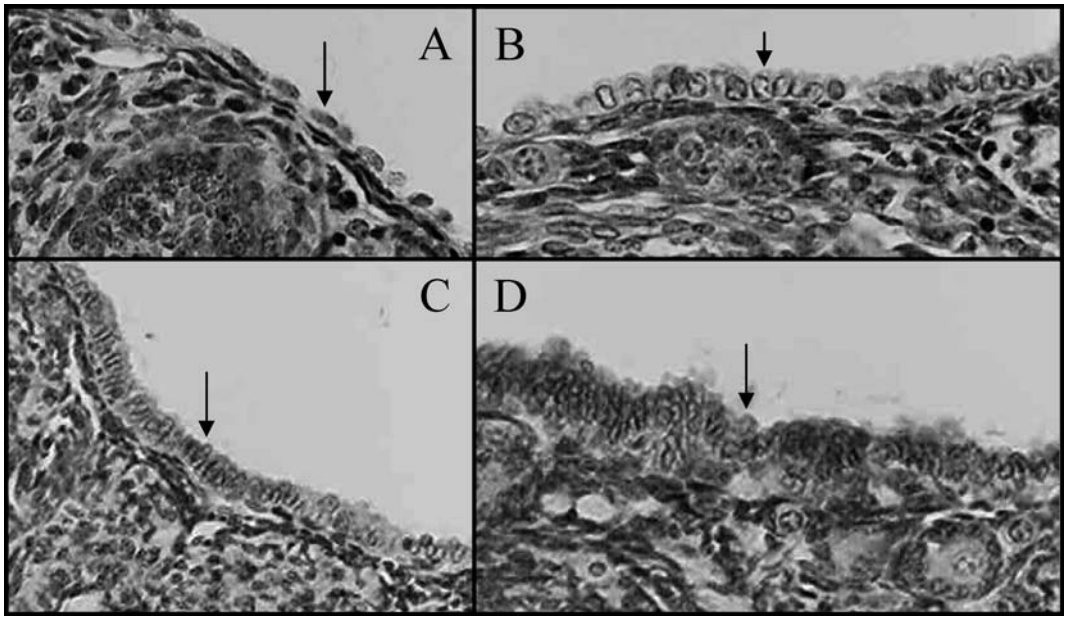


**Figure 1.** Representative photomicrographs of ovarian surface epithelial classifications (arrows): (A) Simple squamous. (B) Simple cuboidal. (C) Simple/pseudostratified columnar. (D) Stratified.

required for DNA repair/proofreading or engagement of the apoptotic pathway (3, 8, 9).

Acute interactive effects of ovulation and steroid hormonal milieu on morphologic responses of the ovarian surface epithelium have not been delineated. We quantified epithelial subtypes in histologic sections of ovaries of immature mice treated with a superovulatory regimen of gonadotropins and estradiol or progesterone.

## Materials and Methods

Reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless indicated otherwise.

**Mice.** C57BL/6J mice were maintained under controlled temperature (24°C) and lighting (12:12-hr light:dark cycle) conditions. Rodent chow and water were supplied *ad libitum*. Animals were killed by cervical dislocation (ovary collections). Protocols were approved by the University of Wyoming Animal Care and Use Committee.

**Ovulation Induction.** Follicular development and ovulation were stimulated in prepuberal (26-day-old) animals by intraperitoneal injections with 10 IU equine choriogonadotropin and 48 hrs later (=Day 0) with 10 IU human choriogonadotropin, respectively. The injection vehicle was 0.1 ml sterile phosphate-buffered saline (PBS). Ovulation rates as determined in a preliminary study were 35.4 ± 2.5 ($n = 5$).

**Ovarian Surface Epithelial Morphometry.** Ovaries were excised, fixed in Histochoice (Amresco, Solon, OH), washed in PBS, dehydrated in a graded series of ethanol, cleared in xylene, infiltrated with and embedded in paraffin, and serially-sectioned (8-μm thickness). Tissue sections were transferred from a water bath onto subbed microscope slides, air-dried, deparaffinized in xylene, rehydrated, stained in hematoxylin and eosin, dehydrated, placed in xylene, and coverslipped with mounting medium.

Eight sections from different regions of each ovary were subjected to light microscopic analysis (×40–100). Subtypes of epithelia present along the ovarian surface were categorized as simple squamous, simple cuboidal, simple/pseudostratified columnar, or stratified (Fig. 1) and respective areas (relative to the total ovarian circumference represented) were quantified using Image J software (http://rsb.info.nih.gov/ij/).

**Effect of Ovulation on the Ovarian Surface Epithelium (Experiment 1).** Animals were treated with gonadotropins or PBS alone (controls) and killed on Days 2, 4, and 8 ($n = 4$).

**Effects of Ovulation and Exogenous Estradiol or Progesterone on the Ovarian Surface Epithelium (Experiment 2).** Ovulated and control mice were implanted subcutaneously on Day 1 with a blank, estradiol-17β (1.5 mg, 21-day release), or progesterone (5 mg, 21-day release) pellet using a stainless steel trochar according to the instructions of the manufacturer (Innovative Research of America, Sarasota, FL). Four animals were included in each of the six treatment groups. Ovaries were collected on Day 8.

**Statistics.** Assignments to treatments and selections of tissue sections for analyses were made at random. Within-animal (subsample) data were averaged. Treatment mean comparisons were made by analysis of variance and protected least significant difference. Contrasts were considered significantly different at $P < 0.05$.

## Results

**Experiment 1.** Ovarian surface epithelia of prepuberal mice were predominately of the simple squamous and

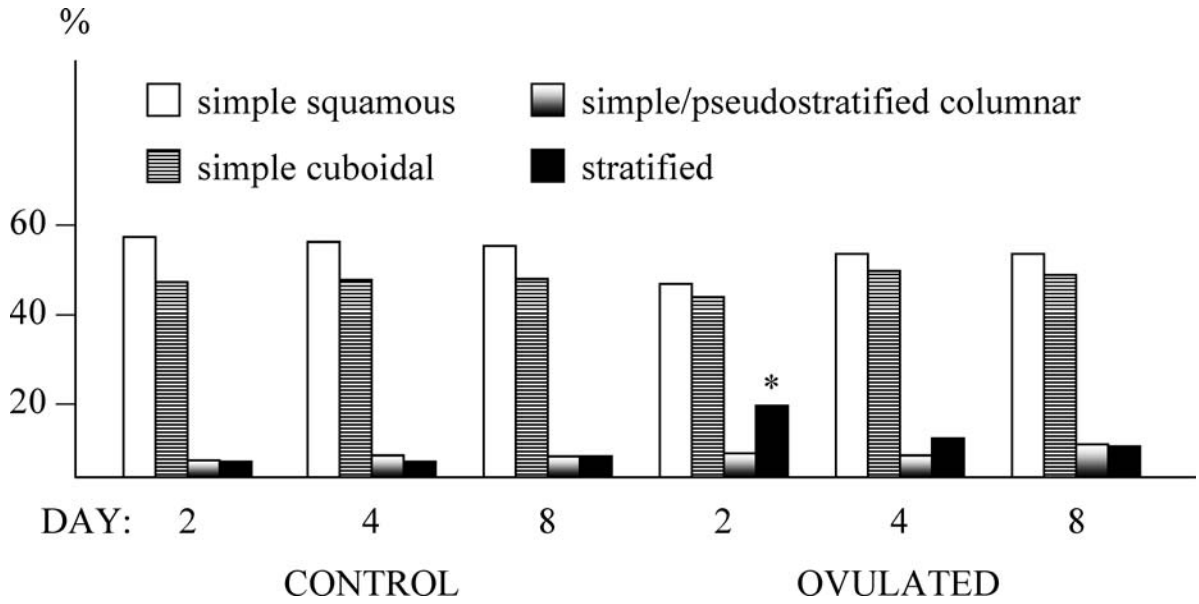


**Figure 2.** Effect of ovulation on the morphology of the ovarian surface epithelium of mice. The asterisk denotes a significant (stratified) increase. Pooled SE = 3.2.

cuboidal types. Epithelial stratifications became more apparent immediately upon ovulation (Day 2) and subsided thereafter (Days 4 and 8). There were no significant effects of time or treatment on the simple/pseudostratified columnar classification of epithelium (Fig. 2).

**Experiment 2.** Relative cumulative percentages along the ovarian surface represented by the different categories of epithelia in control and ovulated mice were comparable to those observed on Day 8 in the preceding experiment. Areas occupied by stratifications of epithelial cells were increased by treatment with estradiol; this response was most prevalent in ovulated animals. Estradiol also increased the frequencies of columnar cells. It followed, then, that incidences of simple squamous and cuboidal epithelial cells were least prevalent in mice exposed to estradiol. In contrast, surface epithelia of animals treated with progesterone were exclusively of the simple squamous or cuboidal types; no columnar or stratified layers of cells were observed (Fig. 3). There was no evidence in either study that surface epithelial cells were in the process of, or had infiltrated into, the ovarian cortical interstitium.

## Discussion

Although the surface epithelium represents only a small fraction of the diverse cell types that populate the ovary, it

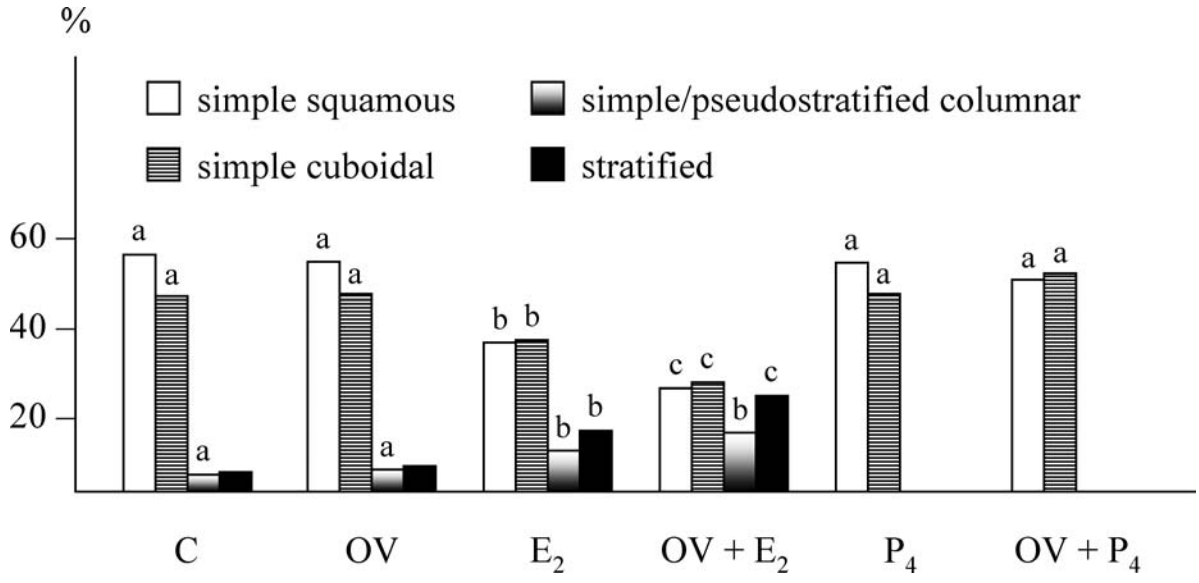


**Figure 3.** Effects of ovulation and steroid hormonal treatments on the morphology of the ovarian surface epithelium of mice (Day 8). C, control; OV, ovulated; $E_2$, estradiol; $P_4$, progesterone. Different letters (a, b, c) indicate significant (within classification) differences. Pooled SE = 2.6.

accounts for upward of 90% of malignancies attributed to this organ (2). Perhaps the surface epithelium is vulnerable to genomic disturbances that are not repaired or deleted (by apoptosis) because it has not been under a strong evolutionary pressure to respond to repeated ovulations (10). Positive correlations clearly exist between increasing numbers of lifetime ovulations, ovarian precursor lesions, and carcinoma in women. Additionally, conditions that circumvent ovulation, namely pregnancy/lactation and oral contraceptive use, safeguard against ovarian cancer (11, 12).

There are four basic stages of advancement in common ovarian cancer. Stage I is defined by the formation of a cyst that contains surface epithelial cells that have invaded the ovarian cortex. Transformed cells, typically exhibiting a Mullerian epithelial morphology, are extruded into and seed the abdominal cavity when an inclusion cyst ruptures. Pelvic spread of malignant cells and generation of ascites fluid are the hallmarks of Stage II disease. Stage III is characterized by tumor implants involving the small bowel, mesentery, and superficial liver. Distant disseminated metastasis to the parenchymal liver and pleura occurs in Stage IV (2).

That proliferation of the murine ovarian surface epithelium (as measured by bromodeoxyuridine incorporation) occurs following one superovulatory event was reported recently (13). It appears that the proliferative effect of ovulation toward the ovarian epithelium is compounded by a subsequent exposure to estradiol. There is evidence in rodents that surface epithelial stratification and ovarian invaginations/cysts are related to total lifetime ovulations (4, 6) and repetitive cycles of ovulation induction (5). Additional experiments will be needed to determine whether the acute phenotypic responses to ovulation + estradiol (i.e., a shift from a simple squamous/cuboidal epithelium toward columnar cells and stratifications) would eventually advance to preneoplasia/neoplasia. Progression to cancer occurred in superovulated rats whose ovaries were subjected to a carcinogen (dimethylbenzanthracene) that caused point mutations in the tumor suppressor TP53 and Ki-Ras genes (14).

The consensus from *in vitro* studies of normal (and transformed) ovarian surface epithelial cells is that estrogens stimulate and progesterone suppresses proliferation (7, 8, 15). It appears that progesterone restores the epithelium of postovulatory ovaries to a resting/nonproliferative state; this lends support to the notion that the protective action of progesterone against epithelial ovarian cancer extends beyond its ovulation-inhibiting property.

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