Loss of tubal ciliated cells as a risk for “ovarian” or pelvic serous carcinoma

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Research

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Abstract

Background

Recent advances suggest the fallopian tube as the main anatomic site for high-grade ovarian or pelvic serous carcinoma (O/PSC). Human fallopian tube is mainly lined by two cell types, secretory and ciliated cells. Large number of studies on the biologic role of tubal secretory cells in O/PSC have been performed in the last decade. However, the role of tubal ciliated cells in relation to the development of O/PSC has rarely been explored. The purpose of this study was to determine if change of the tubal ciliated cells shows difference in age and location and to examine their association with serous neoplasia.

Methods

Three groups (low-risk or benign control, high-risk, and O/PSC) of patients were age matched. The age data was stratified by 10-year intervals ranging from age 20 to older than 80. Ciliated cells from both tubal fimbria and ampulla segments were counted by microscopy and by tubulin immunohistochemical staining. The data was analyzed by standard contingency table, Poisson distribution methods, nonparametric Mann–Whitney U-tests and Spearman correlation analysis after age justification.

Results

The study revealed that the absolute number of tubal ciliated cells decreased significantly with age within each group. A reduction in ciliated cells within the fallopian tube remained a significant risk factor for serous neoplasia after age adjustment. A dramatic decrease of tubal ciliated cells in both tubal segments was identified in patients with high-risk and with O/PSC compared to those in the low-risk (benign control) group ($p < 0.001$). Further, within the fimbria segment, a reduced number of tubal ciliated cells was more prevalent in the high-risk group when compared to those in O/PSC group.

Conclusion

Our findings suggest that reduced number of ciliated cells within the fallopian tube represents a hallmark of early serous carcinogenesis. Findings also support a relationship between loss of tubal ciliated cells and aging, the presence of high-risk factors, and co-existing ovarian or pelvic high-grade serous cancers. This represents an early study identifying the role of tubal ciliated cells in the process of high-grade O/PSC development.

Introduction

Ovarian cancer is the most lethal gynecologic malignancy, with more than 20,000 new cases and about 14,000 associated deaths each year (1). Epithelial ovarian cancer is the most prevalent subtype,

accounting for ~ 80–90% of cases. Amongst epithelial ovarian cancers, serous carcinomas (OSCs) are the most common histologic subtype (~ 70%), followed by mucinous, endometrioid and clear cell carcinomas (2, 3). Hisologically, OSCs are generally classified into two groups: high-grade serous carcinomas (HGSCs) and low-grade serous carcinomas (LGSCs). HGSCs account for ~ 90% of OSC cases and account for approximately 70% of all ovarian cancer-related deaths. These cancers typically present as metastatic disease, involving pelvic and upper abdominal organs. Unclear etiology and lack of early effective detection methods for HGSC remain the major obstacles to curing this deadly disease (2).

The concept of HGSC covers primary sites of ovary, fallopian tube, and peritoneal cavity. By convention, they are lumped into ovarian or pelvic serous carcinoma (O/PSC) category. Previously, O/PSCs were thought to originate from ovarian surface epithelium (4). However, Important advances in recent years suggest that precancerous lesions of O/PSC may commonly originate from the fallopian tube, rather than the ovary or peritoneal surface (2, 5–12). Within the fallopian tubal mucosa, there are two major morphologically different cell types, ciliated and non-ciliated cells. The latter are also called secretory cells. It is believed that the secretory cell of the fallopian tube serve as the cell of origin for the majority of O/PSC (2, 5, 6, 13, 14).

With confidence that tubal secretory cells may be the origin of O/PSC, many investigators have focused attention on this particular group of cells within the tubal epithelia. Tubal secretory cell expansion (SCE) and secretory cell outgrowths (SCOUTs), defined at the cellular level as non-interrupted growth of at least 10 and 30 secretory cells, respectively have been correlated to patients at high-risk for ovarian cancer as well as sporadic ovarian cancer (13, 15). In the study of SCE by our own group, we found that the number of tubal secretory cells increases with age and clearly shows a risk for the development of O/PSC (15). An increase of secretory cells was observed in high-risk individuals and sporadic serous cancer cases (15). Although SCE may represent a biomarker for early serous carcinogenesis, mostly in tubal fimbria, in patients with coexisting O/PSC, SCE is prevalent in both fimbria and ampulla tubal segments in fallopian tubal regions. In the study of SCOUTs by Crum's group at Brigham and Women's pathology, among patients with high-risk factors such as BRCA + or family history of ovarian cancer, they concluded that SCOUTs may represent an indirect precursor with further signal alterations in gene function leading to O/PSC (6, 13). This was supported by the observation that SCOUTs were found in a significantly higher frequency in tubal mucosa from patients with high-grade serous carcinoma than women without cancer (13). Both SCEs and SCOUTs may be used as surrogate biomarkers for O/PSC screening and targets for potential cancer prevention.

The physical and functional alterations of tubal ciliated cells in relationship to the development of O/PSC are relatively unknown. Similarly, the biologic function of tubal ciliated cells during the process of serous carcinogenesis is also unknown. Recently we used single cell sequencing approaching to address the roles of tubal epithelial cells in the process of O/PSC development, we supprisingly found that the tubal ciliated cells have stem cell like nature and many other enexpected findings (manuscript in preparation), further indicating there are many undiscovered biologic function of the tubal ciliated cells. The aim of this study was to determine if the O/PSC precursor model could be further scrutinized through the study of

physical changes of the number of tubal ciliated cells in detail. We addressed the following questions: 1) the overall normal number of tubal ciliated cells within the different tubal segments and correlation to patient age; 2) the extent of change in the number of ciliated cells in high-risk patients compared to normal or low-risk controls) as well as patients with O/PSC when compared to high-risk or to low-risk controls; 3) whether these changes are independent of aging process; and 4) if a specific number of ciliated cells observed in the tubal segments may represent an early morphologic marker for the risk of ovarian or pelvic serous cancer development.

Materials And Methods

Case collection

A total of 240 cases of the fallopian tubes, which were surgically removed from 2007 to 2015 were identified from pathology files of University of Arizona Medical Center in Tucson, Arizona. The study was approved by institutional review board. Cases were divided into three groups of patients: low-risk (n = 120), high-risk (n = 60), and patients with O/PSC (n = 60). Low-risk patients served as the control group and consisted of those patients post hysterectomies and salpingectomies performed for benign disease (leiomyomata, endometriosis or uterine prolapse). Controls were further divided into age groups to determine normal distribution of tubal ciliated and secretory cells. High-risk patients were those with either *BRCA* mutations (n = 32), history of breast cancer (n = 20) or first degree family history of ovarian cancer (n = 8). Typically, these patients underwent at least prophylactic bilateral salpingo-oophorectomy. The median and mean interval between previous breast cancer and prophylactic bilateral salpingo-oophorectomy were 78 and 85 months, respectively. The 60 O/PSC patients represented FIGO stage 2 (n = 6), stage 3 (n = 49) and stage 4 (n = 5). Based on clinicopathologic findings, the primary sites for the O/PSC cases included ovary (n = 40), unilateral fallopian tube (n = 14) and peritoneum (n = 6). Fallopian tubal samples from patients with O/PSC were either intact or with only serosal involvement by high-grade serous carcinoma. To avoid potential compounding factors, samples were excluded if tumor including serous tubal intraepithelial carcinoma involving tubal lumen or extensive tubal mucosa. Age of patients was matched among the three groups.

Tissue handling

For benign controls, at least two representative sections of the fallopian tube, one from ampulla (proximal) and the other from fimbria were submitted. Fallopian tube from benign control cases were processed by embedding all fimbriated ends similar to cancer patients with additional representative 2 cross sections of the ampulla as described previously (5). For patients with high-risk or O/PSC, the fallopian tube was examined by using SEE-FIM protocol (16). Among all sections, 2 sections (one from fimbria and one from ampulla) from each case of the high-risk group were examined under microscope.

All tissues were fixed in 10% buffered formalin and processed routinely for paraffin embedding. Five-micron sections for IHC were cut and placed on Super Plus slides (Fisher Scientific, Pittsburgh, PA)

followed by a section of each specimen that was stained with hematoxylin and eosin and examined microscopically to confirm the diagnosis.

Counting the number of ciliated cells in tubal mucosa

The ciliated cells within the fallopian tubal mucosa were readily identifiable under the light microscopy when cilia are present on the apical part of the cells, while the epithelial cells without cilia are assumed as secretory cells. However, cilia sometimes may not be visible due to different plane orientation since tissue sections are random. The number of ciliated cells within the tubal fimbria and ampulla epithelia was counted in the fallopian tube in each case and evaluated by 2 methods: light microscopy and immunostaining with tubulin (marker for ciliated cells) and PAX8 (marker for secretory cells) as described previously (17, 18). After a defined area was selected under microscope, the absolute number of ciliated cells was derived from 3 different high power fields (400x magnification). Microscopically, the number of tubal epithelial cells ranged from 275 to 500 with an average of 410 epithelial cells in each high power field. For the cases stained with tubulin, the number of ciliated cells was counted based on the cells with positive cilia on the cell apical boarder and the counting method was same as routine microscope for HE slides. The percentage of the ciliated cells were calculated based on the total number of cells counted. The authors performing slide reviews were blinded as to the group status of the tubal sections.

Immunohistochemical analysis

For the immunohistochemical (IHC) analyses, 4-μm-thick sections were cut from formalin-fixed paraffin-embedded tissue blocks, deparaffinized in xylene, and rehydrated through sequential washes of alcohol and distilled water.

Tubulin and Pax-8 were detected using ready-to-use monoclonal antibodies against tubulin and Pax-8 as previously described (17, 18). PAX8 has been considered as a mullerian epithelial marker identifying tubal secretory, but not ciliated cells (5, 19, 20). Alpha-tubulin identifies cellular surface cilia, and is an appropriate marker to identify tubal ciliated cells (5, 14, 21). The slides were heated in an autoclave at 120 °C for 5 min in 0.01 M citrate buffer (pH 6.0) before immunostaining. The slides were incubated with the above-mentioned antibodies for 2 h at room temperature. Antibody binding was visualized using the EnVision + Dual link system and diaminobenzidine as a chromogen (Dako, CA, USA). The slides were counterstained with methyl green or hematoxylin and mounted.

Benign tubal ligated sections from women without known risk for cancer served as positive controls for the antibodies studied in this project. Negative controls were performed by replacing the primary antibody with nonimmune IgG. All slides were reviewed independently by 2 investigators (YiW or WL and WZ). The percentage of positive cells that showed dark brown nuclear or cilia staining was recorded. Only moderate or strong intensity of the IHC staining was considered as positive, while weak stains were considered as negative.

Data evaluation and statistical analysis

We used 2 methods to evaluate the number of ciliated cells in the fallopian tubal mucosa. With the light microscopy methodology or IHC stains, an average number of the ciliated cells as well as the percentage of the ciliated cells within the tubal mucosa for each tubal segment was used for statistical analysis. The following parameters were calculated for the cases studied: 1) The number of ciliated cells and its distribution (fimbria vs ampulla) were calculated by both microscopy counting and tubulin stains. The cellular numbers were arranged according to age organized in 10 year intervals; 2) Comparison of ciliated cells in ampulla and fimbria within the fallopian tubes among the three groups; 3) Comparison of percentage of positive hormone receptor expressions among the control and study groups; and 4) Evaluation if high-risk and/or O/PSC associations is independent of age for the number of ciliated cell changes.

The data were analyzed by standard contingency table methods, nonparametric Mann–Whitney U-tests and Spearman correlation analysis using GraphPad Prism 7.0 for Windows (GraphPad Software, San Diego, CA, USA) and Stat View (SAS Institute, Cary, NC, USA) computer package programs. All *P* values were two-sided, and a value of $P < 0.05$ was considered statistically significant. To adjust for age differences and the varying numbers of section or microscopic fields examined for each case, the data were calculated on the assumption that the number of ciliated cells in each case follows a Poisson distribution, which is commonly used to model count data, with an offset term used to account for the microscopic fields examined. For comparisons with patients' age, the age data were stratified into 10-year intervals ranging from 20–29 to older than 80.

Results

As age increased, the number of ciliated cells decreased
A total of 240 patients' fallopian tube specimens, divided into 10 groups based on age (ie 20–29, 30–39, … and > 80) were studied. These included benign or low risk (n = 120), high-risk (n = 60) and O/PSC (n = 60).

Overall, there was a significantly decreased number of ciliated cells in the fallopian tube with increasing age in the cases studied. Compared with the age 20–29 group, which contained an average of 258 ciliated cells/hpf in fimbria and 260/hpf in ampulla, the average number of ciliated cells decreased 95% and 82% with average number of ciliated cells of 15/hpf in fimbria and 48/hpf in ampulla, respectively at the age group of > 80 years. There was a clear trend that the number of ciliated cells decreases as a function of age in both fimbria and ampulla segments ($p < 0.001$) and the significant reduction of the ciliated cells starting from age of 30 years old (Fig. 1A and B). Compared with ampulla region, number of ciliated cells in fimbria further decreased significantly starting from age of 40 years old. The detailed data are summarized in Supplementary Table S1 and visualized as a bar graph in Figs. 1A to 1C.

The number of ciliated cells was significantly decreased in tubal segments of patients with high-risk or ovarian/pelvic serous carcinoma

Among 120 patients with benign gynecologic diseases or with low risk for ovarian cancers, we selected 60 high-risk patients and additional 60 patients with O/PSCs matched for age to the low-risk group for the comparison of overall number of ciliated cells in the fallopian tube by using both morphologic and IHC methods. With tubulin stains, the average number of ciliated cells in the fimbria region in low-risk tubes was 236/hpf (Supplementary Table S2). This cellular number decreased significantly with an average of 118/hpf in high-risk group and 156/hpf in O/PSC group, respectively ($p < 0.001$), which was translated to a 50% and 34% reduction of the ciliated cells, respectively in tubal fimbria (Supplementary Table S2). A similar trend of reduction of the tubal ciliated cells was found in the ampulla segment in high-risk and O/PSC groups, compared with that of low-risk group ($p < 0.001$) (Fig. 2A, 2B). Although the number of ciliated cells/hpf was slightly higher in the ampulla region than that in the fimbria, it did not reach to a statistical significance in all 3 patient groups (Fig. 2C). We also compared the number of ciliated cells between high-risk and O/PSC group. Interestingly, there was further 25% reduction, from average of 156/hpf of O/PSC group to 118/hpf of high-risk group, of ciliated cells in the tubal fimbia ($p < 0.001$) (Fig. 3A). However, there was no statistical difference of the number of ciliated cells in the ampulla region between the high-risk and O/PSC groups ($p = 0.18$) (Fig. 2B). The detailed data are summarized in Supplementary Table S2.

When the data were arranged based on age distribution within each group, we found that there was also a significantly decreased number of ciliated cells in the fallopian tube with increasing age in all three groups. Compared with the low-risk group, the number of ciliated cells in high-risk and O/PSC groups were further reduced ($p < 0.001$). The detailed data are summarized in Supplementary Table S3 and corresponding bar graph in Fig. 3.

The number of tubal ciliated cells counted by microscopy was comparable to the number of ciliated cells detected by tubulin staining

To evaluate whether tubulin IHC stain can accurately reflect the distribution of ciliated cells, we examined the relationship between the number of tubal ciliated cells calculated with microscopic direct counting (H&E) in tubal tissues and the number calculated with IHC (tubulin) method from 120 matched tissues. A robustly positive correlation was observed. (Fimbria: $r = 0.68$, $P < 0.0001$; Ampulla: $r = 0.72$, $P < 0.0001$; Spearman correlation analysis) (Fig. 4).

Interestingly, Pax-8 stained cells partially overlapped with ciliated cells, while tubulin illustrated the tubal ciliated cells only. This was the reason we did not consider Pax-8 negative cells as ciliated cells. Representative pictures of the tubal ciliated cells identified by morphology and tubulin immunohistochemical stains are illustrated in Figs. 5 to 6.

Decreased number of ciliated cells was significantly associated with age, with high-risk factors, and with the presence of ovary/pelvic serous carcinoma

As we demonstrated above,decreased number of tubal ciliated cells was associated with age and more strikingly with high-risk and O/PSC patients. Therefore, we explored whether the decreased number of

ciliated cells in high-risk or O/PSC patients are independent of age. We addressed this question in a regression model that adjusted for age, as well as the number of cross-sections examined for the number of ciliated cells by linear regression analysis. The three groups (low-risk or control, high-risk, and O/PSC) of patients were divided according to 10-year intervals and average number of ciliated cells for the intervals were compared. There were still significant differences in the decreased number of ciliated cells as a function of increasing age in both case and control groups (Supplementary Table S3 and corresponding bar graph in Fig. 3). When both the cases and controls were combined to provide a greater number for comparison, a significant correlation was observed with a determination coefficient of 0.171. This implies that approximately 17% of decreased ciliated cells in the fallopian tube could be attributed to age. Both high-risk and O/PSC groups showed more reduction of the number of ciliated cells than low-risk controls ($p < 0.001$), an average decrease of 0.83 log counted for O/PSC cases vs controls and 0.90 log for high-risk cases vs controls. Therefore, age, high-risk factors, and O/PSC were all independent risk factors for decreased number of ciliated cells. High-risk cases showed the strongest association with decreased number of ciliated cells in the fallopian tube, while age showed the weakest, although all findings were statistically significant. The number of ciliated cells was noted to be slightly higher in ampulla region than that in fimbria (Fig. 1C, Supplementary Table S1).

Discussion

The mammalian fallopian tube mucosa consists primarily of two cell types, ciliated cells and secretory cells. Cilia are usually classified into primary cilia and motile cilia according to the components of their axonemal microtubules (22). Motile cilia extend from the basal bodies in multi-ciliated cells of the ciliated epithelium in the fallopian tube. Ciliated cells occur throughout the fallopian tube but predominant at the apex of mucosal folds. Physiologically, the coordinated beating of multiple motile cilia is thought to provide the fluid flow needed to move an oocyte through the fallopian tube to the uterus. Multiple motile cilia have a canonical “9 + 2” ultrastructure of microtubules in the ciliary axoneme, consisting of 9 peripheral doublet microtubules surrounding 2 single central microtubules (23). In this study, we examined motile cilia of the tubal epithelia, which can be identified microscopically and immunohistochemically by tubulin stain.

Tubal ciliated cells are thought in general to represent terminally differentiated cells from either tubal secretory cells or stem-like cells within the fallopian tube and induced by estrogen stimulation (24–26). Thus, it is not surprising that there is a reduction of ciliated cells with age, in particular over age 40. In reality, tubal ciliated cells *in vitro* may reversely differentiate into non-ciliated cells (morphologically show no difference from the secretory cells), which is commonly observed in tubal primary culture experiments (Zheng et al., unpublished data). It is unclear how this happens and whether those ciliated cells have potential to initiate serous neoplasia after becoming non-ciliated cells. One early study about the role of tubal epithelia in supporting early embryo development showed that tubal motile cilia is lost in vitro when estrodial is withdrawn (24). But overall, there is limited understanding of the tubal ciliated cells on the aspects of cellular differentiation and neoplasia. As the tubal mucosa is mainly composed of ciliated and secretory cells, people may have a general impression that the increased density of tubal secretory cells

reflects the decreased number of ciliated cells. In reality, such relationship has never been systemically studied.

In this study, we have examined the global change of tubal ciliated cells and its relationship with the aging process by counting the number of ciliated cells in patients with low-risk (benign group), high-risk, and O/PSC. Although the study is descriptive in nature, it explores a novel approach to evaluate the risk for ovarian or pelvic serous carcinogenesis. This is the first study to describe the change in number of tubal ciliated cells in relation to age. For patients in the low-risk (control group) for ovarian or pelvic serous cancers, the number of tubal ciliated cells decreased with age, starting at age 30 s and decreasing by 94% after age 80. It appears that the reduction of ciliated cells is consistent with the increment of tubal secretory cells with aging as we demonstrated earlier (2, 15). However, it is unclear if the reduction of tubal ciliated cells and increased the number of secretory cells are independent or reciprocal. Our observations of loss of tubal ciliated cells with aging also go along with the well-known epidemiologic findings that O/PSC increases with age and shows a peak incidence after menopause (27–29). A more dramatic decrease of tubal ciliated cells is observed in patients with high-risk factors, such as *BRCA* mutations or family history of ovarian cancer, and in patients with O/PSC. This decreased the number of tubal ciliated cells is also closely associated with age in both high-risk and O/PSC groups, indicating that decreased number of tubal ciliated cells is linked to “ovarian” or pelvic serous neoplasia.

Since decreased number of tubal ciliated cells was associated with age and more strikingly with high-risk as well as with O/PSC patients, we examined if the decreased number of ciliated cells in high-risk or O/PSC patients are independent of age in the study. By linear regression analysis, when both the cases and controls were combined, we found that all three factors namely age, high-risk status, and patients with O/PSC, are independent risk factors for the decreased number of tubal ciliated cells. Approximately 10–17% of decreased ciliated cells in the fallopian tube are attributed to age, while 83–90% attributed to O/PSC and high-risk status, respectively. Patients with BRCA mutations show the strongest association with decreased number of ciliated cells in the fallopian tube, consistent with its known risk for O/PSC development (28, 30–32). While there is still an association found of reduction of ciliated cells as well in the O/PSC group, this group is not restricted to cases with serous neoplasia. Thus, diverse tumor microenvironmental changes exist which could impact number of neighboring ciliated cells by other mechanisms. From these findings we speculate that patients with high-risk factors and/or with O/PSCs have an unidentified mechanism to cause reduction of tubal ciliated cells in addition to the aging process and such changes are not influenced by hormonal change since our study is age-matched.

Fallopian tube serves as the cellular source for the majority of O/PSC, while tubal fimbria is considered as the anatomical site of origin of these non-uterine high-grade serous cancers in women (33). Tubal ciliated cells are usually distributed evenly within the tubal fimbria and ampulla segments. In this study, we examined the number of ciliated cells and their distributions in both tubal segments. We found that there is no significant difference between the two tubal segments for the number of ciliated cells. However, the number of tubal ciliated cells are significantly less in high-risk group that that in O/PSC group within the tubal fimbria (Fig. 2A). It is unclear how to explain this phenomenon. But the findings are consistent with

that patients with BRCA mutations develop HGSCs at an earlier age than those who develop sporadic cancer (34) as well as with the well accepted concept of tubal fimbria as the main anatomic site for ovarian or pelvic serous neoplasia (35, 36).

The functional role of cilia in human carcinogenesis is unclear. There are no studies on the role of multiple motile cilia for the tubal ciliated cells in the process of ovarian carcinogenesis. Through literature search, however, a few studies on the biologic function of primary cilia of the fallopian tube in the process of cancer development come to our attention (23, 37). Egeberg et al., suggested that defects of primary cilium in ovarian tumorigenesis may be related to deregulation of cilia signaling pathways such as Hedgehog, platelet-derived growth factor, aurora A kinase signaling (37). Some other earlier studies showed that primary cilia may play a critical role in tumorigenesis and cancer progression by functioning as a tumor suppressor organelle that regulates cell cycle/proliferation, differentiation, polarity, and migration (38, 39). On the other hand, loss of tubal ciliated cells may also reduce the capacity of removing follicular fluid induced genotoxicity within the fallopian tube (40). In one of our studies about ovarian serous carcinogenesis, we also notice that gradual motile cilia loss from serous cystadenoma to serous borderline tumor and finally complete loss of cilia in low-grade serous carcinoma (5). More recently, we have studied tubal epithelial cells from BRCA1 mutation carriers and benign controls without known history of BRCA1 mutation by using single cell sequencing technology. We found that tubal ciliated cells express SOX2 biomarker, which is known to be one of the stem cell markers, while secretory cells do not (manuscript in preparation). It would be interesting to study biologic function of tubal ciliated cells and their motile cilia in the process of serous carcinogenesis.

Tubal mucosa consists of both secretory and ciliated cells, arranged in a recurring pattern of alternating each other in normal fallopian tube of reproductive aged women. PAX-8 is a member of the pair-box (PAX) family of transcription factor genes. Studies have shown that PAX8 is a biomarker of tubal secretory cells and is used to distinguish gynecologic cancers from non-gynecologic malignancies (41). Therefore, PAX-8 has been widely used in the clinic. In this study, we used PAX-8 to distinguish tubal secretory cells from tubal ciliated cells. However, not infrequently we have found that not only secretory cells, but also some of the ciliated cells are positively stained by PAX-8 (Fig. 5). This is the reason for us to use tubulin highlighting the ciliated cells in the study. Although morphologically tubal ciliated cells are easily distinguished from secretory cells because of the presence of multi-motile cilia on the cellular apex, these two cell types may be interchangeable, which is supported by ciliation changes of the tubal epithelia in the menstrual cycle (42). That can explain why some of the ciliated cells are positive for PAX-8 expression in current study. Studies to identify regulatory factors for the transitions between ciliated and secretory cells are needed to help us uncover the role and functions of these tubal epithelial cells.

Conclusion

In summary, our findings offer a novel perspective on the initial mechanisms involved in the development of O/PSC, which can foster experimental research on the impact of tubal ciliated cell clearance. Tubal ciliated cells are morphologically distinct and therefore reduced number of tubal ciliated cells can be used

as an early biomarker for serous carcinogenesis. Single cell sequencing study illustrating the relationship between secretory and ciliated cells are ongoing in our lab. Molecular mechanism studies of tubal ciliated cells may facilitate strategies of O/PSC prevention and early intervention.

Abbreviations

O/PSC, ovarian or pelvic serous carcinoma;

CC, ciliated cells;

IHC: immunohistochemistry

Declarations

Ethics approval and consent to participate

All experiments regarding human tissue slides in this study were performed in according to ethical standards. Informed consent was obtained from all patients.

Consent for publication

All authors involved in the study had given their consent for submitting this article for publication.

Availability of data and materials

All experimental data generated or analysed during this study are included in this published article and its supplementary files.

Competing interests

The authors declare that they have no competing interests.

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Author’s contribution

WZ conceived the study design and experiments. YuW, YiW, WL, and YaW carried out experiments and data analysis. WZ, YiW, and WL performed data analysis. YiW and WZ wrote the manuscript. SKC provided the majority of the high-risk cases, with relevant clinical information. All authors were involved in editing and approving the final manuscript.

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Figures

Figure 1

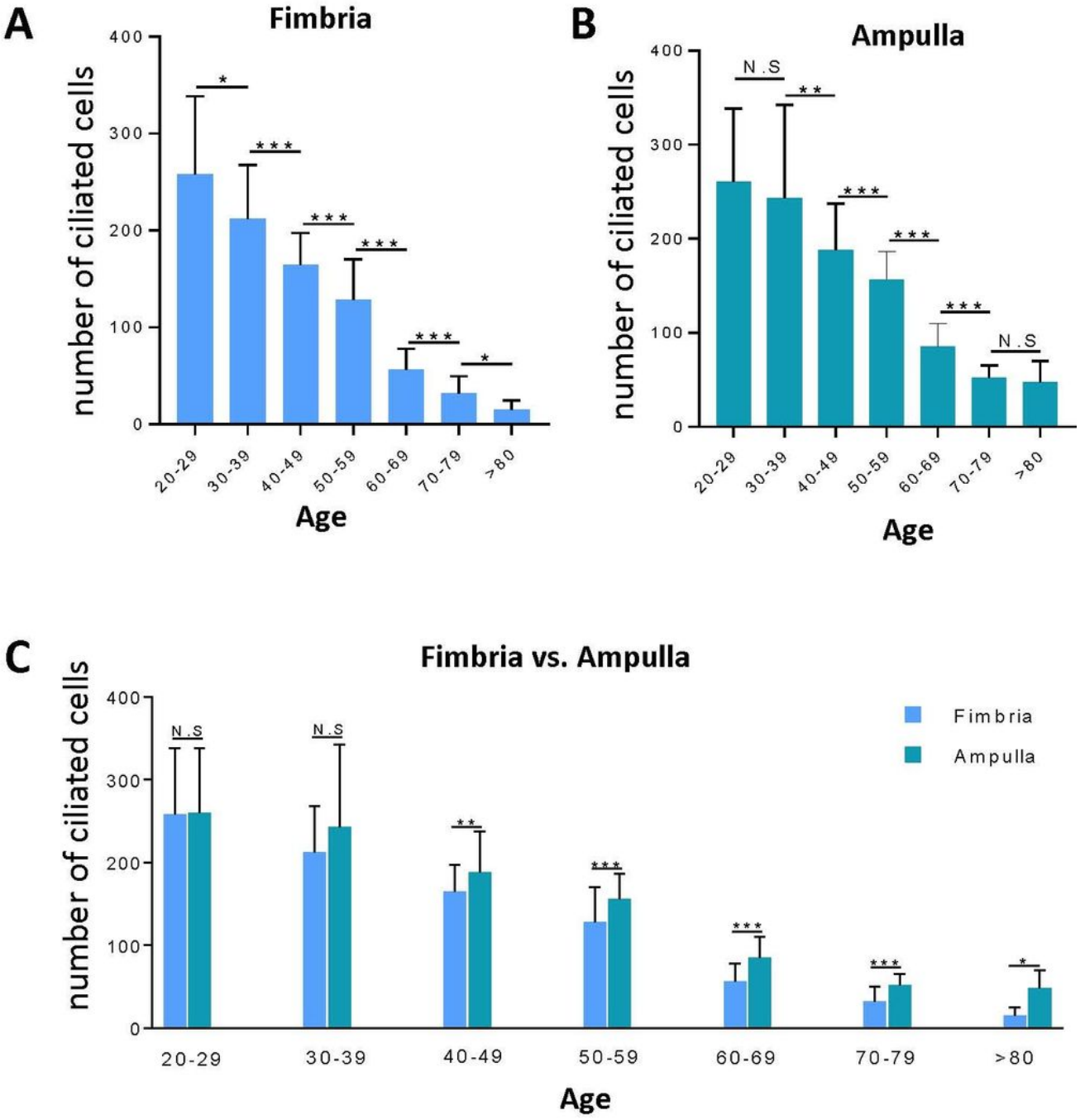

Figure 1

Ciliated cells distribution trend among different age groups, calculated by microscopic direct counting (H&E). A clear trend of reduction of the number of tubal ciliated cells was present in the aging process. A significant reduction started at age 30s in both fimbria and ampulla segments. Compared with ampulla region, further reduction of the tubal ciliated cells was detected in the fimbria. A. The number of ciliated cells in tubal fimbria region; B. The number of ciliated cells in tubal ampulla region; C. Comparison of

ciliated cells distribution between ampulla and fimbria. Statistically significant differences were determined using the Mann-Whitney U test. N.S, no significance; *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 2

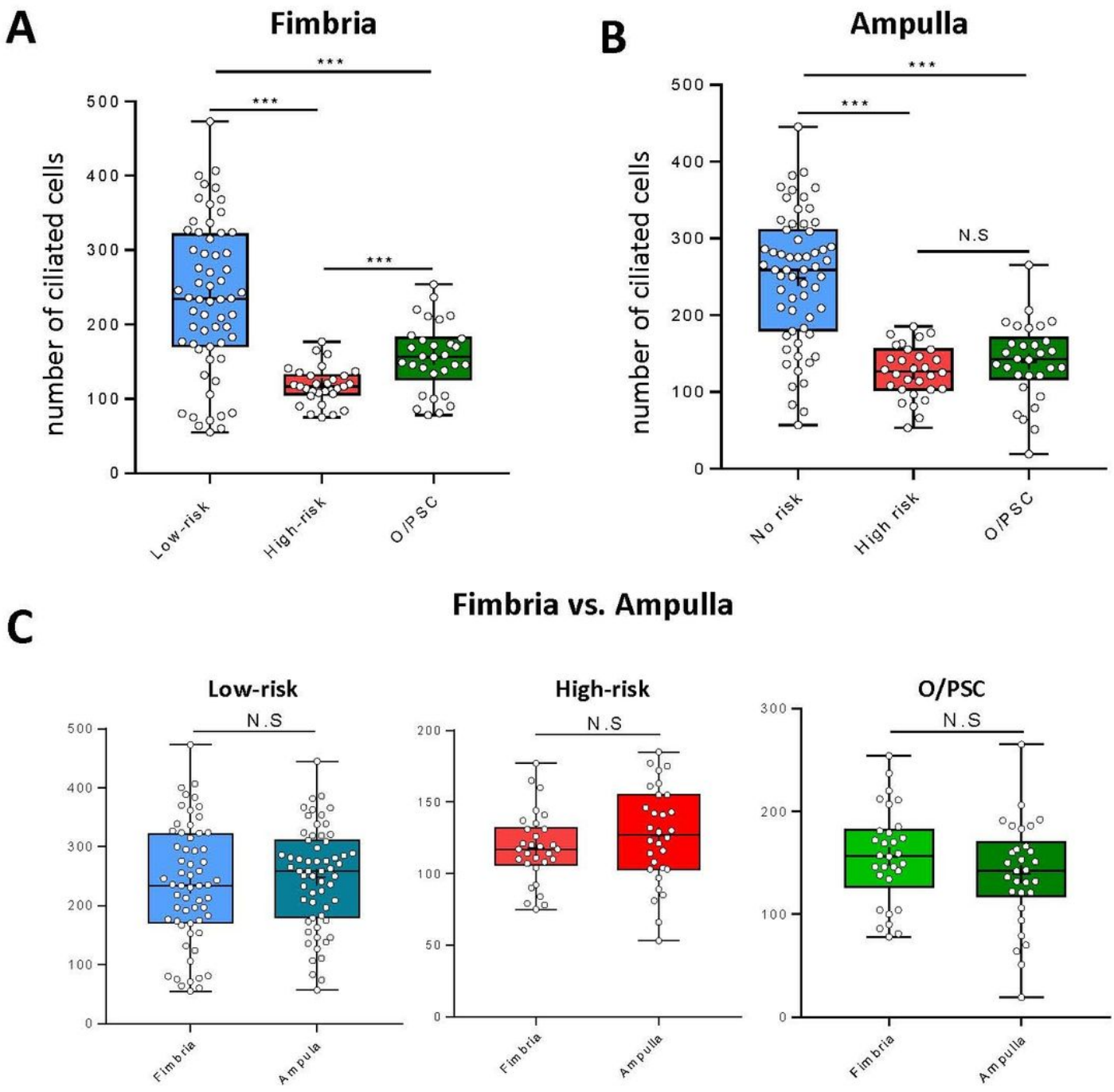

Figure 2

Comparison of the number of ciliated cells calculated by tubulin staining method among control (low-risk) and study groups (high-risk or O/PSC). Compared with the control group, the number of tubal ciliated cells was significantly reduced in both study groups in both tubal segments (A, B). There was no

statistical significant difference detected when the number of tubal ciliated cells was compared between fimbria and ampulla in the same group (C). Statistically significant differences were determined using the Mann-Whitney U test. N.S, no significance; ***P < 0.001.

Figure 3

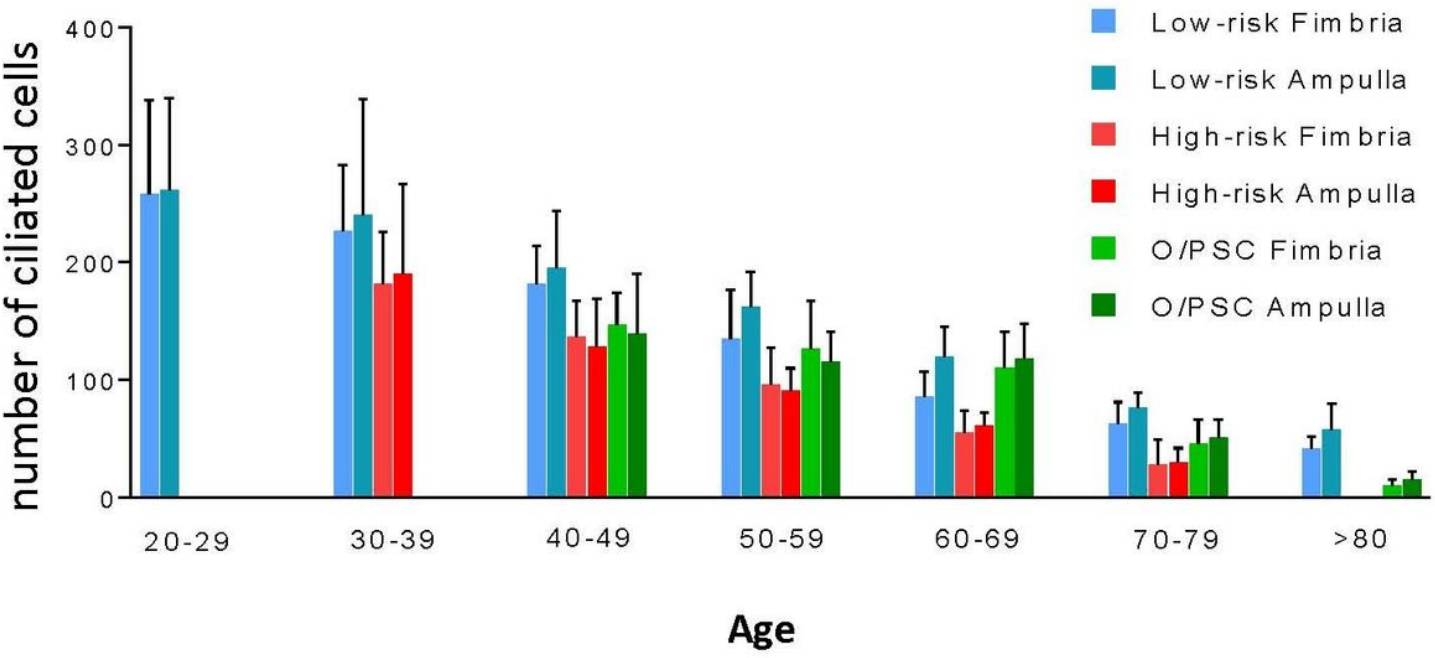

Figure 3

Overview of ciliated cells distribution in fimbria and ampulla with age and among patients with low-risk, high-risk, and ovarian/pelvic serous carcinoma (O/PSC). A clear trend of tubal ciliated cell reduction was observed in the aging process.

Figure 4

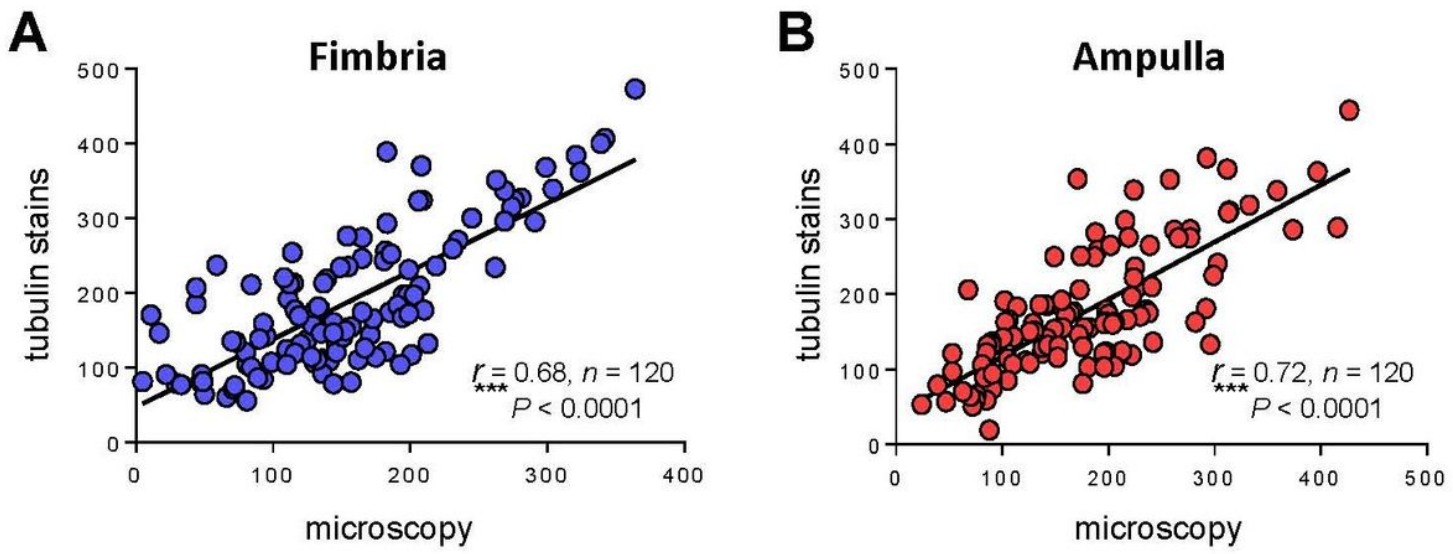

Figure 4

Correlation of the number of tubal ciliated cells between the method of microscopic direct counting (HE) and the method with tubulin staining (IHC) from matched tubal tissue sections. A robust correlation between the two methods was present. A. Ciliated cells distribution in tubal ampulla region; B. Ciliated cells distribution in tubal Ampulla region; Spearman rank correlation (r) was used for the correlation analysis; ***P < 0.001.

Figure 5

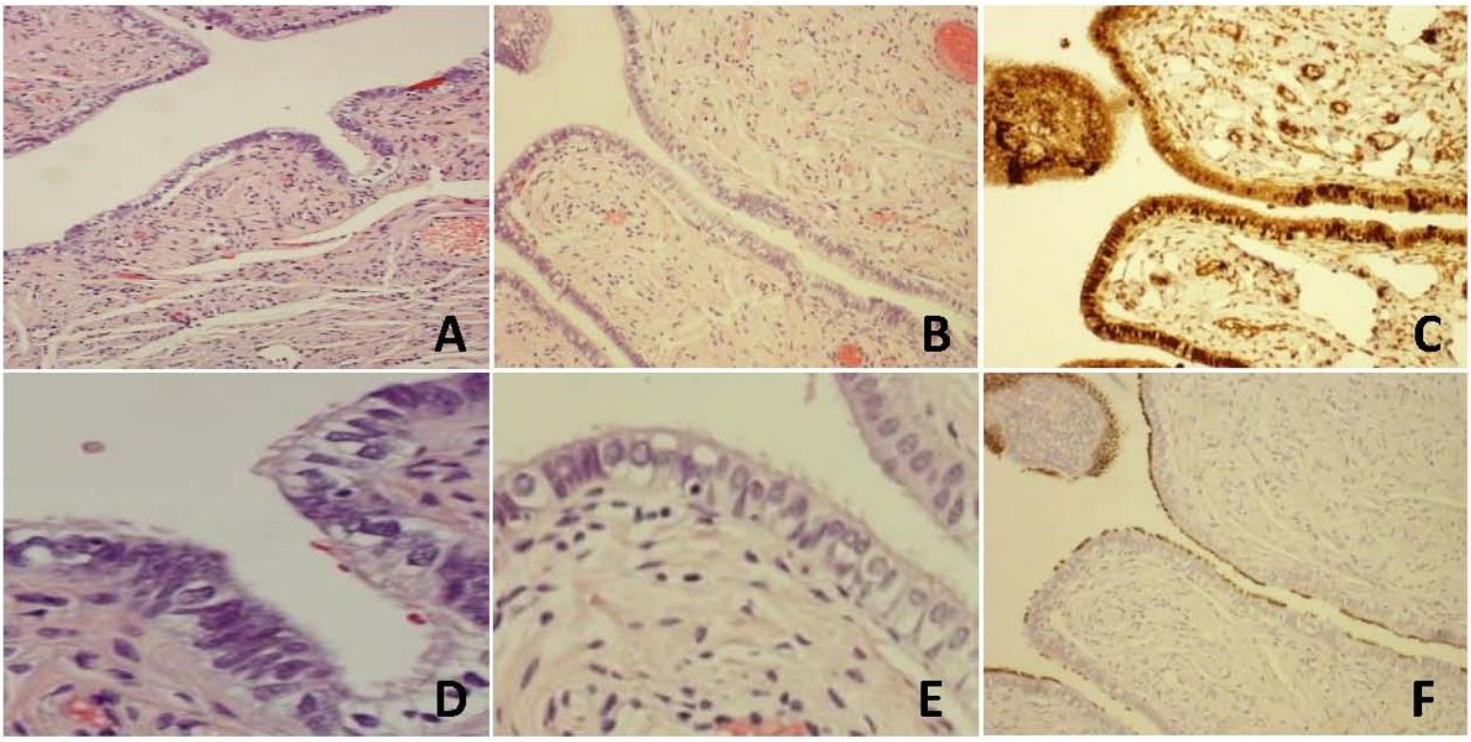

Figure 5

Example of tubal ciliated cells detected by microscopy and tubulin staining in a patient of 45 years old. Tubal ampulla segment (A, B) showed ciliated cells, which are easily visible in a high power (upper right corner of B). Tubal fimbria region (C, D) showed cilia on the apical cellular border under a high power view (D). The same fimbria region (C) stained with PAX8 for tubal secretory cells (E) and tubulin for ciliated cells (F). Apparently, PAX8 stained both secretory and ciliated cells (E) as the ciliated cells were illustrated by tubulin stain (F). Original magnifications: A, C, E, and F 100x; B and D, 400x

Figure 6

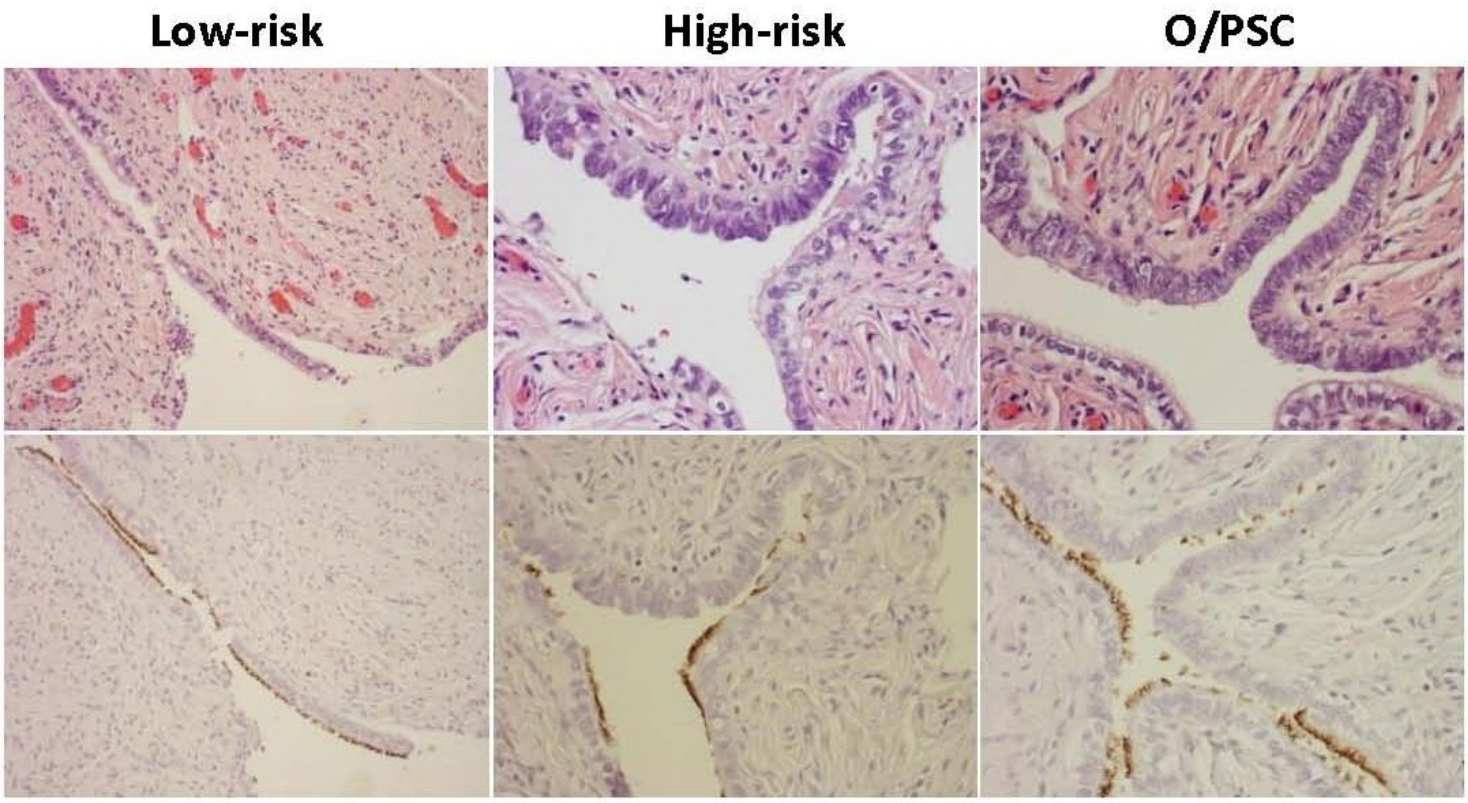

Figure 6

Morphologic and immunohistochemical identification of tubal ciliated cells in tubal fimbria. One representative section of tubal fimbria from patients in an age group of 40s was presented. Top panel shows morphologic picture of tubal fimbria, while bottom panel shows corresponding tubulin stains. The number of ciliated cells (tubulin+) was 180, 128, and 141 for low-risk, high-risk, and O/PSC patients, respectively. Original magnifications: left 100x, middle 200x, right 200x

Supplementary Files

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