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Original Article

Ultrastructural study of human ampullary endosalpinx in different stages of ovarian cycle

Sudipto Konar [a,*], *Kalyan Brata Singha* [b], *Maloy Kumar Mondal* [c], *Tapas Ghosh* [c]

[a] *Assistant Professor, Dept of Anatomy, Murshidabad Medical College & Hospital, West Bengal, India*
[b] *Assistant Professor, Dept of Anatomy, Midnapore Medical College & Hospital, West Bengal, India*
[c] *Associate Professor, Dept of Anatomy, Burdwan Medical College & Hospital, West Bengal, India*

ARTICLE INFO

Article history:
Received 27 July 2014
Accepted 10 November 2014
Available online 28 November 2014

Keywords:
Fallopian tubes
Ultrastructure
Ovarian cycle

ABSTRACT

Introduction: Efficient tubal transportation of gamete and embryo is essential for successful pregnancy. Tubal pathologies are among the leading causes of ectopic pregnancy and infertility. Scanning electron microscopy [SEM] was used to study the luminal surface morphology of the ampullary part of human Fallopian tube, paying particular attention to the different changes occurring with the progression of ovarian cycle.
Methods: The tubal samples were prepared from 15 women of different stages of ovulatory cycle, undergoing bilateral tubectomy operation for sterilization. After proper processing, the samples were examined under SEM.
Results: The results demonstrated that, in the free luminal surface of Fallopian tube there were presence of both ciliated and nonciliated cells. In both of these cell types, obvious structural and quantitative cyclical changes were detected during different stages of ovarian cycle. In the follicular phase the ciliated cells were abundant and there was gradual increase in the cilia length. In this phase the nonciliated cells also progressively increased in height to reach their maximal height in the periovulatory period. Subsequently, as the cycle advanced towards the end of luteal phase, both the cell types reduced in height and there was partial deciliation.
Discussion: Primed by a period of estrogen dominance at ovulation and a subsequent shift to progestogen dominance with permissive estrogenic action, the endometrial epithelium undergoes the structural and physiological changes necessary for blastocyst attachment and implantation in utero.

1. Introduction

The contribution of the Fallopian tubes towards successful reproduction can not be overlooked. Human endosalpinx plays important roles in sperm transportation and capacitation, ovum transportation, fertilization and early embryogenesis. In human ampullary segment of Fallopian tube is the most common site of *in vivo* fertilization. The lining epithelium of the ampulla is comprised of single layered ciliated

* *Corresponding author*. Officers' Colony, P.O. – Sripally, Burdwan, West Bengal 713103, India. Tel.: +91 9433209144.
E-mail address: sudiptokonar@gmail.com (S. Konar).
http://dx.doi.org/10.1016/j.jasi.2014.11.007

cells along with nonciliated secretory tall columnar cells. Ciliated cells are mainly involved in transportation of ova towards the uterus, while the secretory cells maintain the nourishment for the gametes & early embryo and also lend a hand in capacitation of spermatozoa. Derangements of these functions may lead to infertility or ectopic pregnancy.

The ovarian cyclicity in Human is divided into a follicular and a luteal phase.[1] The estrogen governed follicular phase begins on day 1 of the cycle and is variable in length. The progesterone driven luteal phase starts after ovulation and its length is relatively constant to 12–15 days.[2]

In literature there is very little material on cyclical changes of the ultrastructure of human ampullary endosalpinx in a particular age group. It was observed that with increase in age fallopian tubes were also showing atrophic changes.[3] This observation urges the need of uniformity in age distribution while selecting study population. Most of the previous ultrastructural studies on Fallopian tube employed techniques other than critical point drying method during specimen preparation. But it has been clearly pointed out that techniques such as air drying or even freeze drying by and large consequence in very much distortion of surface microstructure.[4]

In the present study we have attempted to cram a scanning electron microscopic survey of the mucosal lining of the ampulla of human Fallopian tube, giving particular attention to the changes that happen during the progression of ovarian cycle through the follicular phase and the luteal phase. Moreover we have used the critical point drying method to curtail the distortion of the surface microstructure during tissue preparation.

2. Methods

Ampullae of the Fallopian tubes were obtained from fifteen women, aged between 28 and 32 years. They were selected for bilateral tubectomy operation for female sterilization. Specimens were collected in a manner that they represented different timeframe of ovulatory cycle (distributed in follicular and luteal phases). So that, if any cyclical change happens with progression of ovarian cycle, can easily be recorded. All the fifteen women had history of normal menstrual cycle and consents for the study were taken as per rule.

Soon after collection, specimens were thoroughly rinsed in freshly prepared phosphate buffer solution. Then primary fixation was done in 2.5% glutaraldehyde at 5 °C for 4 h and subsequently after washing the specimens in buffer solution they were post fixed in 2% osmium tetraoxide (OsO_4) for 90 min. After post fixation tissues were again properly bathed in phosphate buffer solution. Then specimens were gradually dehydrated through a series of ascending concentration of ethanol to 100% amyl acetate. The final solvent was flushed from the tissue when the specimens were transferred to liquid carbon dioxide for critical point drying by Hitachi HCP-2 Apparatus. Finally dried samples cut into small pieces and mounted on an aluminum stub by using a double-sided adhesive tape. Then it was placed in an ion coater unit [IB2 ion coater Sputter Device] and coated with a thin layer of gold. All the specimens were examined and photographed using S530-Hitachi SEM instrument operated at 20 kV (University Science Instrumentation Centre, The University of Burdwan).

3. Results

The mucosal surface of the ampullae was lined by ciliated cells as well as nonciliated secretory cells having microvilli on their surface. The distribution and characteristics of both ciliated and secretory cells changed along with follicular phase and the luteal phase of ovulatory cycle. Characteristic features of different ovulatory phases are summarized in Table 1.

In the follicular phase, progressive ciliogenesis was noticed. The mucosal surface was covered by groups of ciliated cells in the company of secretory cells (Fig. 1: Day 8). The cilia were densely distributed over the cell surface. Often the cilia were not bent in the same direction during observation (Fig. 2: Day 11). Whether the dissimilar orientation among the cilia was either real or due to preparatory relic, is not clear. During initial part of this phase, the cilia were approximately 5 micron in length. With progression of this phase, increase in cilia length was noticed along with increase in number of ciliated cells (Fig. 3: Day 11). Small population of secretory cells could also be seen in between the ciliated cells. The surfaces of secretory cells were polygonal in shape and were projecting into the lumen of Fallopian tube as globular processes. Such processes were studded with typical short microvilli pattern on the surface. The short microvilli were approximately 0.2 micron in length.

The micrographs belonging to the pre-ovulatory period (late follicular phase), suggested a grater quantitative and qualitative changes in secretory cells than the ciliated cells. The secretory cells were not only projected further into the Fallopian tube lumen but also very densely covered with well developed microvilli (Fig. 4: Day 13). The heights of both ciliated and secretory cells became almost equal, with the secretory cells forming domes between the tufts of cilia. Even sometimes ciliated cells became almost covered by secretory cells (Fig. 5: Day 14). So in some field the cilia of the ciliated cells were hardly discernable because they were covered by well developed microvilli of the secretory cells.

Table 1 – Characteristic features of different ovulatory phases.

Phase of ovulatory cycle	Ciliated cell	Nonciliated cell
Follicular phase	Progressive increase in number along with increased cilia length	Small cell population with short microvilli
Pre-ovulatory period (late follicular phase)	Cilia were hardly discernable as covered by well developed microvilli of the secretory cells	Densely covered with well developed microvilli projecting into the lumen of the Fallopian tube
Luteal phase	Progressive quantitative reduction in cell number with marked deciliation	Predominant cell type but drawn away from the lumen and decrease in of microvilli size

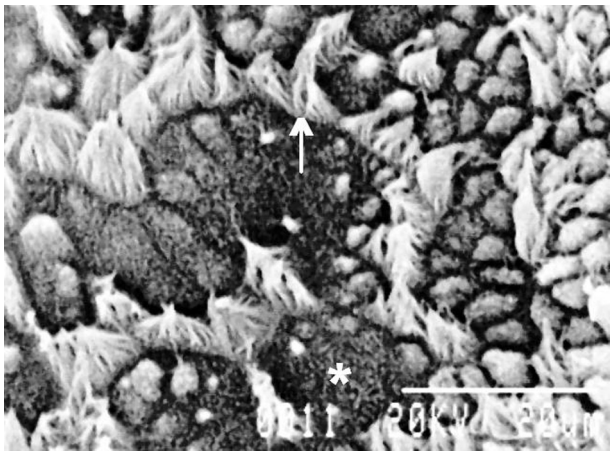

Fig. 1 – Human endosalpinx (Day 8) [Magnification 1500×]. → Ciliated cell, * Secretory cell.

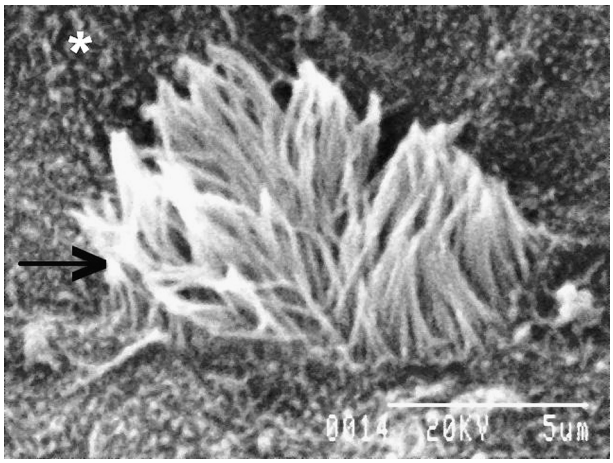

Fig. 2 – Human endosalpinx (Day 11) [Magnification 8000×]. → Ciliated cell, * Secretory cell.

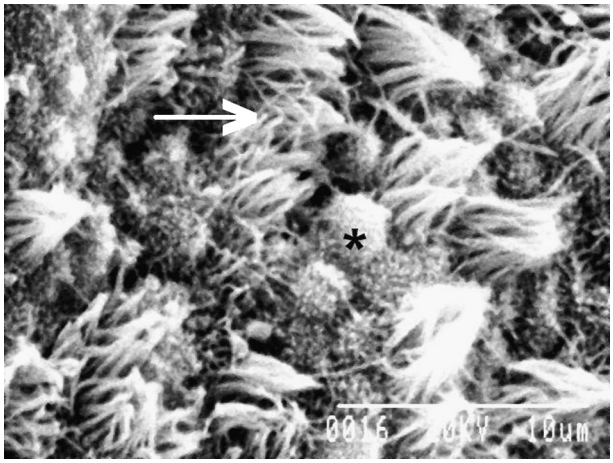

Fig. 3 – Human endosalpinx (Day 11) [Magnification 5000×]. → Ciliated cell, * Secretory cell.

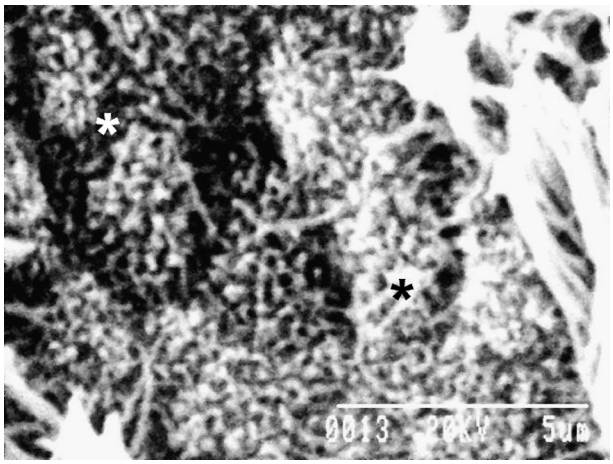

Fig. 4 – Human endosalpinx (Day 13) [Magnification 10,000×]. * Secretory cells with microvilli.

The luteal phase was characterized by progressive quantitative diminution in ciliated cells. Though the secretory cells were predominant in the field, they receded from the lumen and the sizes of the microvilli were also decreased. At the beginning of this phase, in the post ovulatory period cilia became more prominent as there was faster decrease in height of nonciliated secretory cells (Fig. 6: Day 15 and Fig. 7: Day 16). On further progression of this phase ciliated cells were also hugely reduced in number; they could be seen hither and thither in very small groups or even singly within the large population of secretory cells with hardly any microvilli, reminds bird's eye view of offshore yacht racing (Fig. 8: Day 19). At the end of luteal phase marked deciliation was noted along with hardly discernable microvilli over the secretory cells (Fig. 9: Day 24).

4. Discussion

The Fallopian tube, like other organs of the female reproductive tract, is under hormonal influence of the ovary. Cyclical changes of the ampullary endosalpinx in different stages of

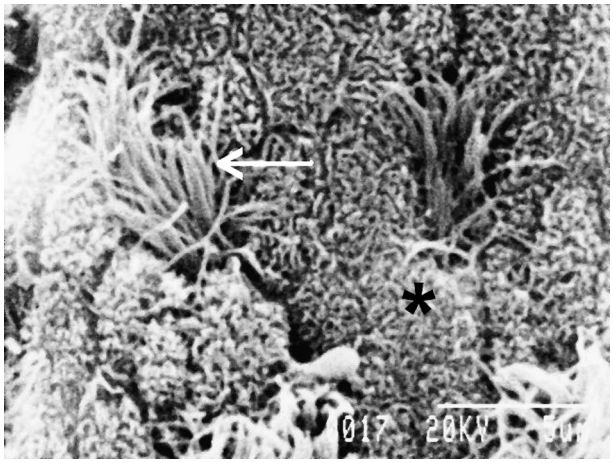

Fig. 5 – Human endosalpinx (Day 14) [Magnification 6000×]. → Ciliated cell, * Secretory cell.

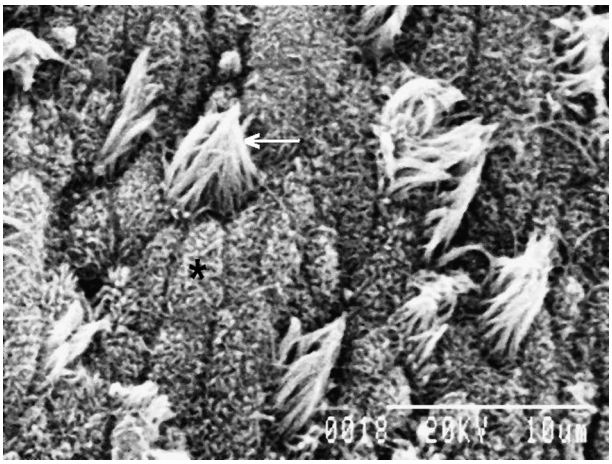

Fig. 6 – Human endosalpinx (Day 15) [Magnification 4000×]. → Ciliated cell, * Secretory cell.

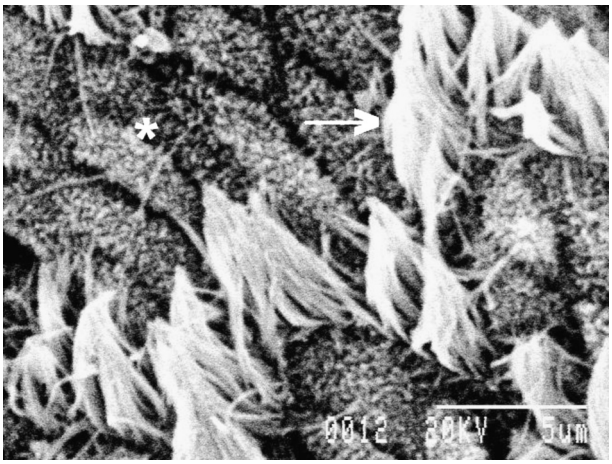

Fig. 7 – Human endosalpinx (Day 16) [Magnification 6000×]. → Ciliated cell, * Secretory cell.

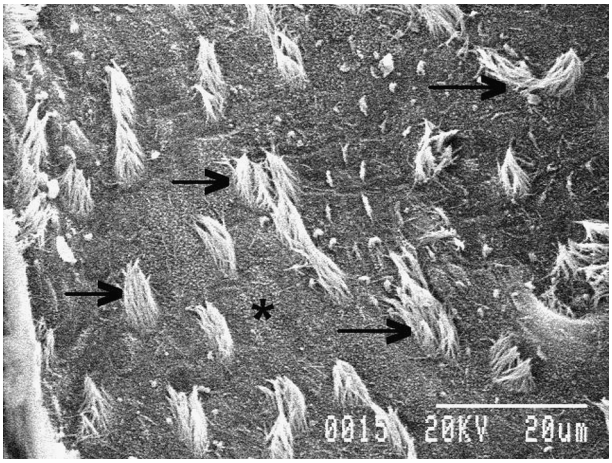

Fig. 8 – Human endosalpinx (Day 19) [Magnification 1500×]. → Ciliated cell, * Secretory cell.

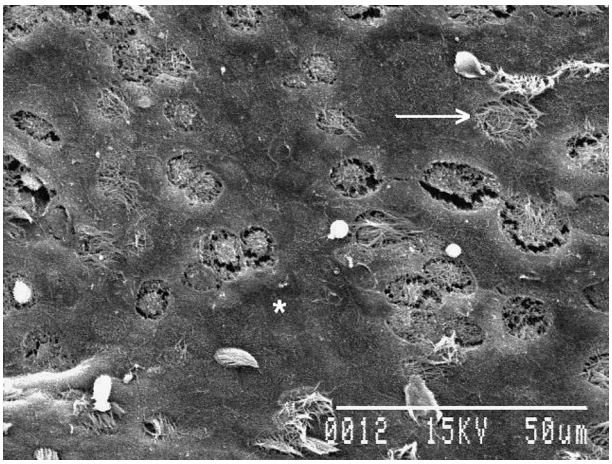

Fig. 9 – Human endosalpinx (Day 24) [Magnification 1500×]. → Ciliated cell, * Secretory cell.

ovarian cycle are one of the key events for reproduction. Authors [Rumery and Eddy, 1974] have reported that the Fallopian tube of ovariectomized rabbits had undergone extensive loss of cilia.[5] Among the different parts of the human Fallopian tube, only the ampullary and fimbrial regions seemed to be more responsive to hormonal stimulation.[6] Changes in the activities of the mucosal surface, varies with the stages of menstrual cycle as well as with the age. The height of the epithelium regresses in postmenopausal age group and also towards the end of the ovulatory cycle, when the number of the ciliated cells are also reduced. Though SEM study is helpless to recognize cellular heights but it can be done by TEM (Transmission electron microscopy). The hormonal regulation of Gαi2 expression in human oviductal epithelium cell line OE-E6/E7 by estradiol had already been demonstrated. Hormonal regulation of the novel, G protein coupled membrane receptor for progesterone mPRα, which was shown to be up-regulated by progesterone and down-regulated by estradiol in OE-E6/E7 cell line. The ERβ receptor to mediate the estradiol induced regulation of Gαi2 and mPRα expression had also been suggested.[7]

Around the time of ovulation, the secretory cells reach peak activity and discharge their contents into the lumen of the tube, consequently reducing in height relative to the ciliated cells. This results in greater prominence of the cilia and may enable them to move particulate material or viscous secretions more effectively. Transport of the ocytes into the uterine tube is thus encouraged by the ciliary beats and defective ciliary beats may result in failure of migration of ocytes or zygote which may result in implantation of the blastocyst within the wall of uterine tube.

Changes in the surface architecture of the ampullary epithelium during the different stages of the ovarian cycle, is quite interesting. With the raising titer of estradiol during follicular phase to ovulatory phase of the ovulatory cycle, microvilli of the secretory cells also expresses mostly. As the cycle proceeds from ovulatory phase to luteal phase, the microvilli reduce in height probably due to increased level of progesterone hormone. With the raising titer of progesterone, degeneration of the cilia has been observed in the luteal phase, only to renew following an elevation of estrogen production as

the next ovulatory cycle proceeds from beginning of the follicular phase to the ovulatory phase. It may be inferred from the scanning electron microscopic images that both the microvillus containing secretory cells and the non-secretory ciliated cells perform their main function at the time of ovulation to maintain one of the nature's greatest event, the reproduction.

Conflicts of interest

All authors have none to declare.

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