Effect of Clomiphene Citrate on the Fallopian Tube of Rats. Histological Considerations and Clinical Implications

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Abstract: Background: Fallopian tube is a dynamic, steroid responsive tissue that facilitates fertilization of the gamete and early embryo transport. Clomiphene citrate is considered the first line therapy for induction of ovulation in women with anovulatory infertility. There is marked increase in the rate of ectopic tubal pregnancy with clomiphene citrate therapy. **Objective:** To clarify the histological changes in the tubal mucosa that accompany clomiphene citrate therapy and may predispose to ectopic tubal pregnancy. **Material and Methods:** Twenty prepubertal female rats were used, they were divided into control group and another group treated with intraperitoneal injections of 10 mg/kg body weight/day of clomiphene citrate for 6 consecutive days. **Results:** Hypertrophy and hyperplasia of the tubal epithelium with marked ciliogenesis. Marked disturbance of tubal mucosa with shedding of parts of tubal mucosa and mucosal folds into the lumen of the tube. Accumulation of structureless masses in the lumen of the tube. The changes were seen in the tubal ampulla as well as the isthmus. **Conclusion:** Mucosal disturbance and shedding, epithelial hyperplasia and hypertrophy, luminal masses of shedded epithelium and structurless tissues and disturbance in the function of both epithelial and secretory cells. All the preceeding features may hinder the movement of fertilized ovum and consequently increase the chance for occurrence of ectopic tubal pregnancy.

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Key words: Fallopian tube, mucosa, epithelium, clomiphene citrate, histological considerations, rats.

1. Introduction

Clomiphene citrate, a non steroidal synthetic hormone with estrogenic effect, has been the first line therapy for induction of ovulation in women with anovulatory infertility and in couples with unexplained infertility[1,2,3]. Its action seems to involve hypothalamic pituitary axis and ovaries[4,5]. Although clomiphene citrate has limited dose-dependent side effects, prolonged treatment is associated with an increased frequency of tubal ectopic pregnancy in humans[6,7] raising the possibility of fallopian tube disorders. It is tissue-selective estrogen receptor modulator[8,9]. Clomiphene citrate causes the pituitary to secrete higher levels of FSH and LH. These hormones stimulate the ovary to produce and release a mature egg. Structurally similar to estrogen, clomiphene binds to estrogen receptor sites in the brain. When these receptor sites are filled with clomiphene, they can't bind with estrogen circulating in the blood in essence, the brain is tricked thinking that the amount of estrogen in the blood is too low. In response, the pituitary produces more FSH. The increased level of FSH inturn stimulates maturation of an egg within an ovarian follicle[10,11].

Fallopian tube is a dynamic steroid responsive tissue that facilitates fertilization of the gamete and early embryo transport[12]. Ciliary beating is thought to regulate gamete and embryo transport[13]. Abnormalities in the structure and function of the fallopian tube could interfere with the transport process, leading to tubal ectopic pregnancy[14]. It is unclear why rate of ectopic tubal pregnancy increases with clomiphene citrate therapy. In this work, some of the unclear points about this phenomenon will be clarified.

2.Material and Methods

The study was carried out on twenty prepubertal female rats of about 25 days old. They were obtained from the animal house, Faculty of Medicine, Zagazig University. They weighing 110-135 gm at the start of the study. They were divided into two groups, ten rats for each group. The first group, control one, received daily intraperitoneal injections of sterile normal saline, of the same volume as the second group, for 6 consecutive days. The second group, clomiphene citrate-treated group received daily intraperitoneal injections of clomiphene citrate 10 mg/kg body weight for 6 consecutive days[15] (Global Napi Pharmaceutical-Egypt, Underlicence of Sanofi Aventis-France). They were treated according to the guidelines approved by the animal use committee of Zagazig University. Twenty four hours after the last injection, rats were anaesthetized by ether inhalation, careful laparotomy was done and fallopian tubes were collected and divided anatomically into small specimens which were processed for light and electron

microscopic study. Specimens for light microscope were fixed in 10% neutral formol saline for 24 hours. They were processed to prepare 5 µm thick paraffin sections for Hematoxylin and Eosin (H & E) stain[16]. Specimens for electron microscope were immediately fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for 2 hours at 4°C, then they were dehydrated with ascending grades of ethanol and then were put in propylene oxide for 30 minutes at room temperature, impregnated in a mixture of previous reagents at 48°C for another one hour. The specimens were embedded in EM bed-812 resin in BEEM capsules at 60°C for 24 hours[17]. By using Leica ultra cut UCT we obtained ultrathin sections (1 µm) which were stained with uranyl acetate and lead citrate and were examined and photographed by JEOL 1200 EX-II in Electron Microscopic Center, Faculty of Science, Ain Shams University.

3.Results

1- Control rats:

A- Light microscopy:

The ampulla of the fallopian tube appears with thin wall, wide lumen and long mucosal folds. Its wall is formed of mucosa formed of epithelium and lamina propria and thin muscle layer. The lining epithelium is mainly formed of secretory and ciliated cells (Figs. 1, 2). The isthmus of the fallopian tube is different from the ampulla in that, its wall is thicker, more muscular, with narrow lumen and short mucosal folds (Fig. 3).

B- Electron microscopy:

The ciliated epithelial cells are short and broad with large ovoid euchromatic nuclei. Their cytoplasm contains mitochondria (Fig. 4). The secretory epithelial cells are long narrow cells, their nuclei are euchromatic oval and slightly elongated. Variable-electron dense granules and mitochondria are present in their cytoplasm (Figs. 5, 6).

2- Clomiphene citrate-treated rats:

A- Light microscopy:

In the ampulla; mucosal folds are either short, irregular with disturbed appearance or loss their regular structural pattern. The mucosa shows cleavage at the level of lamina propria with accumulation of shedded epithelial tissues from mucosa and mucosal folds into the lumen of the tube. Hypertrophy and hyperplasia of epithelium are evident. Remnants of tissues are also present in the lumen of the tube (Figs. 7, 8). The isthmus is less affected, with shedded parts of the mucosa into the lumen of the tube (Fig. 9).

B- Electron microscopy:

The lumen of the ampulla contains structureless masses of separated tissue remnants with many cilia running in different directions. The lumen of the isthmus contains separated tissues containing nuclei and secretory granules with subsequent luminal narrowing. The ciliated and secretory cells of the ampulla and isthmus show normal ultrastructural appearance with some opacities and densities in their apical parts (Figs. 10- 13).

Fig. (1): A photomicrograph of a section in the fallopian tube of a control prepubertal female rat showing the ampulla of the tube with thin wall (arrow), long mucosal folds (arrow head) and wide lumen. (H & E X 100)

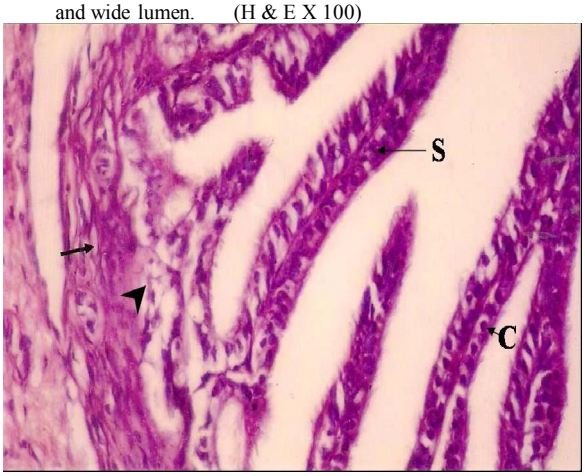

Fig. (2): Higher magnification of the previous section showing the thin muscle layer of the wall (arrow), lamina propria (arrow head), secretory (S) and ciliated (C) epithelial cells of the mucosa. (H & E X 400)

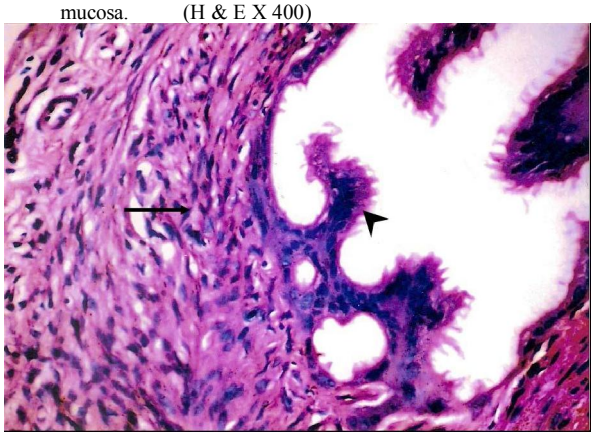

Fig. (3): A photomicrograph of a section in the fallopian tube of a control prepubertal female rat showing the isthmus of the fallopian tube with thick wall (arrow), short mucosal folds (arrow head) and narrow lumen. (H & E X 400)

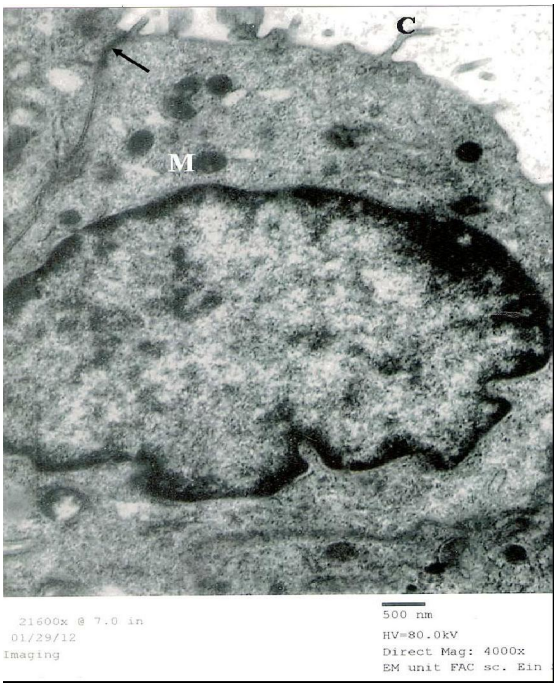

Fig. (4): An electron micrograph of a section in the fallopian tube of a control prepubertal female rat (isthmus) showing ciliated epithelial cell with large, ovoid euchromatic nucleus. The apical part of the cell contains cilia (C), with tight junction (arrow) with the neibouring cell. The cytoplasm contains mitochondria (M). (X 21600)

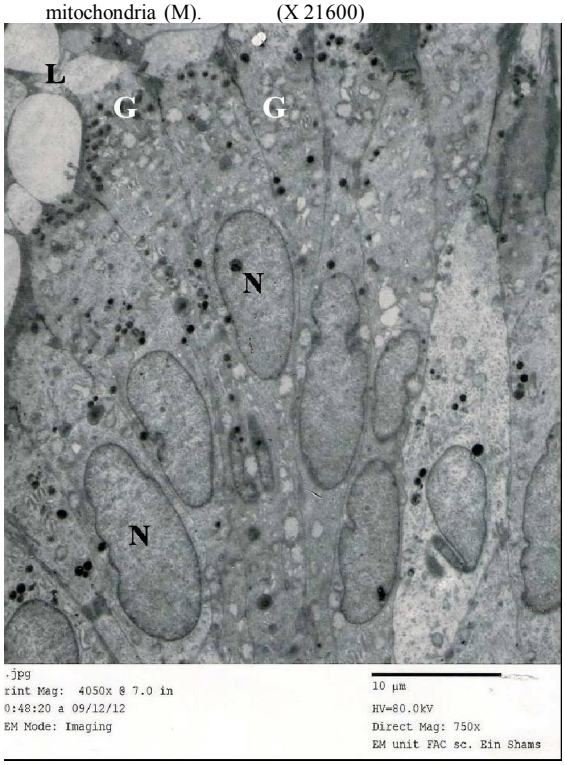

Fig. (5): An electron micrograph of a section in the fallopian tube of a control prepubertal female rat (ampulla) showing group of secretory epithelial cells with oval, slightly elongated, euchromatic nuclei (N) and secretory granules (G) of various electron density in their cytoplasm. The lumen of the tube (L) appears. (X 4050)

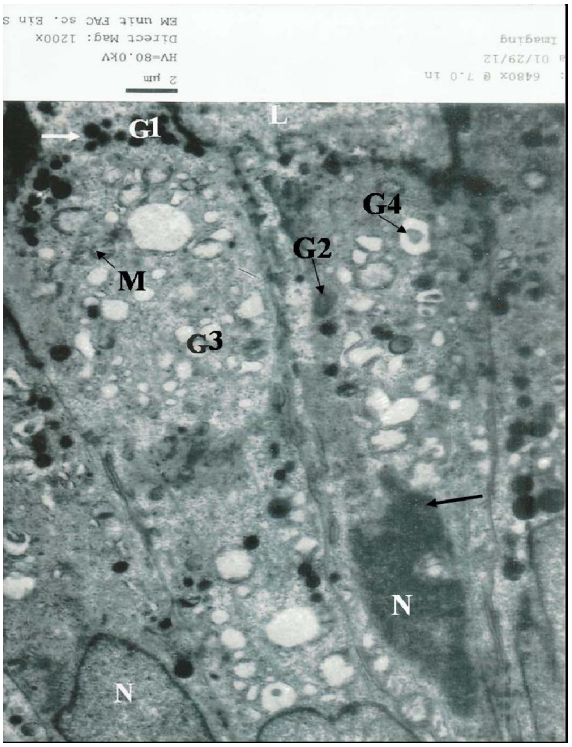

Fig. (6): An electron micrograph of a section in the fallopian tube of a control prepubertal female rat (isthmus) showing the apical parts of secretory epithelial cells. High-electron dense (G1), moderate-electron dense (G2) and low-electron dense (G3) granules are present in the cytoplasm. Some granules (G4) show mixed appearance. Mitochondria (M) are also present in the cytoplasm. The lumen of the tube appears (L). The nucleus (N) of one secretory cell is more-electron dense (arrow) than other nuclei.
(X 6480)

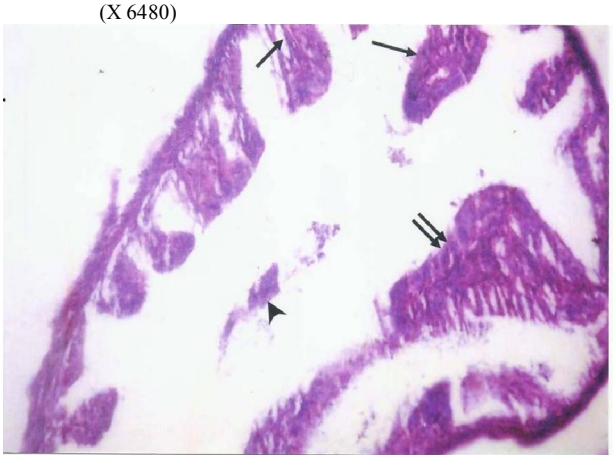

Fig. (7): A photomicrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat showing the ampulla of the tube with short, irregular, disturbed mucosal folds (arrow). There is massive separation of the mucosa and mucosal folds into the lumen of the tube (double arrows). Tissue remnants are also present in the lumen of the tube (arrow head). (H & E X 200)

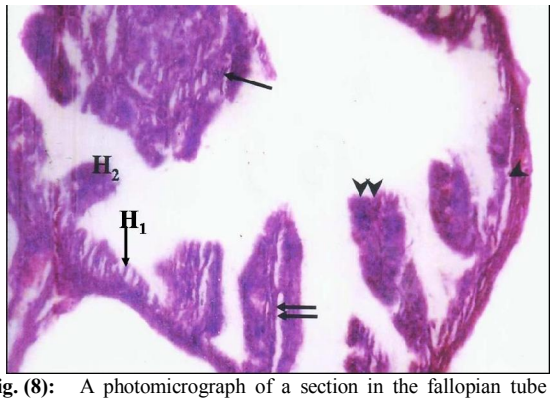

Fig. (8): A photomicrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat showing accumulation of shedded epithelial tissues (arrow) in the lumen of the ampulla of the tube. The line of cleavage appears to be at lamina propria (arrow head). Mucosal folds may be also disturbed at lamina propria (double arrows) or may loss their regular structural pattern (double arrow heads). Hypertrophy (H_1) and hyperplasia (H_2) of the epithelium are evident. (H & E X 200)

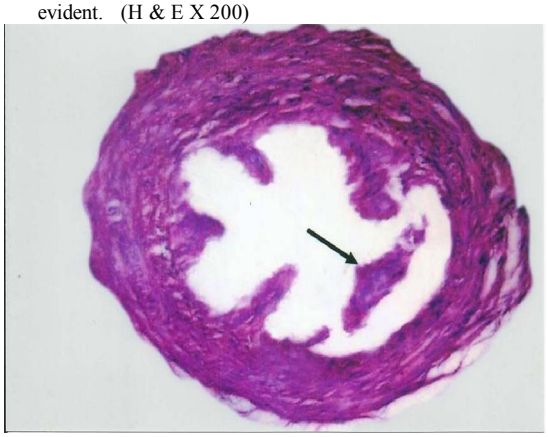

Fig. (9): A photomicrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat showing the isthmus with shedded part of the mucosa (arrow). (H & E X 100)

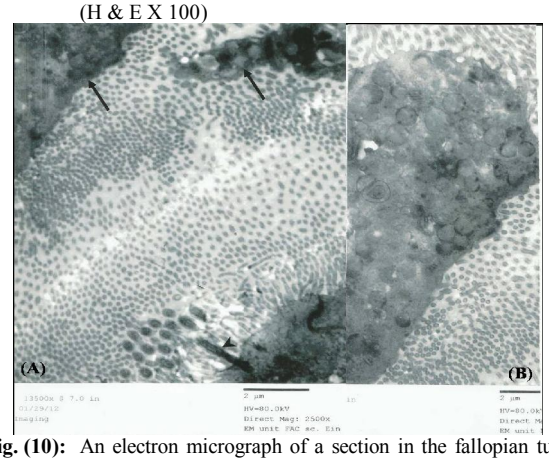

Fig. (10): An electron micrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat (ampulla) showing (A) the lumen of the tube contains two structureless masses of separated tissue remnants (arrow). The most apical part of ciliated cell appears with intact cilium (arrow head). The lumen contains many cilia. (B) Higher magnification of the separated structurless mass. (A X 13500) (B X 16200)

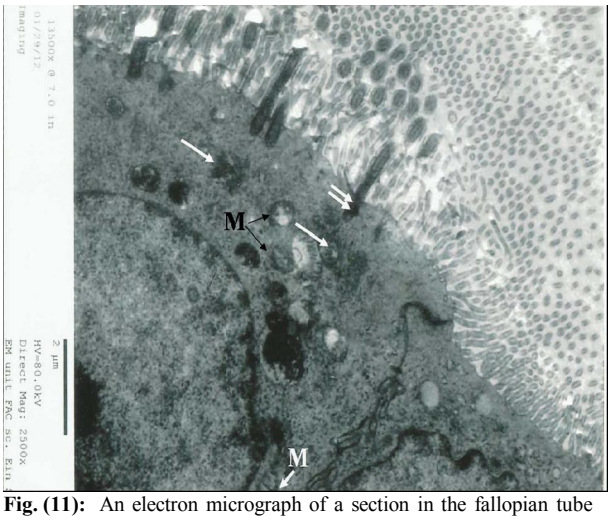

Fig. (11): An electron micrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat (ampulla) showing the apical part of the ciliated cell of previous figure with nearly normal cytoplasmic structures as mitochondria (M) and cilial origin from the apical cytoplasm (double arrows). Only some irregular opacities (arrow) appear in the cytoplasm. A part of the euchromatic normal nucleus appears. The lumen contains ciliary parts. (X 13500)

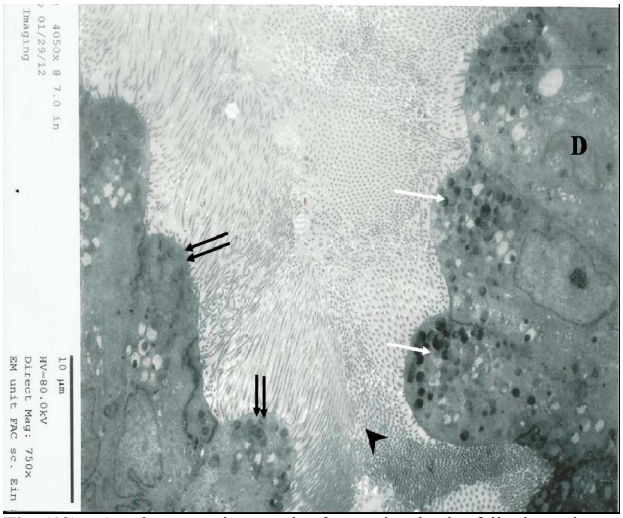

Fig. (12): An electron micrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat (isthmus) showing secretory epithelial cells with accumulation of secretions in the apical parts of the cells (arrow). Separated tissues containing nuclei and secretory granules are present in lumen of the tube (double arrows) with narrowing of the tubal lumen inbetween (arrow head). The lumen of the tube contains many cilia running in different directions. Disturbed nucleus of secretory cell (D) is seen. (X4050)

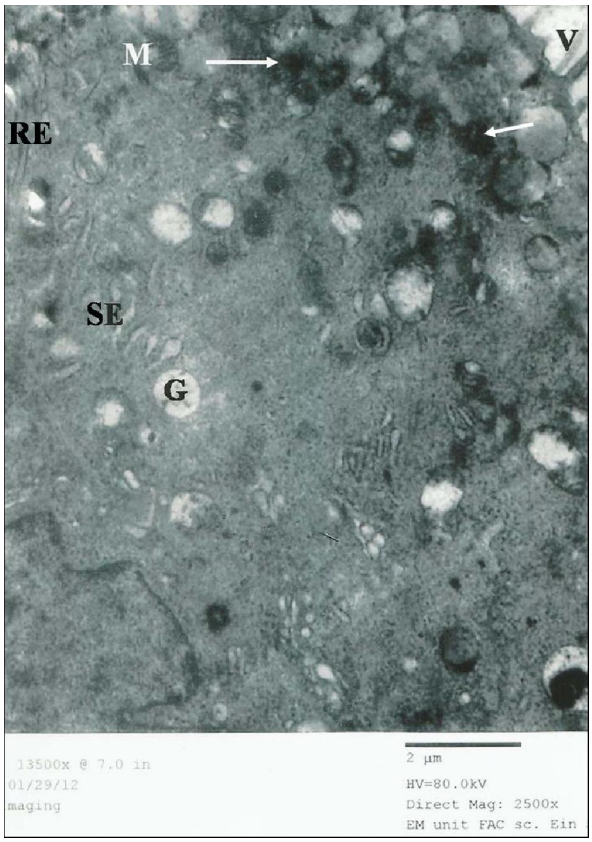

Fig. (13): An electron micrograph of a section in the fallopian tube of clomiphene citrate-treated prepubertal female rat (isthmus) showing the apical part of secretory epithelial cell containing secretory granules (G). The cytoplasm contains also mitochondria (M), smooth endoplasmic reticulum (SE), rough endoplasmic reticulum (RE) and surface microvilli (V). Only some irregular densities (arrow) appear in the cytoplasm. A part of the euchromatic normal nucleus appears. (X 13500)

4.Discussion

Clomiphene citrate therapy for anovulatory infertility increases the risk of tubal ectopic pregnancy which is a life-threatening disorder[18]. Destruction of the tubal epithelial cell layers and impairment of epithelial cell function have been suggested as a cause of tubal ectopic pregnancy through blockage of gamete and embryo transport[19].

Most tubal ectopic pregnancies in human occur in isthmus. Histological analysis of the fallopian tissue revealed hypertrophy and hyperplasia of luminal epithelial cells in the isthmus in rats treated with chronic dose of clomiphene citrate[20].

Chronic treatment with clomiphene citrate causes severe disruption and disorganization of the fallopian tube epithelium which are not specific to the isthmus but more marked in the tubal ampulla. Hypertrophy and hyperplasia of the tubal epithelium, and consequently functional disturbance as well as shedding of multiple parts of the tubal mucosa into its lumen are features of clomiphene citrate therapy.

Epithelial separation and shedding are more marked than apoptosis and commonly proceed it as the remaining ciliated and secretory epithelial cells showed only densities and opacities in their apical parts and some disturbance of nuclei of secretory cells. Also masses of epithelial cells containing nuclei and secretory granules are seen in the lumen of the tube.

Ciliogenesis with multiple cilia running in different directions and consequently disturbance of their function and disturbed function of secretory cells with accumulation of structureless masses of tissue debris in the lumen of the tube, all these features lead to hindering of the ovum transport and consequently predispose to ectopic tubal pregnancy.

The results of this study are in agreement with those obtained by **Hulas and Gawron**[21] concerning function of cilia and disturbed activity of secretory cells but they added that, clomiphene citrate activates estrogen receptors (ESRs), particularly cilia-localized ESR2A so, isthmus-specific apoptosis of epithelial cells and activation of cilia-ESR2A act in parallel to block gamete embryo passage through the fallopian tube, eventually resulting in tubal ectopic pregnancy.

The structureless masses appeared in the lumen of the tube may result from apoptotic cells lost and accumulated with each other to form such masses.

Chronic treatment with clomiphene citrate induces tubal apoptosis but not necrosis, through an intrinsic mitochondria-dependent signaling pathway in vivo. The apoptosis was specific to epithelial cells in the isthmus and the damage was reversed with 17 beta estradiol (E_2)[22], however, in this study, the destructive effects are more obvious in the epithelium of the ampulla than the isthmus.

Cilia are critical in gamete and embryo transport so clomiphene citrate influences the transport of gamete by altering the function of ESR2 receptors in the cilia or modulating cilia-localized ESR2 activity[23,24].

Collectively, this study reveals that, epithelial shedding, hypertrophy, hyperplasia and ciliogenesis are the common disorders of clomiphene citrate therapy. Additionally, accumulated structureless masses appeared in the lumen of the tube may result from separated apoptotic epithelial cells. The changes are more marked in the ampulla than the isthmus, so, the recorded rate of ectopic tubal pregnancy which is more frequent in the isthmus with clomiphene citrate therapy may be due to its more narrower lumen and more intact epithelial lining which is more suitable for implantation of the fertilized ovum.

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