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Histopathological study of the oviduct in female mice treated with levetiracetam

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Abstract

The present study was conducted to investigate the effects of levetiracetam (LEV) at a concentration of 350 mg/kg/day of body weight on oviduct tissue in female albino mice (*Mus musculus*). The experimental animals were divided into three groups and administered orally once daily for 14, 30, and 45 days. Each treatment group was matched to its own control group. The results of the present study showed that ciliated columnar cells transformed into cuboidal cells, with reduced cilia density, shortened secretory cells, hemorrhage, necrosis, and cell death of the mucosal folds, decreased tissue hyperplasia with the appearance of vacuoles, the presence of inflammatory cells in the lumen, and hyperplasia and hypoplasia of the tunica muscularis when compared to the control group.

Keywords: levetiracetam, Histopathological, muscularis, Swiss mice, and TUM

Introduction

Levetiracetam (LEV) is a new antiepileptic drug, widely used for its antiepileptic efficacy, offering an alternative to valproic acid (VPA) for many patients which discovered in the early 1980s. Safety data indicate that LEV is well tolerated by the vast majority of patients and has no endocrine side effects (Harden, 2001) [6].

In 1991, studies were reported clinical trials in patients with epilepsy, and it was considered an adjunctive treatment for the onset of partial seizures (Rossetti and Bromfield, 2005; Garcia and Rubio, 2009) [11, 3]. LEV was first approved by the US Food and Drug Administration (FDA) in 1991 as a new second-generation antiepileptic drug, and was approved for use as tablets in India in 2005 (Kavita *et al.*, 2011) [8]. LEV which knows (S-alpha-ethyl-2-oxo-1-pyrrolidine acetamide) also used to treat non-epileptic conditions including bipolar disorder and to prevent hemiplegia, myoclonus, and myoclonus. LEV as a single enantiomer, was reported by pharmacological studies "the therapeutic effects of antiepileptic drugs on sexual function since 1850, when bromides were introduced as a treatment for epilepsy". Later research showed the development of impotence and decreased libido in 11-22% of patients after initiating treatment with carbamazepine (CBZ), phenytoin (PHT), phenobarbital, or primidone. These drugs are known as older or first-generation drugs, as they were widely used. Second-generation drugs have gained favor due to their fewer side effects. However, several studies have demonstrated that the endocrine effects of valerian (VPA) in females occur in the ovary, where it acts as an enzyme inhibitor that prevents the conversion of testicular fat to estradiol (Perucca *et al.*, 2004) [10]. This hypothesis has been supported by numerous studies on porcine ovarian follicle cells, where it exerts a significant sterologenic effect in both gonadotropin-stimulated and non-gonadotropin-stimulated genital glands (Gregoraszczuk *et al.*, 2000; Taubøll, 2003) [4, 14]. High levels of testicular fat in the ovary inhibit the maturation of ovarian follicles and promote polycystic ovarian syndrome (PCOS) (Nandedkar and Munshi, 1981) [9]. (LEV) has been widely used for its antiepileptic efficacy, representing an alternative to VPA for many patients. Safety data indicate that LEV is well-tolerated and safe in many patients, and it has no endocrine side effects (Harden, 2001) [6]. However, it has an effect on basal steroid hormone secretion in ovarian follicular cells in prepubertal pigs, suggesting its effect on endocrine function and hormonal balance by increasing progesterone levels or decreasing follicle-stimulating hormone (FSH) levels (Gustavsen, 2008; Svalheim, 2010) [5, 13].

Thus, the aim of this study was to identify the histopathological changes in oviductal tissue resulting from the use of the drug and compare them with the control group.

Materials and Methods

Levetiracetam: In the current study, the drug Levetiracetam (LEV) was used. The drug was administered to mice orally at a concentration of 350 mg/kg/day. The dose was multiplied by the average mouse weight (28) g and the drug concentration in the package (100) mg/ml. The dose used in the experiment was (0.1) ml/day (FDA, 2013).

The study samples: consisted of 40 *Mus musculus* (Swiss mice) of the Balb/C strain, obtained from the Drug Control Center. The mice were 8-12 weeks old and weighed an average of 24-25 grams. The animals were divided into four groups, each containing 10 mice. The female animals were dosed as follows: the first group received a dose for 14 days, the second group for 30 days, and the third group for 44 days, while the control group was treated with distilled water. Vaginal swabs were taken from the females used in this study for two consecutive estrous cycles to ensure the integrity of the estrous cycle.

Animal house: which present in Department of Biology/College of Education for Pure Sciences (Ibn Al-Haitham) at the University of Baghdad plastic and that consist of cages covered with metal mesh lids, measuring 48 x 15 x 7 cm. The house containing wooden shore sawdust and tap water. They were supplied with standard food and were kept under standard laboratory conditions, with uniform temperature, lighting, and ventilation.

Samples of swab: A swab was taken from the vaginal lining using a metal loop, which was sterilized over a flame until red, and then immersed in normal saline (NaCl). The swabs were placed on clean glass slides and left to dry. They were then stained with methylene blue for three minutes. The slides were then washed with top water and allowed to dry (Husman, 1967) [7]. They were then examined under a light compound microscope.

Anatomy process: After completing the treatment, each group was prepared to anatomy process, by fixing the animal's head and pulling the tail to make a longitudinal incision in the middle region of the lower abdomen up to the sternum of the female mic. The viscera were then removed,

the oviduct was liberated and transferred to normal saline, and the surrounding fatty material was removed. The oviduct was also cleaned of the surrounding tissue, and its convolutions were opened so that they extended along its length. Then, the ampulla, isthmus, and uterus regions were separated. They were dried with filter paper and transferred to the fixative for the purpose of preparing microscopic slides (Butler and Jurrlink, 1987) [1].

Microscopic studies: Microscope slides were prepared according to the method of Bancroft and Stevens (1982) [21], where the oviduct was fixed in Bouin's solution for 24 hours, after which it was washed several times in a 70% ethanol solution. After that, the samples were passed through a series of ascending concentrations of ethyl alcohol starting from (70%, 100%) for half an hour, then the samples were incubated in xylene for 15 minutes, then the samples were placed in a mixture of xylene and molten paraffin wax at a temperature of (56-58) °C in a ratio of (1:1) for 15 minutes in a convection oven at a temperature of 60°C. After that, the samples were impregnated by placing them in the molten wax twice for an hour each time. Then the samples were stained in the center of the wax and left until the wax hardened. After that, the samples were cut with a thickness of 5 micrometers, and the transverse sections were carried in the oviduct for the regions (the annulus and the isthmus). The sections were stained with hematoxylin-eosin (Eosin- (hematoxylin) Histological and histopathological changes were studied using a compound imaging microscope of type M.

Results and Discussion

Regarding the histopathological changes in the amniotic cavity, the present results indicate that LEV causes histopathological changes in the amniotic cavity wall of animals treated with the drug for periods of 14, 30, and 45 days, respectively. These changes were characterized by a decrease in the height of the folds and a lack of branching into secondary and tertiary folds. According to the figure 1 and 2, the height of the ciliated epithelial cells decreased, as they appeared low columnar to cuboidal, with the nucleus at the apex of the cells, with an eccentric location. All sections were reported in the two figures 1 and 2, which was tunica mucosa TM, tunica muscularis TUM, tunica serosa TS, media mesothelium ME, vasa pellucida BV, columnar epithelium LE, lamina propria LP, columnar cells COC, secretory cells SCE, cilia C, mucosal fold MF, degeneration D, tear DI, and stained (H & E).

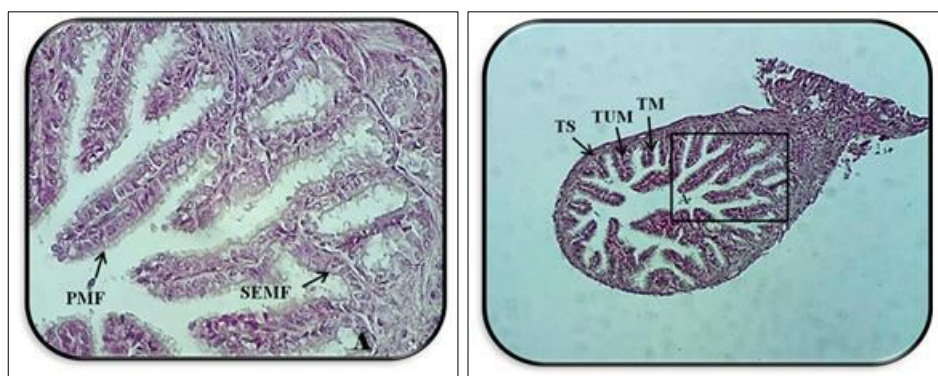


Fig 1: A transverse section of the oviduct of a female white mouse from the control group showing the histological structure the two images was 100x

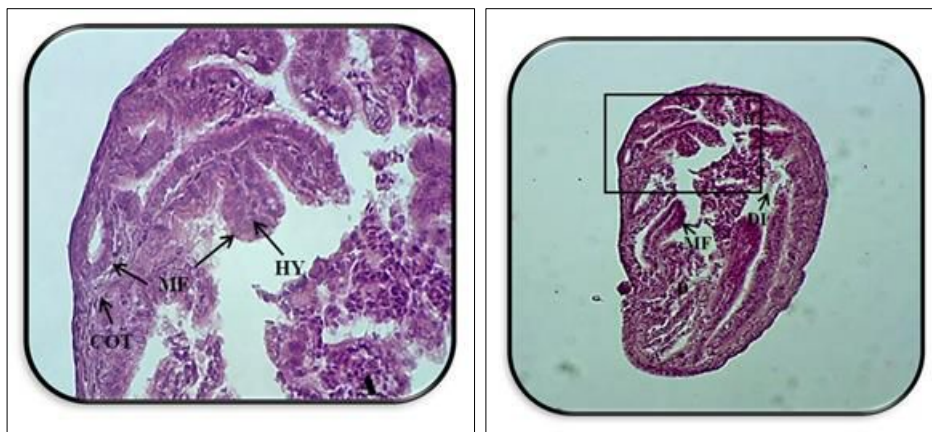


Fig 2: A cross-section of the (ovary) of a female white mouse in the group treated with (LEV) at a concentration of 350 mg/kg/day for 14 days of dosing, showing the histological changes (40x).

The non-ciliated secretory cells appeared thin and columnar. Some of these folds extended into the connective tissue and sometimes into the tunica muscularis, especially in groups dosed for a long period. Furthermore, hemorrhage was found, along with the presence of mononuclear cells and inflammatory cells in the connective tissue and in the amniotic cavity of the oviduct. Degeneration and disruption of some mucosal folds were also observed, as well as the scattering of cells. The mucous membrane in the middle of the lumen of the duct, and the shape of the ciliated cells changed to resemble secretory cells, and the appearance of vacuolation within the epithelial tissue and clusters of epithelial cell nuclei in a syncytium with necrosis and cell death.

Figure 3 and 4 refers to, observed in the loose connective tissue surrounding the mucous membrane in the muscular and serous layers of the epithelial tissue of the mucous membrane, as a result of the use of the drug under study, with an increase in the duration of treatment when compared with the control group. Figure 3 and 4 shown that damage to the mucosal folds (MF), and small number and lack of branching, DI tearing of the mucosal folds and scattering of their cells in the middle of the lumen of the oviduct, stained (H & E). Magnified section of rectangle which include hypoplasia H, muscular and serous layers, media ME, epithelial lining LE, cell death DC, cilia C.

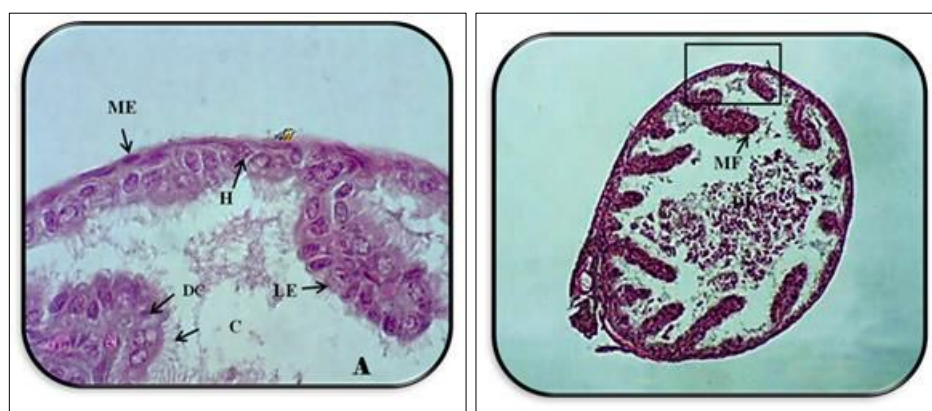


Fig 3: A cross-section of the oviduct (ovary) of a female white mouse in the group treated with the drug (LEV) at a concentration of 350 mg/kg/day for 30 days of dosing, showing the histological changes (100x).

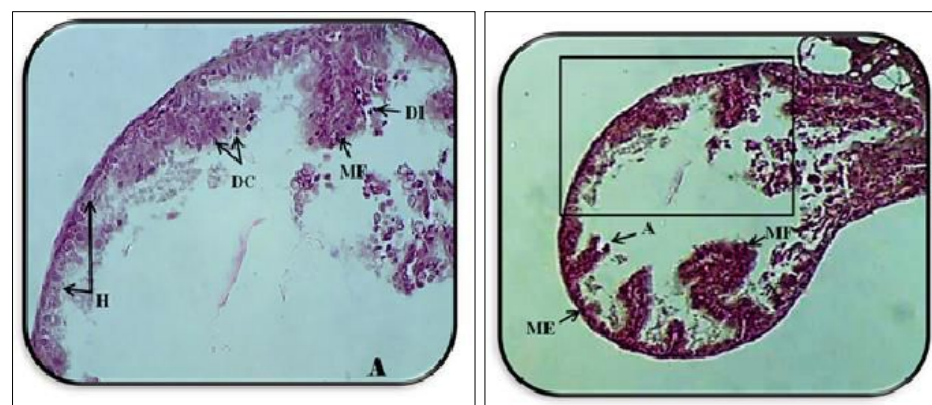


Fig 4: A cross-section of the oviduct (ovary) of a female mouse in the group treated with the drug (LEV) at a concentration of 350 mg/kg/day for 45 days of dosing, showing the histological changes (40x).

These results are consistent with what Wordinger and Morrill (1985) ^[20] reached regarding the negative effect of the oviduct upon treatment with the hormone diethylstilbestrol (DES), which is used as a treatment for abortion, as it causes an increase in the number of epithelial cells lining the mucosal folds, and the nuclei of the cells become central and change to a location close to their apical edge.

Newbold *et al.* (1983) ^[12] demonstrated that the epithelium lining the mucosal folds in the wall of the ampulla region of the oviduct of animals treated with diethylstilbestrol (DES) suffered from hyperplasia of the muscularis and serosa tunica. This is consistent with the present finding, as the mucosal folds extended into the muscularis layer in some ampulla sections, as shown in figure 5 and 6, the lamina

propria, which consists of loose connective tissue and blood vessels, was affected and had less tissue in the groups treated with LEV for a prolonged period compared to the control group. This may be due to the death of cells affected by the drug under study. The Magnified part 40x refers to epithelial tissue LE, lamina propria LP, columnar cell COC, secretory cell SCE, lumen L., figure 6 with magnified 100x shown that damage to the membranes of the isthmus wall, especially the tunica mucosa TM, rupture of DI, degeneration of D, mucosal fold MF, tunica muscularis TUM, tunica serosa TS, stained (H & E), 10x. The Enlarged portion of rectangle degeneration of D tunica muscularis, rupture of DI mucosal fold MF, separation of DE tunica mucosa from the muscularis, N necrosis, dead cells DC, .

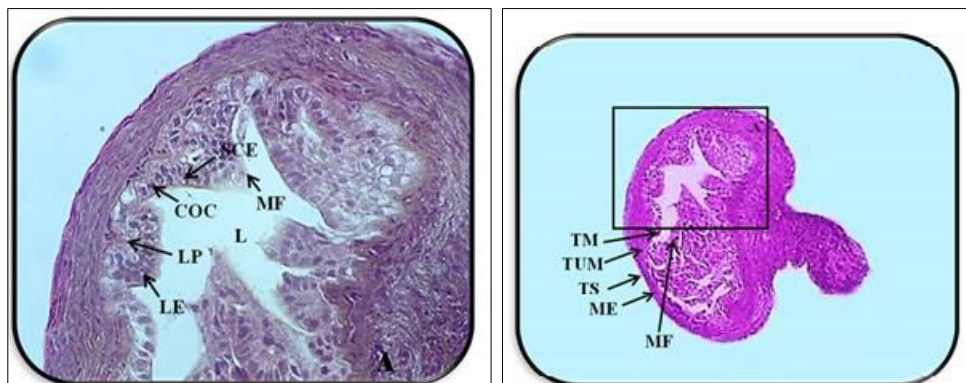


Fig 5: A cross-section of the oviduct (isthmus) of a female white mouse of the control group showing the histological structure. Note: mucosal folds MF, tunica mucosa TM, tunica muscularis TUM, tunica serosa TS, media ME, stained (H & E), 10x-40x.

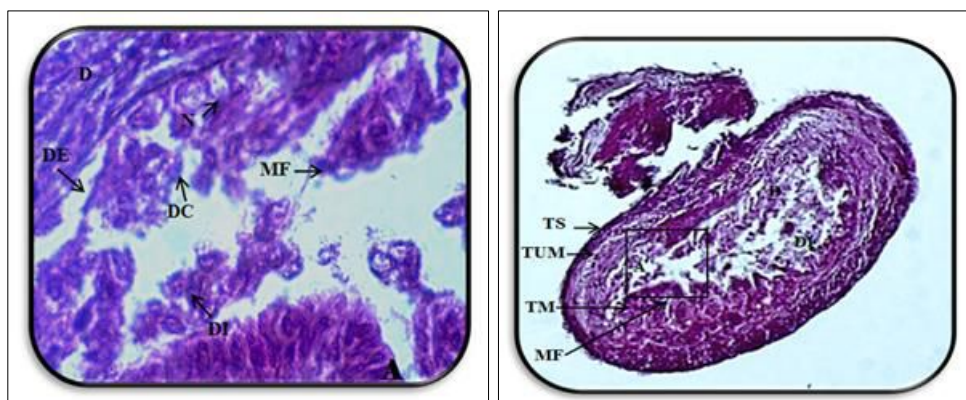


Fig 6: A cross-section of the oviduct (isthmus) of a female white mouse in the group treated with LEV at a concentration of 350 mg/kg/day for 14 days of dosing, showing the histological changes.

This is consistent with what Wordinger and Morrill (1985) ^[20] reported, who indicated that the oviduct wall was affected in rats treated with drugs. The current result also showed a decrease in the smooth muscle tunica with increasing duration of treatment with LEV, as it thinned in animals treated with the drug for 45 days to a single row of smooth muscle cells. This is inconsistent with what Treuting *et al.* (2012) ^[18] indicated, which is that the tunica muscularis is formed through the oviduct in the amniotic region. As for the serous tunica, the current result showed that it consists of a layer of loose connective tissue containing blood vessels and covered by simple squamous epithelium. This is consistent with what was shown by Codon and Casanave (2009) ^[17], who explained that this

tunica consists of a thin layer of loose connective tissue containing blood vessels. Figure 7 and 8 refers to mucosal folds and the absence of the typical arrangement (atypia) of the isthmus wall, especially the mucosal folds that suffered atrophy, degeneration, and sloughing of their mucosal sheath, in addition to their disruption. Figure 7 include Magnified section A. HY hyperplasia of muscularis cells, MF mucosal fold, LE columnar epithelium, TUM muscularis, 100x While, TS serous tunica, TUM muscularis, DI tear, MF mucosal fold, H&E stained, 40x. Figure 8 represent by TS serous tunica, TUM muscularis, TM mucosa, MF mucosal fold, H hypoplasia, D degeneration, EX sloughing of tunica intima cells, DIS epithelial cells translocation, stained (H & E), 10x.

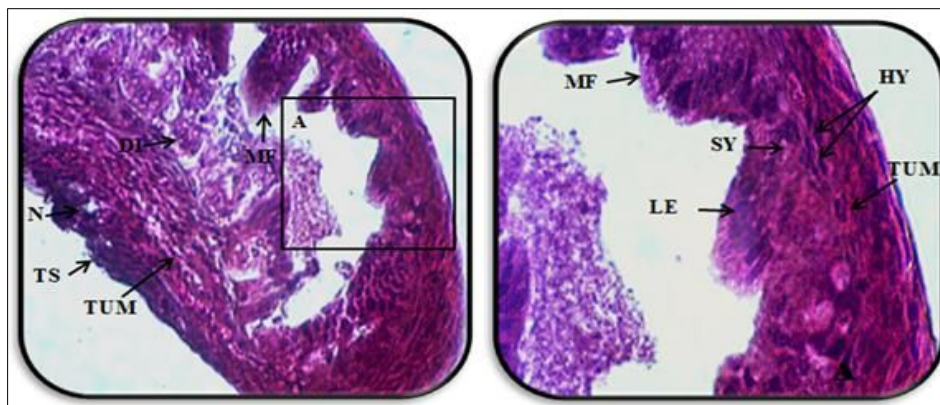


Fig 7: A cross-section of the oviduct (isthmus) of a female white mouse in the group treated with LEV at a concentration of 350 mg/kg/day for 30 days of dosing

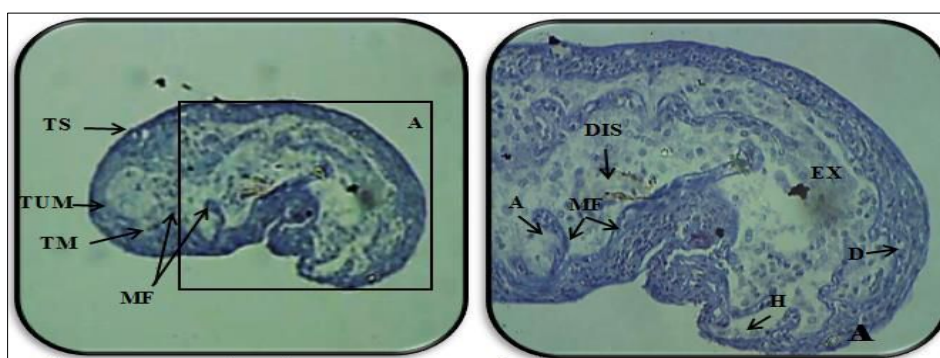


Fig 8: Transverse section of the oviduct (isthmus) of a female albino mouse from the group treated with LEV at a concentration of 350 mg/kg/day for 45 days of dosing.

The epithelial lining of the mucosal folds suffered a lot of damage represented by hyperplasia with degeneration and necrosis and the scattering of its cells in the middle of the cavity. However, the death of a large portion of its cells and their degradation and sloughing, and their presence in a compact cell form (synaptic), swelling, and a lack of cilia in a large portion of them, with changes occurring in their nuclei represented by their karyolysis.

The effect of the number of columnar and secretory epithelial cells lining the mucosal folds in the current result may be related to the effect of hormone concentration levels resulting from the use of the drug (LEV) used in this study. This was shown by Gustavsen (2008)^[5] and Taubøll *et al.* (2009)^[15]. LEV has an important role in reducing estrogen, and this hormone plays a very important role in the integrity of the ovarian wall. The epithelial cells lining the mucosal folds are also affected by diethylstilbestrol when adult mice are exposed to it, as an increase in the number of secretory cells was observed, and the nucleus was close to the cell surface (Newbold *et al.*, 1983; Wordinger and Morril, 1985)^[12, 20]. This is also consistent with the current result, as indicated by Chit *et al.* (2013) indicated that the epithelial cells lining the mucosal folds in the oviduct are affected by varying levels of ovarian hormone concentrations, estrogen and progesterone. As for the presence of gaps between epithelial cells in the isthmus region in the current result, this was also indicated by Wordinger *et al.* (1980)^[19] through their study of the oviduct at the cellular level. The presence of a number of white blood cells (lymphocytes), macrophages, and inflammatory cells in this result may indicate an immune response by the animal treated with the drug. This was also indicated by Newbold *et al.* (1983)^[12], who noted the accumulation of lymphocytes and plasma

cells in the oviduct wall of drug-treated animals. Wordinger and Morril (1985)^[20] and King (1987) also explained that inflammation in the oviduct is represented by the presence of inflammatory cells, which are macrophages, which are multinucleated giant cells, with the occurrence of hemorrhage, degeneration, and necrosis.

Conclusion

According to the work the number of columnar was influence in addition to secretory epithelial cells lining the mucosal folds in the current result which may be related to concentration levels of hormone when effect on the results when used of the drug (LEV). The studies also agree with some research which include effect of an accumulation of neutrophil white blood cells in the lumen of the oviduct, as well as glomerular and lymphatic macrophages. A lot of damage represented by hyperplasia with degeneration and necrosis and the scattering of its cells in the middle of the cavity due to effect of the epithelial lining of the mucosal folds suffered. According to this studies the death of a large portion of its cells and their degradation and sloughing, and presence in a compact cell form (synaptic), swelling, and a lack of cilia in a large portion of them, causing reduce in the activity of LEV .

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