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Research Article

Histopathological Changes in Ovarian Tissues of Rats Underwent Ovarian Ischemia-Reperfusion Injury

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Abstract

This study investigates histopathological changes in ovarian tissues of rats subjected to ischemia-reperfusion (I/R) injury, a condition where restored blood flow causes cellular stress and potential damage. Rats were divided into three groups: control, ischemia, and ischemia-reperfusion. Histological analysis revealed that ischemic ovaries exhibited increased follicular degeneration, atretic follicles, and inflammatory infiltration. The ischemia-reperfusion group showed even more pronounced damage, including disrupted cellular structures and severe vascular congestion. These findings highlight the exacerbated tissue damage caused by reperfusion, underscoring the complex impact of I/R injury on ovarian tissue integrity.

Keywords: ovary, ischemia, reperfusion, histology

INTRODUCTION

Ovarian torsion is one of the most common gynecological emergencies and occurs when the ovary and fallopian tube partially or completely rotate around the infundibulopelvic ligament. Ovarian ischemia-reperfusion (I/R) injury is a complex process that occurs when blood flow to the tissue is suddenly interrupted and then restored. Hypoxia and metabolic stress that occur during ischemia can trigger oxidative stress and cellular damage as a result of oxygen recovery with reperfusion. This process plays a decisive role in biological events such as autogenic cellular responses, autophagy mechanisms, and hypoxia adaptation [1]. Understanding the responses that cells develop against this situation is critical for the pathophysiology of I/R injury and the determination of treatment strategies. In this context, autophagy mechanisms and hypoxia response processes play a key role in maintaining the balance during ischemia-reperfusion in ovarian tissue. The consequences of torsion can be serious; ischemic damage develops with the interruption of arterial blood flow and subsequent disruption in venous flow. This condition can lead to infertility, necrosis and life-threatening complications if left untreated [2,3]. Therefore, early diagnosis and immediate surgical intervention are critical for the preservation of the ovaries and adnexa. The reperfusion process following ischemia helps restore normal functions of the tissue to a large extent by restoring oxygen, but it can also cause unwanted side effects called reperfusion damage [4,5]. Although the treatment methods for torsion are still a matter of debate, many studies in this area recommend conservative surgical approaches; laparoscopic detorsion and, in some cases, oophorexy are generally preferred. The reperfusion process following ischemia helps restore normal functions of the tissue to a large extent by restoring oxygen, but it can also cause unwanted side effects called reperfusion damage [6].

This study aimed to investigate histopathological changes in ovaries of rats which underwent ovarian ischemia reperfusion injury by histochemical staining.

MATERIALS AND METHODS

Study design

This study was approved by the Dicle University Animal Experimentation Center (DUSAM) with ethical approval number 2023/16. A total of 24 Wistar albino female rats

were used and these animals were divided into three groups (n=8). The experimental animals were provided with unlimited access to water and food; they were kept in a 12-hour day/12-hour dark cycle and at an ambient temperature of 23±2 °C. Before the experiment, the animals were anesthetized with 90 mg/kg ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey) intramuscularly.

Sham Group: No surgical intervention was performed on the animals in this group, only their abdomens were opened in accordance with the surgical protocol and then closed

Ischemia Group: Under general anesthesia, a 2 cm lower midline incision was made in the abdomen. The ovarian adnexa, which contain the tube uterine and ovarian tissues, was exposed and rotated 360° clockwise. At this stage, the ovaries were fixed to the abdominal wall and torsion will be applied for 1 hour to create ischemia. When the ischemia period was over, the animals were sacrificed without returning the ovaries to their previous positions and the ovaries were excised.

Ischemia/Reperfusion group: Under general anesthesia, the abdomens of the animals in this group were opened with a 2 cm lower midline incision. After exposing the ovarian adnexa, they was rotated 360° clockwise and torsioned for 1 hour. Then, the ovaries were returned to their original positions and placed in their anatomical locations, and reperfusion was performed by detorsion for 2 hours.

Tissue Follow-up

After the experimental protocol was completed, the animals were sacrificed by withdrawing blood from the heart under general anesthesia. Ovarian tissue samples obtained from animals that underwent ovarian ischemia-reperfusion were transferred to be processed under laboratory conditions. The ovarian tissues removed after the experiment were prepared for routine histological tissue follow-up. The tissues were fixed for 24 hours by placing them in 10% Formalin fixative (catalog no: Z2902-3.75L, Sigma, Germany). After the fixation process, the tissues were kept under running water overnight and dehydrated by passing through increasing alcohol series (50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol). For the transparency process, the tissues were kept in xylene for 30 minutes 3 times and the alcohol were removed. Then, the tissues were taken to the oven for paraffin incubation at 58 °C and incubated 3 times for 45 minutes and embedded in paraffin blocks. 4 µm thick sections from the obtained blocks were taken on a positively charged slide with the help of a microtome (catalog no: Leica RM2265, Wetzlar, Germany) and histochemical and immunohistochemical staining procedures were performed [7].

Hematoxylin-Eosin Staining

Ovarian tissue sections obtained from paraffin blocks were placed in a bain-marie set at 37 °C. In order to melt the excess paraffin on the slide, the sections were kept in a 58-62 °C oven for 6 hours. Then, the sections were deparaffinized in xylene for 15 minutes, 3 times. Then, they were taken into distilled water after passing through decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes each and kept for 5 minutes. Hematoxylin-eosin (H&E) was applied to the sections for histopathological evaluation. After staining, the sections were quickly immersed in increasing alcohol series (80%, 90%, 96% ethyl alcohol) and then kept in absolute alcohol for 2 minutes. Finally, the sections were kept in xylene for 15 minutes three times and then the tissue sections were covered with a coverslip by dropping Entellan on them. This procedure was performed to provide histopathological analysis of ovarian tissues subjected to ischemia-reperfusion [8].

RESULTS

Control group

In the control group hematoxylin eosin findings, it was observed that ovarian follicles were generally located in the cortex and that the follicles were in different developmental stages. Primordial, primary, antral and Graafian follicles were evident. Granulosa cells located around the follicles drew attention with their tightly arranged, oval-shaped nuclei and cytoplasm. Theca cells with darker staining were observed around the follicles and were adjacent to the stroma. Connective tissue cells located in the medulla and between the follicles were relatively loosely located in the stromal regions. Oocytes with large nuclei were seen in the middle of the developing follicles. Theca lutein and granulosa lutein cells in the corpus luteum were evident (Figure 1).

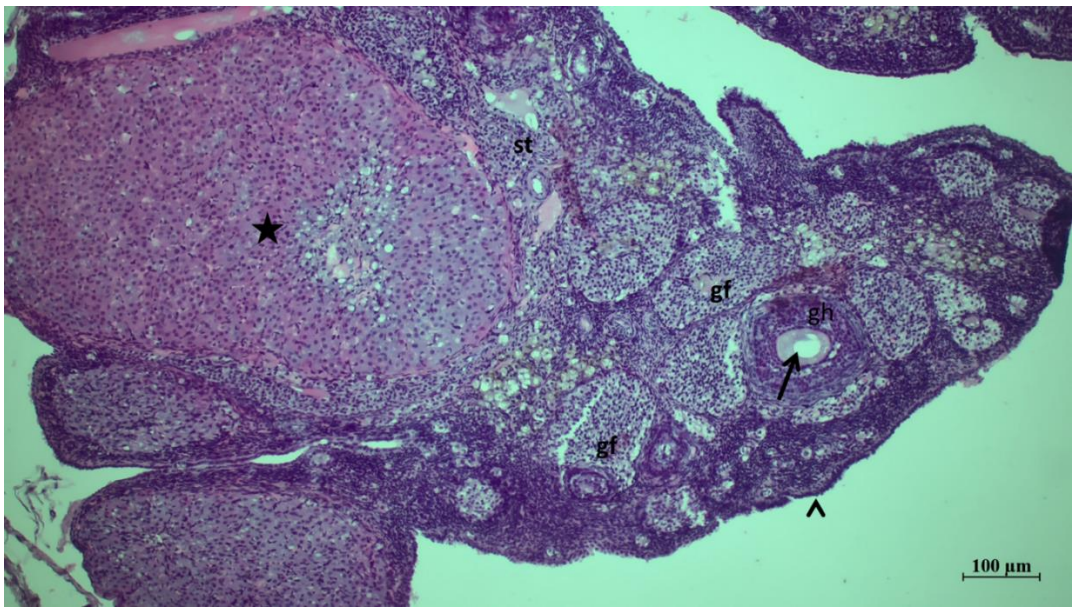

Figure 1: Ovarian section showing H-E staining of the control group. Arrow: oocyte, star: corpus luteum, gf: developing follicles, gh: granulosa cells, stroma, arrowhead: germinal epithelium

Ischemia group

In the ischemia group, follicular degeneration and the number of atretic follicles were quite high. High degeneration, pyknotic nuclear appearance, and distorted cytoplasm were observed in oocyte cells, granule cells, corpus luteum cells, and theca cells. High levels of hemorrhage and fibrotic formation were observed in follicular and stromal areas. Widespread inflammatory cell infiltration was observed in all areas. Congestion, dilatation, and damage to the vascular wall were observed in vascular structures (Figure 2).

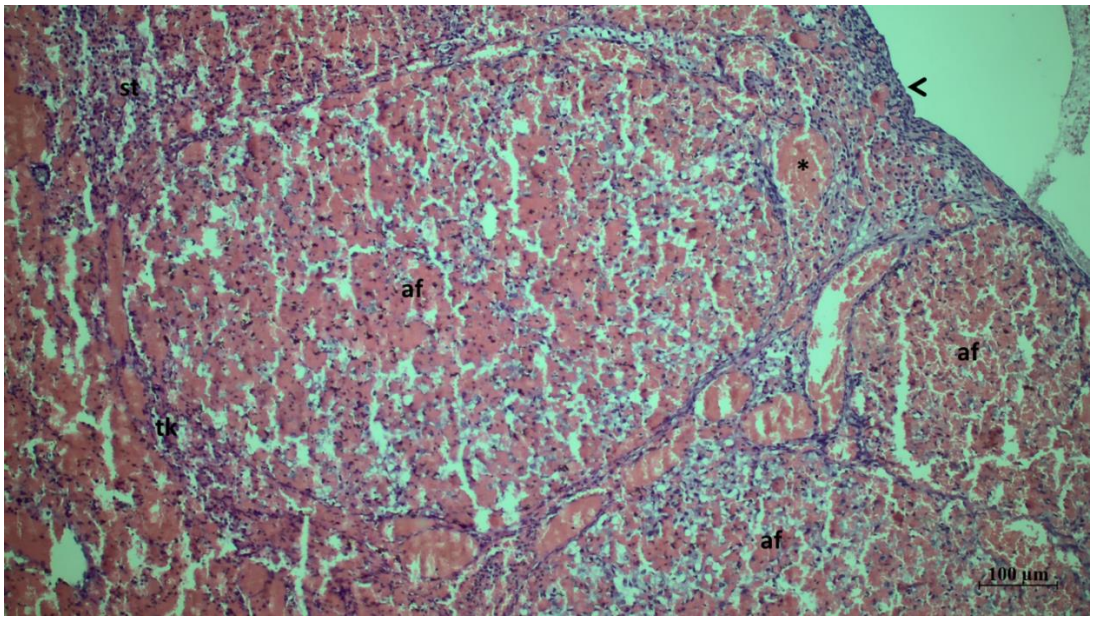

Figure 2: Ovarian section showing H-E staining of the ischemia group. af: atretic follicles, st: stroma, arrowhead: germinal epithelium, tk: theca layer, *: dilatation/congestion

Ischemia Reperfusion Group

Histopathological findings similar to the ischemia group were observed in the ischemia reperfusion group. An intense increase in the number of atretic follicles was observed. It was observed that folliculogenesis processes were impaired. It was observed that cellular structures were largely disrupted, cytoplasmic details were not clear, and nuclear density was reduced. The presence of abundant erythrocytes was striking. In the tissue, especially in damaged areas, fibrotic structures were formed, leukocyte accumulation and increased inflammatory cell presence, as well as cellular irregularities and vascular wall damage around the blood vessels were observed (Figure 3).

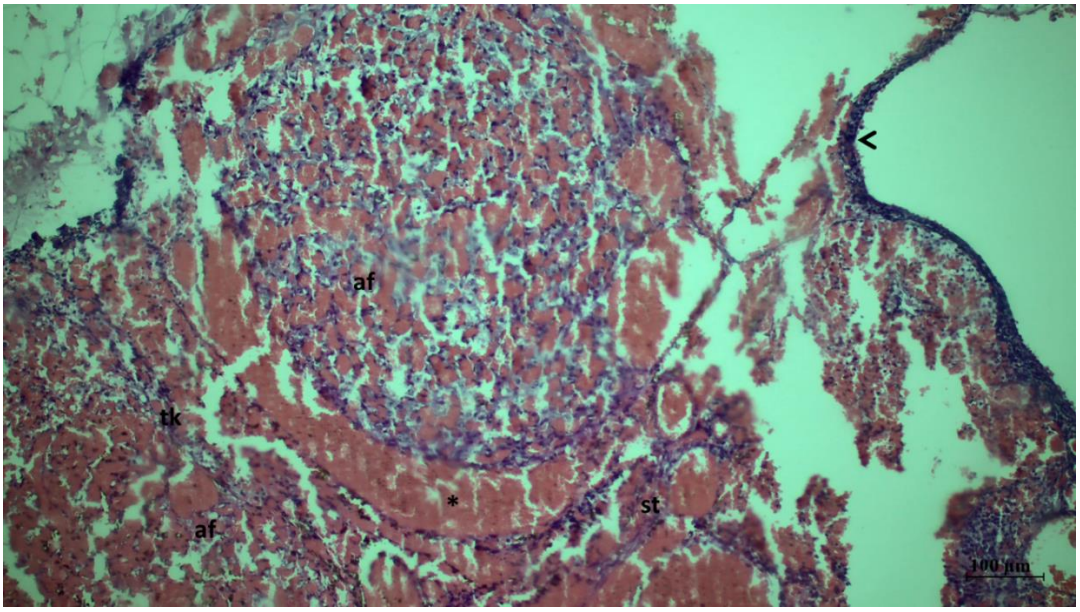

Figure 3: Ovarian section showing H-E staining in the ischemia-reperfusion group. af: atretic follicles, st: stroma, arrowhead: germinal epithelium, tk: theca layer, *: dilatation/congestion

DISCUSSION

Reversible damage to cells resulting from ischemia can be corrected by restoring blood flow. However, in some cases, restoring blood flow can increase damage rather than reduce it. This process can cause further cell loss in addition to tissues already damaged by the ischemic event [9]. When blood flow is restored, cells that have not yet restored their ionic balance are exposed to high calcium levels. Increased intracellular calcium triggers the activation of ATPases, phospholipases, proteases, and endonucleases, leading to disruption of cell integrity. Reperfusion also causes increased infiltration of inflammatory cells into tissues. These cells release oxygen derivatives that increase membrane damage and affect mitochondrial permeability [10]. Ovarian torsion is among the common gynecological emergencies that require urgent treatment. Although it can be seen in all age groups, it is especially common in women of reproductive age. Since ovarian torsion has no specific symptoms, delays in diagnosis and treatment may occur [11]. Ovarian ischemia occurs due to insufficient blood flow to the ovary as a result of torsion, and this process leads to cell death. Reperfusion of ischemic tissue is called reperfusion. However, contrary to expectations, reperfusion can cause more tissue damage than ischemia alone, and this is defined as ischemia-reperfusion injury (IRD). In conclusion, the effects of ischemia and reperfusion processes on tissue are complex and must be addressed carefully [12].

The effects of ovarian ischemia reperfusion injury have been investigated in the studies. Hortu and colleagues applied 3 hours of ischemia followed by 3 hours of reperfusion to examine ovarian ischemia reperfusion injury in their study on 30 female Sprague Dawley albino rats. In histopathological examinations, it was determined that findings such as follicular degeneration, vascular congestion, edema, hemorrhage and leukocyte infiltration observed in the ischemia-reperfusion group were significantly increased compared to the sham group [13]. Similarly, Melekoğlu and colleagues applied 3 hours of ischemia and 3 hours of reperfusion in the ovarian ischemia-reperfusion model they created in female rats. Histopathological analyses revealed that pathological conditions such as severe vascular congestion, edema, hemorrhage, and follicular degeneration were observed in the ischemia-reperfusion group [14].

In this study, histopathological changes observed in ovarian tissues in control, ischemia and ischemia-reperfusion groups and Hif1α and Beclin1 expressions were compared. In the control group, it was determined that follicles developed healthily, granulosa and theca cells had regular structures, minimal degeneration and no atretic follicles were present. These findings reflect healthy ovarian functions and the folliculogenesis process. In the ischemia group, the increase in follicular degeneration, the number of atretic follicles and inflammatory cell infiltration indicate tissue damage due to ischemia. Oocyte and granulosa cell degeneration observed in follicles indicates that the folliculogenesis process is impaired and the ovarian tissue is seriously damaged. In the ischemia-reperfusion group, similar but more severe histopathological findings were recorded in the ischemia group. The increase in the number of atretic follicles and the serious damage to vascular structures indicate that reperfusion after ischemia causes more damage to the tissue.

CONCLUSION

In our study, the negative effects of ischemia and ischemia-reperfusion on ovarian tissue were clearly observed. Increased follicular degeneration in the ischemia group indicates that the folliculogenesis process is impaired and this poses a significant threat to fertility. In the ischemia-reperfusion group, it was noted that these impairments were even more severe and the damage after reperfusion reached serious levels.

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