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Histopathology of ipsilateral and contralateral ovaries and plasma interleukin 6 levels after unilateral ovarian torsion

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Summary

Objective: The aim of the present study was to evaluate the time-dependent histopathologic changes in both ovaries and to determine the time-dependent levels of plasma interleukin 6 (IL-6) after unilateral ovarian torsion. *Materials and Methods:* An experimental animal study included 48 female Sprague-Dawley rats which were distributed to six groups: control group (Group 1), sham-operated control group (Group 2), and four unilateral ovarian torsion groups with torsion duration of three, six, 12, and 24 hours (Group 3, 4, 5, and 6, respectively). Histopathologic criteria (follicular degeneration, vascular congestion, hemorrhage, inflammatory cell infiltration, and total tissue damage score) were evaluated in both ovaries, and plasma IL-6 levels were measured. *Results:* At 24 hours after torsion began, mean total tissue damage score was similar between ovaries that had torsion and contralateral ovaries. Mean plasma IL-6 level did not change during the 24 hours after torsion began ($p = 0.584$). *Conclusions:* In addition to ovaries that had torsion, histopathologic abnormalities also occurred in contralateral ovaries. These results suggest that contralateral ovaries are not quiescent after unilateral ovarian torsion. Plasma IL-6 levels did not change significantly during the 24 hours after ovarian torsion began, resulting in a limitation of its diagnostic use in the early course of the disease.

Key words: Acute abdominal pain; Surgical emergency; Biomarkers; Cytokines.

Introduction

Ovarian torsion is a rare but important surgical emergency that comprises 2.7% of gynecologic emergencies [1]. Torsion of the ovarian vascular pedicle causes ischemia and infarction because of compromised blood supply to the ovary and obstruction of the venous and lymphatic drainage. Therefore, early accurate diagnosis with prompts treatment is crucially important to salvage ovarian tissue and preserve future fertility. However, the diagnosis of ovarian torsion mostly relies on clinical suspicion and non-specific clinical and laboratory findings, and incorrect or late diagnosis may occur. An accurate preoperative diagnosis is made in only 38% of patients, and definitive diagnosis usually is established only by intraoperative findings [2].

Histologic and ultrastructural changes may occur in the contralateral ovary after unilateral ovarian ischemia [3]. However, the time-dependent pattern of the histologic changes that develop within both ovaries after unilateral ovarian torsion has not been clearly investigated.

There is no specific laboratory biomarker for the preoperative diagnosis of ovarian torsion. A specific laboratory biomarker may improve diagnosis and minimize delayed or incorrect diagnosis. Interleukin 6 (IL-6) is a proinflammatory cytokine and an acute phase protein. Serum IL-6 levels increase during ischemic events such as myocardial and intestinal ischemia [4, 5]. In addition, IL-6 levels are increased in ovarian torsion and may be helpful in making a preoperative diagnosis [6-9]. However, the pattern and extent to which IL-6 levels increase in the early course of ovarian torsion are unknown. Such information may improve the specificity and usefulness of this serum biomarker in early preoperative diagnosis.

The purpose of this experimental study was to evaluate time-dependent histopathologic changes in both ovaries and determine the time-dependent levels of IL-6 after unilateral ovarian torsion.

Materials and Methods

This study was performed with 48 female Sprague-Dawley rats (weight: 200 to 220 grams). Before the experiment, the rats were maintained in standard laboratory conditions. Animals were randomly distributed to six groups (eight rats each): control group with-

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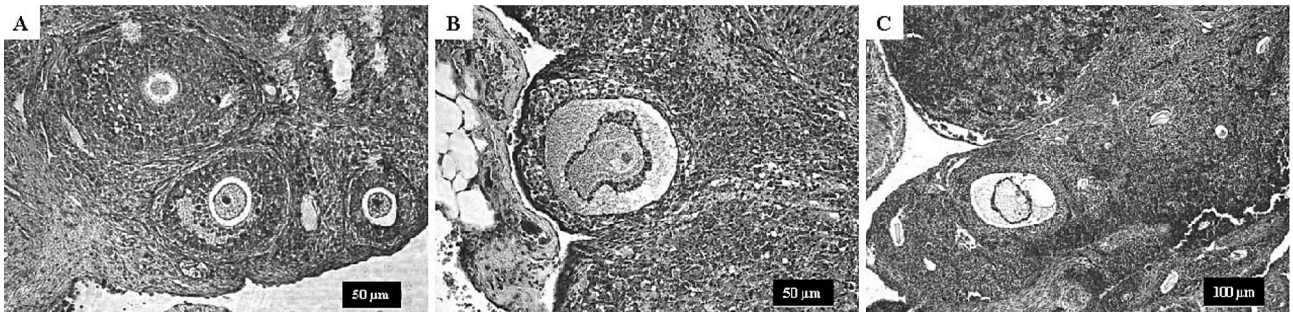

Figure 1. — Light micrographs of rat ovaries. A) In sham-operated control rats, normal follicular structure with different stages of developing follicles and no pathologic changes were noted (Masson trichrome, original magnification ×20). B) At 12 hours after ovarian torsion, the ovary with torsion had severe follicular degeneration, severe vascular congestion, and severe hemorrhage (Masson trichrome, original magnification ×5). C) At 24 hours after ovarian torsion, the contralateral ovary had severe follicular degeneration, moderate vascular congestion, severe hemorrhage, and moderate inflammatory cell infiltration (Masson trichrome, original magnification ×10).

out laparotomy (Group 1), sham-operated control group (Group 2), and four unilateral ovarian torsion groups with torsion duration of three, six, 12, and 24 hours (Group 3, 4, 5, and 6, respectively). The researchers who performed histologic and biochemical studies were blinded to the randomized group identification until the end of the study. The study was approved by the ethical committee of the Hacettepe University, Medical Faculty and the ethical guidelines for care and use of the animals in experimental studies were followed.

Laparotomy was performed in all except in the Group 1 rats (only blood was obtained for IL-6 measurements) and unilateral ovarian torsion was created in all except in the Group 1 and 2 rats.

Each rat was anesthetized with ketamine hydrochloride (50 mg/kg, intraperitoneal) and xylazine hydrochloride (ten mg/kg, intraperitoneal). Rats were placed in a dorsal recumbent position and covered with sterile drapes before surgery. The skin area for the incision was shaved and disinfected. A longitudinal lower abdominal incision (length: two cm) was made and both uterine horns and ovaries were identified. In the sham-operated control group, the abdomen was opened and both ovaries were surgically removed without torsion. In the remaining four study groups, unilateral left ovarian torsion was created by using vascular clips just above and below the left ovary as previously described [10]. The abdomen was surgically closed in two layers with silk 3-0 sutures.

At three, six, 12, or 24 hours after ovarian torsion in the four study groups, repeated laparotomy was performed through the previous incision and both ovaries (left ovary with torsion; contralateral right ovary without torsion) were removed for light and electron microscopic evaluation. Immediately after the ovaries were excised, blood (two to three ml) was obtained by aspiration from the heart for determination of plasma IL-6 levels, and the rat was sacrificed.

Ovaries were rapidly fixed in 10% phosphate-buffered formalin, dehydrated in graded alcohols, and processed for light microscopy. All specimens were embedded in paraffin blocks. Sections (five µm thickness) were cut and stained with hematoxylin-eosin and Masson trichrome stains. Sections were examined with a light microscope and photographed.

A single histologist assessed the histologic changes in a blinded manner. The four criteria for ovarian histopathologic injury were follicular degeneration, vascular congestion, hemorrhage, and inflammatory cell infiltration. Each specimen was scored semiquantitatively for each criterion with a scale ranging from 0 to 3 (0: none, 1: mild, 2: moderate, and 3: severe) as previously described [11]. The total tissue damage score was defined as the sum of the scores of the four histologic parameters (total range, 0 [no damage] to 12 [most severe damage]).

For electron microscopy, tissue samples were immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for two hours at room temperature. After washing three times for ten minutes in 0.1 M phosphate buffer (pH 7.4), samples were postfixed with 1% osmium tetroxide. After dehydrating in an ethanol gradient at room temperature, the tissue samples were infiltrated and embedded in epoxy resin. For light microscopy, semithin (one µm thickness) sections were stained with toluidine blue-azure II stain before examination. An ultramicrotome was used to cut two blocks per ovary. The ultrathin (70 nm thickness) sections were double-stained with uranyl acetate and lead citrate before viewing with an electron microscope operating at 80 kV.

All blood samples were studied simultaneously in the same assay, and the laboratory staff members were blinded to the identity of the samples (control, sham-operated or study groups). For plasma IL-6 measurements blood samples collected in tubes with ethylenediaminetetraacetic acid were centrifuged for 15 minutes at 2,000 g at room temperature.

The plasma was immediately stored in pyrogen-free tubes at -80°C until use. The IL-6 plasma levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to instructions from the manufacturer. The wells of microtiter strips were coated with antibodies specific to rat IL-6. The samples and known rat standards were added to the wells, incubated, and washed. The intensity of absorbance was determined at 450 nm with an ELISA reader. The IL-6 levels were expressed in pg/ml.

Data analysis was performed with statistical software (SPSS version 20. Data were reported as mean ± SD. The data were assessed with visual (histogram and probability plot) and analytical methods (Shapiro-Wilk test) to evaluate normal distribution. The nonparametric Kruskal-Wallis test was used to compare scores for follicular degeneration, vascular congestion, hemorrhage, and inflammatory cell infiltration at the different times because these variables were not normally distributed. After performing the Kruskal-Wallis test, the Mann-Whitney test was performed to test the significance of the pairwise differences, using a Bonferroni adjustment for multiple comparisons. Plasma IL-6 levels were normally distributed between the study groups, and one-way analysis of variance was used to compare IL-6 levels between the groups. The Mann-Whitney test was applied to compare the total tissue damage score between the ipsilateral and contralateral ovaries at different times. Statistical significance was defined by $p \leq 0.05$.

Table 1. — *Comparison of semiquantitative scores[a] of four histopathologic parameters and total tissue damage scores between the ovary that had torsion and the contralateral ovary in sham-operated control rats and rats that had unilateral ovarian torsion.*

	Contralateral ovary		Ovary with torsion		*p* value[b]
	Mean ± SD	Median (quartiles)	Mean ± SD	Median (quartiles)	
Sham					
Follicular degeneration	0.1 ± 0.4	0.0 (0.0-0.0)	0.0 ± 0.0	0.0 (0.0-0.0)	0.721
Vascular congestion	0.0 ± 0.0	0.0 (0.0-0.0)	0.0 ± 0.0	0.0 (0.0-0.0)	1.000
Hemorrhage	0.1 ± 0.4	0.0 (0.0-0.0)	0.1 ± 0.4	0.0 (0.0-0.0)	1.000
Infiltration [c]	0.4 ± 0.5	0.0 (0.0-1.0)	0.6 ± 0.7	0.5 (0.0-1.0)	0.574
Total tissue damage score	0.6 ± 0.7	0.5 (0.0-1.0)	0.8 ± 1.0	0.5 (0.0-1.0)	0.959
3-hour					
Follicular degeneration	0.6 ± 0.5	1.0 (0.0-1.0)	1.0 ± 0.5	1.0 (1.0-1.0)	0.279
Vascular congestion	0.0 ± 0.0	0.0 (0.0-0.0)	1.0 ± 0.5	1.0 (1.0-1.0)	0.002
Hemorrhage	0.0 ± 0.0	0.0 (0.0-0.0)	1.3 ± 0.5	1.0 (1.0-1.8)	< 0.001
Infiltration	0.3 ± 0.5	0.0 (0.0-0.8)	0.8 ± 0.5	1.0 (0.3-1.0)	0.105
Total tissue damage score	0.9 ± 0.8	1.0 (0.0-1.8)	4.0 ± 0.9	4.0 (4.0-4.8)	< 0.001
6-hour					
Follicular degeneration	0.9 ± 0.6	1.0 (0.3-1.0)	1.5 ± 0.9	1.5 (1.0-2.0)	0.195
Vascular congestion	0.4 ± 0.5	0.0 (0.0-1.0)	1.8 ± 1.0	2.0 (1.0-2.8)	0.001
Hemorrhage	0.6 ± 1.1	0.0 (0.0-1.0)	2.0 ± 1.3	2.0 (1.0-3.0)	0.038
Infiltration	0.5 ± 0.5	0.5 (0.0-1.0)	1.1 ± 0.6	1.0 (1.0-1.8)	0.105
Total tissue damage score	2.4 ± 1.8	2.0 (1.3-3.0)	6.4 ± 3.0	7.0 (5.3-8.0)	0.015
12-hour					
Follicular degeneration	1.4 ± 0.7	1.0 (1.0-1.8)	2.4 ± 0.5	2.0 (2.0-3.0)	0.015
Vascular congestion	0.9 ± 0.4	1.0 (1.0-1.0)	2.0 ± 0.8	2.0 (1.3-2.8)	0.007
Hemorrhage	0.6 ± 0.7	0.5 (0.0-1.0)	1.5 ± 0.9	1.0 (1.0-2.5)	0.083
Infiltration	1.3 ± 0.7	1.0 (1.0-2.0)	1.6 ± 0.7	1.5 (1.0-2.0)	0.442
Total tissue damage score	4.1 ± 2.0	4.0 (3.3-4.8)	7.5 ± 2.2	6.5 (6.0-9.8)	0.030
24-hour					
Follicular degeneration	2.0 ± 0.5	2.0 (2.0-2.0)	2.3 ± 0.7	2.0 (2.0-3.0)	0.505
Vascular congestion	1.0 ± 0.9	1.0 (0.3-1.0)	1.3 ± 1.0	1.0 (0.3-2.0)	0.645
Hemorrhage	0.5 ± 0.5	0.5 (0.0-1.0)	0.9 ± 1.0	0.5 (0.0-2.0)	0.574
Infiltration	1.5 ± 0.5	1.5 (1.0-2.0)	1.6 ± 0.5	2.0 (1.0-2.0)	0.721
Total tissue damage score	5.0 ± 1.5	5.0 (4.3-6.0)	6.0 ± 2.1	5.5 (4.3-8.0)	0.442

Note: SD = standard deviation.

In contralateral ovaries, three parameters and total tissue damage scores changed significantly with time: follicular degeneration ($p \leq 0.001$), vascular congestion ($p \leq 0.001$), infiltration ($p \leq 0.001$), and total tissue damage score ($p \leq 0.001$); there was no significant change in hemorrhage with time ($p = 0.110$). In ovaries that had torsion, all four histopathologic parameters and total tissue damage scores changed significantly with time: follicular degeneration ($p \leq 0.001$), vascular congestion ($p \leq 0.001$), hemorrhage ($p \leq 0.003$), infiltration ($p \leq 0.001$), and total tissue damage score ($p \leq 0.001$).

[a] The scoring was done semiquantitatively for each criterion with a scale ranging from 0 to 3 (0: none; 1: mild, 2: moderate, and 3: severe);

[b] *p* value according to Mann-Whitney U test; [c] Infiltration of the tissue by inflammatory cells.

Results

On gross inspection, most ovaries that had torsion had a cherry-red to black color and appeared hemorrhagic and edematous. Histologic examination of the sham-operated ovaries showed normal ovarian structure (Figure 1A). In all ovaries from rats that had ovarian torsion, there were various degrees of follicular degeneration, vascular congestion, hemorrhage, and inflammatory cell infiltration both in torsioned and contralateral side (Figures 1B and C). In all ovaries that had torsion, there were free-floating follicular cells within the antral space. In most rats that had ovarian torsion, the torsioned ovaries showed a decreased number of follicular cell layers and enlarged intercellular spaces between follicular cells.

In the ovaries that had torsion, the scores of all four histopathologic parameters increased significantly with time after torsion. The contralateral ovaries had a significant increase in three histologic parameters (follicular degeneration, vascular congestion, and infiltration), but there was no significant change in hemorrhage. The torsioned ovaries had mean total tissue damage scores higher from contralateral ovaries in three, six, and 12 hours; however, the mean total tissue damage scores were similar at 24 hours between torsioned and contralateral ovaries (Table 1).

Electron microscopic examination showed normal ovarian ultrastructure in the sham-operated group (Figure 2A). At 12 hours after torsion began, the ovaries that had torsion had separated granulosa cells with enlarged granulated en-

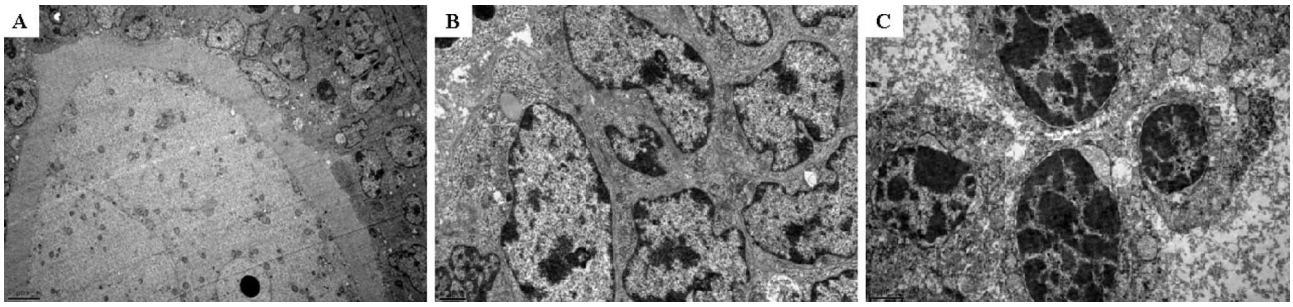

Figure 2. — Electron micrographs of rat ovaries. A) In sham-operated control rats, normal oocytes, zona pellucida, and mitochondria were noted (uranyl acetate-lead citrate, original magnification ×3,000). B) In sham-operated control rats, normal granulosa cells were noted with mitochondria, abundant Golgi apparatus, and uniform distribution of chromatin structure (uranyl acetate-lead citrate, original magnification ×12,000). C) At 12 hours after ovarian torsion, the ovary with torsion had separated granulosa cells with enlarged granulated endoplasmic reticula, dilated perinuclear cisternae, accumulation of electron lucent material in perinuclear cisternae of granulosa cells, and lipid droplets (uranyl acetate-lead citrate, original magnification ×15,000).

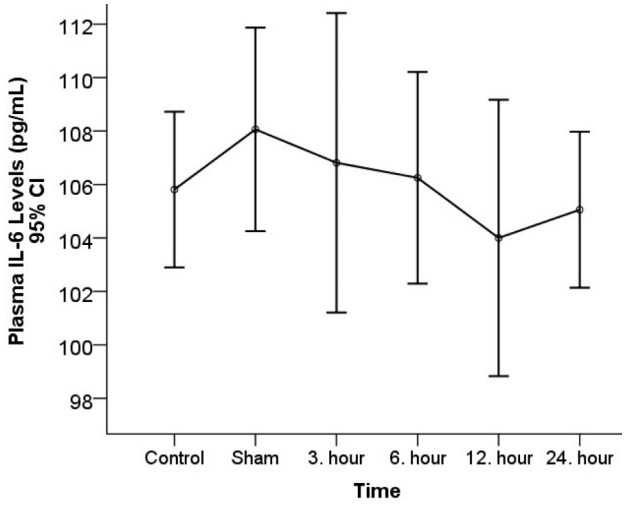

Figure 3. — Time-dependent changes in mean interleukin 6 (IL-6) levels within 24 hours after experimental unilateral ovarian torsion in rats. Abbreviation: CI: confidence interval. There was no significant change in mean plasma IL-6 level within 24 hours after unilateral ovarian torsion ($p = 0.584$).

doplasmic reticulum, distorted perinuclear cisternae (accumulation of electron lucent material), and lipid droplets (Figures 2B and C).

Mean plasma IL-6 levels did not change significantly from 0 to 24 hours after torsion began (Figure 3).

Discussion

The present results showed that histopathologic changes of similar severity occurred by 24 hours after torsion, both in torsioned and in contralateral ovaries. There were distinctive ultrastructural changes noted in the torsioned ovaries after 12 hours. In addition, plasma IL-6 levels did not change significantly during 24 hours after ovarian torsion.

Ischemia and histopathologic changes were expected to be more severe in torsioned ovaries than contralateral ovaries. However, the mean total tissue damage score was higher in torsioned ovaries than contralateral ovaries only from three to 12 hours, and after 24 hours the score was similar between torsioned and contralateral ovaries (Table 1). A previous study that evaluated ischemia-modified albumin as a marker of ovarian torsion also showed significantly higher total tissue damage scores after three hours from torsion [12]. Another study that assessed the protective effect of the drug iloprost in an ovarian torsion model showed that the ipsilateral ovaries that had torsion had significantly higher total tissue damage scores than the rats without torsion after four hours [13]. However, the study that evaluated iloprost showed no significant change in the mean total tissue damage score in the contralateral ovaries between the animals that had torsion and control animals after four hours [13]. In addition, contrary to the present findings, another study of unilateral ovarian torsion showed that the histology of the contralateral ovaries was unaffected, with normal ovarian cortices, from four to 36 hours after torsion; histologic sections of the ovaries that had torsion had negligible changes, with an intact ovarian structure similar to controls in the four to 24 hour groups [14]. In contrast, the present study showed that all histopathologic parameters except hemorrhage in contralateral ovaries changed significantly within 24 hours after torsion for both torsioned and contralateral ovaries (Table 1). The discrepancy between studies may have occurred because of different examiners and histologic parameters in different studies.

There are some studies that concluded that histologic, ultrastructural, and functional changes may develop in the contralateral, uninvolved ovary after unilateral ovarian

torsion [14, 15]. The present study showed that histopathologic changes are similar between the torsioned and contralateral ovaries after 24 hours. The mechanisms for these changes in the contralateral ovary are unknown. After unilateral ovarian torsion, histologic and ultrastructural changes and decreased hormone production in the contralateral ovary may occur because of neuroendocrine and neuroregulatory mechanisms [14, 15]. Tissue ischemia in the torsioned ovary may stimulate the sympathetic nervous system, and this may decrease regional blood flow and cause hypoxia in the contralateral ovary [15, 16]. There may be an indirect or direct connection between the sympathetic nervous systems of the ovaries. After unilateral testicular torsion, a similar reduction in blood flow to the contralateral testis occurs, with a mechanism that may be similar to the mechanism in ovarian torsion [17]. The results of the present study suggest that the hypoxia caused by sympathetic activation may cause histopathologic changes in the contralateral ovary. However, further experimental and clinical studies are necessary to clarify the mechanisms that cause histopathologic and functional changes in the contralateral ovary.

The proinflammatory cytokine IL-6 may be another serum marker of ovarian torsion. However, the present study showed no significant change in plasma IL-6 levels during the first 24 hours after torsion (Figure 3). Previous clinical studies had suggested that plasma IL-6 levels may have diagnostic value in ovarian torsion and may facilitate prompt diagnosis and treatment [7-9]. When clinical signs of ovarian torsion are inconclusive, serum IL-6 level might help to distinguish the patients that should undergo diagnostic laparoscopy [9]. A meta-analysis of the previous studies showed increased serum IL-6 levels in patients who had ovarian torsion, and serum IL-6 level may have good sensitivity (86%) and positive likelihood ratio for the diagnosis of ovarian torsion in patients who have abdominal pain and ultrasonographic evidence of an ovarian cyst [6, 8, 9]. The present study was limited to 24 hours after ovarian torsion; it is possible that IL-6 levels may increase after 24 hours torsion.

The absence of a change in plasma IL-6 levels observed in this study (Figure 3) was different than previous findings in rats with ovarian torsion. In a previous experimental study, the study design was similar to the current study and serum IL-6 levels were measured at 0 and 3 hours after ovarian torsion. A significant increase in serum IL-6 level was observed at three hours after torsion [18]. Another study also reported significantly elevated serum IL-6 levels at three hours after ovarian torsion [19]. It is unknown why the present and previous studies had different results because the study designs and laboratory methods were similar for the first three hours after torsion. Further studies may clarify the time-dependent changes and usefulness of IL-6 plasma levels in the early diagnosis of ovarian torsion.

Limitations of this study include the animal model that may not necessarily simulate ovarian torsion in clinical practice. Serial blood measurements from the same animal were not performed after ovarian torsion and may be useful in evaluating changes in IL-6 levels. In addition, the study was limited to 24 hours after ovarian torsion, and measurements after 24 hours were not performed. Nevertheless, the study extends the available information about ovarian torsion because it included time-dependent data about plasma IL-6 levels, which may be useful in the early and correct diagnosis of the disease.

In conclusion, the present study showed that histopathologic changes were similar in torsioned and contralateral ovaries at 24 hours. Therefore, contralateral ovaries are not quiescent after unilateral ovarian torsion. In addition, plasma IL-6 levels did not change significantly during the 24 hours after ovarian torsion, and it is unknown whether IL-6 levels may increase after 24 hours. More studies are needed to reveal the time-dependent changes in IL-6 levels after 24 hours.

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