Effect of detorsion alone and in combination with enoxaparin therapy on ovarian reserve and serum antimüllerian hormone levels in a rat ovarian torsion model

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Objective: To investigate the effect of enoxaparin on ovarian reserve and serum antimüllerian hormone (AMH) levels in a rat ovarian torsion model.
Design: Experimental study.
Setting: Experimental surgery laboratory in a training and research hospital.
Animal(s): Fourteen female Wistar Hannover rats.
Intervention(s): 1) Control group received no special treatment other than abdominal exposure; 2) detorsion-only group received bilateral adnexal torsion (3-hour ischemia), and then after 3-hour torsion period, detorsion (reperfusion) was performed; and 3) detorsion-enoxaparin group received 0.5 mg/kg enoxaparin subcutaneously 2 hours before the same surgery as the detorsion-only group and a second 0.5 mg/kg dose of enoxaparin 24 hours after the first surgeries. Apart from the surgeries, preoperative and postoperative 1-mL blood samples were drawn from the right jugular vein of each rat.
Main Outcome Measure(s): Preoperative and postoperative serum AMH levels, histopathologic damage scores, and follicle counts in the ovarian tissue of the rats.
Result(s): Vascular congestion and hemorrhage scores were higher in the detorsion-enoxaparin group than in the detorsion-only and control groups. The number of small antral follicles was smaller in the detorsion-only group than in the control group. The difference in the pre- and postoperative AMH levels was higher in the detorsion-only group than in the control and detorsion-enoxaparin groups.
Conclusion(s): The combination of enoxaparin therapy with conventional ovarian detorsion is more effective in protecting the ovarian reserve than detorsion alone. (Fertil Steril® 2014;102: 878–84. ©2014 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, enoxaparin, ischemia, ovarian follicle, ovary

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Adnexal torsion is defined as the twisting of the ovary and the tube around its vascular pedicle. It is relatively frequent in women of reproductive age, accounting for 2.7% of all gynecologic emergencies (1, 2). Because of the risk of thromboembolism and the notion that the twisted ovary is nonviable, the traditional treatment approach for this condition in the past was salpingo-oophorectomy (3). Recently, the most successful strategy for preserving the ovarian tissue has been detorsion by early surgical intervention after a rapid diagnosis, but there are still some concerns regarding local and systemic effects associated with the detorsed tissue (3).

Ischemia and reperfusion (I/R) injury is thought to be the major cause of ovarian tissue damage associated

Received May 5, 2014; revised and accepted June 4, 2014; published online July 1, 2014.
C.K. has nothing to disclose. H.T. has nothing to disclose. H.C. has nothing to disclose. A.T. has nothing to disclose. M.E. has nothing to disclose. L.Y. has nothing to disclose.
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Fertility and Sterility® Vol. 102, No. 3, September 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.06.007

with torsion and detorsion. In earlier studies, ovarian tissue damage was reported to have increased even further after detorsion (4, 5). It is also known that decreased blood flow causes tissue hypoxia, leading to ischemia and reperfusion. On detorsion of the ovary, the level of reactive oxygen species (ROS) increases in the damaged tissue region. This excessive release of ROS and their toxic products causes lipid peroxidation in the cellular and mitochondrial membranes, nuclear damage, and ultimately cell death (6).

I/R injury leads to a decline in ovarian hormonal functions and diminishes the ovarian reserve. Several studies have evaluated the protective effect of antiinflammatory and antioxidant agents (7–9). In a study by Ozler et al. (7), treatment by detorsion only was found to be insufficient to prevent the decline in the ovarian reserve, and the authors speculated that an effective antioxidant and antiinflammatory treatment may be efficient in complementing the conservative surgery.

Based on this hypothesis, in the present study we used enoxaparin, a low-molecular-weight heparin (LMWH) known to act as an anticoagulant and antioxidant agent, in addition to the standard detorsion treatment for preserving the ovarian reserve.

MATERIALS AND METHODS

The study was approved by the local Ethical Review Board of the Bagcilar Training and Research Hospital in Istanbul, Turkey, for the use of laboratory animals, and was performed at the experimental surgery training and education center of the same hospital (approval number: 2013-33).

Animal Maintenance and Treatment

Rats weighing ~180–260 g were housed in compliance with the Institutional Review Board's guidelines for animal care using a 14-hour light cycle with free access to food and water. A power analysis performed to calculate the minimum sample size required for the study (alpha error = 0.05; 1 − beta = 0.8) suggested that ≥ 12 ovaries were required for each study group. Because 10% of the animals are lost during the procedures, we included 14 ovaries per surgery group. To comply with the goal of reducing the number of animals in experimental studies, we used the pre- and postoperative blood samples as well as 12 ovarian histopathologic specimens from six similar rats that had been used as a control group in our previous study (approval number: 2013-18, same Ethical Review Board). This control group had received no special treatment other than abdominal exposure. Apart from the control samples, the rats included in this experimental study were randomly assigned to two groups, each consisting of seven rats, with the use of computer-based randomization. Fourteen mature nonpregnant female Wistar Hannover rats (ages 8–10 weeks) were used as models of experimental ovarian torsion as described by Ergun et al. (10). The stage of the estrous cycle of the rats was determined by performing daily vaginal smears after acclimation. Rats determined to have at least three consecutive 4-day estrous cycles were prepared for the surgical procedure. The animals were anesthetized by administering 50 mg/kg 10% ketamine hydrochloride (Ketasol; Richter Pharma) and 5 mg/kg 2% xylazine (Rompun; Bayer Health Care) intramuscularly. One rat in each group died after anesthesia induction. A 1-mL preoperative blood sample was drawn from the right jugular vein of each rat for the measurement of serum antimüllerian hormone (AMH) levels. Before the operation, the abdominal skin was shaved and disinfected with the use of 10% povidone-iodine solution (Batticon; Adeka Laboratories). A 3-cm midline incision was made, and the ovaries and uterine horns were exposed (Fig. 1A). For the detorsion-only group, bilateral adnexal torsion (3-hour ischemia) was performed. This procedure was carried out as follows: the adnexa, including the tubo-ovarian vessels, were rotated 360° clockwise, and then fixed to the abdominal sidewall (Fig. 1B). After a 3-hour torsion period, detorsion (reperfusion) was performed. The detorsion-enoxaparin group received 0.5 mg/kg enoxaparin subcutaneously 2 hours before the same surgery. In all animals, abdominal incisions were closed by two layers of 4-0 polyglycolic acid suture (Vicryl; Johnson and Johnson Medical, Ethicon) for the peritoneum and 3-0 polyglactin suture for the skin. After the animals recovered from the surgery, they were housed separately at a controlled temperature of 22 ± 2°C and a 14-hour light cycle, with food and water ad libitum. The surgery time was limited to ~15 minutes for each rat to prevent tissue drying at room temperature. All surgical procedures were performed by the same researchers. Twenty-four hours after the first surgeries, the detorsion-enoxaparin group received a second 0.5 mg/kg dose of enoxaparin subcutaneously.

Tissue Sample Collection and Histopathology

After the 14-day recovery period, postoperative 1-mL serum samples were drawn from the right jugular vein of each rat for the measurement of serum AMH levels. Both ovaries were surgically removed at the end of the second surgical procedure. The rats were killed by breaking their necks. All tissue samples were evaluated by a single histologist who did not know their origin. For histologic analyses, the excised tissues were fixed in 10% buffered formalin solution for 24 hours. After fixation, a routine tissue-processing procedure was performed, and the samples were embedded in paraffin. Paraffin wax blocks were cut into 4-μm-thick sections with the use of a microtome (Leica RM2125RTS) and stained with hematoxylin and eosin. The histologic method for the follicle count was created based on the studies by Alexandra et al. (7). At least five microscopic areas were examined for each specimen with a light microscope (Nikon Eclipse 80i). The follicles were divided into four groups based on the mean diameter: primordial (diameter <20 μm), preantral (20–220 μm), small antral (221–310 μm), and large antral (311–370 μm; Fig. 2A–2C). Atretic follicles were defined according to the description of Osman et al. (11). The ovarian damage score was determined based on the extent of follicular cell degeneration (granulosa cells), vascular congestion, hemorrhage, and inflammation (Fig. 2D–2F). Each specimen was scored for each criterion with the use of a scale of 0–3 (0: none; 1: mild; 2: moderate; and 3: severe) (12).

Measurements of the Serum Levels of AMH

All collected blood samples were immediately centrifuged at 4,000 rpm for 10 minutes, and the collected sera were