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Assessing the potential of nifedipine and resveratrol to enhance ovarian viability in conjunction with detorsion treatment: A rat ovarian torsion model study

Detorsiyon tedavisine ek olarak nifedipin ve resveratrolün serum anti-müllerian hormon seviyesi ve over histopatolojisi üzerindeki etkileri: Rat over torsiyon modeli çalışması

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Abstract

Objective: Evaluating the therapeutic effect of detorsion, resveratrol, and nifedipine on ovarian viability assessed by biochemical, histopathological and immunohistochemical parameters and markers of oxidative stress.

Materials and Methods: Twenty-four Sprague-Dawley rats were included in 4 groups, namely: sham operation, ischemia-reperfusion (I/R), I/R+10 mg/kg nifedipine (NIF), I/R+100 mg/kg resveratrol (RSV). In the study groups, bilateral 720º ovarian torsion was performed and continued for 3 hours, followed by detorsion for another 3 hours. Thirty minutes before the detorsion, NIF and RSV groups received respective treatments. Adnexectomy was performed, and evaluations were made for the expression of anti-müllerian hormone (AMH), vascular endothelial growth factor receptor 2 (VEGFR-2), markers of oxidative stress, and follicle counts. Blood AMH levels were measured.

Results: No change in AMH levels was detected. Although the expression of AMH was significantly reduced following I/R alone, it remained similar to the control group in the NIF group. Meanwhile, the RSV group exhibited slightly lower expression than the control, although it was still higher than that observed with the I/R injury group. VEGFR-2 staining was similar in the I/R and NIF groups, but reduced in the RSV group. Markers of oxidative stress were similar between groups. Primordial follicle count was lower in the untreated I/R injury group compared to the control group ($p<0.05$). The NIF group had more secondary follicles than the I/R injury and RSV groups ($p<0.05$).

Conclusion: Nifedipine and resveratrol treatments did not influence AMH levels and antioxidant-oxidant system parameters in rats exposed to I/R injury. However, nifedipine had a positive effect on AMH expression and secondary follicle count, and resveratrol decreased the expression of VEGFR-2 in tissues, which can be an indicator of their clinical potential.

Keywords: Ovary, follicle count, anti-müllerian hormone, VEGFR-2, immunohistochemical analysis, oxidative stress

PRECIS: We evaluated the protective effect of nifedipine and resveratrol in an experimental rat adnexal I/R injury model by creating subsequent adnexal torsion and detorsion.

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Öz

Amaç: Bu çalışmanın amacı, detorsiyon, resveratrol ve nifedipin tedavisinin anti-müllerian hormon (AMH) seviyeleri ve over histopatolojisi üzerindeki etkisinin değerlendirilmesidir.

Gereç ve Yöntemler: Yirmi dört Sprague-Dawley rat; kontrol, iskemi-reperfüzyon (I/R), I/R+10 mg/kg nifedipin (NIF), I/R+100 mg/kg resveratrol (RSV) gruplarına ayrıldı. Çalışma gruplarında, bilateral 720º over torsiyonu yapıldı ve 3 saat boyunca tutuldu, ardından 3 saat boyunca detorsiyon uygulandı. Detorsiyondan otuz dakika önce, NIF ve RSV gruplarına ilgili tedaviler uygulandı. Adneksiyal ekizyon yapıldı ve AMH ve vasküler endotelyal büyüme faktörü reseptörü 2 (VEGFR-2) immünohistokimyasal ekspresyonu, oksidatif stres belirteçleri ve folikül sayıları değerlendirildi. Kan AMH seviyeleri ölçüldü.

Bulgular: AMH seviyelerinde değişiklik tespit edilmedi. AMH ekspresyonu I/R grubunda azalmış olmasına rağmen, NIF grubunda kontrol ile benzerdi. RSV grubunda ekspresyon, I/R grubuna göre daha yüksek, ancak kontrol grubuna göre daha düşüktü. VEGFR-2 boyaması, I/R ve NIF gruplarında kontrol grubuna benzerdi, ancak RSV grubunda azalmıştı. Gruplar arasında oksidatif stres belirteçlerinde istatistiksel olarak anlamlı bir fark tespit edilmedi. Kontrol grubundaki primordial folikül sayısı, I/R grubundan daha yüksekti ($p<0,05$). NIF grubundaki sekonder folikül sayısı, I/R ve RSV gruplarından daha yüksekti ($p<0,05$).

Sonuç: Nifedipin ve resveratrol tedavileri, ratlarda I/R hasarında AMH seviyeleri ve antioksidan-oksidan sistem parametrelerini etkilemedi. Ancak, nifedipin AMH ekspresyonu ve sekonder folikül sayısı üzerinde olumlu bir etkiye sahipti ve resveratrol, dokularda VEGFR-2 ekspresyonunu azalttı, bu da bu ajanların klinik potansiyellerinin bir göstergesi olabilir.

Anahtar Kelimeler: Yumurtalık, folikül sayısı, anti-müllerian hormon, VEGFR-2, immünohistokimyasal analiz, oksidatif stres

Introduction

Adnexal torsion is a serious gynecological emergency defined by the ovary rotating partially or fully around its pedicle or vascular axis. Torsion creates lymphatic and arterial blockage that may result in gangrene and necrosis in adnexa[1]. Ovarian cysts represent the predominant cause of ovarian torsion[2]. A sudden occurrence of abdominal pain, often with nausea or vomiting, frequently indicates ovarian torsion[3]. In case of a suspected ovarian torsion, the initial imaging technique used is ultrasound. Impaired or absent blood flow in an enlarged and edematous ovary on ultrasound is findings that assist in the diagnosis of ovarian torsion[4]. Historically, adnexectomy was considered to be the only option. Nevertheless, results from contemporary studies suggest that detorsion of the adnexa can be a more conservative treatment option with similar success[1]. However, concerns have been raised about increased production of reactive oxygen radicals due to reperfusion after detorsion, which can inflict further damage to the adnexa[5]. Various agents have been suggested to reduce this ischemia-reperfusion (I/R) injury, including nifedipine and resveratrol[6-8].

Resveratrol, naturally a phytoalexin, is typically found in plants such as grapes and berries, and is thought to have an antioxidant effect due to the hydroxyl group it contains[9]. Meanwhile, nifedipine is a calcium channel blocker, and its effect on reducing oxygen radicals formed after I/R injury has been tested in several studies on the heart and testis[7,10-12]. Few studies have examined the antioxidant effect of resveratrol in protecting the adnexa from I/R injury following a detorsion, and none have investigated nifedipine, to the best of our knowledge[8].

Our objective was to examine the effect of nifedipine and resveratrol on ovarian viability in a rat model of adnexal I/R injury, induced through consecutive adnexal torsion and detorsion procedures. Ovarian viability was evaluated with biochemical, histopathological and immunohistochemical parameters and markers of oxidative stress.

Materials and Methods

The protocol was reviewed and approved by the Gazi University Animal Experiments Local Ethics Committee of animal studies (approval number: G.Ü.ET-20.034, date: 09.07.2020). All experiments were conducted in accordance with the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act of 1986 and its associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Research Council's Guide for the Care and Use of Laboratory Animals.

In the study, we included 24 female Sprague-Dawley rats weighing 255-290 grams and about 3 months old, divided into 4 groups with the same number of rats in each group using the RAND function in Microsoft Excel (v16.0, Microsoft Corporation, Redmond, U.S.). Rats were taken under standard laboratory conditions with a temperature of 22±2° C, 60% relative humidity, and 12 hour light and dark photoperiod before the experiment at a standard temperature of 22° C for at least 48 hours prior to randomization, being fed with tap water ad libitum and standard rat chow.

Allocation concealment was maintained until allocation. Animal care staff were unaware of allocation groups. The surgeon performing the adnexectomy was blind to which treatment each rat received. During the analysis of the outcome, investigators knew which rats were grouped to enable group comparisons; however, they were unaware of which specific treatment each group received.

Groups were determined as follows: Group I, control (sham operation); Group II, torsion-detorsion (I/R); Group III, torsion-detorsion + nifedipine (I/R + NIF); Group IV, torsion-detorsion + resveratrol (I/R + RSV).

Surgical Procedure

After weighing, anesthesia was performed with xylazine (5 mg/kg) and ketamine (45 mg/kg) intramuscular injections. The operation field was sterilized, and all rats were placed in the operation area on their backs. Blood samples were taken from all rats preoperatively for anti-müllerian hormone (AMH)

measurement. The torsion-detorsion model was performed according to Parlakgumus et al.'s[13] study. We twisted ovaries two complete circles around their pedicle as described in the method. Laparotomy started with a 2-cm midline abdominal incision, and then intestines were gently retracted. The subsequent procedures were implemented for each group:

• Group I (sham operation): Bilateral adnexal exploration was performed without intervention following the midline laparotomy. Six hours after the laparotomy, a blood sample for AMH measurement was collected, and bilateral ovaries were surgically removed.

• Group II (I/R): After midline laparotomy, both adnexa were explored and then rotated clockwise by 720°. Then, both twisted adnexa were sutured to the lateral abdominal wall with a 5.0 polyglactin suture. The skin was closed with a 4.0 poliglecapron suture. Three hours after torsion, a secondary laparotomy was performed to achieve detorsion of the adnexa to provide reperfusion (Figure 1). The abdomen was closed after detorsion. Three hours following reperfusion, blood samples were taken for AMH measurement, and bilateral adnexa were surgically removed.

• Group III (I/R + NIF): Exact steps from Group II were followed, except, 30 minutes before detorsion, this group received intraperitoneal nifedipine (10 mg/kg). The dosage of nifedipine was based on Chander and Chopra[14] study, which assessed its protective effect against cyclosporine-induced oxidative stress in rats.

• Group IV (I/R + RSV): Exact steps from Group II were followed, except, 30 minutes before detorsion, this group received intraperitoneal resveratrol (100 mg/kg). The dosage of resveratrol was determined according to Aydın et al.'s[15] study, which evaluated its protective effect against sepsis-induced oxidative stress in rats.

Biochemical AMH Evaluation

Serum samples were obtained by centrifuging blood samples at 4000 rpm for 10 minutes. Then all samples were preserved at -80 ºC in Eppendorf tubes in an ultra-low temperature freezer until analysis. AMH levels were analyzed on the same day with an automatic ELISA kit.

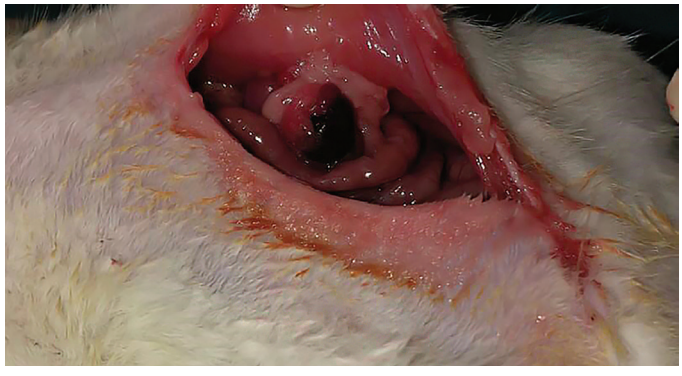

Figure 1. During secondary laparotomy three hours after torsion, ischemia is visible on the torsioned ovary

Histopathological Examination

Ovaries were placed in fixation fluid containing 10% neutral buffered formalin. Afterwards, samples were embedded in paraffin and were processed using routine histological light microscopy techniques. Once embedded, sections were taken from each paraffin block to create 4-micron-thick slices. Ovary sections were stained with hematoxylin and eosin. Histomorphological analyses and follicle counting were performed using a light microscope and a computer-enhanced visualization system (Leica DM 4000, Germany). Primordial follicles, antral follicles, atretic follicles, and corpus luteum were counted. The number of follicles from five randomly selected cross-sections from both experimental and control groups was quantified, and the results were compared.

Immunohistochemical Procedure

Anti-AMH antibody and anti-vascular endothelial growth factor receptor 2 (VEGFR-2) antibody were used for immunohistochemical evaluation based on the avidin-biotin peroxidase method. Ovarian tissue blocks were sectioned with a microtome into 4 micrometer-thick cross-sections. After deparaffinization, the cross sections underwent retrieval processing by incubation in citrate buffer (pH 6.0) (Lab Vision, Thermo Scientific) and exposure to 3% hydrogen peroxide (Lab Vision, Thermo Scientific) to block endogenous hydrogen peroxidase enzyme activity. Ultra V block (Lab Vision, Thermo Scientific, Fremont) was used to restrict non-selective interaction of enzymes or primary antibodies with tissue. Following the blocking stage, primary antibody (VEGFR-2 polyclonal antibody, bs10412R, Bios, AMH polyclonal antibody, D1201A, AFG Bioscience, respectively) was applied to tissue sections at a dilution ratio of 1:150 for a duration of 90 minutes. Following the primary antibody incubation, sections were washed with phosphate-buffered saline (PBS) three times for three minutes each. Afterwards, the secondary antibody (Lab Vision, Thermo Scientific, Fremont) was used for 10 minutes. The tissues were washed again with PBS three times for three minutes each. The immunohistochemical reaction components were made visible using streptavidin peroxidase complex (Lab Vision, Thermo Scientific, Fremont) with DAB. Counter-staining was performed with Mayer's hematoxylin. The immunostaining intensity in adnexal sections was evaluated semi-quantitatively as follows: (0) very weak; (1) weak; (2) moderate; and (3) strong[16,17].

Evaluation of Oxidative Stress

Total antioxidant status (TAS) and total oxidant status (TOS) of ovarian tissues were measured using Erel's method[18]. The oxidative stress index (OSI) was calculated as OSI=0.25 TOS/TAS.

Statistical Analysis

SPSS for Windows 22.0 (Statistical Package for the Social Sciences) software was utilized for statistical analysis. Normal distribution was evaluated by the Shapiro-Wilk test. Standard

deviations were used to present normally distributed data, while medians and percentiles were used for the rest. Categoric data were reported as percentages. Independent groups were compared with independent samples t-test for normally distributed data and with Mann-Whitney U test for non-parametric data. ANOVA was used to compare multiple groups, and post-hoc tests were conducted. The threshold for statistical significance was established at p<0.05.

Results

Biochemical AMH Evaluation

Preoperative to postoperative AMH level change was similar among all groups (p=0.058; p=0.089; p=0.756; p=0.206, respectively) (Table 1).

Histopathological Examination

Number of follicles was similar, except that in primordial and secondary follicles (p=0.047, p=0.031, respectively) (Table 2). Post-hoc tests showed that the control group had a higher primordial follicle count than the I/R group (p=0.014). The nifedipine-treated group had a higher secondary follicle count compared to the I/R and I/R+RSV groups (p<0.013, p<0.017, respectively) (Table 3). Visualization of changes in follicle morphology and vascular dilatation is presented in Figure 2.

Immunohistochemical Procedure

Immunohistochemical evaluation showed a remarkable decrease in AMH reactivity in the I/R group (Figure 3). Although AMH staining was more prominent in the resveratrol-treated group compared to the I/R group, it was observed to be weaker than in the control group. It was noted that in the nifedipine-treated group, tissue-wide uptake in the AMH immunostainings was similar to that in the control group.

Immunostaining for VEGFR-2 showed significantly increased immunoreactivity in all regions of the I/R group (Figure 4). VEGFR-2 levels were low in the resveratrol group, as in the control group. Conversely, levels in the nifedipine-treated group were close to those in the I/R group.

Evaluation of TAS, TOS, and OSI

Assessment of TAS, TOS, and OSI in harvested ovarian tissues yielded no significant difference between groups (p=0.094, p=0.089, p=0.162, p=0.611) (Table 4).

Discussion

We investigated the effects of nifedipine and resveratrol on enhancing ovarian viability following I/R injury due to torsion-detorsion. Histopathological findings indicated that the nifedipine-treated group exhibited a higher secondary follicle count compared to the I/R and I/R+RSV groups. Additionally, the AMH immunostaining grade in the nifedipine group was similar to the control group. Shown by VEGFR-2 staining, resveratrol also demonstrated protective attributes.

AMH level is an important parameter for predicting the reproductive period and evaluating long-term ovarian function in women; however, in many studies in the literature, only the postoperative AMH level was evaluated[19,20]. We hypothesized that evaluating the change of AMH level from the preoperative to the postoperative period can better portray the extent of I/R injury. Like our study, Karakaş et al.[21] investigated the effect of metformin on I/R injury in ovaries, focusing on blood AMH levels. The researchers found a higher AMH change in

Table 1. Preoperative to postoperative AMH change within each group

	Group 1 (SD)	Group 2 (SD)	Group 3 (SD)	Group 4 (SD)
Preoperative AMH (ng/mL)	1.94 (0.3)	2.17 (0.4)	2.23 (0.6)	1.93 (0.2)
Postoperative AMH (ng/mL)	1.64 (0.2)	1.81 (0.4)	2.32 (0.7)	1.75 (0.5)
AMH change (ng/mL)	0.3 (0.3)	0.35 (0.4)	0.9 (0.6)	0.17 (0.3)
p-value	0.058	0.089	0.756	0.206

SD: Standard deviation, AMH: Anti-müllerian hormone

Table 2. Evaluation of primordial, primary, secondary, antral corpus luteum and atretic follicle count

	Group 1 (SD)	Group 2 (SD)	Group 3 (SD)	Group 4 (SD)	p-value
Primordial	8.8 (2.6)[a]	4.8 (1.9)[a]	6.3 (2.5)	6.3 (1.9)	0.047
Primary	3.6 (0.8)	2.8 (1.3)	4.8 (0.9)	4.8 (2.7)	0.13
Secondary	3.6 (2.2)	1.5 (0.7)[b]	2.8 (0.7)[b, c]	1.8 (0.4)[c]	0.031
Antral	4.3 (0.8)	2.6 (1.2)	3.5 (1.3)	3 (1.2)	0.246
Corpus luteum	5.5 (2.07)	5.1 (1.7)	4.8 (1.07)	5 (3.09)	0.96
Atretic	17 (6.2)	23 (11.1)	16.8 (9.1)	18.6 (6.5)	0.541

SD: Standard deviation, ANOVA test. Superscript letters ([a, b, c]) indicate statistically significant difference between groups, detected in post-hoc analysis

the torsion/detorsion group, while the addition of metformin maintained the levels of the control. In the study of Sakin et al.[19], the effect of phosphodiesterase-5 inhibitors on ovarian I/R injury was investigated, and the postoperative AMH level was measured 6 hours after surgery, without measuring the preoperative AMH level. They reported that administration of vardenafil was associated with higher postoperative AMH levels; however, it could be argued that this result may be unreliable due to preoperative AMH levels being unknown.

Follicle count is an important parameter in most of the studies evaluating ovarian I/R injury. The increase in oxidative stress during ovarian torsion causes tissue damage, and reduction in follicle numbers in almost all stages of oocyte maturation[22]. Shokri et al.[23] analyzed the effect of Galea officinalis extract on

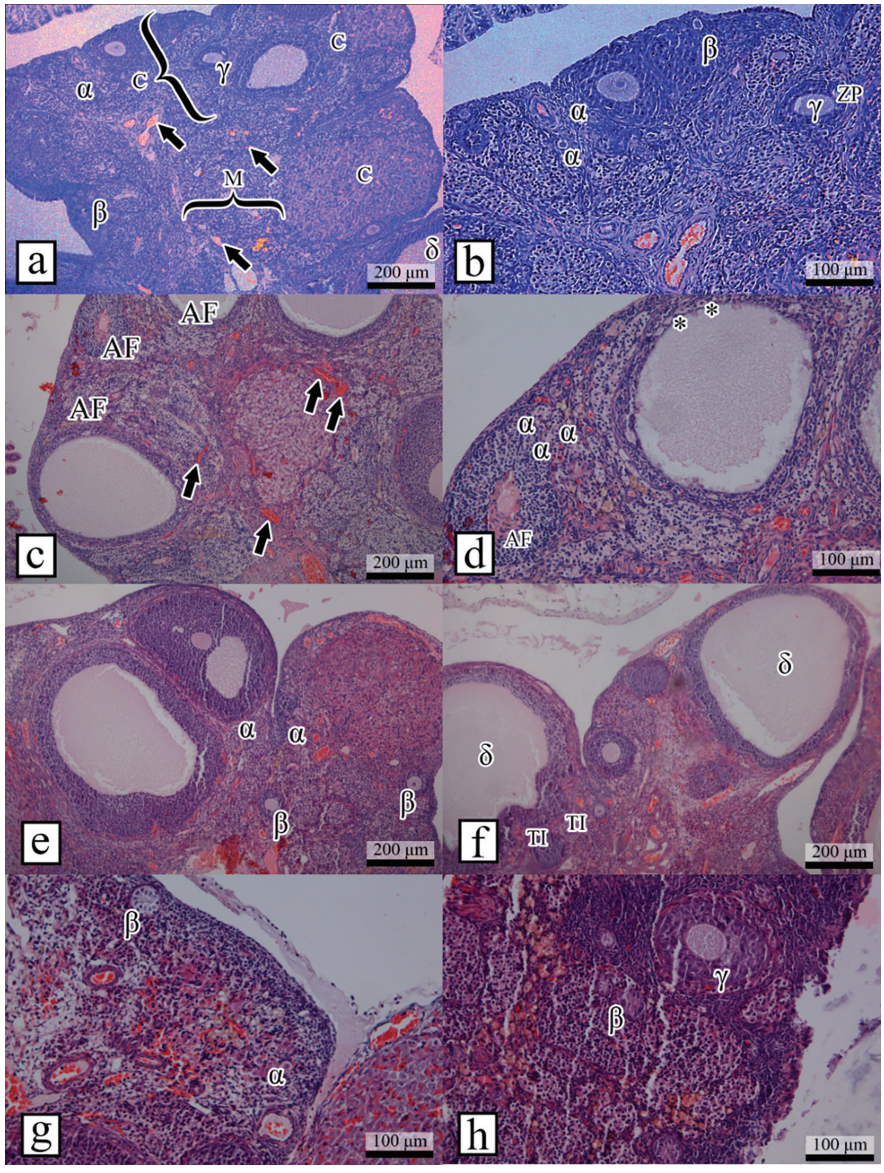

Figure 2. Representative micrographs of H&E-stained ovarian tissue sections captured under 100 x and 200 x magnifications, in all experimental groups: Group I (2a, 2b); Group II (2c, 2d); Group III (2e, 2f); Group IV (2g, 2h). Primordial (α), primary (β), secondary (γ), and antral (δ) follicles appear normal in the control group (2a, 2b). Zona pellucida (ZP) is observed around the secondary follicle (γ). Cortex (C) and medulla (M) are identified with normal vasculature (arrows) (2a, 2b). The I/R group shows vascular dilatation (arrows), atretic follicles (AF) with degenerated ZP, apoptotic bodies (asterisk), and primordial follicles with edema (α) (2c, 2d). I/R+NIF group shows vascular dilatation (arrows) however follicles appeared close to the control group (2e, 2f) with well-vascularized structures within theca interna of antral follicles (δ) (2f). There were no AF. I/R+RSV group showed normal Primordial (α), and primary (β) follicles (2g) but, primary (β) and antral follicles (δ) were atretic (2h)

H&E: Hematoxylin and eosin, NIF: Nifedipine, RSV: Resveratrol, I/R: Ischemia-reperfusion

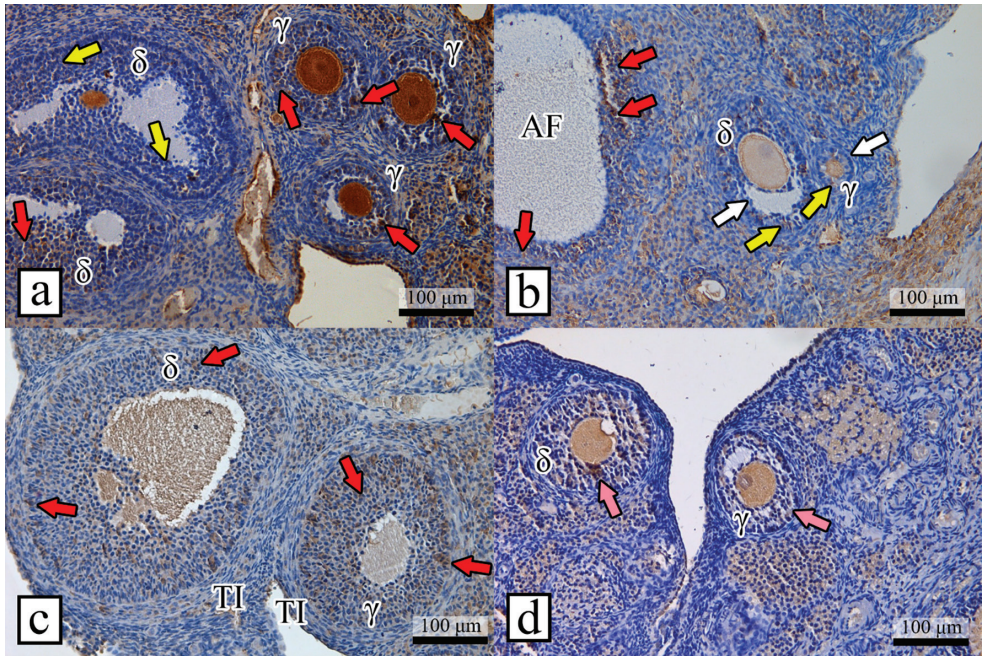

Figure 3. Representative micrographs of AMH-immunostained ovarian tissue sections captured under 200 x magnification, in all groups: Group I (3a); Group II (3b); Group III (3c); Group IV (3d). Secondary follicles (γ) show high (red arrows), antral follicles (δ) show low (yellow arrows) and high (red arrows) AMH reactivity in the control group (3a). The I/R group showed markedly decreased AMH reactivity from low (yellow arrows) to negative (white arrows) with a low number of reactive secondary (γ) and antral (δ) follicles as well as atretic follicles with high immunoreactivity (3b). The I/R+NIF group was similar to the control group with high reactivity (red arrows) in secondary (γ) and small antral follicles (δ), with more reactivity closer to theca interna (3c). Although the I/R+RSV group showed better immunoreactivity (pink arrows) than the I/R group in secondary (γ) and antral follicles (δ), it remained lower than the control group (3d)

AMH: Anti-müllerian hormone, NIF: Nifedipine, RSV: Resveratrol, I/R: Ischemia-reperfusion

I/R injury and reported the primary, antral, graafian, and atretic follicle count were higher, indicating a positive effect. Santa-Helena et al.[24], also observed that nifedipine reduced reactive oxygen radicals and increased the viability of cardiomyoblasts after I/R injury. This indicates that nifedipine may have a role against I/R injury. In another study on the same topic, the effect of enoxaparin was investigated, and although primordial, preantral, corpus luteum and atretic follicle counts were not found to be different, the number of small and large antral follicles was significantly lower in the torsion/detorsion group compared to the control[25]. We found that the nifedipine-treated group had a higher secondary follicle count than groups 2 and 4, a finding which warrants further investigation in clinical studies.

We performed AMH and VEGFR-2 immunostaining in ovarian tissues. Parlakgumus et al.[13] studied atorvastatin and expression of AMH and VEGF-A in I/R damaged ovaries, and they found that AMH was higher in the torsion/detorsion + atorvastatin group. The expression of AMH originates in granulosa cells

Table 3. Comparison of groups for primordial and secondary follicles

	Primordial follicle	Secondary follicle
Group 1/Group 2	0.014	0.05
Group 1/Group 3	0.123	0.41
Group 1/Group 4	0.092	0.078
Group 2/Group 3	0.273	**0.013**
Group 2/Group 4	0.213	0.374
Group 3/Group 4	1	**0.017**

Independent t-test. P<0.05 was considered statistically significant

Table 4. Evaluation of TAS, TOS and OSI (±: standard deviation)

	Group 1 (SD)	Group 2 (SD)	Group 3 (SD)	Group 4 (SD)	p-value
TAS (mmol/L)	0.37 (0.17)	0.4 (0.12)	0.58 (0.13)	0.45 (0.14)	0.094
TOS (µmol/L)	3.4 (1.3)	5.6 (5.1)	9.0 (3.5)	8.6 (6.7)	0.162
OSI	1.1 (0.8)	1.3 (0.8)	1.6 (0.7)	1.7 (1.1)	0.611

SD: Standard deviation, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index

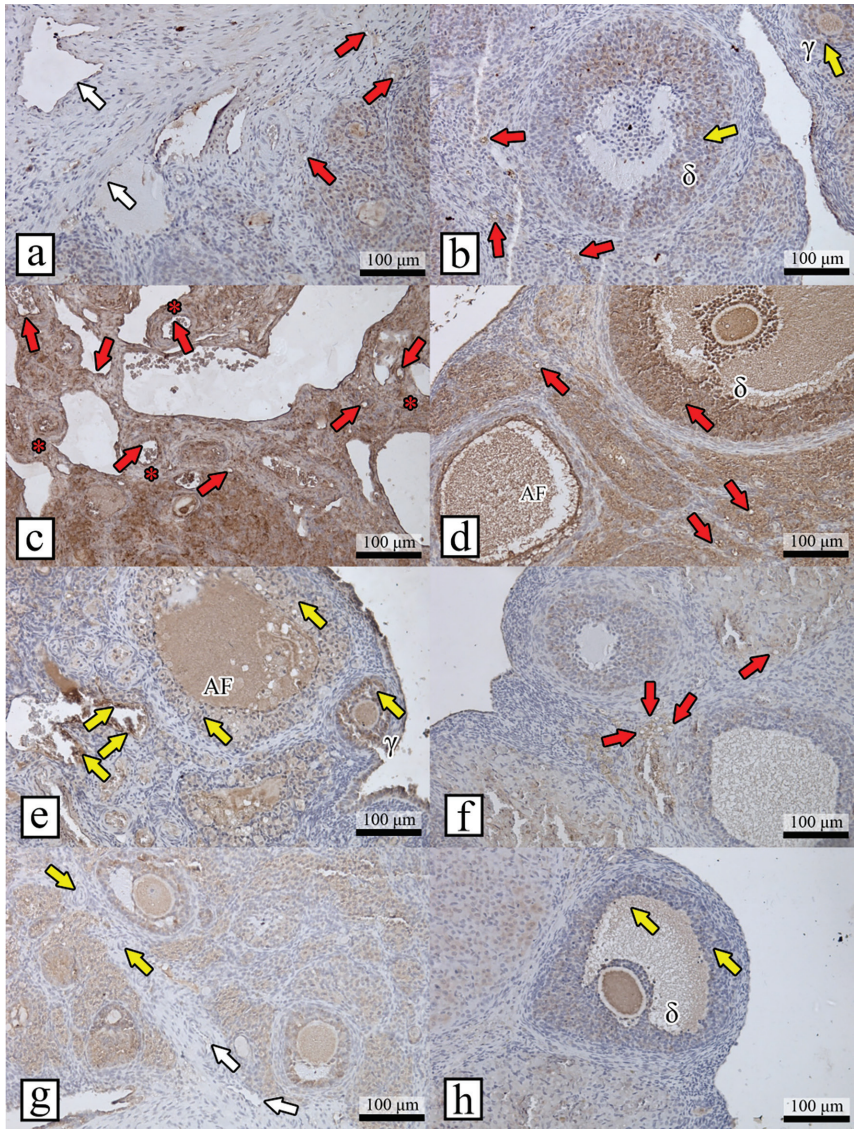

Figure 4. Representative micrographs of VEGFR-2-immunostained ovarian tissue sections captured under 200 x magnification, in all groups: Group I (4a, 4b); Group II (4c, 4d); Group III (4e, 4f); Group IV (4g, 4h). No uptake was detected around large vessel endothelial cells (white arrows), but capillary uptake was present (yellow arrows) in endothelial cells in the medulla in the control group (4a). Viewing the cortex, secondary (γ) and antral (δ) follicles in this section showed weak uptake (yellow arrows), while capillary here showed similar uptake (red arrows) (4b). The I/R group was different with tissue-wide, strong uptake in the medulla, with capillary as well as large vessel (red arrows) endothelial uptake, and high uptake in smooth muscle cells (asterisk) (4c). Cortex also showed high reactivity around capillary endothelial cells, antral (δ), and atretic follicles (AF) (red arrows) (4d). The I/R+NIF group showed medium uptake, closer to the I/R group rather than the control, with medullary uptake (yellow arrows) around large vessel endothelial cells, granulosa of secondary (γ) and AF (4e) and high capillary uptake (red arrows) in the cortex (4f). The I/R+RSV group showed a potentially better effect against oxidative stress than the previous group with negative (white arrows) to weak (yellow arrows) uptake in both large vessels and capillaries in the medulla (4g) as well as weak (yellow arrows) uptake similar to the control group in antral follicles (δ) (4h)

VEGFR-2: Vascular endothelial growth factor receptor 2, NIF: Nifedipine, RSV: Resveratrol, I/R: Ischemia-reperfusion

of early antral follicles, and the follicles expressing the most AMH are preantral and small antral follicles[26,27]. Ischemia is a factor that reduces AMH expression. Cells expressing higher AMH are thought to have higher proliferative and steroidogenic activity[28]. In our study, the staining of preantral and antral follicles for AMH was weak in groups II and IV, although it was close to control in the nifedipine-treated group. This suggests that nifedipine may preserve the proliferative capacity of cells.

Hypoxia is one of the best stimulators of angiogenesis, thus increased expression of VEGF receptors during I/R can be expected. Kanellis et al.[29] showed that after renal I/R, VEGFR-2 immunostaining surged in renal tissue. In our study,

although the staining of VEGFR-2 was increased in group 2, it was decreased in the resveratrol-treated group. The lower VEGF receptor level in group 4 suggests that resveratrol can help in tolerating oxidative stress and its effects. Evidence suggests that calcium channel blockers may increase VEGF concentration[30,31]. Our findings indicate that the staining of VEGFR-2 in the nifedipine group was similar to that in the I/R group alone. Therefore, it is suggested that nifedipine has no significant effect on VEGF.

Oxidative stress was similar among groups in the current study. Gungor et al.[32] evaluated the possible beneficial effects of a clinically available omega-3 fatty acid emulsion on I/R injury by measuring TAS, TOS, and OSI, and they did not find any positive effect. Although resveratrol and nifedipine were shown to have antioxidant effects in several studies, we did not observe any evidence of this effect in our study[33].

Study Limitations

There are several limitations that should be noted. First, we performed torsion for only 3 hours. However, in clinical ovarian torsion scenarios, the exact duration from torsion to detorsion is often unknown. As the torsion duration increases, the damage to the ovary might become irreparable. Moreover, we administered the agents at doses used in previously published studies and have not tested other doses, which may have allowed more optimal dosing regimens.

Conclusion

Although nifedipine and resveratrol did not demonstrate a concrete protective effect on every parameter evaluating I/R injury after ovarian torsion/detorsion, increased immunohistochemical AMH expression and secondary follicle count in the nifedipine treated group, and decreased VEGFR-2 expression in the resveratrol treated group, can be regarded as indicators of their potential.

Ethics

Ethics Committee Approval: The protocol was reviewed and approved by the Gazi University Animal Experiments Local Ethics Committee of animal studies (approval number: G.Ü.ET-20.034, date: 09.07.2020).

Informed Consent: Not necessary.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Concept: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Design: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Data Collection or Processing: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Analysis or Interpretation: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Literature Search: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö., Writing: A.S., A.A.E.S., G.T.K., C.K., E.D., M.F.C.A., M.E., A.E., M.Ö.

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References

1. Bouquet de Joliniere J, Dubuisson JB, Khomsi F, Fadhlaoui A, Grant G, Ali N Ben, et al. Laparoscopic adnexectomy for ovarian torsion during late pregnancy: case report of a non-conservative treatment and literature analysis. Front Surg. 2017;4:50.
2. Varras M, Tsikini A, Polyzos D, Samara Ch, Hadjopoulos G, Akrivis Ch. Uterine adnexal torsion: pathologic and gray-scale ultrasonographic findings. Clin Exp Obstet Gynecol. 2004;31:34-8.
3. Gasser CRB, Gehri M, Joseph JM, Pauchard JY. Is it ovarian torsion? A systematic literature review and evaluation of prediction signs. Pediatr Emerg Care. 2016;32:256-61.
4. Wilkinson C, Sanderson A. Adnexal torsion -- a multimodality imaging review. Clin Radiol. 2012;67:476-83.
5. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: the evolution of a concept. Redox Biol. 2015;6:524-51.
6. Borekci B, Gundogdu C, Altunkaynak BZ, Calık M, Altunkaynak ME, Unal D, et al. The protective effect of dehydroepiandrosterone on ovarian tissues after torsion-detorsion injury: a stereological and histopathological study. Eurasian J Med. 2009;41:22-7.
7. Meštrović J, Drmić-Hofman I, Pogorelić Z, Vilović K, Šupe-Domić D, Šešelja-Perišin A, et al. Beneficial effect of nifedipine on testicular torsion-detorsion injury in rats. Urology. 2014;84:1194-8.
8. Hascalik S, Celik O, Turkoz Y, Hascalik M, Cigremis Y, Mizrak B, et al. Resveratrol, a red wine constituent polyphenol, protects from ischemia-reperfusion damage of the ovaries. Gynecol Obstet Invest. 2004;57:218-23.
9. Lu Z, Cheng B, Hu Y, Zhang Y, Zou G. Complexation of resveratrol with cyclodextrins: solubility and antioxidant activity. Food Chem. 2009;113:17-20.
10. Okamatsu S, Lefer AM. The protective effects of nifedipine in the isolated cat heart. J Surg Res. 1983;35:35-40.
11. Ruigrok TJC, van Echteld CJA, de Kruijff B, Borst C, Meijler FL. Protective effect of nifedipine in myocardial ischemia assessed by phosphorus-31 nuclear magnetic resonance. Eur Heart J. 1983;4(Suppl. C):109-13.
12. Mak IT, Boehme P, Weglicki WB. Antioxidant effects of calcium channel blockers against free radical injury in endothelial cells. Correlation of protection with preservation of glutathione levels. Circ Res. 1992;70:1099-103.
13. Parlakgumus HA, Aka Bolat F, Bulgan Kilicdag E, Simsek E, Parlakgumus A. Atorvastatin for ovarian torsion: effects on follicle counts, AMH, and VEGF expression. Eur J Obstet Gynecol Reprod Biol. 2014;175:186-90.
14. Chander V, Chopra K. Nifedipine attenuates changes in nitric oxide levels, renal oxidative stress, and nephrotoxicity induced by cyclosporine. Ren Fail. 2005;27:441-50.
15. Aydın S, Şahin TT, Bacanlı M, Taner G, Başaran AA, Aydın M, et al. Resveratrol protects sepsis-induced oxidative DNA damage in liver and kidney of rats. Balkan Med J. 2016;33:594-601.
16. Ginnavaram V, Vasugi A, Sundaram S. Immunohistochemical evaluation of notch1 in ovarian tumours and its prognostic implications. Cureus. 2023;15.

17. Goel A, Rao NM, Santhi V, Byna SS, Grandhi B, Conjeevaram J. Immunohistochemical characterization of normal ovary and common epithelial ovarian neoplasm with a monoclonal antibody to cytokeratin and vimentin. Iran J Pathol. 2018;13:23-9.
18. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38:1103-11.
19. Sakin O, Anğin AD, Akalin EE, Cikman MS, Basak K, Kaptanagasi AO. Comparison of the protective effects of sildenafil, vardenafil and tadalafil treatments in ischemia-reperfusion injury in rat ovary. Ginekol Pol. 2019;90:513-9.
20. Abdel-Gaber SAW, Atta M, Abdel-Hafez SMN, Abdelzaher WY. Ameliorative effect of nicorandil in ovarian ischemia-reperfusion-induced injury in rats: role of potassium channel. Naunyn Schmiedebergs Arch Pharmacol. 2020;393:1599-610.
21. Karakaş S, Kaya C, Güraslan H, Sakiz D, Süzen Çaypinar S, Cengız H, et al. Effect of metformin and detorsion treatment on serum anti-müllerian hormonelevels and ovarian histopathology in a rat ovarian torsion model. Turk J Med Sci. 2020;50:455-63.
22. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reproductive Biology and Endocrinology. 2012;10.
23. Shokri F, Shokoohi M, Roudi Rasht Abadi A, Kalarestaghi H. The ameliorative effect of Galega officinalis extract on histological damages, oxidative stress induced by torsion-detorsion in adult rats' ovarian. International Journal of Women's Health and Reproduction Sciences. 2018;7:119-23.
24. Santa-Helena E, Teixeira S, Castro MR de, Cabrera D da C, D'Oca CDRM, D'Oca MGM, et al. Protective role of the novel hybrid 3,5-dipalmitoyl-nifedipine in a cardiomyoblast culture subjected to simulated ischemia/reperfusion. Biomed Pharmacother. 2017;92:356-64.
25. Kaya C, Turgut H, Cengiz H, Turan A, Ekin M, Yaşar L. Effect of detorsion alone and in combination with enoxaparin therapy on ovarian reserve and serum antimüllerian hormone levels in a rat ovarian torsion model. Fertil Steril. 2014;102.
26. Durlinger ALL, Gruijters MJG, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Anti-müllerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002;143:1076-84.
27. Durlinger ALL, Visser JA, Themmen APN. Regulation of ovarian function: the role of anti-müllerian hormone. Reproduction. 2002;124:601-9.
28. Baarends WM, Uilenbroek JTJ, Kramer P, Hoogerbrugge JW, Van Leeuwen ECM, Themmen APN, et al. Anti-müllerian hormone and anti-müllerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle, and gonadotropin-induced follicle growth. Endocrinology. 1995;136:4951-62.
29. Kanellis J, Paizis K, Cox AJ, Stacker SA, Gilbert RE, Cooper ME, et al. Renal ischemia-reperfusion increases endothelial VEGFR-2 without increasing VEGF or VEGFR-1 expression. Kidney Int. 2002;61:1696-706.
30. Kumar A, Mutter S, Parente EB, Harjutsalo V, Lithovius R, Mathavan S, et al. L-type calcium channel blocker increases VEGF concentrations in retinal cells and human serum. PLoS One. 2023;18.
31. Miura SI, Fujino M, Matsuo Y, Tanigawa H, Saku K. Nifedipine-induced vascular endothelial growth factor secretion from coronary smooth muscle cells promotes endothelial tube formation via the kinase insert domain-containing receptor/fetal liver kinase-1/NO pathway. Hypertens Res. 2005;28:147-53.
32. Gungor ANC, Turkon H, Albayrak A, Ovali M, Islimye M, Gencer M, et al. Does omegaven have beneficial effects on a rat model of ovarian ischemia/reperfusion? Eur J Obstet Gynecol Reprod Biol. 2014;181:240-5.
33. Dokuyucu R, Karateke A, Gokce H, Kurt RK, Ozcan O, Ozturk S, et al. Antioxidant effect of erdosteine and lipoic acid in ovarian ischemia-reperfusion injury. Eur J Obstet Gynecol Reprod Biol. 2014;183:23-7.