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Sildenafil as a Salvage Treatment for Ovarian Torsion: A Rat Model

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ABSTRACT

OBJECTIVE: In this study, we aimed to investigate the effect of sildenafil, acting as an anti-oxidant on the ischemic rat ovarian torsion model by pointing out ovarian reserve via AMH levels which is the best marker for oocyte pool.

STUDY DESIGN: This study is an experimental animal study conducted according to the principles of the Declaration of Helsinki. Twenty-seven rats were assigned into three groups, each consisting of nine individuals. Group 1: Sham operation (laparotomy only) was performed. Group 2: The torsion group had bilateral torsion and detorsion of the ovaries. Group 3: In Sildenafil and torsion group the rats received 0.7 mg/kg sildenafil (Viagra, Pfizer, New York, ABD) intraperitoneally one hour before torsion and detorsion operation. One month after the initial operation, blood was drawn via the intra-cardiac route, and serum antimullerian hormone (AMH) concentrations were studied.

RESULTS: The mean weights of the rats were similar 224 g, 229 g, and 222 g for groups 1, 2, and 3 respectively. Only one rat from Group 2(T/D) died during the experiment leaving eight rats for analysis. The mean value for AMH was 24.9227±12.19124 ng/ml and the mean value for weight was 226.22±19.200. The differences in AMH groups were not statistically significant (p=0.873)

CONCLUSIONS: As a result, as is shown in our study, the ovarian reserve may not be influenced by ischemia developed by ovarian torsion or any influences could not change AMH levels. Sildenafil which was given to the rats before detorsion to protect against reperfusion ischemia was found to be ineffective on ovary reserve according to AMH assessment.

Keywords: Ovarian torsion, Rat model, Sildenafil

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Introduction

Ovarian torsion is defined as the complete or partial loss of arterial and/or venous blood flow of the adnexa which happens as a result of ovarian vascular pedicle twisting. It constitutes 2.7% of gynecologic emergencies (1). Its treatment is rapid detorsion of the twisted pedicle to restore blood flow. A delay in diagnosis leads to damage in ovarian tissue that is associated with oocyte loss. As a result, infertility may be confronted. Delay in diagnosis is frequent because it presents with many vague symptoms such as abdominal pain and distension, nausea, vomiting, low-degree fever, tachycardia, and leukocytosis which are common for many diseases. So the diagnosis mainly depends on a high degree of clinical suspicion (1,2).

Although deteriorating the ovary restores the vascularization of the ovary, it inevitably may result in the risk of ischemia-reperfusion injury. Ischemia-reperfusion injury diminishes the oocyte pool via increased oxygen radicals in the ovarian tissue (3-6). Any substance to reverse the effects of

ischemia-reperfusion would be a great contribution to inhibiting the loss of ovarian reserve. Researchers have studied many substances including montelukast, growth hormone, atorvastatin, enoxaparin, dehydroepiandrosterone (DHEA), proanthocyanidin, curcumin, and selenium in preventing ischemia-reperfusion injury (3,7-13).

Sildenafil is a phosphodiesterase type-5 (PDE5) inhibitor. It was first developed in 1989 for coronary artery disease and received approval from the Food and Drug Administration of the USA in 1998 for the treatment of erectile dysfunction. This PDE5 inhibitor is a vasoactive drug that causes smooth muscle relaxation on the artery wall. It has been used for erectile dysfunction, pulmonary artery disease, coronary syndrome, and endothelial dysfunction until today (14). Anti-oxidant effect of sildenafil was shown in ischemia of the lung, liver, testis, and ovary histopathologically (5,15-17).

Many parameters have been proposed as a marker of diminished oocyte pool. Anti-mullerian hormone (AMH) is the best parameter showing primordial follicle pool (18). It is a glycopeptide from the transforming growth factor beta (TGFβ) family and is excreted from preantral and small antral follicles (18). Its level is minimally affected by intra-cycle, inter-cycle, pregnancy, and hormone supplementation.

In this study, we aimed to investigate the effect of sildenafil, acting as an anti-oxidant on the ischemic rat ovarian torsion model by pointing out ovarian reserve via AMH levels which is the best marker for oocyte pool.

Material and Method

This study was conducted in The Animal Research Laboratory of Baskent University, Ankara, Turkey. Twenty-seven Wistar albino rats (6 months old, 180-250 g) were kept at 20-24°C temperature and 50-60% humidity, 12 hours of light, and 12 hours of dark for one week. Rats were fed with water and pellet food ad libitum. On the day of the surgical operation, rats were arbitrarily assigned into three groups, each consisting of nine individuals. All rats were weighed on the day of the surgery before starting the experiment.

Group 1: Sham operation (laparotomy only) was performed

Group 2: The torsion group had bilateral torsion and detorsion of the ovaries

Group 3: In the Sildenafil and torsion group the rats received 0,7 mg/kg sildenafil (Viagra, Pfizer, New York, ABD) intraperitoneally one hour prior to torsion and detorsion operation

Rats were anesthetized with ketamine (60 mg/kg, Ketasol, Richter Pharma, Wels, Austria), and xylazine hydrochloride (7 mg/kg, Rompun, Bayer, Leverkusen, Germany) administered intraperitoneally. After anesthesia, rats were placed surgically in a supine position; the surgical site was shaved and disinfected. In the first group; a sham operation; a 2.5 cm midline incision exposing uterine horns and adnexa was made and the abdominal layers and skin were covered with 3/0 polysorb (Covidien®) suture material.

The second group was the torsion-detorsion group. After the 2.5 cm midline incision, the ovarian pedicles were twisted twice in a clockwise direction and fixed to the abdominal wall with a 4/0 silk suture. Then, abdominal layers and skin were closed with a 3/0 polysorb suture and rats were anesthetized for three hours. Throughout this period one rat from the torsion-detorsion group died. Eight remaining rats had re-laparotomy after three hours, sutures of torsioned adnexa were taken, adnexa was detorsioned and the abdomen was closed with a 3/0 polysorb suture. In the third group, 0.7 mg/kg of sildenafil was administered intraperitoneally (17,19) one hour prior to surgery and the rats had a similar surgical procedure as those in the second group. Fentanyl (0.02 mg/kg) was administered as an analgesic for three days after the operations. Recovery time was set as one month (20,21). One month after the initial operation the rats were anesthetized again with cetamin and xylazine hydrochloride (7 mg/kg). Blood was drawn via the intra-cardiac route. Rats were sacrificed with cervical dislocation under anesthesia.

Blood samples were centrifuged for 10 minutes with 5000 revolutions in the NF 415 device and serum was collected. Serum samples were preserved at -80 °C. Serum AMH concentrations were studied by Cusabio commercial kit (Wuhan P.R.) (Catalogue no: CSB-E11162r) by ELISA technique. AMH levels were presented as ng/mL. The sensitivity of the method is less than 0.1 ng/mL. Data were analyzed with Statistical Package for Social Sciences, version 17.0 (SPSS Inc., Chicago, IL, USA). Since the sample size is less than thirty Kruskal-Wallis Test was used for the analysis. Statistical significance level p below 0.05 was accepted as significant.

This study was approved by the Baskent University Medical Faculty Ethical Committee of Animal Experiments (Project laboratory No: DA 15/33) and supported by the Baskent University Fund. This study is an experimental animal study conducted according to the principles of the Declaration of Helsinki.

Results

The mean weights of the rats were similar and 224 g, 229 g, and 222 g for groups 1, 2, and 3 respectively. Only one rat from Group 2 (T/D) died during the experiment leaving eight rats for analysis. The mean value for AMH was 24.9227± 12.19124 ng/mL and the mean value for weight was 226.22± 19.200. The differences in AMH groups were not statistically significant (p=0.873) (Table I).

Table I: *Kruskal-Wallis test for rat weights and AMH levels*

	Group/N		Mean Rank	Minimum	Maximum	Mean	Median	SD	p
AMH (ng/m	1. Sham	9	14.56	1.31	43.25	24.9227	27.2850	12.19124	0.873
	2.T/D	8	12.75						
	3.T/D+Sildenafil	9	13.11						
Rat weight (gram)	1. Sham	9	13.33	182	262	226.22	226.00	19.200	0.554
	2.T/D	9	16.28						
	3.T/D+Sildenafil	9	12.39						

SD: Standart deviation, T/D: Torsion – Detorsion

Discussion

Initially, we hypothesized that the oxidative stress created by ischemia-reperfusion injury would damage ovarian reserve and diminish the oocyte pool, which would probably be reflected in serum AMH levels. In our study which is an ovarian torsion model of rats, sildenafil prior to the ovarian detorsion has not been found to affect ovarian reserve measured by AMH.

Many antioxidant agents have been tested for the treatment of oxidative damage in different tissues. Sildenafil was shown to have some protective effect against oxidative damage in different tissues and organs. In the rat model of ovarian torsion, sildenafil was found to reduce the damage to the tissues and reduce the blood levels of oxidative stress markers significantly without a dose-dependent manner. In the intestinal ischemia, sildenafil was found to protect liver functions from ischemia. In lung ischemia, sildenafil caused a significant decrease in caspase, TNF-alfa, IL-6, and p53 levels, which are responsible for cell death. In testicular torsion, malondialdehyde (MDA) lipid peroxidation levels were found to be lower in those treated with sildenafil (5,15-17).

In another experimental animal model of ovary torsion assessing the effect of a different PDE inhibitor vardenafil, ovary tissue was excised after surgery, and edema, vascular congestion, and follicular cell degeneration were checked histopathologically and the total status of oxidant and antioxidant of the tissue was calculated. In the group of animals those vardenafil was applied has been found to have significantly better histopathological parameters ($p<0.05$). Follicular damage was reduced with the use of vardenafil in a dose-dependent manner. However, there was no significant change in the antioxidant status of the tissue with vardenafil medication (22). In the rat models conducted by Kalyoncu et al., unlike vardenafil, the use of melatonin, octreotide, and lanreotide in ovarian tissue was observed to reduce oxidative damage scores and increase antioxidant status. From a histopathological perspective, the use of these agents also led to a decrease in follicular degeneration, vascular congestion, hemorrhage, and inflammation. Similar studies with different medical agents, such as metformin, which show the same effects, have also been presented in the literature (23-25).

However, these studies are related to histopathological oxidative damage and do not indicate the direct effect of sildenafil on ovarian reserve. Therefore AMH, accepted as the best indicator of ovarian reserve, was used in our study (18). Unlike the other studies, no significant difference was detected between the groups in terms of AMH levels (26).

In another AHM study, where Kaya and his friends used enoxaparin against ischemia-reperfusion injury on ovarian tissue, it was observed that vascular congestion and hemorrhage scores were increased in the enoxaparin group (9). This situation can be explained by the anticoagulant effect of enoxaparin. There were no differences between groups in terms of follicular cell degeneration and inflammatory score. Post-operative AMH levels were lower in all groups compared to pre-operative levels, but, this reduction was more apparent in the detorsion group. Thus, enoxaparin medication in addition to detorsion can be practiced in the treatment of ovarian torsion (9).

Current studies in the literature show that histopathological and biochemical injuries take place in the ovary with torsion detorsion (5,9,22). Nevertheless, our study did not point to any change in AMH levels due to torsion detorsion. We performed AMH blood level tests one month after the torsion detorsion operation. In Soyman's study, AMH levels were checked 3 hours after detorsion and AMH levels in the torsioned group were found to be lower than those of the control group (27). In the study of Sahin Ersoy et al, preoperative levels of AMH decrease at the end of 24 hours after detorsion compared to the control group (28). In the study of Çalış et al., rat ovaries were torsioned on the 2nd, 4th, and 16th hours. They found that the primary and primordial follicle numbers were similar within the groups after 28 days. From this point of view, as AMH is secreted from primary and primordial follicle cells, as usual, we demonstrated that AMH levels were found to be similar after 28 days in parallel with this study (29). The prolonged time (one month) of checking AMH levels after surgery may lead the ovarian tissue to ameliorate which may be the fact to equalize two group values.

In the study of Sugita A et al., after cystectomy of the human ovary, AMH levels were followed for one year. Although the AMH levels dropped after the first month, they rose to the initial levels after the first year following surgery (30). Therefore, recovery of ovarian tissue after a one-month interval can explain the equality of AMH levels of the two groups in our study.

In our study, the duration of torsion was parallel with the other studies (27,28) and was considered to be sufficient since it was 3 hours. However, there are also studies suggesting that the torsion process does not affect the reserve. The study of Oelsner et al. suggests that the ovarium reserve does not deteriorate with torsion, as it is preserved in 91% (31). In Pena's Doppler study, there was no significant difference between patients with normal or abnormal Doppler findings for necrotic adnexa in terms of ovary reserve (32). In this case, it can be considered that the blood flow of the ovary is supplied from different arteries, the blood flow does not deteriorate in the torsion, the edema and congestion may be related to venous and lymphatic stasis and this may not affect the viability of the ovary.

Limitations of our research are that histopathological injury was not shown after torsion and detorsion and AMH blood levels were not checked before the operation. One of the limitations of the study is that oxidant and antioxidant status was not evaluated in the study.

As a result, as is shown in our study, ovarian reserve may not be influenced by ischemia developed by ovarian torsion or any influences could not change AMH levels. Sildenafil which was given to the rats prior to detorsion to protect against reperfusion ischemia was found to be ineffective on ovary reserve according to AMH assessment. The decrease in ovarian follicular numbers and AMH levels after torsion and detorsion rat models in other studies may recover after enough intervals of time to restore the number of primordial and primary follicles. To identify changes in ovary reserve, homogenous groups of more subjects with specific demographic properties and shown ischemia-reperfusion injury are needed. Animal experiments in this context are going to shed light on human research in the future.

Declarations

Ethics Committee Approval and Fundings: This study was approved by the Baskent University Medical Faculty Ethical Committee of Animal Experiments (Project laboratory No: DA 15/33) and supported by the Baskent University Fund. This study is an experimental animal study conducted according to the principles of the Declaration of Helsinki.

Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request. / The datasets and code used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

Protocol development, writing: DAY, HAP, PCA

Data collection: DAY, HAP, SY, HEOO, DAA

Data analysis: DAY, HAP, PCA

Editing: DAY, HAP, PCA, EBK

References

1. Andre IB, Anne KW. Adnexal torsion: Can the adnexa be saved? Am J Obstet Gynecol. 1994;171(6):1506-10. Doi: 10.1016/0002-9378(94)90393-x. PMID: 7802059
2. Ashwal E, Krissi H, Hiersch L, Less S, Eitan R, Peled Y. Presentation, diagnosis and treatment of ovarian torsion in premenarchal girls. J Pediatr Adolesc Gynecol. 2015; 28(6):526-9. Doi: 10.1016/j.jpag.2015.03.010. PMID: 26168769
3. 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. Doi: 10.1016/j.ejogrb.2014.01.017. PMID: 24507756
4. Ozkisacik S, Yazici M, Gursoy H, Culhaci N. Does gradual detorsion protect the ovary against ischemia-reperfusion injury in rats? Pediatr Surg Int. 2014;30(4):437-40. Doi: 10.1007/s00383-014-3480-3. PMID: 24519483
5. Incebiyik A, Seker A, Camuzoglu H, Kocaaslan S, Camuzoglu A, Hilali NG, et al. Does sildenafil have protective effects against ovarian ischemia-reperfusion injury in rats? Arch Gynecol Obstet. 2015;291(6):1283-8. Doi: 10.1007/s00404-014-3554-4. PMID: 25416202
6. Kurtoglu E, Kokcu A, Danaci M. Asynchronous bilateral ovarian torsion. A case report and mini review. J Pediatr Adolesc Gynecol. 2014;27(3):122-4. Doi: 10.1016/j. jpag. 2013.06.016. PMID: 24075085
7. Oral A, Odabasoglu F, Halici Z, Keles ON, Unal, B, Coskun AK, et al. Protective effects of montelukast on ischemia-reperfusion injury in rat ovaries subjected to torsion and detorsion: biochemical and histopathologic evaluation. Fertil Steril. 2011;95(4):1360-6. Doi: 10.1016/j. fertnstert.2010.08.017 PMID: 20850724.
8. Yigiter M, Halici Z, Odabasoglu F, Keles ON, Atalay F, Unal B, et al. Growth hormone reduces tissue damage in rat ovaries subjected to torsion and detorsion: biochemical and histopathologic evaluation. Eur J Obstet Gynecol Reprod Biol. 2011;157(1):94-100. Doi: 10.1016/j.ejogrb. 2011.02.012. PMID: 21439711.
9. Kaya C, Turgut H, Cengiz H, Turan A, Ekin M, Yasar L, et al. Effect of detorsion alone and in combination with enoxaparin therapy on ovarian reserve and serum anti mullerian hormone levels in a rat ovarian torsion model. Fertil Steril. 2014;102(3):878-884.e1. Doi: 10.1016/j.

fertnstert.2014.06.007. PMID: 24996496

10. 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(1):22-7. PMID: 2561 0059; PMCID: PMC4261661

11. Yıldırım Ş, Topaloğlu N, Tekin M, Küçük A, Erdem H, Erbaş M, et al. Protective role of Proanthocyanidin in experimental ovarian torsion. Med J Islam Repub Iran. 2015;29:185. PMID: 26034738, PMCID: PMC4431445.

12. Sak ME, Soydinc HE, Sak S, Evsen MS, Alabalik U, Akdemir F, et al. The protective effect of curcumin on ischemia-reperfusion injury in rat ovary. Int J Surg. 2013;11(9):967-70. Doi: 10.1016/j.ijsu.2013.06.007. PMID: 23796447

13. Bozkurt S, Arikan DC, Kurutas EB, Sayar H, Okumus M, Coskun A, et al. Selenium has a protective effect on ischemia/reperfusion injury in a rat ovary model: biochemical and histopathologic evaluation. J Pediatr Surg. 2012;47(9):1735-41. Doi: 10.1016/j.jpedsurg.2012.03. 053 PMID: 22974615

14. Kukreja RC, Salloum F, Das A, Ockaili R, Yin C, Bremer YA, et al. Pharmacological preconditioning with sildenafil: Basic mechanisms and clinical implications. Vascul Pharmacol. 2005;42(5-6), 219-32. Doi: 10.1016/j.vph. 2005.02. 010. PMID: 15922255

15. Inan M, Uz YH, Kizilay G, Topcu-Tarladacalisir Y, Sapmaz-Metin M, Akpolat M, Aydogdu N. Protective effect of sildenafil on liver injury induced by intestinal ischemia/reperfusion. J Pediatr Surg. 2013;48(8):1707-15. Doi: 10.1016/j.jpedsurg.2012.12.054. PMID: 23932610.

16. Shih PK, Cheng CM, Li HP, Huang MF, Chiu CW, Chen JX, et al. Pretreatment with sildenafil alleviates early lung ischemia-reperfusion injury in a rat model. J Surg Res. 2013;185(2):e77-83. Doi: 10.1016/j.jss.2013.07.010. PMID: 23953793

17. Beheshtian A, Salmasi AH, Payabvash S, Kieumehr S, Ghazinezami B, Rahimpour S, et al. Protective effects of sildenafil administration on testicular torsion/detorsion damage in rats. World J Urol. 2008;26(2):197-202. Doi: 10.1007/s00345-008-0243-6. PMID: 18265987

18. Gruijters MJG, Visser JA, Durlinger ALL, Themmen APN. Anti mullerian hormone and its role in ovarian function. Mol Cell Endocrinol. 2003;211(1-2):85-90. Doi: 10.1016/j.mce.2003.09.024. PMID: 14656 480

19. Ockaili R, Salloum F, Hawkins J, Kukreja RC. Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K(ATP) channels in rabbits. Am J Physiol Heart Circ Physiol. 2002;283(3):H1263-9. Doi: 10.1152/ajpheart.00324.2002. PMID: 12181158

20. Hubscher CH, Brooks DL, Johnson JR. A quantitative method for assessing stages of the rat estrous cycle. Biotech Histochem. 2005;80(2):79-87. Doi: 10.1080/ 10520290500138422. PMID: 16195173

21. Li H, Yan B, Wang Y, Shu Z, Li P, Liu Y, et al. The optimal time of ovarian reserve recovery after laparoscopic unilateral ovarian non-endometriotic cystectomy. Front Endocrinol (Lausanne). 2021;12:671225. Doi: 10.3389/ fendo.2021.671225. PMID: 34630317, PMCID: PMC 849 8098

22. Yurtcu E, Togrul C, Ozyer S, Uzunlar O, Karataş YH, Seckin KD. Dose dependent protective effects of vardenafil on ischemia-reperfusion injury with biochemical and histopathologic evaluation in rat ovary. J Pediatr Surg. 2015;50(7):1205-09. Doi: 10.1016/j. jpedsurg. 2014.12.013. PMID: 25783344.

23. Kalyoncu Ş, Yilmaz B, Demir M, Tuncer M, Bozdağ Z, Ince O, et al. Melatonin attenuates ovarian ischemia reperfusion injury in rats by decreasing oxidative stress index and peroxynitrite. Turk J Med Sci. 2020;50(6):1513-22. Doi: 10.3906/sag-2004-135. PMID: 32927928, PMCID: PMC7605088.

24. Kalyoncu S, Yilmaz B, Demir M, Tuncer M, Bozdag Z, Ince O, et al. Octreotide and lanreotide decrease ovarian ischemia-reperfusion injury in rats by improving oxidative and nitrosative stress. J Obstet Gynaecol Res. 2020;46 (10):2050-8. Doi: 10.1111/jog.14379. PMID: 32748523

25. Demir M, Yilmaz B, Kalyoncu S, Tuncer M, Bozdag Z, Ince O, et al. Metformin reduces ovarian ischemia reperfusion injury in rats by improving oxidative/nitrosative stress. Taiwan J Obstet Gynecol. 2021;60(1):45-50. Doi: 10.1016/j.tjog.2020.10.004. PMID: 33495007

26. Ozler A, Turgut A, Soydinç HE, Sak ME, Evsen MS, Alabalik U. The biochemical and histologic effects of adnexal torsion and early surgical intervention to unwind detorsion on ovarian reserve: an experimental study. Reprod Sci 2013;20:1349-55. Doi: 10.1177/19337191 13485300. PMID: 23585344

27. Soyman Z, Kelekci S, Sal V, Sevket O, Bayındır N, Uzun H. Effects of Apigenin on Experimental Ischemia/ Reperfusion Injury in the Rat Ovary. Balkan Med J. 2017;34(5):444-9. Doi: 10.4274/balkanmedj.2016.1386. PMID: 28443590, PMCID: PMC5635632

28. Sahin Ersoy G, Eken M, Tal R, Oztekin D, Devranoglu B, Isik Kaygusuz E, Cevik O. N-acetylcysteine leads to greater ovarian protection than enoxaparin sodium in a rat ovarian torsion model. Reprod Biomed Online. 2016; 33(1):93-101. Doi: 10.1016/j.rbmo.2016.03.009. PMID: 27083693.

29. Calis P, Bozdag G, Karakoc Sokmensuer L, Kender N. Does ischemia-reperfusion injury affect ovarian reserve and follicle viability in a rat model with adnexal torsion? Eur J Obstet Gynecol Reprod Biol. 2015;185:126-30. Doi: 10.1016/j.ejogrb.2014.12.006. PMID: 25575140.

30. Sugita A, Iwase A, Goto M, Nakahara T, Nakamura T, Kondo M, et al. One-year follow-up of serum antimüllerian hormone levels in patients with cystectomy: are dif-

ferent sequential changes due to different mechanisms causing damage to the ovarian reserve? Fertil Steril. 2013;100(2):516-22.e3. Doi: 10.1016/j.fertnstert.2013.03.032. PMID: 23579006.

31. Oelsner G, Cohen SB, Soriano D, Admon D, Mashiach S, Carp H. Minimal surgery for the twisted ischaemic adnexa can preserve ovarian function. Hum Reprod. 2003; 18(12):2599-602. Doi: 10.1093/humrep/deg498. PMID: 14645177

32. Pena JE, Ufberg D, Cooney N, Denis AL. Usefulness of doppler sonography in the diagnosis of ovarian torsion. Fertil Steril. 2000;73(5):1047-50. Doi: 10.1016/s0015-0282(00)00487-8. PMID: 10785237