Research Journal of Pharmacology and Toxicology 02[03] 2016
www.asdpub.com/index.php/rjpt

Original Article

The effect of erythropoietin on ovarian epithelium edema during ischemia reperfusion injury in rats

C. Tsompos[*1], C. Panoulis[2], K Toutouzas[3], A. Triantafyllou[4], G. Zografos[5] and A. Papalois[6]

[1]Consultant A, Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece
[2]Assistant Professor, Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece
[3]Assistant Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
[4]Associate Professor, Department of Biologic Chemistry, Athens University, Attiki, Greece
[5]Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
[6]Director, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, attiki, Greece

***Corresponding Author**
Tsompos Constantinos
Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece
E-mail: Tsomposconstantinos@gmail.com

Keywords:
Ischemia,
Ovarian epithelium edema,
Reperfusion,
Erythropoietin

Abstract

Objective: This experiment investigated a probable recessing effect of erythropoietin (Epo) in a rat model of ovarian ischemia-reperfusion (IR) injury concerning the mean ovarian epithelium edema (OE) lesions.
Methods: 40 rats of mean weight 247.7 g were used totally. The OE lesions scores were evaluated at 60 min (groups A and C) and at 120 min (groups B and D) of reperfusion; after Epo administration in groups C and D.
Results: Epo administration non-significantly decreased the OE scores by 0.3 without lesions [-0.8356043 - 0.2356043] (p= 0.2803). Reperfusion time kept non-significantly increased the OE by 0.35 without lesions [-0.2356043 - 0.8356043] (p=0.2557). However, erythropoietin administration and reperfusion time together non-significantly decreased the OE scores by 0.1272727 without lesions [-0.4530022 - 0.1984567] (p=0.4339).
Conclusion: Epo administration showed a non-significant short-term recessing trend for OE scores without lesions alteration. Perhaps, a longer study time than 2 hours or a higher Epo dosage may reveal clearer and significant effects.

1. Introduction

Erythropoietin (Epo) is a glycoprotein hormone that controls the erythropoiesis, or red blood cell (RBC) production. It is about a cytokine (protein signaling molecule) for rbc precursors production in bone marrow. This is the reason that Epo implicates over 29,116 known biomedical studies. 1003 (3.44%) at least of these studies concern experiments of tissue ischemia-reperfusion (IR). Further research interests occupy whether Epo is able to reverse any injuries of targeted IR tissues as well adjacent organs and of course the patients' health. The investigation of basic answers as, its reaction velocity, the time of administration and the the dosage height fill in this context. The benefits of this cytokine may be further than the original action of Epo. However, the more specific matters, the fewer related reports are found. A numeric outcome of the Epo efficacy was yielded by a meta-analysis of 32 published serum variables. These outcomes from the same experimental setting, for the same endpoints (Table 1).

The certain aim of this biomedical work was to test the effect of Epo on a rat model and mainly in an ovarian IR protocol. The Epo effect was particularly tested on mean ovarian epithelium edema (OE) lesions.

2. Materials and methods

2.1 Pats preparation

The vet Address of East Attiki Prefecture licensed this experimental work by 3693/12-11-2010 No and 14/10-1-2012 No decisions. ELPEN Pharmaceuticals Co Inc. S.A. granted all substances, equipment and consumable. Humanistic care was adopted for the female albino Wistar rats. This care started already pre-experimentally by 7 days housing in laboratory with *ad libitum* diet. Intra-experimental prenarcosis of animals, continuous general anesthesia [1-7], electrocardiogram, acidometry, oxygen supply and post-experimental euthanasia were followed. The rats were randomly classified into four groups of 10 animals each one. These 4 groups were submitted into preceded ischemia of 45 min induced by laparotomic clamping of inferior aorta over the renal arteries. Then, the clamp removal restored the reperfusion after inferior aorta patency reestablishment. Reperfusion lasted 60 min for group A, 120 min for group B, 60min along with Epo intravenous (IV) administration for group C and 120 min along with Epo IV administration for group D. The dosage height for cytokine was 10 mg/kg body mass of animal. Epo administration at the time of reperfusion set was inserted through inferior vena cava catheter. The OE lesions evaluations were performed 60 min after reperfusion for A and C groups and 120 min after reperfusion for B and D groups. The mean of the forty (40) female Wistar albino rats mass used was 247.7 g [Standard Deviation (SD): 34.99172 g], with min weight 165 g to max weight 320 g. Thus, pathologic evaluation[8] and classification of OE findings was outcome as scores: 0 for none lesions, 1 for mild ones, 2 for moderate ones and 3 for serious ones. The previous classification was transformed as follows: (0-0.499) without OE lesions, (0.5- 1.499) the mild ones, (1.5 - 2.499) the moderate ones and (2.5-3) the serious ones since score ranges replaced the point ones. The OE scores were evaluated by the 1st Pathology Department of Clinical – Laboratory Sector in Faculty of Medicine of Athens University.

2.2 The model of ovarian ischemia-reperfusion

2.2.1 Control branch

This branch included 20 control rats of mean weight 252.5 g [SD: 39.31988 g] submitted into ischemia for 45 min followed by reperfusion. The 2 sub-branches consisted of the group A reperfused by 60 min for 10 controls rats of mean weight 243 g [SD: 45.77724 g] and mean mild OE lesions score 0.7 [SD: 0.8232726] and the group B reperfused by 120 min for 10 controls rats of mean weight 262 g [SD: 31.10913 g] and mean mild OE lesions score 1.1 [SD: 0.9944289] (Table 2).

2.2.2 Erythropoietin branch

This branch included 20 Epo rats of mean weight 242.9 g [SD: 30.3105 g] submitted into ischemia for 45 min followed by both reperfusion and 10 mg Epo/kg body weight IV administration. The 2 sub-branches consisted of the group C reperfused by 60 min for 10 Epo rats of mean weight 242.8 g [SD: 29.33636 g] and mean mild OE lesions score 0.5 [SD: 0.7071068] and the group D reperfused by 120 min for 10 Epo rats of mean weight 243 g [SD: 32.84644 g] and mean mild OE lesions score 0.7 [SD: 0.8232726] (Table 2).

2.3 Statistical analysis

The statistical analysis was performed twice. The one way included the statistical standard t-tests and Wilcoxon signed-rank test for mass and OE lesions scores groups respectively (Table 3). If any probable significant difference among OE lesions scores was revealed, it would be tested whether along any respective probable significant mass one exists (Table 3). The other way was the generalized linear models (glm). They included the OE lesions scores as dependant variable and the Epo administration or no, the reperfusion time and their interaction as the 3 independent variables. Rats' mass could be probably a confusing factor, e.g. the more obese rats to have higher OE lesions scores. This assumption was rejected since the insertion of rats' mass as independent variable at glm, yielded a non significant correlation with OE lesions scores (p=1.0000). The statistical tests were performed by Stata 6.0 statistical software [Stata 6.0, StataCorp LP, Texas, USA].

3. Results

The glm resulted in: Epo administration non-significantly decreased the OE scores by 0.3 without lesions [-0.8356043 - 0.2356043] (p=0.2639). This finding was in accordance with the results of Wilcoxon signed-rank test (p=0.2967). Reperfusion time kept non-significantly increased the OE by 0.3 without lesions [-0.2356043 - 0.8356043] (P= 0.2639), approximately in accordance with Wilcoxon signed-rank test; increased by 0.4 without lesions (-1.014921 - 0.214921) (p= 0.2475). However, erythropoietin administration and reperfusion time together non-significantly decreased the OE scores by 0.1272727 without lesions [-0.4530022 - 0.1984567] (p=0.4339). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the alteration influence of Epo in connection with reperfusion time.

Table 1: The erythropoietin (Epo) influence (±SD) on the levels of some seric1 variables concerning reperfusion (rep) time

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of Epo and rep	p-value
White BCC	+24.01%±13.38%	0.1012	+22.09%±9.11%	0.0163	+20.17%±12.94%	0.0902	+14.63%±5.40%	0.0080
Red BCC	+1.45%±3.31%	0.6589	+0.37%±3.02%	0.9048	-0.70%±4.68%	0.8844	+0.81%±1.79%	0.6446
Hematocrit	+0.14%±2.89%	0.9626	-0.61%±2.37%	0.8072	-1.37%±4.05%	0.7485	+0.24%±1.38%	0.8586
Hemoglobi[6]	+4.09%±5.20%	0.3350	+2.15%±2.63	0.4527	+0.20%±5.08%	0.9584	+1.31%±1.59%	0.3984
MCH	+0.01%±1.29%	0.9904	+0.67%±0.80%	0.3549	+1.34%±1.08%	0.1509	-0.36%±0.47%	0.4430
MCV[5]	+0.01%±1.08%	0.9904	+0.56%±0.66%	0.3549	+1.12%±0.91%	0.1509	+0.30%±0.39%	0.4430
MCHC[3]	+1.82%±0.56%	0.0076	+1.73%±0.50%	0.0016	+1.65%±0.92%	0.0721	+0.89%±0.31%	0.0061
RbcDW	-1.85%±4.24%	0.6703	-1.64%±2.53%	0.5159	-1.43%±3.34%	0.6078	-1.06%±1.43%	0.4733
Plt C[2]	-7.32%±13.11%	0.5219	-2.14%±8.04%	0.7581	+3.04%±10.78%	0.7204	-0.16%±4.76%	0.9725
Platelet DW	+1.60%±0.80%	0.0765	+1.36%±0.58%	0.0205	+1.13%±0.74%	0.1152	+0.37%±0.37%	0.0615
Platelet-crit	-16.47%±10.40%	0.0921	-13.74%±7.01%	0.0158	-11.01%±7.34%	0.0882	-6.88%±3.69%	0.0615
Glucose[7]	+0.75%±8.11%	0.9307	+5.59%±6.46%	0.3208	+10.44%±10.99%	0.3491	+4.94%±3.81%	0.1892
Urea	+21.42%±7.84%	0.0115	+20.11%±7.25%	0.0059	+18.80%±9.44%	0.0709	+15.64%±4.04%	0.0003
Creatinine	-0.10%±9.78%	0.9904	-4.84%±5.78%	0.3721	-9.59%±7.74%	0.1509	-2.62%±3.49%	0.4430
Uric acid	+10.13%±15.10%	0.4917	+15.86%±10.21%	0.1408	+21.59%±15.45%	0.1940	+9.33%±6.16%	0.1264
Total protei	-0.02%±2.47%	0.9904	-1.27%±1.51%	0.3721	-2.52%±2.03%	0.1509	-0.68%±2.48%	0.4430
Albumins	-4.61%±4.21%	0.2530	-9.28%±3.20%	0.0054	-13.96%±5.03%	0.0095	-5.37%±2.73%	0.0072
ALT	+18.89%±12.42%	0.1372	+7.63%±18.94%	0.6396	-3.63%±25.19%	0.8617	+8.03%±11.36%	0.4698
AST	+29.53%±9.72%	0.0096	+26.71%±13.17%	0.0235	+23.89%±21.59%	0.1709	+19.73%±7.70%	0.0119
γGT	-19.35%±18.58%	0.2362	-12.70%±13.11%	0.3541	-6.06%±19.96%	0.7800	-4.62%±7.97%	0.5534
ALP	+0.20%±18.57%	0.9904	+10.70%±12.78%	0.3549	+21.20%±17.11%	0.1509	+5.79%±7.72%	0.4430
ACP	+0.06%±5.79%	0.9904	+3.11%±3.71%	0.3172	+6.16%±4.97%	0.1509	+1.68%±2.23%	0.4430
CPK	+0.15%±14.09%	0.9904	+7.91%±9.44%	0.3549	+15.67%±12.65%	0.1509	+4.28%±5.70%	0.4430
CK-MB[4]	+0.08%±7.90%	0.9904	+4.28%±5.11%	0.3721	+8.49%±6.85%	0.1509	+2.32%±3.09%	0.4430
LDH	+0.08%±7.92%	0.9904	+4.48%±5.35%	0.3549	+8.89%±7.17%	0.1509	+2.42%±3.22%	0.4430
Sodium	+0.72%±0.74%	0.3054	+0.21%±0.63%	0.7136	-0.29%±1.09%	0.7670	-0.11%±0.38%	0.7531
Potassium	-6.17%±4.94%	0.1540	-2.21%±3.66%	0.5134	+1.74%±5.43%	0.7299	+0.18%±2.22%	0.9338
Calcium	0.28%±1.19%	0.8065	-0.56%±1.13%	0.5761	-1.41%±2.08%	0.4100	-0.34%±0.68%	0.6095
Phosphorus	+1.92%±5.25%	0.6982	+3.95%±3.35%	0.2100	+5.98%±4.81%	0.2930	+2.45%±2.01%	0.2168
Magnesium	+1%±6.20%	0.8596	-1.09%±3.34%	0.7248	-3.19%±3.90%	0.3729	-0.19%±1.93%	0.9197
Amylase	+6.50%±9.15%	0.4161	+5.04%±6.12%	0.3831	+3.59%±8.42%	0.6649	+4.36%±3.65%	0.2258
Progesteron	-0.20%±18.65%	0.9904	-8.86%±10.58%	0.3549	-17.53%±14.15%	0.1509	-4.79%±6.39%	0.4430
Mean	+2.15%±9.92%	0.5824	+2.67%±9.06%	0.3645	+3.2%±10.34%	0.3583	+2.26%±5.91%	0.4041

Table 2: Weight and ovarian epithelium edema (OE) score mean levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	OE	mild 0.7	0.8232726
B	Weight	262 g	31.10913 g
	OE	mild 1.1	0.9944289
C	Weight	242.8 g	29.33636 g
	OE	mild 0.5	0.7071068
D	Weight	243 g	32.84644 g
	OE	mild 0.7	0.8232726

Table 3: Statistical significance of mean values difference for groups (DG) after statistical paired t test application for weight and Wilcoxon signed-rank test for scores

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	OE	without lesions -0.4	0.3307
A-C	Weight	0.2 g	0.9900
	OE	without lesions 0.2	0.6371
A-D	Weight	0 g	1.0000
	OE	without lesions 0	1.0000
B-C	Weight	19.2 g	0.0478
	OE	mild 0.6	0.1511
B-D	Weight	19 g	0.2113
	OE	without lesions 0.4	0.3095
C-D	Weight	-0.2 g	0.9883
	OE	without lesions -0.2	0.6190

Table 4: The decreasing influence of erythropoietin in connection with reperfusion time

			p-values	
Decrease	**95% c. in.**	**Reperfusion time**	**Wilcoxon**	**glm**
without lesions 0.2	-0.9210105 - 0.5210105	1h	0.6371	0.5673
without lesions 0.3	-0.8356043 - 0.2356043	1.5h	0.2967	0.2639
without lesions 0.4	-1.257698 - 0.4576978	2h	0.3095	0.3402
without lesions -0.3	-0.2356043 - 0.8356043	reperfusion time		0.2639
without lesions -0.4	-1.014921 – 0.214921	reperfusion time	0.2475	
without lesions	0.1272727 -0.4530022	-0.1984567	interaction	0.4339

Table 5: Concise presence of the decreasing influence of erythropoietin in connection with reperfusion time

Decrease	95% c. in.	Reperfusion time	p-values
without lesions 0.2	-0.9210105 - 0.5210105	1h	0.6022
without lesions 0.3	-0.8356043 - 0.2356043	1.5h	0.2803
without lesions 0.4	-1.257698 - 0.4576978	2h	0.3248
without lesions -0.35	-0.62526265 – 0.52526265	reperfusion time	0.2557
without lesions 0.1272727	-0.4530022 - 0.1984567	interaction	0.4339

4. Discussion

The following clinical situations show the association between ischemia and OE. Ergenoglu *et al* [9] revealed higher scores for follicular degeneration and edema ($p < 0.0001$) in IR-induced ovarian sections treated with edaravone compared with sham ones in female Sprague Dawley rats. Chuderland *et al*[10] explained that ovarian hyperstimulation syndrome (OHSS) is induced by the ovarian release of a vasoactive, angiogenic substance that results in vascular hyperpermeability, leakage, and shift of fluids from blood vessels into the extravascular space with consequent ascites and edema, attributed to vascular endothelial growth factor (VEGF) in mice. Kline *et al*[11] included massive edema among other nonneoplastic conditions of the ovary that may present as adnexal masses. Seidman *et al* [12] noticed edema, congestion, and dilated lymphatic channels characterizing the tubal-peritoneal junction as a potential site of carcinogenesis in fallopian tube specimens. Cansu *et al* [13] determined that valproic acid (VPA) and oxcarbazepine (OXC) increased apoptosis and intracytoplasmic edema in female prepubertal Wistar rats 21-24 days old. Hosfield *et al* [14] noticed that long-term peritoneal dialysis develops edema of the peritoneal membrane, but the morphologic effects on the organs of female genital tract are obscure. Lubo-Palma *et al* [15] evaluated the edema of cadmium both in ovary cortex and in interfollicular zone of Swiss albino healthy females mice. Khalbuss *et al* [16] described the first case of an ovarian serous cystadenoma associated with a massive ovarian edema in a 17-year-old female. The solid mass had an intact capsule and diffuses interstitial edema, preserving the overall structure of the ovary and sparing the outer cortex. Gordon *et al*[17] noticed one patient (1.12%) among ten who withdrew because of edema related with the drug stealth liposomal doxorubicin in platinum- and paclitaxel-refractory ovarian cancer. Young *et al* [18] discussed tumors that occur in pregnancy which often have prominent intercellular edema. Espey *et al*[19] detected loosening of the connective tissue elements and some indication of edema at the apex of the mature follicles during the ovulatory process in indomethacin-treated rabbits compared with normal prostaglandin synthesis ovulatory follicles. Volkova OV *et al* [20] demonstrated that the structure of follicular components such as internal ovulation is accompanied with increasing interstitial edema. Motta *et al* [21] observed that fluidlike material was to (1) infiltrate the connective tissue of the tunica albuginea, (2) accumulate under the basal lamina, (3) distend intercellular spaces of the superficial epithelium and (4) a local increase of fluids (edema) may be an important factor in the final decomposition of the distended and weakened apex of the preovulatory follicle. Cherney *et al*[22] noticed extensive edema followed by rupture of the follicular wall within 10 hours after copulation in the apex of rabbit ovarian follicles.

The following situations show the association between Epo and ischemic ovaries. Mahmoodi *et al* [23] found that Epo reduced IR injury and free radical production, increasing follicle survival and function in transplanted ovarian tissue. Sayyah-Melli *et al*[24] determined that rEpo was effective in reducing the oxidative damage of ovarian torsion in operated patients, 18-35 years old, with signs and symptoms of ovarian torsion. Karaca *et al*[25] evaluated the Epo administration as effective in reversing tissue damage induced by IR in ovaries of adult female rats. Suzuki *et al* [26] demonstrated that administration of asialo Epo could effectively enhance the survival of the follicles of transplanted cryopreserved ovaries in frozen-thawed canine ovarian xenotransplantation. However, David *et al* [27] did not detect expression of Epo mRNA in porcine ovaries. Kristiansson *et al* [28] concluded that females with carbohydrate-deficient glycoprotein syndrome type I have primary ovarian failure, but the syndrome does not affect the terminal charged carbohydrate portion in Epo. Hyttinen JM *et al* [29] generated a transgenic calf from in vitro produced bovine embryos microinjected with a gene construct consisting of genomic sequences encoding human Epo. Kamiński M [30] claimed that apoptosis regulates the atrophy of completely developed organs, e.g. thymus, and the hormonal restructuring of ovaries and others but on the other hand,

the development of apoptosis is arrested by so called "survival factors" as Epo.

5. Conclusion

Epo administration showed a non-significant short-term recessing trend for OE scores without lesions alteration. Perhaps, a longer study time than 2 hours or a higher Epo dosage may reveal clearer and significant effects.

Acknowledgment

This study was funded by Scholarship by the Experimental Research Center ELPEN Pharmaceuticals (E.R.C.E), Athens, Greece. The research facilities for this project were provided by the aforementioned institution.

References

[1] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The Effect of Erythropoietin on Aspartate Aminotransferase Levels during Ischemia Reperfusion Injury in Rats. *Electronic Journal of Biology*, 2016, 12(2): 161-167.

[2] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The acute trend of erythropoietin on platelet count during hypoxia reoxygenation injury in rats. *Medical Channel Karachi* 2016; 22 (1): 7-13.

[3] Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The acute effect of erythropoietin on mean corpuscular hemoglobin concentration levels during hypoxia - reoxygenation injury in rats. *J Med Soc* 2016;30:79-83.

[4] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The effect of erythropoietin on creatine kinase MB levels during hypoxia reoxygenation injury in rats. *Research Journal of Pharmacology and Toxicology* 2015; 1(5): 51-53.

[5] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The acute effect of erythropoietin on mean corpuscular volume levels during hypoxia-reoxygenation injury in rats. *Journal of Cellular Biotechnology* 2016; 2: 77–83.

[6] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The short-term effect of erythropoietin on hemoglobin during ischemia reperfusion injury in rats. *Fiziologia* 2013; 23.3 (79): 26-32.

[7] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The Acute Effect of Erythropoietin on Glucose Levels during Ischemia Reperfusion Injury. *FMI* 2016;52:14-18.

[8] Osmanağaoğlu MA, Kesim M, Yuluğ E, Menteşe A, Karahan SC. Ovarian-protective effects of clotrimazole on ovarian ischemia/reperfusion injury in a rat ovarian-torsion model. *Gynecol Obstet Invest.* 2012;74(2):125-30.

[9] Ergenoglu M, Erbaş O, Akdemir A, Yeniel AÖ, Yildirim N, Oltulu F, Aktuğ H, Taskiran D. Attenuation of ischemia/reperfusion-induced ovarian damage in rats: does edaravone offer protection? *Eur Surg Res.* 2013;51(1-2):21-32.

[10] Chuderland D, Ben-Ami I, Kaplan-Kraicer R, Grossman H, Ron-El R, Shalgi R. The role of pigment epithelium-derived factor in the pathophysiology and treatment of ovarian hyperstimulation syndrome in mice. *J Clin Endocrinol Metab.* 2013;98(2):E258-66.

[11] Kline RC, Bazzett-Matabele LB. Adnexal masses and malignancies of importance to the colorectal surgeon. *Clin Colon Rectal Surg*. 2010 Jun;23(2):63-71.

[12] Seidman JD, Yemelyanova A, Zaino RJ, Kurman RJ. The fallopian tube-peritoneal junction: a potential site of carcinogenesis. *Int J Gynecol Pathol*. 2011 Jan;30(1):4-11.

[13] Cansu A, Erdogan D, Serdaroglu A, Take G, Coskun ZK, Gurgen SG. Histologic and morphologic effects of valproic acid and oxcarbazepine on rat uterine and ovarian cells. *Epilepsia.* 2010 Jan;51(1):98-107.

[14] Hosfield EM, Rabban JT, Chen LM, Zaloudek CJ. Squamous metaplasia of the ovarian surface epithelium and subsurface fibrosis: distinctive pathologic findings in the ovaries and fallopian tubes of patients on peritoneal dialysis. *Int J Gynecol Pathol.* 2008; 27(4):465-74.

[15] Lubo-Palma A, Nava-Leal C, Villasmil V, Quevedo AL, Montiel M, Simoes D, Faría C. Effects of cadmium on the ovarian parenchyma in Swiss albino mice. Invest Clin. 2006 Sep;47(3):219-31.

[16] Khalbuss WE, Dipasquale B. Massive ovarian edema associated with ovarian serous cystadenoma: a case report and review of the literature. *Int J Gynecol Cancer*. 2006 Jan-Feb;16 Suppl 1:326-30.

[17] Gordon AN, Granai CO, Rose PG, Hainsworth J, Lopez A, Weissman C, Rosales R, Sharpington T. Phase II study of liposomal doxorubicin in platinum- and paclitaxel-refractory epithelial ovarian cancer. *J Clin Oncol.* 2000 Sep;18(17):3093-100.

[18] Young RH. Sertoli-Leydig cell tumors of the ovary: review with emphasis on historical aspects and unusual variants. *Int J Gynecol Pathol.* 1993 Apr;12(2):141-7.

[19] Espey LL, Coons PJ, Marsh JM, LeMaire WJ. Effect of indomethacin on preovulatory changes in the ultrastructure of rabbit Graafian follicles. *Endocrinology*. 1981 Mar;108(3):1040-8.

[20] Volkova OV, Alkadarskaia IM, Milovidova NS. Morphologic changes in the ovary during ovulation. *Arkh Anat Gistol Embriol.* 1980 Aug;79(8):5-18.

[21] Motta P, Van Blerkom J. A scanning electron microscopic study of the luteo-follicular complex. II. Events leading to ovulation. *Am J Anat*. 1975 Jun;143(2):241-63.

[22] Cherney DD, Didio LJ, Motta P. The development of rabbit ovarian follicles following copulation. *Fertil Steril.* 1975 Mar;26(3):257-70.

[23] Mahmoodi M, Soleimani Mehranjani M, Shariatzadeh SM, Eimani H, Shahverdi A. Effects of erythropoietin on ischemia, follicular survival, and ovarian function in ovarian grafts. *Reproduction*. 2014 Apr 10;147(5):733-41.

[24] Sayyah-Melli M, Rashidi MR, Kaseb-Ganeh M, Rashtchizadeh N, Taghavi S, Ouladsahebmadarek E, Ghojazadeh M. The effect of erythropoietin against oxidative damage associated with reperfusion following ovarian detorsion. *Eur J Obstet Gynecol Reprod Biol*. 2012 Jun;162(2):182-6.

[25] Karaca M, Odabasoglu F, Kumtepe Y, Albayrak A, Cadirci E, Keles ON. Protective effects of erythropoietin on ischemia/reperfusion injury of rat ovary. *Eur J Obstet Gynecol Reprod Biol.* 2009 Jun;144(2):157-62.

[26] Suzuki H, Ishijima T, Maruyama S, Yanagimoto Ueta Y, Abe Y, Saitoh H. Beneficial effect of desialylated erythropoietin administration on the frozen-thawed canine ovarian xenotransplantation. *J Assist Reprod Genet.* 2008 Nov-Dec;25(11-12):571-5.

[27] David RB, Blom AK, Sjaastad OV, Harbitz I. The porcine erythropoietin gene: cDNA sequence, genomic sequence and expression analyses in piglets. *Domest Anim Endocrinol*. 2001; 20(2):137-47.

[28] Kristiansson B, Stibler H, Wide L. Gonadal function and glycoprotein hormones in the carbohydrate-deficient glycoprotein (CDG) syndrome. *Acta Paediatr*. 1995; 84(6):655-9.

[29] Hyttinen JM, Peura T, Tolvanen M, Aalto J, Alhonen L, Sinervirta R, Halmekytö M, Myöhänen S, Jänne J. Generation of transgenic dairy cattle from transgene-analyzed and sexed embryos produced in vitro. *Biotechnology* (N Y). 1994 Jun;12(6):606-8.

[30] Kamiński M. Processes of cell necrosis--apoptosis--and their modification by toxic substances. *Med Pr*. 1994;45(3):267-77.

