Journal of Genetics and Genetic Engineering
Volume 2, Issue 2, 2018, PP 6-10

The co-evaluation of endosalpingeal edema and oviductal congestion after the erythropoietin effect on fallopian ischemia reperfusion injury

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

[1]*Constantinos Tsompos: Consultant A, Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios" Thessaloniki, Greece*

[2]*Constantinos Panoulis: Assistant Professor, Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, Greece*

[3]*Konstantinos Toutouzas: Assistant Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Greece*

[4]*Aggeliki Triantafyllou: Associate Professor, Department of Biologic Chemistry, Athens University, Athens, Attiki, Greece*

[5]*George C. Zografos: Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Greece*

[6]*Apostolos Papalois: Director, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Greece*

[7]*Kalliopi Tsarea: Researcher, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Greece*

[8]*Maria Karamperi: Researcher, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Greece*

***Corresponding Author:** Tsompos Constantinos, Department of GynecologyGeneral Hospital of Thessaloniki "St. Dimitrios" 2 Elenis Zografou street,Thessaloniki 54634 GreeceTel: 00302313322171 & 00306946674264Fax: 00302106811215Tsomposconstantinos@gmail.com*

ABSTRACT

Aim: *This study co-evaluated the 2 quoted histologic variables after the cytokine erythropoietin (Epo) administration. The calculation was based on the results of 2 preliminary studies, each one evaluating a respective histologic variable of endosalpingeal edema (EE) or oviductal congestion (OC) in an induced ischemia reperfusion (IR) animal experiment.*

Materials and methods: *The 2 main experimental endpoints at which the EE and OC scores were evaluated, were the reperfusion 60th min (for A & C groups) and the reperfusion 120th min (for B & D groups). Specially, the groups A and B were processed without drugs, whereas the groups C and D after Epo administration.*

Results: *The first preliminary study showed that Epo non significantly inflated the EE scores by the grade "without lesions" 0.1818182 [-.0111079 - 0.3747442] (p-value=0.0640). The second preliminary study showed that Epo non significantly deflated the OC scores by the grade "without lesions" 0.1090909 [-0.3078424 - 0.0896606] (p-value=0.2735). Both studies were co-estimated since they belong to the same experimental setting. This study co-evaluated the combined diagnostic values of both variables together.*

Conclusions: *Epo hardly non significantly inflated both scores for these histologic parameters at the grade of "without lesions" 0.0363636 [-0.1017439 - 0.1744712] (p-value=0.5971) since they were co-evaluated together.*

Keywords: *ischemia, erythropoietin, endosalpingeal edema, oviductal congestion, reperfusion*

INTRODUCTION

Erythropoietin (Epo) was investigated whether having antioxidant capacities. 2 histologic variables in a fallopian ischemia reperfusion (FIR) experiment were tested for this purpose. The one variable was that of endosalpingeal edema (EE) which was non significantly

inflated by the grade "without lesions" 0.1818182±0.09843166 (p-value=0.0640) [1]. The other variable was that of oviductal congestion (OC) which was non significantly deflated by the grade "without lesions" 0.1090909±0.10140383 (p-value=0.2735) [2]. Although Epo is met in over 29,992 published biomedical studies, only a 3.53% of them negotiate its antioxidant capacities. The present experimental work tried to co-evaluate these EE and OC variables together and to compare its outcome with each one separately, from the same rat induced FIR protocol.

MATERIALS AND METHODS

Animal Management

The Vet No 3693/12-November-2010 & 14/10-January-2012 licenses, the auspices company, the experimental location and the Pathology Department are mentioned in preliminary references[1,2]. The human animal care of female *Wistar* Albino rats, the one week pre-experimental *ad libitum* diet, the intra-experimental anesthesiologic techniques, the acidometry, the electrocardiogram and the oxygen supply and post-experimental euthanasia are also described in preliminary references. Rats were 16 – 18 weeks old. They were randomly assigned to four (4) groups consisted in N=10. The common stage of 45 min ischemia was preceded in all 4 groups. Afterwards, 60 min reperfusion was followed in group A; 120 min in group B; immediate Epo intravenous (IV) administration and 60 min reperfusion in group C; and immediate Epo IV administration 120 min in group D. The dose height was assessed at pre-experimental phase as 10 mg/Kg body mass.

The Ischemia-Reperfusion Injury Model

Ischemia was induced by laparotomic clamping the inferior aorta upper the renal arteries level with forceps for 45 min. The forceps removal was restoring the inferior aorta blood patency and reperfusion. Epo was administered at the time of reperfusion; through an inferior vena cava catheter. The EE and OC scores were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (for B and D groups). The pathologic score grading was maintained the same as in preliminary studies: (0-0.499) grade without lesions, (0.5-1.499) grade mild lesions, (1.5 - 2.499) grade moderate lesions and (2.5-3) grade serious lesions damage. Relation was rised between animals' mass with neither EE scores (p-value= 0.9834) nor with OC ones (p-values= 0.0585).

Table1.*Endosalpingeal edema (EE), oviductal congestion* (OC) *and their mean and SD scores*

	Mean EE score ±SD	**Mean OC score ±SD**	**Mean EE&OC score ±SD**
Group A	without lesions 0±0.00	mild lesions 0.5±0.5270463	Without lesions 0.25±0.2635231
Group B	without lesions 0.2±0.421637	without lesions 0.4±0.6992059	without lesions 0.3±0.421637
Group C	mild lesions 0.6±0.6992059	without lesions 0±0.00	without lesions 0.3±0.3496029
Group D	Without lesions 0.4±0.5163978	without lesions 0.3±0.4830459	without lesions 0.35±0.4116363

Placebo groups

The 20 placebo rats were the same for preliminaries and this study.

Group A

60 min reperfusion concerned 10 placebo rats of combined EE and OC (EE&OC) score as the mean of EE score and OC one (Table 1).

Group B

120 min reperfusion concerned 10 placebo rats of combined EE&OC (cEE&OC) score as the mean of EE and OC one (Table 1).

Epo group

The 20 Epo rats were the same for preliminaries and this study.

Group C

60 min reperfusion concerned 10 Epo rats of cEE&OC score as the mean of EE score and OC one (Table 1).

Group D

120 min reperfusion concerned 10 Epo rats of cEE&OC score as the mean of EE score and OC one (Table 1).

Statistical Analysis

Successive comparisons among the 4 cEE&OC groups were performed applying Wilcoxon

signed-rank test (Table 2). Then, the generalized linear models (glm) were applied with dependant variable the cEE&OC scores. Epo administration or no, the reperfusion time and their interaction were used as independent variables.

Table2.*The values difference for groups (DG) after Wilcoxon signed-rank test for mean EK&S scores.*

DG	Difference	p-value
A-B	-0.05	0.8717
A-C	-0.05	0.7055
A-D	-0.1	0.3173
B-C	0	0.8732
B-D	-0.05	0.6547
C-D	-0.05	0.9068

RESULTS

Epo administration hardly non significantly inflated the cEE&OC scores by the "without alterations" grade 0.05 [-0.15685335 - 0.25685335] (p=0.6125) by both Wilcoxon signed-rank test and glm methods respectively. Reperfusion time non significantly deflated the cEE&OC scores by "without alterations" grade 0.125 [-0.0936119 - 0.335681] (p=0.3555) by the similar methodology. Finally, Epo administration and reperfusion time together also hardly non significantly inflated the cEE&OC scores by the "without alterations" grade 0.0363636 [-0.1017439 - 0.1744712] (p-valus=0.5971). A concise form of the above findings is depicted at table 4.

Table3.*The alteration influence of Epo in connection with reperfusion time. p-values*

Alteration	95% c. in.	Reperfusion time	wilkoxon	glm
without alterations 0.05	-0.2631815 - 0.3631815	1h	0.7055	
without alterations 0.05	-0.2803343 0.3803343	1h		0.7541
without alterations 0.05	-0.1792929 0.2792929	1.5h		0.6614
without alterations 0.05	-0.1344138 - 0.2344138	1.5h	0.5637	
without alterations 0.05	-0.2139183 - 0.3139183	2h	0.6547	
without alterations 0.15	-0.2019001 0.5019001	2h		0.3823
without alterations 0.05	-0.1792929 0.2792929	reperfusion		0.6614
without alterations 0.2	0.0079309 - 0.3920691	reperfusion	0.0497	
without alterations 0.0363636	-0.1017439 0.1744712	interaction		0.5971

Table4. *Concise form of the table 3.*

Increase	95% c. in.	Reperfusion time	p-value
without alterations 0.05	-0.2717579 0.3717579	1h	0.7298
without alterations 0.05	-0.15685335 0.25685335	1.5h	0.6125
without alterations 0.1	-0.2079092 0.4079092	2h	0.5185
without alterations 0.125	-0.0936119 0.335681	reperfusion	0.3555
without alterations 0.0363636	-0.1017439 0.1744712	interaction	0.5971

DISCUSSION

Adamyan LV et al considered[3] the principal advantage of fibrin glue anastomoses than microsurgical anastomoses to reduce surgical trauma to oviduct stumps and absence of tissue ischemia. These features promote reparative regeneration and decrease adhesion formation, resulting in complete recanalization of fallopian tubes. Castadot RG protected against salpingitis, other pelvic infections and against tubal pregnancies after combined oral contraceptives administration[4]. Estrogens are clearly responsible for some of the complications, apparently due to a weakening of the fibrinolytic systems, but progestagens or estrogen-progestagen combinations are also implicated. Guennoun A et al reported[5] the case of a pregnant presenting with acute lateropelvic pain. Normal adnexal torsion is rare during pregnancy. Çılgın H et al indicated[6] that plasma heat shock protein 70 level could be used as a serum marker in the early detection of adnexal torsion since its significant increase in the study group was 1.50-fold and 1.47-fold respectively (P = 0.001) than that in the laparotomy and control groups, following 12 h of adnexal torsion. Ayachi A et al reported[7] two cases of adnexal torsion during the second trimester of pregnancy; presenting with appendix syndrome the one and acute left iliac fossa pain the other. Early treatment could avoid irreversible damages due to ischemia which could be fertility-threatening. Laparotomy revealed the

torsion of a hydatid of Morgagni whose necrotic appearance due to twisting required hydatid ablation. Sukkong K et al evaluated[8] clinical risk factors predictive of torsion with gangrenous adnexa estimated at ≃ 46.2%. Adnexal torsion results in ischemia of structures distal to twisted pedicle and acute onset of pain is responsible for about 3% of all gynecologic emergencies especially in young nulliparous women. Lee MH et al reviewed[9] all computed tomography signs of adnexal torsion with the exception of deviation of the uterus to the twisted side. However, for a twisted vascular pedicle, there was moderate agreement in patients with a mass and no agreement for patients without a mass. Damasceno RW et al concluded[10] a decrease in elastic fibers with ultrastructural abnormalities and an overexpression of elastin-degrading enzymes as the consequence of local ischemia, inflammation, and/or chronic mechanical stress. Aging with progressive loss of tone and laxity may affect the adnexal tissues, resulting in different clinical symptoms and signs. Spinelli C et al described[13] the conservative treatment for adnexal torsion, consisting of detorsion, as the best surgical approach to guarantee the future reproductive capacity of patients. Tunç SY et al observed[12] degeneration of epithelium, loss of cilia, dilation of blood vessels, and hemorrhages in sections of the ischemic group in the fallopian tube structure following ovarian torsion. The studied fallopian section revealed a significant decrease in density of desmin in the torsion group. Moreover, strong positive cytoplasmic CD68 expression was observed in the torsion group. Türk E et al found that adnexal torsion and detorsion significantly increased[13] the tissue level of malondialdehyde, superoxide dismutase and reduced glutathione, whereas hypothermia inhibited their production as well the histopathological changes in rats. Calis P et al found[14] only the loss of cohesion to be significantly different by 1.28-fold than control sides (p=0.017) in terms of the means of total tissue damage. Significantly lower PCNA counts were revealed in the 16-hour torsion group only in a rat model with adnexal torsion. PCNA confirms the viability of the counted follicles and appears to be a more precise approach necessary for demonstrating the functional status than net mean primordial+primary follicle count which were comparable in twisted and control sides. Navve D et al associated[15] the lateral whirlpool sign with enlarged masses the mean volume of which among cases was significantly greater by 2.81-fold than those with the medial whirlpool sign (P = 0.035). Sánez HA et al described that adnexal torsion over its pedicle produces lymphatic and venous stasis, later it develops into ischemia and necrosis, when is not treated. Hirth D et al identified[17] cell necrosis by high mobility group box 1 protein and apoptosis by Caspase 3a staining of tissue samples taken at 3 endpoints postburn. Furthermore, endothelial cell necrosis was deeper than interstitial cell necrosis at 1 hour ($p < 0.001$). Endothelial cell necrosis at 1 hour divided the zone of injury progression (Jackson's zone of stasis) into an upper subzone with necrotic endothelial cells and initially viable adnexal and interstitial cells at 1 hour that progressed to necrosis by 24 hours and a lower zone with initially viable endothelial cells at 1 hour but necrosis and apoptosis of all cell types by 24 hours in a validated porcine model of vertical burn injury progression. Ozler A et al found[18] the mean number of preantral and small antral follicles lower and only AMH levels significantly decreased following the 3-hour IR ($P < .05$) in detorsion group than those of the sham group ($P < .01$). After torsion, anti-Müllerian hormone (AMH), estradiol, and inhibin B levels were decreased significantly than preoperative and postoperative periods (P = 0.032). A numeric evaluation[19] of the Epo efficacies was provided by a meta-analysis of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion time coming from the same experimental setting (table 5).

Table5. *The Epo influence (±SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time*[19]

35 Variables	**1h rep**	**p-value**	**1.5h rep**	**p-value**	**2h rep**	**p-value**	**interaction of Epo and rep**	**p-value**
Mean	+3.39%±12.15 %	0.5636	+4.44%±14.50 %	0.3711	+5.49%±18.55 %	0.3496	+2.83%±7.13 %	0.4045

CONCLUSION

Epo hardly non significantly inflated the cEE&OC scores by the "without alterations" grade (p-valus=0.5971) creating a suspicion for beneficial usage in situations such as tubal pregnancies, fertility, elastic and desmin ultrastructure, aging, tone, laxity and cohesion,

regeneration of epithelium, conservation of cilia, blood vessel diameter regulation and lymphatic and venous stasis, cytoplasmic CD68, antioxidant markers, PCNA counts, mobility group box 1 protein, caspase 3a staining, anti-Müllerian hormone, estradiol and inhibin B presence or absence, ischemia, cell necrosis and apoptosis.

ACKNOWLEDGEMENT

Acknowledged in preliminary studies

REFERENCES

[1] Tsompos C., Panoulis C., Toutouzas K., Triantafyllou A., Zografos G., Papalois A. The effect of erythropoietin on endosalpingeal edema during ischemia reperfusion injury in rats. E-Biology International Journal.2017;1(1):01-07.

[2] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G and Papalois A. The Effect of Erythropoietin on Oviductal Congestion during Ischemia Reperfusion Injury in Rats. JOJ Material Sci 1(4): JOJMS.MS.ID.555569 (2017).

[3] Adamyan LV, Myinbayev OA, Kulakov VI. Use of fibrin glue in obstetrics and gynecology: a review of the literature. Int J Fertil. 1991 Mar-Apr;36(2):76-7, 81-8.

[4] Castadot RG. Oral contraception in 1983 (author's transl). Contracept Fertil Sex (Paris). 1982 Nov;10(11):753-7.

[5] Guennoun A, Krimou Y, Mamouni N, Errarhay S, Bouchikhi C, Banani A. Normal adnexal torsion and pregnancy: about a case. Pan Afr Med J. 2017 Jul 14;27:197.

[6] Çılgın H, Şimşek M, Bal R. Can adnexal torsion be predicted by measuring plasma heat shock protein 70 level? An experimental study. Arch Gynecol Obstet. 2017 Nov;296(5):941-946.

[7] Ayachi A, Blel Z, Khelifa N, Mkaouer L, Bouchahda R, Mourali M. Adnexal torsion during the second trimester of pregnancy: about two cases. Pan Afr Med J. 2016 Oct 25;25:113.

[8] Sukkong K, Sananpanichkul P, Teerakidpisan P, Bhamarapravatana K, Suwannarurk K. High Rate of Gangrenous Adnexal Torsion: Dilemma of a Missing Silent Cancer. Asian Pac J Cancer Prev. 2016 Nov 1;17(11):4981-4984.

[9] Lee MH, Meyers N, Raptis CA, Mellnick VM. Interobserver reliability for computed tomography findings of adnexal torsion. Emerg Radiol. 2017 Feb;24(1):21-24.

[10] Damasceno RW, Avgitidou G, Belfort R Jr, Dantas PE, Holbach LM, Heindl LM. Eyelid aging: pathophysiology and clinical management. Arq Bras Oftalmol. 2015 Sep-Oct;78(5):328-31.

[11] Spinelli C, Piscioneri J, Strambi S. Adnexal torsion in adolescents: update and review of the literature. Curr Opin Obstet Gynecol. 2015 Oct;27(5):320-5.

[12] Tunç SY, Ağaçayak E, Yaman NS, Deveci E, Kalkanlı S, Özler A. Effects of adnexal torsion on the Fallopian tube in rats: a histologic and immunohistochemical study. Anal Quant Cytopathol Histpathol. 2014 Oct;36(5):285-9.

[13] Türk E, Karaca İ, Ozcinar E, Celebiler A, Aybek H, Ortac R, Güven A. The effect of hypothermia on adnexal torsion/detorsion injury in a rat ovary model. J Pediatr Surg. 2015 Aug;50(8):1378-81.

[14] 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 Feb;185:126-30.

[15] Navve D, Hershkovitz R, Zetounie E, Klein Z, Tepper R. Medial or lateral location of the whirlpool sign in adnexal torsion: clinical importance. J Ultrasound Med. 2013 Sep;32(9):1631-4.

[16] Sánez HA, Taboada-Pérez GC, Hernández-Arroyo L, Mateo-Madrigal M, Mateo-Madrigal V. Adnexal torsion: three cases. Ginecol Obstet Mex. 2013 May;81(5):272-8.

[17] Hirth D, McClain SA, Singer AJ, Clark RA. Endothelial necrosis at 1 hour postburn predicts progression of tissue injury. Wound Repair Regen. 2013 Jul-Aug;21(4):563-70.

[18] Ozler A, Turgut A, Soydinç HE, Sak ME, Evsen MS, Alabalik U, Basarali MK, Deveci E. The biochemical and histologic effects of adnexal torsion and early surgical intervention to unwind detorsion on ovarian reserve: an experimental study. Reprod Sci. 2013 Nov;20(11):1349-55.

[19] C. Tsompos, C. Panoulis, K. Toutouzas, A. Triantafyllou, G. Zografos, A. Papalois. The effect of erythropoietin on chloride levels during hypoxia reoxygenation injury in rats. Signa Vitae 2017; 13(2):97-101.