Free Radicals and Antioxidants. 2022;12(2):70-73
A Multifaceted Journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.phcog.net

Original Article

The Rat Fallopian Tubes after Erythropoietin Process

C Tsompos[1,*], C Panoulis[2], A Triantafyllou[3], CG Zografos[4], E Gerakis[5], S Gerakis[5] A Papalois[6]

ABSTRACT

Aim: The capability of erythropoietin (EPO) as antioxidant is exported by the results of 2 preliminary studies, totally evaluating 4 histologic variables of endosalpingeal edema (EE) and oviductal congestion (OC) of the first one and endosalpingeal karyorrhexis (EK) and salpingitis (S) of the other one. **Materials and Methods:** The antioxidant capability was evaluated upon the 60th reperfusion min (for groups called A and C) and upon the 120th reperfusion min (for groups called B and D). The groups A and B were placebo ones, whereas the groups C and D included EPO as antioxidant. **Results:** The first pillar study showed that EPO has an oxidant non-significant potency for EE and OC together (*p*-values=0.5971); grade: "without lesions" alterations 0.0363636 [-0.1017439 - 0.1744712]. The second pillar study showed also an oxidant non-significant potency for EK and S; grade: "without lesions" alterations 0.0090909 [-0.0339659 - 0.0521478] (*p*-values=0.6715). The 2 above studies were added trying to calculate a common diagnostic value for all the four variables. **Conclusion:** EPO as antioxidant is a significant recessing agent for the total of the 4 histologic variables; grade: "without lesions" alterations score 0.0136364 [-0.0887489+0.0614762] (*p*-value=0.7153).

Keywords: Ischemia, Erythropoietin, Endosalpingeal edema, Oviductal congestion, Endosalpingeal karyorrhexis, Salpingitis, Reperfusion.

C Tsompos[1,*], C Panoulis[2], A Triantafyllou[3], C G Zografos[4], E Gerakis[5], S Gerakis[5] A Papalois[6]

[1]Department of Gynecology, Anticancer Hospital of Thessaloniki "Theageneio", Thessaloniki, HELLAS (GREECE).
[2]Department of Obstetrics and Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, HELLAS (GREECE).
[3]Department of Biological Chemistry, Athens University, Athens, Attiki, HELLAS (GREECE).
[4]Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, HELLAS (GREECE) .
[5]Experimental Research Centre, ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, HELLAS (GREECE).
[6]Experimental, Educational and Research Center, ELPEN, European University Cyprus, School of Medicine, CYPRUS.

Correspondence

Dr. Tsompos Constantinos,

Department of Gynecology, Anticancer Hospital of Thessaloniki "Theageneio", Thessaloniki 54639, HELLAS (GREECE).

Email id: constantinos1tsompos@gmail.com

History
- Submission Date: 23-10-2022;
- Review completed: 17-11-2022;
- Accepted Date: 05-12-2022.

DOI : 10.5530/fra.2022.2.12

Article Available online
http://www.antiox.org

INTRODUCTION

Erythropoietin (EPO) is rumored as an antioxidant agent. 4 histologic variables were studied in a fallopian ischemia reperfusion (FIR) experiment for this purpose. Two variables, those of endosalpingeal edema (EE) and oviductal congestion (OC) were studied separated showing a hardly oxidant capacity (*p*-values=0.5971); grade: "without lesions" alterations 0.0363636 [-0.1017439 - 0.1744712].[1] The other two variables, those of endosalpingeal karyorrhexis (EK) and salpingitis (S) were also studied separated showing a non-significantly oxidant capacity; grade: "without lesions" 0.0090909 [-0.0339659 - 0.0521478] (*p*-values=0.6715).[2] Although Epo is involved in over 33,433 published biomedical studies, only a 1.12% of them mention its antioxidant capacities. This study co-evaluates thus all the 4 EE, OC, EK and S variables together so as to export a unique outcome, since it is about a common FIR setting.

MATERIALS AND METHODS

Animal preparation

Two successive ethics committee approvals numbers were received: 3693/12-11- 2010 and 14/10-1-2012 fully complied with the tenants of the Declaration of Helsinki. The grant company, the experiment position and the Pathology Laboratory are referred in pillar references.[1,2] The reader is referred also at the same references about the Albino female Wistar rats, their *ad libitum* diet, the anesthesiologic techniques [acidometry, electrocardiograms, oxygen supply, post-experimental euthanasia], their age, their random assignation to four 10 members groups, their predecessor stage of 45 min ischemia, as well the selective placebo reperfusion of 60 min in group A; 120 min in group B; also selective Epo intravenous (IV) administration and reperfusion of 60 min in group C; EPO IV administration and reperfusion of 120 min in group D and finally the dose height assessment as this of 10 mg/Kg body mass.

The same references provide details for the laparotomic clamping of the inferior aorta over renal arteries as well the procedure of restoring. Epo was administered when selected at the time of reperfusion; through an inferior vena cava catheter. The EE, OC and EK, S scores were written down at 60th min of reperfusion (A&C groups) and at 120th min of reperfusion (B&D groups). No relation was found between animals' mass with either EE scores (*p*-value=0.9834), or with OC ones (*p*-values= 0.0585), or with EK scores (*p*-value=0.7202), or with S ones (*p*-values=1). The pathologic system score grading was kept same with pillar 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.

Cite this article: Tsompos C, Panoulis C, Triantafyllou A, Zografos CG, Gerakis E, Gerakis S, Papalois A. The Rat Fallopian Tubes after Erythropoietin Process. Free Radicals and Antioxidants. 2022;12(2):70-3.

Model of Ischemia-reperfusion Injury

Control: 20 rats were used as control for all the pillar and this study.

Group A: Selected 60 min reperfusion concerned 10 controls rats of combined EE, OC and EK, S fallopian score (cFS) as the means of the respective variables (Table 1).

Group B (Erythropoietin group): Selected 120 min reperfusion concerned 10 controls rats of combined EE, OC and EK, S fallopian score (cFS) as the means of the respective variables (Table 1).

20 rats were used as Epo for all the pillar and this study.

Group C: Selected 60 min reperfusion concerned 10 Epo rats of combined EE, OC and EK, S fallopian score (cFS) as the means of the respective variables (Table 1).

Group D: Selected 60 min reperfusion concerned 10 Epo rats of combined EE, OC and EK, S fallopian score (cFS) as the means of the respective variables (Table 1).

Statistical Analysis

Wilcoxon signed-rank test was used to compare every cFS group score with each other from 3 remained groups (Table 2). Then, the generalized linear models (glm) were following: dependent variable: The cFS scores, independent variables: the Epo administration or no, the reperfusion time and their interaction.

RESULTS

Epo was proved a non-significant antioxidant agent for the 4 histologic variables: grade "without lesions" alterations score 0 [-0.11607555 +0.11607555] (p-value=0.8503), after co-calculation by both Wilcoxon signed-rank test and glm methods (Table 3). Similarly, placebo reperfusion was proved a non-significant oxidant agent accentuating the 4 histologic variables: grade "without lesions" alterations score 0.025 [-0.08615235 +0.13615235] (p-value=0.6636), after the same tests co-calculation (Table 3). Totally, Epo administration and placebo reperfusion time were proved together as a borderline significant antioxidant agent recessing the 4 histologic variables: grade "without lesions" alterations score 0.0136364 [-0.0887489 +0.0614762] (p-value=0.7153) (Table 3). A condensed form of the above outcomes is depicted at Table 4.

Table 1: Endosalpingeal edema (EE), oviductal congestion (OC), endosalpingeal karyorrhexis (EK) and salpingitis (S) and their mean and SD scores.

	Mean EE&OC score ±SD	Mean EK&S score ±SD	Mean EE&OC&EK&S score ±SD
Group A	Without lesions 0.25 ±0.2635231	Without lesions 0	Without lesions 0.125 ±0.1317616
Group B	Without lesions 0.3 ±0.421637	Without lesions 0	Without lesions 0.15 ±0.2108185
Group C	Without lesions 0.3 ±0.3496029	Without lesions 0.1 ±0.2108185	Without lesions 0.2 ±0.2297341
Group D	Without lesions 0.35 ±0.4116363	Without lesions 0	Without lesions 0.175 ±0.2058182

Table 2: The values difference for groups (DG) after Wilcoxon signed-rank test for all histologic variables mean scores.

DG	Difference	p-value
A-B	+0.025	0.8717
A-C	+0.075	0.3601
A-D	+0.05	0.3173
B-C	+0.05	0.5632
B-D	+0.025	0.6547
C-D	-0.025	0.4126

Table 3: The altering influence of erythropoietin in connection with reperfusion time.

Alteration	95% c. in.	Reperfusion time	Wilcoxon	Glm
Without lesions alterations 0.075	-0.0946618 +0.2446618	1h	0.3601	0.3823
Without lesions alterations 0	-0.11607555 +0.11607555	1.5h	0.7006	1.0000
Without lesions alterations 0.025	-0.1235632 +0.1735632	2h	0.6547	0.7541
Without lesions alterations 0	-.1247812 +0.1247812	Reperfusion		1.0000
Without lesions alterations +0.05	-0.0475235 +0.1475235	Reperfusion	0.3273	
Without lesions alterations -0.0136364	-0.0887489 +0.0614762	Interaction		0.7153

Table 4: Concise form of the Table 3.

Alteration	95% c. in.	Reperfusion time	p-value
Without lesions alterations +0.075	-0.0946618 +0.2446618	1h	0.3712
Without lesions alterations 0	-0.11607555+0.11607555	1.5h	0.8503
Without lesions alterations +0.025	-0.1235632 +0.1735632	2h	0.7044
Without lesions alterations +0.025	-0.08615235+0.13615235	Reperfusion	0.6636
Without lesions alterations -.0136364	-0.0887489 +0.0614762	Interaction	0.7153

DISCUSSION

Viveiros *et al.* reduced[3] back a strangulated femoral hernia including a tube into the pelvic cavity. As the hernia was repaired, the patient was discharged without complications, 3 days after operation in a 61-year-old female patient. Merhi *et al.* suggested[4] that ozone therapy could have beneficial effect on tubal occlusion, from endometritis, vaginitis, ovaries from ischemia and oocyte loss and pelvic adhesions. Ahmed *et al.* increased[5] awareness of deep venous thrombosis (DVT) after a sigmoid colon and left fallopian tube and ovary resection with colostomy diversion performance in a 39-year-old female. Posto-perative mesenteric ischemia caused by heparin-induced thrombocytopenia (HIT) occurred and finally, the patient ended up with short bowel syndrome. Sahin *et al.* caused[6] a greater than 2-fold increase in bone marrow-derived cells (BMDC) recruitment localized to the stroma of the oviduct. They demonstrated that IR injury to oviduct recruits BMDCs to this tissue and suggested that BMDCs have function in the healing process in oviducts of reproductive age female mice. Zhang *et al.* reported[7] that hypoxia-inducible factor 1-alpha (HIF-1-alpha) promoted cancer progression, enhanced tumor invasion and metastasis via activating downstream

Table 5: The erythropoietin (Epo) influence (±SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time.[20]

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

genes, such as matrix metalloproteinases (MMPs) more in ovarian cancer tissues and metastatic lesions than in normal fallopian tissues. HIF-1alpha and MMP13 expression were closely related. Colina *et al*. thought[8] high grade serous ovarian cancer (HGSOC) to progress from a series of precursor lesions in the fallopian tube epithelium (FTE). One of the preneoplastic lesions found in the FTE is called a secretory cell outgrowth (SCOUT), which is partially defined by a loss of PAX2. Loss of PAX2 alone in the murine oviductal epithelium (MOE) did not induce changes in proliferation, migration, survival in hypoxia, or contribute to resistance to first line therapies. MOE PAX2shRNA cells had higher estrogen signaling activity and higher expression of putative estrogen responsive genes both in the presence and absence of exogenous estrogen in the fallopian tube. Huang *et al*. found[9] that the effect of HIF-1-alpha on cancers may be correlated with autophagy and some signaling pathways, such as PI3K/AKT/mTOR, in several tumors. HIF-1-alpha was expressed at higher levels in epithelial or metastatic ovarian cancer tissue than in normal fallopian tube tissue. When HIF-1-alpha was knocked down by siRNA in A2780 and SKOV3 cells, the apoptosis and autophagy were strengthened. Accordingly, autophagosome formation increased and the expression of autophagy-related proteins LC3 and P62 increased in HIF-1alpha knockdown cells. Chang *et al*. recently implicated[10] a regulated in development and DNA damage response (REDD1), a gene responding to hypoxia or multiple DNA damage events, in cancer development and progression. High cytoplasmic expression of REDD1 was correlated with ominous poorer outcomes ($p < 0.001$). Dorayappan *et al*. found[11] that hypoxic ovarian cancer cells derived exosomes (HEx) are proficient in re-programming the immortalized fallopian tube secretory epithelial cells (FT) to become pro-tumorigenic in mouse fallopian tubes. Blocking exosome release by known inhibitor Amiloride or STAT3 inhibitor and treating with cisplatin resulted in a significant increase in apoptosis, decreased colony formation and proliferation. Asci H. *et al*. counted[12] oxidative stress (OS) markers increased in fallopian tubes in the cigarette smoking (CS) group. Marked cilial loss in the fallopian tubes and HIF-1α immunoreactions were observed in tubal epithelial cells of the CS group. CS has negative effects on the female reproductive system via HIF-1α in tuba uterina and alpha lipoic acid (ALA) could protect against the negative effects of CS in a rat model. Mitamura *et al*. found[13] that the microseminoprotein, prostate-associated (MSMP) secretion from cancer cells was induced by hypoxia, triggering MAPK signaling in endothelial cells to promote tube formation *in vitro*. These findings imply that MSMP inhibition combined with the use of antiangiogenesis drugs may be a new strategy to overcome resistance to antiangiogenesis therapy. Aiken *et al*. showed[14] that exposure to chronic gestational hypoxia leads to accelerated ageing of the oviduct in early adulthood and they help us understand how exposure to hypoxia during development could influence reproductive health across generations. Specifically, the oviducts of female rats exposed to chronic hypoxia in utero have reduced telomere length, decreased mitochondrial DNA biogenesis and increased oxidative stress. Gestational hypoxia-exposed oviducts also showed evidence of reduced gene expression of Tfam ($p < 0.05$) and Pgc1α ($p < 0.05$). In the hypoxia-exposed oviducts, there was upregulation of mitochondrial-specific anti-oxidant defence enzymes (MnSOD; $p < 0.01$) in Wistar rats. Williamson *et al*. maintained[15] embryos in pre-ovipositional embryonic arrest in the hypoxic oviduct for different lengths of time, allowing longer inter-nesting intervals in olive ridley turtle (Lepidochelys olivacea) eggs. Lim *et al*. described[16] a low oxygen IVF environment of the antral follicle and oviduct/uterus, respectively. Interestingly, hemoglobin (Hb) was recently found in human cumulus and granulosa cells and murine cumulus-oocyte complexes and preimplantation embryos. They explored the potential clinical applications and benefits of Hb supplementation during the *in vitro* culture of gametes and embryos. A numeric evaluation[17] of the Epo antioxidant efficacies was provided by a meta-analysis of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion time coming also from the above experimental setting (Table 5).

CONCLUSION

Epo ia a significant antioxidant agent recessing the 4 histologic variables within the grade: "without lesions" alterations score 0.0136364 [-0.0887489 - +0.0614762] (p-value=0.7153). this encourages for a beneficial usage in femoral hernias, tubal occlusion, endometritis, pelvic adhesions, heparin-induced thrombocytopenia, IR injured oviducts, cancer development and progression, enhanced tumor invasion and metastasis, precursor lesions recession in the fallopian tube epithelium, secretory cell outgrowth, estrogen signaling activity and expression of putative estrogen responsive genes in the fallopian tube, immortalizing fallopian tube secretory epithelial cells as pro-tumorigenic, decreased dsDNA damage and increased cell survival and apoptosis, decreased colony formation and proliferation, cilial loss in fallopian tubes, immunoreactions in tubal epithelial cells, protection on the female reproductive system, overcome resistance to antiangiogenesis therapy, ageing of the oviduct, upregulation of mitochondrial-specific anti-oxidant defence enzymes, fertility, oocyte maturation and preimplantation embryo development in assisted reproductive technologies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos C, Tsarea K, *et al*. The co-evaluation of endosalpingeal edema and oviductal congestion after the erythropoietin effect on fallopian ischemia reperfusion injury. Genet Genet Eng. 2018;2(2):6-10.
2. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos CG, Papalois A, *et al*. The co-evaluation of endosalpingeal karyorrhexis and salpingitis after the erythropoietin effect on fallopian ischemia reperfusion injury. J Surg Med. 2018;2(2):65-8.
3. Viveiros D, Lázaro A, Carvalho H. Femoral hernia containing the right Fallopian tube: A rare finding. Rev Bras Ginecol Obstet. 2019;41(8):520-2. doi: 10.1055/s-0039-1693055, PMID 31450259.
4. Merhi Z, Garg B, Moseley-LaRue R, Moseley AR, Smith AH, Zhang J. Ozone therapy: A potential therapeutic adjunct for improving female reproductive health. Med Gas Res. 2019;9(2):101-5. doi: 10.4103/2045-9912.260652, PMID 31249259.
5. Ahmed M, Habis S, Samotowka M, Mahmoud A, Chin M. Mesenteric ischemia caused by heparin-induced thrombocytopenia: A case report. Cureus. 2019;11(1):e3900. doi: 10.7759/cureus.3900, PMID 30911456.
6. Sahin C, Mamillapalli R, Taylor HS. Bone marrow-derived cells trafficking to the oviduct: Effect of ischemia-reperfusion injury. Reprod Sci. 2018;25(7):1037-44. doi: 10.1177/1933719118770552, PMID 29658434.
7. Zhang H, Yang Q, Lian X, Jiang P, Cui J. Hypoxia-inducible factor-1α (HIF-1α) promotes hypoxia-induced invasion and metastasis in ovarian cancer by targeting matrix metallopeptidase 13 (MMP13). Med Sci Monit. 2019;25:7202-8. doi: 10.12659/MSM.916886, PMID 31587013.

8. Colina JA, Varughese P, Karthikeyan S, Salvi A, Modi DA, Burdette JE. Reduced PAX2 expression in murine fallopian tube cells enhances estrogen receptor signaling. Carcinogenesis. 2020;41(5):646-55. doi: 10.1093/carcin/bgz127, pii: bgz127. PMID 31271204.
9. Huang J, Gao L, Li B, Liu C, Hong S, Min J, *et al.* Knockdown of hypoxia-inducible factor 1α (HIF-1α) Promotes Autophagy and Inhibits phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) Signaling Pathway in Ovarian Cancer Cells. Med Sci Monit. 2019;25:4250-63. doi: 10.12659/MSM.915730, PMID 31175269.
10. Chang B, Meng J, Zhu H, Du X, Sun L, Wang L, *et al.* Overexpression of the recently identified oncogene REDD1 correlates with tumor progression and is an independent unfavorable prognostic factor for ovarian carcinoma. Diagn Pathol. 2018;13(1):87. doi: 10.1186/s13000-018-0754-4, PMID 30428884.
11. Dorayappan KDP, Wanner R, Wallbillich JJ, Saini U, Zingarelli R, Suarez AA, *et al.* Hypoxia-induced exosomes contribute to a more aggressive and chemoresistant ovarian cancer phenotype: A novel mechanism linking STAT3/Rab proteins. Oncogene. 2018;37(28):3806-21. doi: 10.1038/s41388-018-0189-0, PMID 29636548.
12. Asci H, Erol O, Ellidag HY, Tola EN, Savran M, Ozmen O. Pathology of cigarettes on the reproductive system and ameliorative effects of alpha lipoic acid: A rat model study. Toxicol Ind Health. 2018;34(6):385-95. doi: 10.1177/0748233718755160, PMID 29591887.
13. Mitamura T, Pradeep S, McGuire M, Wu SY, Ma S, Hatakeyama H, *et al.* Induction of anti-VEGF therapy resistance by upregulated expression of microseminoprotein (MSMP). Oncogene. 2018;37(6):722-31. doi: 10.1038/onc.2017.348, PMID 29059175.
14. Aiken CE, Tarry-Adkins JL, Spiroski AM, Nuzzo AM, Ashmore TJ, Rolfo A, *et al.* Chronic fetal hypoxia disrupts the peri-conceptual environment in next-generation adult female rats. J Physiol. 2019;597(9):2391-401. doi: 10.1113/JP277431, PMID 30791124.
15. Williamson SA, Evans RG, Robinson NJ, Reina RD. Synchronised nesting aggregations are associated with enhanced capacity for extended embryonic arrest in olive ridley sea turtles. Sci Rep. 2019;9(1):9783. doi: 10.1038/s41598-019-46162-3, PMID 31278292.
16. Lim M, Brown HM, Kind KL, Thompson JG, Dunning KR. Hemoglobin: Potential roles in the oocyte and early embryo†. Biol Reprod. 2019;101(2):262-70. doi: 10.1093/biolre/ioz078, PMID 31058953.
17. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The effect of erythropoietin on chloride levels during hypoxia reoxygenation injury in rats. Signa Vitae. 2017;13(2):97-101. doi: 10.22514/SV132.112017.15.

Cite this article: Tsompos C, Panoulis C, Triantafyllou A, Zografos CG, Gerakis E, Gerakis S, Papalois A. The Rat Fallopian Tubes after Erythropoietin Process. Free Radicals and Antioxidants. 2022;12(2):70-3.