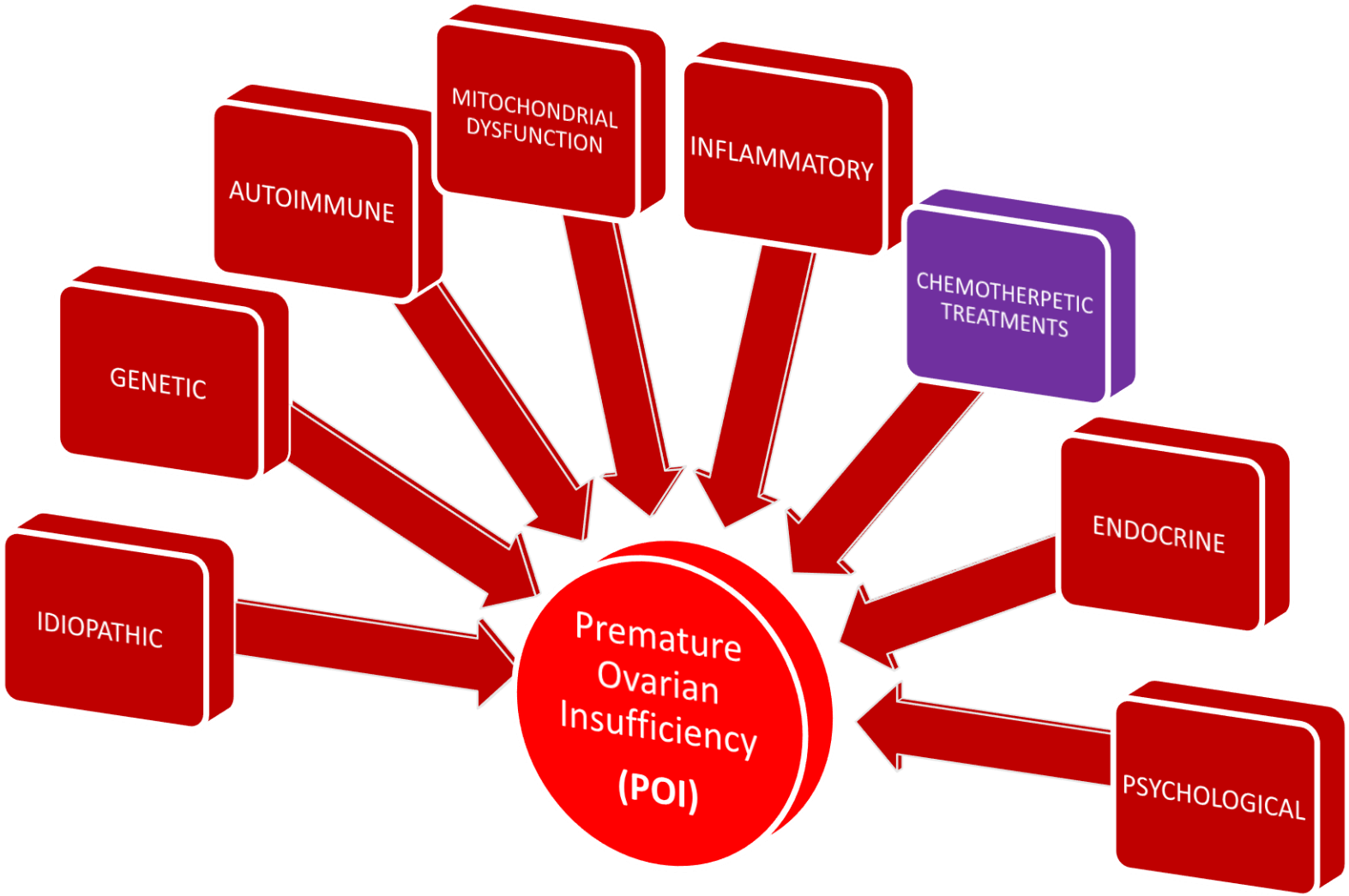
Protective Effect of Glutathione on Ovarian Function in Female Rats with Cyclophosphamide-induced Ovarian Failure

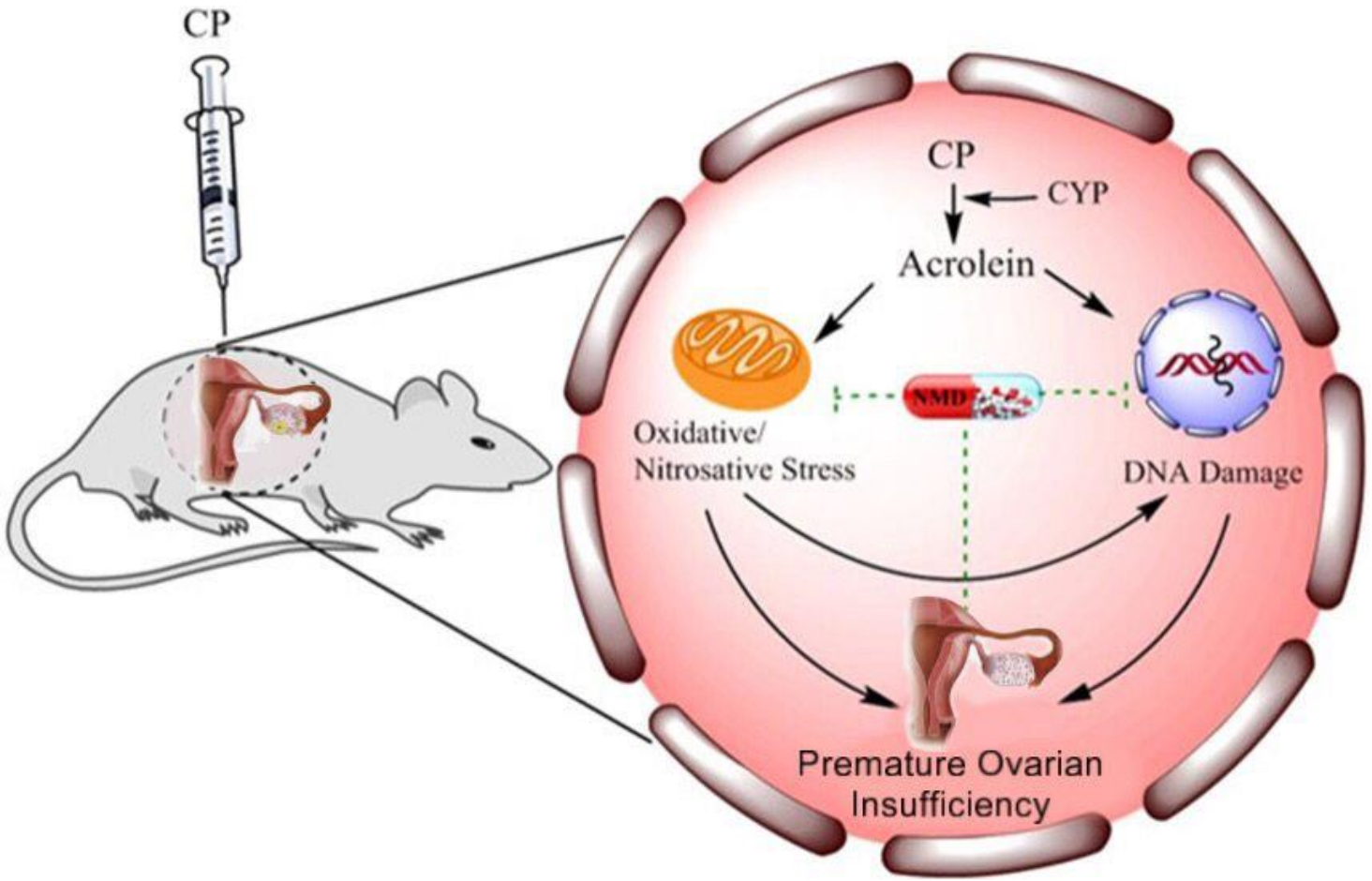
Ebru Tansu Yurttançıkmaz,Pınar Özcan,Fatma Başak Tanoğlu,Olgu Enis Tok



It is seen in approximately 1-3% of women under the age of 40.

Drug	Class (action)
Definitely associated with ovarian damage	
Nitrogen mustard	Nechlorethamine (alkylating agent)
L-phenylalanine mustard	Nechlorethamine (alkylating agent)
Chlorambucil	Chloroethylamine (alkylating agent)
Cyclophosphamide	Chloroethylamine (alkylating agent)
Melphalan	Nechlorethamine (alkylating agent)
Busulfan	Alkylalkane sulfonate (alkylating agent)
Procarbazine	Substituted hydrazine
Dacarbazine	Alkylating agent
Probably associated with ovarian damage	
Vinblastine	Vinca alkaloid
Cytosine arabinoside (Ara-C)	Antimetabolite
Cis-platinum	Heavy metal
Carmustine	Nitrosourea (alkylating agent)
Lomustine	Nitrosourea (alkylating agent)
VP-16 (etoposide)	Podophyllotoxin
Imatinib	Tyrosine kinase inhibitor
Low probability of ovarian damage	
Methotrexate	Antimetabolite
Flucrouracil (5-FU)	Antimetabolite
6-mercaptopurine	Antimetabolite
Vincristine	Vinca alkaloid
Mitomycin	Antibiotic (alkylating agent)
Unknown	
VM-26	Podophyllotoxin
Daunorubicin	Anthracycline
Bleomycin	Peptide
Vindesine	Vinca alkaloid
Doxorubicin	Anthracyclin

How Does Cyclophosphamide Cause the Side Effect?



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FERTILITY PRESERVATION

The protective effect of platelet-rich plasma administrated on ovarian function in female rats with Cy-induced ovarian damage

Pinar Ozcan[1,5] · Taha Takmaz[1] · Olgu Enis Tok[2] · Sevde Islek[1] · Esra Nur Yigit[3] · Cem Ficicioglu[4]

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Abstract

Purpose We evaluated the protective effect of PRP on ovarian function in female rats with cyclophosphamide (Cy)-induced ovarian damage.

Methods Thirty-two adult female Sprague–Dawley rats were randomly divided into four groups. Group 1 (control-sodium chloride 0.9%; 1 mL/kg, single-dose ip injection), group 2 (Cy); 75 mg/kg, single-dose ip injection and sodium chloride 0.9% (1 mL/kg, single-dose ip injection), group 3 Cy plus PRP, Cy (75 mg/kg, single-dose and PRP (200 µl, single-dose) ip injection), group 4 (PRP, 200 µl, single-dose ip injection). Primordial, antral, and atretic follicle counts; serum anti-Müllerian hormone (AMH) levels; AMH-positive granulosa cells; and gene expression analysis of Ddx4 were assessed.

Results Serum AMH levels were significantly lower in group 2 compared to groups 1, 3, and 4 ($p < 0.01$, $p < 0.01$, and $p = 0.04$, respectively). A significant difference was found in the primordial, primary, secondary, antral, and atretic follicle counts between all groups ($p < 0.01$). There was a statistically significant difference in AMH-positive staining primary, secondary, and antral follicles count between the groups ($p < 0.01$). There was a statistically significant difference in primary, secondary, and antral AMH positive staining follicle intensity score between the groups ($p < 0.01$). Ddx4 expression in group 4 was highest compared to other groups.

Conclusion Our study may provide evidence that PRP could protect ovarian function against ovarian damage induced by Cy. It could lead to improved primordial, primary, secondary, and antral follicle numbers.

Keywords Follicle · Ovarian failure · Ovary · Platelet-rich plasma · Stereology

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REPRODUCTIVE PHYSIOLOGY AND DISEASE

Can Coenzyme Q10 supplementation protect the ovarian reserve against oxidative damage?

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Abstract

Purpose We investigated antioxidant effects of CoQ10 supplementation on the prevention of OS-induced ovarian damage and to evaluate the protective effect of such supplementation against OS-related DNA damage.

Methods Twenty-four adult female Sprague–Dawley rats were randomly divided into three groups (8 rats per group): group 1 (control): saline, ip, and orally; group 2 (cisplatin group): cisplatin, 4.5 mg/kg ip, two times with an interval of 7 days; and group 3 (cisplatin + CoQ10 group): cisplatin, 4.5 mg/kg ip, two times with an interval of 7 days, and 24 h before cisplatin, 150 mg/kg/day orally in 1 mL of saline daily for 14 days. Serum concentrations of anti-Mullerian hormone (AMH), number of AMH-positive follicles, the assessment of the intensity of 8'OHdG immunoreactivity, the primordial, antral and atretic follicle counts in the ovary were assessed.

Result(s) The mean serum AMH concentrations were 1.3 ± 0.19, 0.16 ± 0.03, and 0.27 ± 0.20 ng/mL in groups 1, 2, and 3, respectively ($p < 0.01$). Serum AMH levels were significantly higher in group 1 compared to groups 2 and 3 ($p < 0.01$ and $p = 0.01$, respectively). There was a statistically significant difference in AMH-positive follicle count between the groups ($p < 0.01$). Group 1 showed higher numbers of AMH-positive granulosa cells compared to group 2 ($p = 0.01$). A significant difference was found in the primordial, the atretic, and antral follicle counts between the three groups ($p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively). The atretic follicle count was significantly lower in the cisplatin plus CoQ10 group compared to the cisplatin group ($p < 0.01$). The antral follicle counts were significantly higher in the cisplatin plus CoQ10 group compared with the cisplatin group ($p < 0.01$). There was a statistically significant difference in the intensity of staining of the follicles that were positive for anti-8'OHdG between the groups ($p = 0.02$). Group 1 showed a significant lower intensity of staining of the follicles positive for anti-8'OHdG compared with group 2 ($p = 0.03$).

Conclusion(s) CoQ10 supplementation may protect ovarian reserve by counteracting both mitochondrial ovarian ageing and physiological programmed ovarian ageing although the certain effect of OS in female infertility is not clearly known.

Capsule CoQ10 supplementation may protect ovarian reserve by counteracting both mitochondrial ovarian ageing and physiological programmed ovarian ageing.

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Keywords AMH · Ovarian reserve · Oxidative damage · 8'OHdG · Coenzyme Q10

Introduction

Reactive oxygen species (ROS) consisting of superoxide anion, hydroxyl radical (OH), and hydrogen peroxide are naturally generated by normal oxygen metabolism during some physiological conditions. They are generally regulated by enzymatic

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REPRODUCTIVE BIOMEDICINE ONLINE

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Protective effect of resveratrol against oxidative damage to ovarian reserve in female Sprague–Dawley rats

Pınar Özcan · Cem Fıçıcıoğlu · Özge Kızılkale Yıldırım · Ferda Özkan · Hatice Akkaya · İsmail Aslan

Published: June 19, 2015 · DOI: https://doi.org/10.1016/j.rbmo.2015.06.007

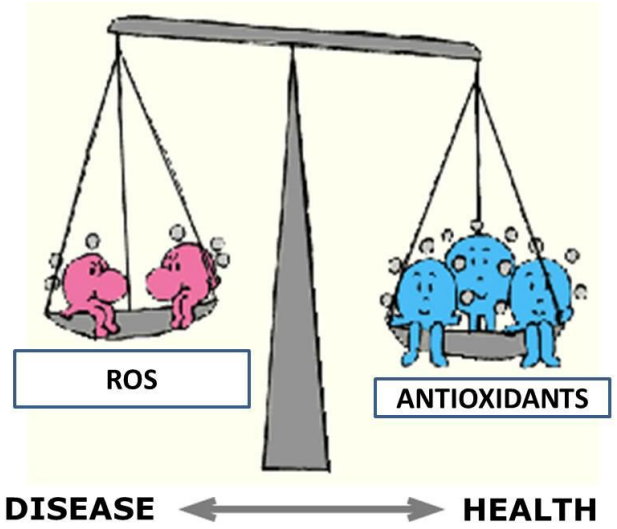
Abstract An increased accumulation of intracellular levels of reactive oxygen species with time may play an important role in the process of ageing. The antioxidant properties of resveratrol are dependent upon the up-regulation of endogenous cellular antioxidant systems. We evaluated whether resveratrol has protective antioxidant effects on ovarian damage related to oxidative stress in a rat model. Twenty-four female rats were randomly divided into three groups and were given saline (group 1: control); intraperitoneal cisplatin, 4.5 mg/kg, two weekly doses in total (group 2); or cisplatin, 4.5 mg/kg plus intraperitoneal resveratrol 10 mg/kg/day, 24 h before the administration of cisplatin (group 3). Serum anti-Müllerian hormone (AMH) concentrations were significantly lower in group 2 than in group 3 ($P < 0.01$ and $P = 0.04$, respectively). The evaluation of the atretic and antral follicle counts revealed statistically significant differences between the groups ($P = 0.04$ and $P < 0.01$, respectively). A statistically significant difference was observed in the follicle count positive for AMH between the groups ($P = 0.01$). Oxidative stress plays an important role in the process of ovarian ageing. Because of its natural antioxidant properties, resveratrol may be an effective option in protecting ovarian tissue against oxidative damage.

KEYWORDS: AMH, ovarian reserve, oxidative damage, resveratrol

A Powerful Antioxidant-Glutathione

- Glutathione is a powerful antioxidant and is necessary for the healthy functioning of mitochondria. It consists of the amino acids **cysteine, glycine and glutamine.**

- It is produced **naturally** in our body.



BEZMİÂLEM VAKIF ÜNİVERSİTESİ

Chmeotherapeutic

Cyclophosphamide

Natural Antioxidant Glutathion

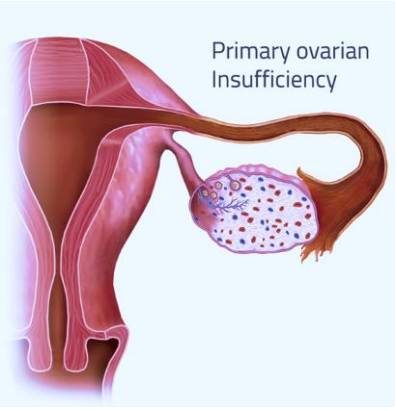
Oksidative Stress

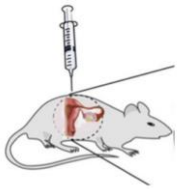
Mitotoxicity

Apoptozis

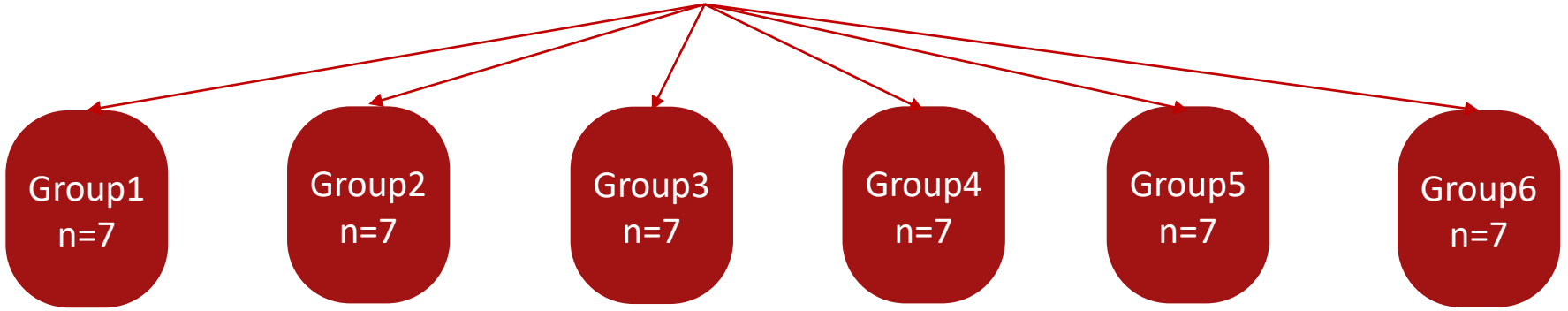
Aim

To evaluate the protective effect of glutathione, an antioxidant, on ovarian function with cyclophosphamide induced ovarian insufficiency, by measuring primordial, primary, secondary, antral and atretic follicle counts, AMH levels, and AMH-positive granulosa.





42 Sprauge-Dawley Rats

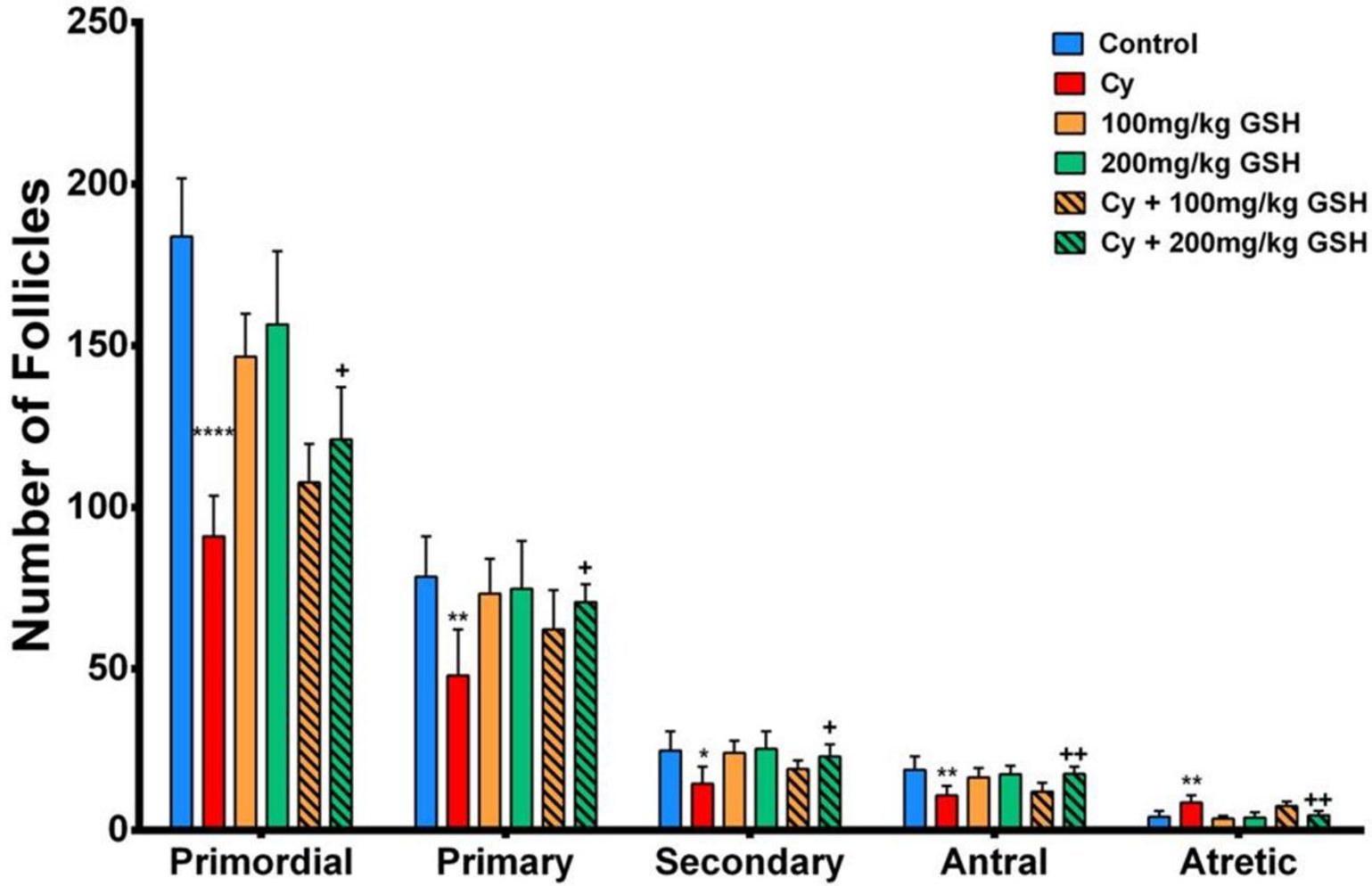


DAY 0	%0.9 NaCl	cy 75 mg/kg	GSH 100 mg/kg	GSH 200 mg/kg	CY 75 m/kg +GSH 100 mg/kg	CY 75 m/kg +GSH 200 mg/kg
DAY 7	%0.9 NaCl	%0.9 NaCl	GSH 100 mg/kg	GSH 200 mg/kg	GSH 100 mg/kg	GSH 200mg/kg
DAY 14	%0.9 NaCl	%0.9 NaCl	GSH 100 mg/kg	GSH 200 mg/kg	GSH 100 mg/kg	GSH 200 mg/kg
DAY 21	Ovarian tissue and blood samples were taken and rats were sacrificed					

Table 1a: Comprasion of primordial, primary, secondary, antral, atretic follicle counts of all groups.

Variables	Control (n=7)	Cy (n=7)	100mg/kg GSH (n=7)	200mg/kg GSH (n=7)	Cy+100mg/kg GSH (n=7)	Cy+200mg/kg GSH (n=7)
Primordial follicle counts	183.8±17.99	91±12.55****	146.6±13.3	156.6±22.61	107.7±11.88	122.7±15.11^{+}
Primary follicle counts	78.5±12.45	48±14.18**	73.2±10.89	74.8±14.84	62.17±12.22	70.67±5.53^{+}
Secondary follicle counts	24.75±5.85	14.4±5.32*	24±3.67	25.2±5.45	19±2.6	22.83±3.81^{+}
Antral follicle counts	18.75±4.11	10.8±3.03**	16.4±2.96	17.4±2.6	12±2.75	17.5±2.16^{++}
Atretic follicle counts	4.25±1.7	8.6±2.3**	3.6±0.89	4±1.58	7.5±1.37	4.66±1.36^{++}

All values are expressed as mean ± SD
* p <0.05, ** p <0.01, ****p<0.0001, compared to control group;
+ p <0.05, ++ p <0.01, compared to cyclophosphamide group.



BEZMİÂLEM VAKIF ÜNİVERSİTESİ

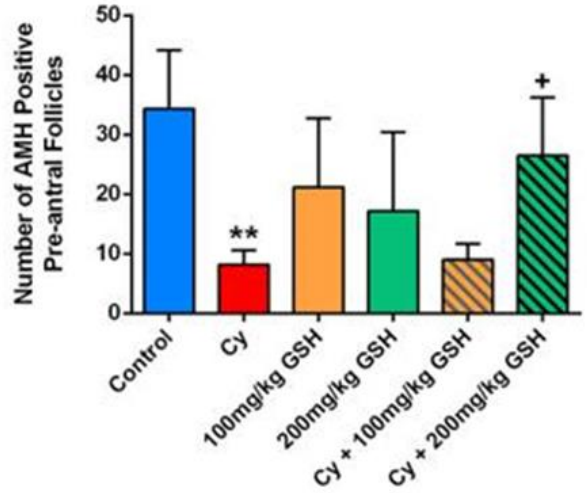
Table 1b: Comparison of the pre-antral and antral AMH-positive staining follicle counts, AMH-positive staining follicle percent, AMH-positive staining follicle intensity

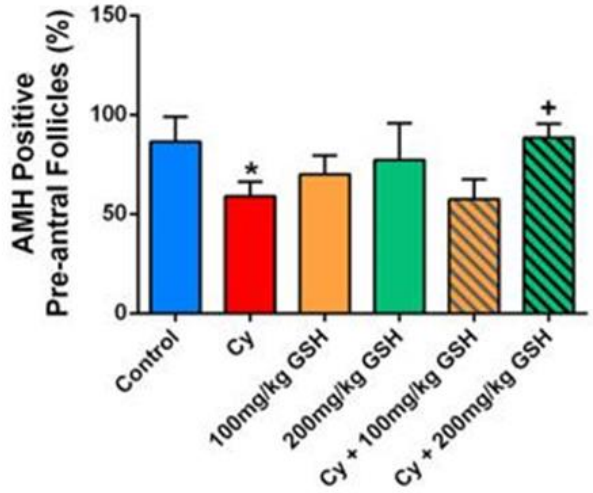
Variables	Control (n=7)	Cy (n=7)	100mg/kg GSH (n=7)	200mg/kg GSH (n=7)	Cy+100mg/kg GSH (n=7)	Cy+200mg/kg GSH (n=7)
Pre-antral AMH positive staining follicle counts	34.33±9.81	8.2±5.4**	21.2±11.52	17.2±13.22	9±2.68	26.5±9.73+
Antral AMH positive staining follicle counts	2.6±0.89	0.4±0.54*	1.6±1.14	1.8±0.83	1.16±0.75	2.33±1.36+
Pre-antral AMH positive staining follicle percent	86.62±12.49	58.9±14.94*	69.88±9.55	77.14±18.64	57.62±10.01	88.48±7.1+
Antral AMH positive staining follicle percent	75±20.41	14.66±14.44*	48.34±38.37	55.34±14.09	43.05±35.13	68.45±8.51+
Pre-antral AMH positive staining follicle intensity score	1.94±0.49	0.53±0.03*	1.08±0.31	1.73±0.9	1.49±0.76	2.26±0.4++
Antral AMH positive staining follicle intensity score	1.3±0.57	0.17±0.23**	0.65±0.54	0.96±0.29	0.47±0.34	1.02±0.3+

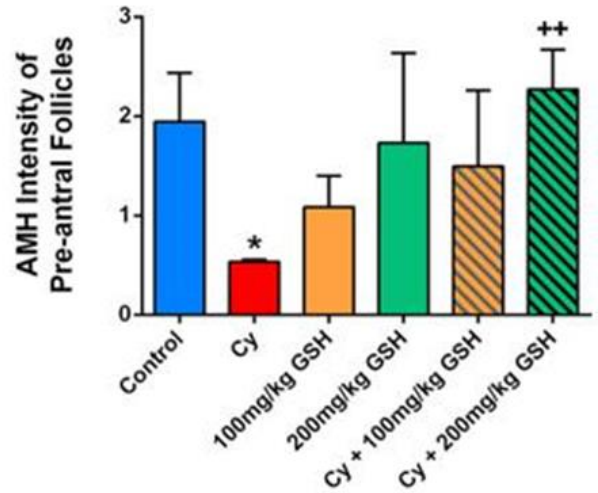
All values are expressed as mean ± SD

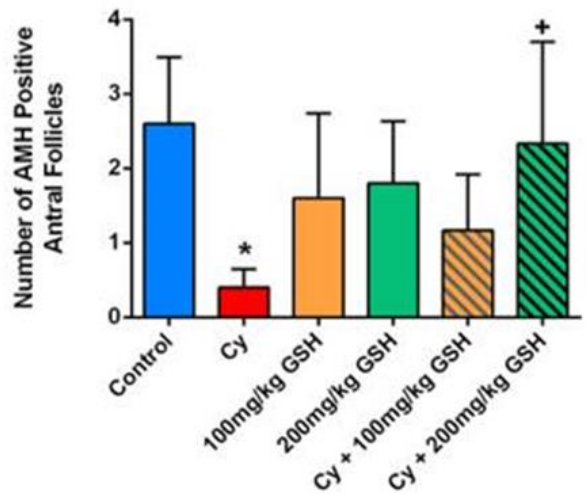
* p <0.05, ** p <0.01, ****p<0.0001, compared to control group;

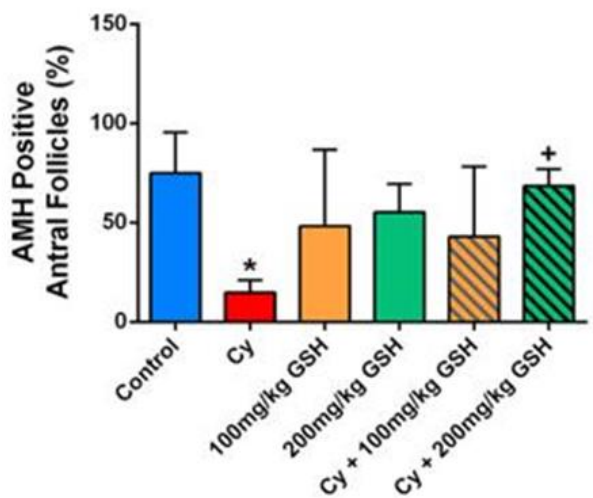
+ p <0.05, ++ p <0.01, compared to cyclophosphamide group.

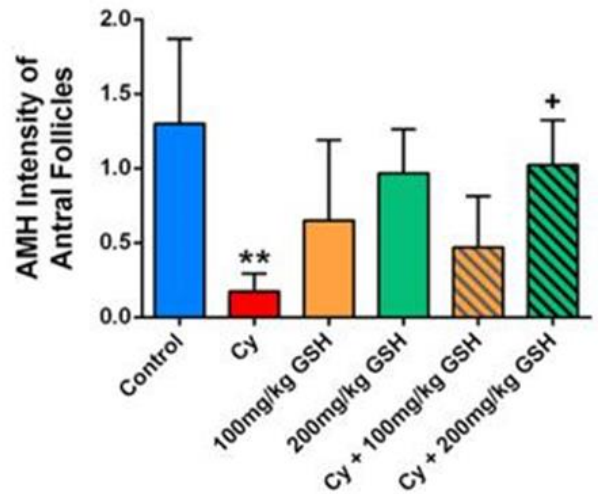












Kontrol

Cy

100mg/kg GSH

200mg/kg GSH

Cy + 100mg/kg GSH

Cy + 200mg/kg GSH

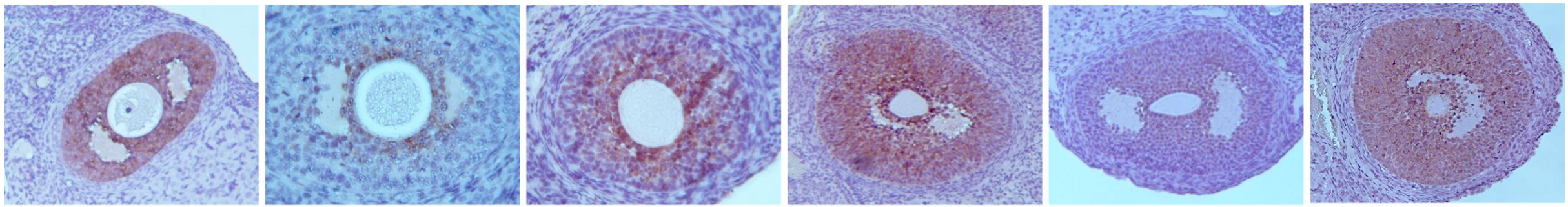
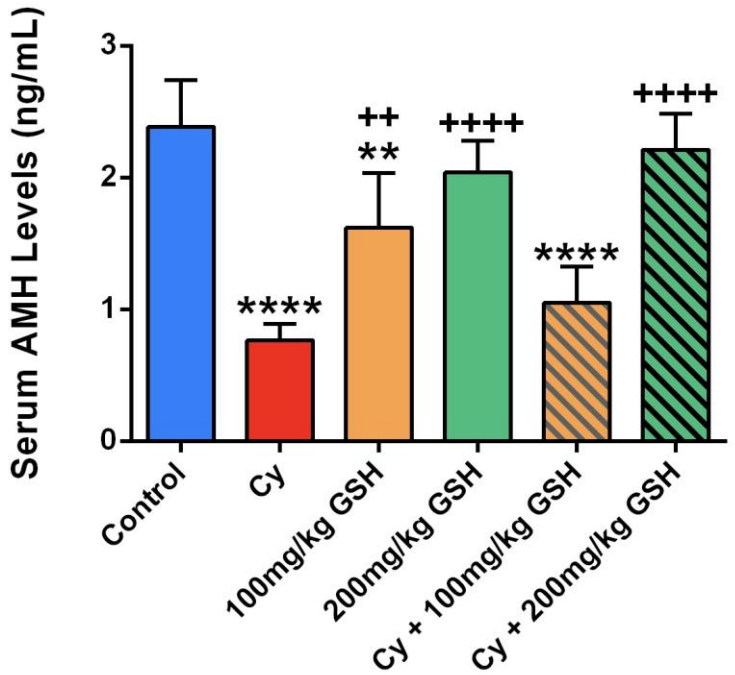


Table 2: Comparison of AMH values between groups

Variables	Group 1 Control Group (n=7)	Group 2 Cyclophosphamide Group (n=7)	Group 3 100mg/kg Glutathion (n=7)	Group 4 200mg/kg Glutathion (n=7)	Group 5 75mg/kg Cyclophosphamide +100mg/kg Glutathion (n=7)	Group 6 75mg/kg Cyclophosphamide+ 200mg/kg/kg Glutathion (n=7)	P
AMH (ng/ml)	2.38± 0.35	0.76±0.28	1.61±0.41	2.03±0.24	1.05±0.27	2.21±0.27	<0.0001



All values are given as mean SD.
*p <0.05 was considered statistically significant.

CONCLUSION

- Our study showed that appropriate dose of glutathione may be protective against cyclophosphamide-induced ovarian failure.

- Other experimental studies ought to be designed to determine the optimum dosage and duration of glutathione treatment to enhance its protective effects.

- The data obtained using an experimental animal model may not predict accurate results on human reproduction directly, future studies are needed to investigate the effect of glutathione on human ovaries.

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THANK YOU FOR YOUR INTEREST!

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