Effect of lycopene on oxidative ovary-damage induced by cisplatin in rats

(Lycopene and oxidative ovary-damage)

Introduction: The anti-cancer drugs, particularly those used in reproductive period, may cause several complications such as ovarian insufficiency and infertility. The mechanism of action of cisplatin toxicity on the ovaries is not fully described. However, further production of free oxygen radicals and reduced production of antioxidants are thought to have an effect on the occurrence of cisplatin toxicity. The aim of this study was to investigate the effects of lycopene on cisplatin-induced ovary-damage, oxidative stres and histological changes in rats.

Materials and methods: Albino Wistar female rats were randomly divided into three groups. The control group (group 1) received sunflower oil; animals in group 2 received only cisplatin; one hour of lycopene pre-treatment was applied to the animals in group 3 before administration of cisplatin. Cisplatin (5 mg/kg/day) was intraperitoneally injected as a single dose and lycopene (0,5 mg/kg/day) was administered by gavage. Biochemical and histopathological methods were utilised for evaluation of the oxidative ovary-damage.

Results: Ovarian tissues of animals administered cisplatin alone, there was an increase in the levels of malondialdehyde, while total glutathione, glutathione reductase, and superoxide dismutase were decreased, but it is observed that these ratios are reversed in the healty control and in the lycopene + cisplatin groups.

Conlusion: Cisplatin treatment induced ovary damage and especially pre-treatment with lycopene provided protective effect against this Cisplatin-induced ovary-damaged.

Key words: Antioxidants, cisplatin, lipid peroxidation, lycopene, oxidative ovary-damage

Introduction

The anti-cancer drugs, particularly those used in reproductive period, may cause several complications such as ovarian insufficiency and infertility [1]. Cisplatin (cis-diamminedichloroplatinum-II, CisPt) is a widely prescribed anticancer drug. The activity against various neoplasms has been shown especially in the head and neck, testis and ovarian, bladder and small cell lung cancers. The mechanism of action of cisplatin toxicity on the ovaries is not fully described. However, further production of free oxygen radicals and reduced production of antioxidants are thought to have an effect on the occurrence of cisplatin toxicity. [2,3]. Moreover, it has been demonstrated that CisPt -associated infertility is caused by the toxic effect on the primordial follicles. Since the primordial follicles are not able to regenerate, the damage caused by the exposure to toxic agents may lead to ovarian insufficiency and infertility [4]. It has been claimed that free radical-related organ damage is the result of deteriorating antioxidant defense mechanisms. Moreover, it was reported that the toxicity caused by CisPt in the tissues was closely related to the increased lipid peroxidation [5]. This literature knowledge suggests that antioxidant treatment might be helpful to prevent CisPt -related ovarian toxicity and therefore infertility due to this toxicity.

Lycopene a naturally occurring carotenoid as tomatoes has attracted considerable attention as a potential chemopreventive agent. Recently, lycopene has received particular attention as a result of studies that have reported that it is a highly efficient antioxidant and has a singlet-oxygen and free radical scavenging capacity [6,7]. A study by Ucar et al. reported that furan-induced oxidative ovarian injury regressed with lycopene in diabetic and non-diabetic rats. [8]. In another study by El-Saad AA et al., It was found that lycopene inhibited dichlorvos-induced hepatic damage [9]. Many studies demonstrated that lycopene has helped to eliminate the adverse efects of risk factors in the case of heart and cancer diseases. [10,11] In literature research, we found no information about preventing oxidative ovarian damage due

to CisPt administration with lycopene in rats. Thus, the purpose of this study was to demonstrate whether lycopene would be efficacious for preventing oxidative ovarian damage due to CisPt administration, and to define the association of oxidative stress in ovarian tissues.

Materials and methods

2.1. Animals. The recommendations of the Declaration of Helsinki (1964) for animal care were taken into account. The experiments were carried out in accordance with‘‘Principles of laboratory animal care’’ (NIH publication no. 86-23, revised 1985). For the experiment, a total of 18 albino Wistar female rats weighing between 250-265 g were used. Rats were provided by Atatürk University Medical Experimental Application and Research Center. The animals were kept in groups at room temperature (22 C.) and fed ad libitum. Animal experiments were performed in accordance with the National Guideline on the Use and Care of Laboratory Animals and approved by the local animal ethics committee of Atatürk University Erzurum, Turkey (Ethics Committee Number: 1800109520 Dated: 04.04.2018)

2.2. Chemical Substances. The thiopental sodiumused in the experiment was provided by Ibrahim Etem Ulagay (Istanbul, Turkey); Cisplatin was provided by Ebewe Liba-(Turkey) and Lycopene was provided by Solgar (USA). Distilled water was used for dissolving cisplatin and lycopene.

2.3. Experiment groups. The rats used in this study were divided into three groups – Healthy Control (HC), 5 mg/kg/day in 14 days Cisplatin alone (CisPt) and 0.5 mg/kg/day lycopene plus 5 mg/kg/day in 14 days cisplatin (LC). The rats in LC group lycopene was administered by oral gavage at a dose of 0.5 mg/kg/day in 14 days. The rats in the CisPt (n-6) and HC (n-6) groups, sunflower oil as the solvent was applied by the same method and same volume (0.5 ml). One hour after administration of lycopene and solvent, the rats were injected with cisplatin at a dosage of 5 mg/kg/day intraperitoneally (i.p.). Lycopene, cisplatin, and solvent were applied

at the indicated dose and volume once a day for 14 days using the same method. In the literatüre, the protective drugs against cisplatin toxicity are usually given to experimental animals one hour before cisplatin [12]. At the end of the period, six rats from each group were sacrificed using high-dose anesthetic (50 mg/kg of thiopental i.p.). Their ovaries were removed, and the levels of malondialdehyde (MDA), total gluthatione (GSH), Glutathione reductase (GR), superoxide dismutase (SOD) were measured. Oxidant and antioxidant parameters were measured to evaluate oxidative stress in the ovary-exposed tissues. The tissues were also examined histopathologically. Biochemical and histopathologic results obtained from lycopene and healthy control groups were compared with those of cisplatin alone group.

2.4. Biochemical Analysis of Ovarian Tissue. Whole ovarian tissue was weighed and homogenizedonice with 2-mL relevant buffer. Buffers were 0.5% hexadecyltrimethyl ammonium bromide (pH 6), potassium phosphate buffer formyelophoroxidase assay, 1.15% potassium chloride solution for malondialdehyde analysis and pH 7.5 phosphate buffer for other analyzes. It was then centrifuged at 4'C, 10,000 rpm for 15 min. The supernatant was used for analysis.

2.4.1. Malondialdehyde (MDA) analysis. (nmol/mg)

The concentrations of ovarian mucosal lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test [13]. 0.5mL homogenate was added to a solution containing 0.2mL of 80 g/L sodium lauryl sulfate, 1.5mL of 200 g/L acetic acid, 1.5mL of 8 g/L of 2-thiobarbiturate, and 0.3mL of distilled water. The mixture was incubated at 98 °C for 1 h. After cooling, 5 mL of n-butanol: pyridine (15: 1) was added. The mixture was vortexed for 1 min and centrifuged at 4000 rpm for 30 min. The absorbance of supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane.

2.4.2.Total Glutathione (GSH) analysis (nmol/mg)

The amount of GSH in the total homogenate was measured according to the method of Sedlak and Lindsay with some modifications [14]. The sample was weighed and homogenized in 2 mL of 50 mmol/L Tris–HCl buffer containing 20 mmol/L EDTA and 0.2 mmol/L sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid and the precipitate was removed after centrifugation at 4200 rpm for 40 minutes at 4 ° C.and the supernatant was used to determine GSH level. A total of 1500 µL of measurement buffer (200 mmol/L Tris–HCl buffer containing 0.2 mmol/L EDTA at pH 7.5), 500 µL of supernatant, 100 µL of DTNB (10 mmol / L) and 7900 µL of methanol were added into a tube. and vortexed and incubated at 37 ° C for 30 minutes. 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) was used as an chromogen and it formed a yellow-colored complex with sulfhydry groups. The absorbance was measured at 412 nm using a spectrophotometer (Beckman DU 500, USA). The standard curve was obtained using reduced glutathione.

2.4.3.Glutathione reductase (GR) analysis **(EC 1.6.4.2)** (nmol/mg)

GR activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm according to Carlberg and Mannervik method [15]. After tissue homogenization, supernatant was used for GR measurement. After the NADPH and GSSG addition, chronometer was on and absorbance was measured for 5 min by 30-min intervals at 340 nm spectrophotometric methods.

2.4.4.Superoxide dismutase (SOD) analysis **(EC 1.15.1.1)** (nmol/mg)

Measurements were performed according to the method of Sun et al [16]. When xanthine is converted into uric acid by xanthine oxidase, SOD forms. Providing that the nitro blue tetrazolium (NBT) is added to this reaction, SOD reacts with NBT and a purple formazan dye is formed. The sample was weighed and homogenized in 2 ml of 20 mmol / L phosphate

buffer containing 10 mmol / L of EDTA at pH 7.8. The sample was centrifuged at 6000 rpm for 10 minutes and than the brilliant supernatant was used as assay sample.

2.5. Histological examination. All samples in each group were evaluated histologically. Histological examination was performed at the pathology department of University Hospital. Following dehydration of the excised ovaries and embedment in paraffin blocks, 4–6 mm slices were prepared and stained with hematoxylin and eosin. The specimens were evaluated under light microscopy simultaneously by two experienced histopathologists. primary, secondary and antral follicle-containing ovarian tissue was considered normal ovary structure. Ovarian damage; including follicular cell degeneration, vascular congestion, hemorrhage, and inflammation (neutrophil infiltration), was evaluated histologically

2.6. Statistical analysis

The software SPSS 16.0 was employed for the statistical analysis (SPSS Inc., Chicago, IL). Descriptive statistical methods: mean, standard deviation were obtained. Differences among the three groups were evaluated with Tuckey analysis. $P < 0,05$ was considered as statistically significant.

Results

The ovarian tissue of rats from the control group showed normal ovarian architecture with an orderly arrangement of follicles (Figure 2A). The ovarian tissue of animals treated with cisplatin exhibited disordered, apoptotic cells with shrunken pyknotic nuclei, hemorrhage and vascular congestion as well as follicular degeneration and edema compared to the control group (Figure 2B). Animals treated with lycopene before cisplatin administration showed improved histological architecture and orderly arrangement (Figure 2C).

Effect of CP or its combination with lycopene on levels of ovary MDA, GSH, GR and SOD activities in rats are presented in Table 1.

As the results of our study demonstrated, in ovarian tissues of animals administered cisplatin, there was an increase in the levels of MDA, while GSH, GR, and SOD were decreased, but it is observed that these ratios are reversed in the HC and in the LC groups (Figure 1). Evaluation of histological ovarian damage results is shown in figure 2. The comparisons between and within groups are presented in table 1.

Table 1: Comparison between groups in terms of variables and Effect of CisPt or its combination with lycopene on levels of ovary MDA, GSH, GR and SOD activities in rats

Dependent Variable	(I) Group	(J) Group	P value	Mean ± SD		
				Control	**Cisplatin alone**	**Lycopene+Cisplatin**
Malondialdehyde (MDA) (nmol/mg)	1	2	0.000	5,583±0,7574	15,5±1,8708	6,4±0,5762
		3	0.784			
	2	1	0.000			
		3	0.000			
	3	1	0.784			
		2	0.000			
Total Glutathione (GSH) (nmol/mg)	1	2	0.000	5,350±0,5010	1,533±0,2066	4,35±0,4806
		3	0.003			
	2	1	0.000			
		3	0.000			
	3	1	0.003			
		2	0.000			
Glutathione reductase (GR) (nmol/mg)	1	2	0.000	6,150±0,3834	2,15±0,3391	5,333±0,5715
		3	0.018			
	2	1	0.000			
		3	0.000			
	3	1	0.018			
		2	0.000			
Superoxide dismutase (SOD) (nmol/mg)	1	2	0.000	9,550±0,3271	4,717±0,6113	8,917±0,3312
		3	0.078			
	2	1	0.000			
		3	0.000			
	3	1	0.078			
		2	0.000			

The mean difference is significant at the 0.05 level

1:Control, 2: Cisplatin alone, 3: Lycopene+Cisplatin

Discussion

Every year, thousands of women undergo chemotherapy, and some face decreased fertility ranging from minimal to complete ovarian failure. CisPt is used in the treatment of many solid tumors, mainly testicular and ovarian tumors. However, it was reported that cisplatin caused severe adverse effects such as nephrotoxicity, neurotoxicity, gastric toxicity, and infertility [17]. It was reported that chemotherapeutic medicines leading to either temporary or permanent infertility severely affected the ovaries and hormonal balance [18]. Limited studies have shown that CisPt damages primordial follicles, which constitute a major part of the ovarian reserve. The number of primordial follicles is positively correlated with reproductive proficiency in female mammals [4]. Meirow et al. examined human ovaries that had been exposed to CisPt, and histologically identified apoptosis in granulosa cells and the destruction of primordial follicles [19]. Antioxidants reduce reactive oxygen radical-induced oocyte and follicular damage. Bas and Pandır [10] have shown that furan induced lung toxicity in the diabetic rats with severe pathological alterations. Emphysematous changes, hemorrhage, changes in connective tissue of the alveolar septa, edema, and desquamation of the epithelial cell of the terminal bronchiole were observed in the diabetic furan group. Lycopene treatment cured these transformations. Emphysematous and hemorrhage were seen in the diabetic furan + lycopene group in moderate level. Unal et al. [11] demonstrated that histological damages of kidney were more severe in diabetic furan group, particularly extensive infammatory cell infltration, glomerular lobulation, glomerular atrophy, tubular degeneration, hemorrhage, and dilatation of Bowmann's space. Lycopene supplementation was protective against furan caused histopathological changes, too. Administration of cisplatin increased severe hemorrhage, edema, follicular degeneration, and vascular congestion in the ovary tissue in this study. However, milder pathological changes

were seen in lycopene + cisplatin group. In this way, the toxicity caused by cisplatin in rat ovarian tissues appears to be inhibited by lycopene.

In chemotherapy-induced ovarian damage, the effects of protective drugs are evaluated by measuring plasma levels of antioxidants and reactive oxygen radicals and and follicle morphology. As the results of our study demonstrated, in ovarian tissues of animals administered cisplatin, there was an increase in the levels of MDA, which is oxidant parameter, while the levels of antioxidants such as GSH, GR, and SOD were decreased. (Figure 1)

MDA is the end-product of lipid peroxidation. Lipid peroxidation is known to be the most harmful effect of free radicals in the cell [20]. It was reported that cisplatin caused oxidative damage in the ovarian tissue increasing MDA concentration and decreasing GSH concentration [20]. In our study, for ovarian tissues of the animals in CisPt group, there was an increase in the levels of MDA. On the other hand, it was clearly seen that this increase observed in the CisPt group was not observed in the LC and HC groups (15,5±1,8708 µmol/gr, 6,4±0,5762 µmol/gr, 5,583±0,7575 µmol/gr, respectively).

GSH is a non enzymatic endogenous antioxidant parameter, Under physiological conditions, the oxidant/antioxidant balance is maintained with predominance of antioxidants. Degradation of this balance causes tissue damage called oxidative stress. Therefore, oxidant/antioxidant balance is used to assess if tissue damage emerges [21]. GSH, an endogenous antioxidant, protects the cells against oxidative damage, keeping the –SH groups of proteins reduced and preventing them from reacting with free radicals [22]. In the present study; In the ovaries of the rats given only cisplatin, GSH level was decreased in comparison with the LC and HC group. (1,533±0,2066 nmol/gr, 4,350±0,4806 nmol/gr, 5,350±0,5010 nmol/gr, respectively).

The most important goal of the reaction catalyzed by the enzyme GR is to maintain the reduced glutathione / oxidized glutathione ratio in the cell medium. Oxidative stress occurs

when this ratio is reduced [22]. In this study, GR level was found to be lower in CisPt group compared with LC and HC group (2,150±0,3391 u/gr, 5,333±0,5715 u/gr, 6,150±0,3834 u/gr respectively)

SOD, another protective enzyme against free oxygen radicals, catalyzes the transformation of superoxide molecule into hydrogen peroxide and molecular oxygen [23]. Low SOD activity found in ovarian tissues of rats given cisplatin alone in comparison with the LC and HC group. (4,717±0,6113 u/gr, 8,917±0,3312 u/gr, 9,55±0,3271 u/gr, respectively)

It has been shown that lycopene has the highest antioxidant activity among carotenoids against, hydrogen peroxide and nitrogen dioxide radicals. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo [24]. The main antioxidant feature depends on upregulating antioxidant systems in the cell, and it also has a direct destructive effect on free radicals. During $^1O_2$ quenching (oxygen $^1O_2$ quenchers) , the energy is converted from $^1O_2$ to the lycopene molecule into an energy-rich triplet state. In contrast, capture of other Reactive oxygen species (ROSs) such as hydroxyl radical (OHs), Nitronium (NO_2^+)or peroxynitrite leads to oxidative degradation of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins and DNA [20]. In the present investigation, pre-treatment with lycopene inhibited the increase in MDA induced by CisPt in ovary, but activated the increase the levels of antioxidants such as GSH, GR, and SOD.

There are some limitations of our study. First, the results of experimental studies on lab animals may not be extrapolated to humans. Thus, our study needs to be verified in future studies in humans. In addition, our results can be verified more ovarian toxic agent, namely alkylating agents that destroys primordial follicle pool. For example, cyclophosphamide can be chosen for future studies instead of cisplatin that is moderately gonadotoxic.

In conclusion, it was shown that CisPt treatment induced ovary damage and especially pre-treatment with lycopene provided protective effect against this CisPt -induced ovary-damaged. But, before a conclusive statement on potential usefulness of lycopene as adjunct to the CisPt therapy there is a need for further studies including human trials.

Conflict of Interest: The authors declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008

Figure 1: MDA, GSH, GR, and SOD levels between groups

Figure 2. (A) Normal histopathologic appearance is observed in overgrowth of control group (H&E X100); (B) The destructive secondary follicle (straight arrow), dilate congestige blood vessel (striated arrow), hemorrhage and edema (bilateral arrow) are observed in the Cis group treated with cisplatin alone (H&E X200); (C) Normal appearance is observed except for the congestige blood vessel (straight arrow) in LC group (H&E X100)

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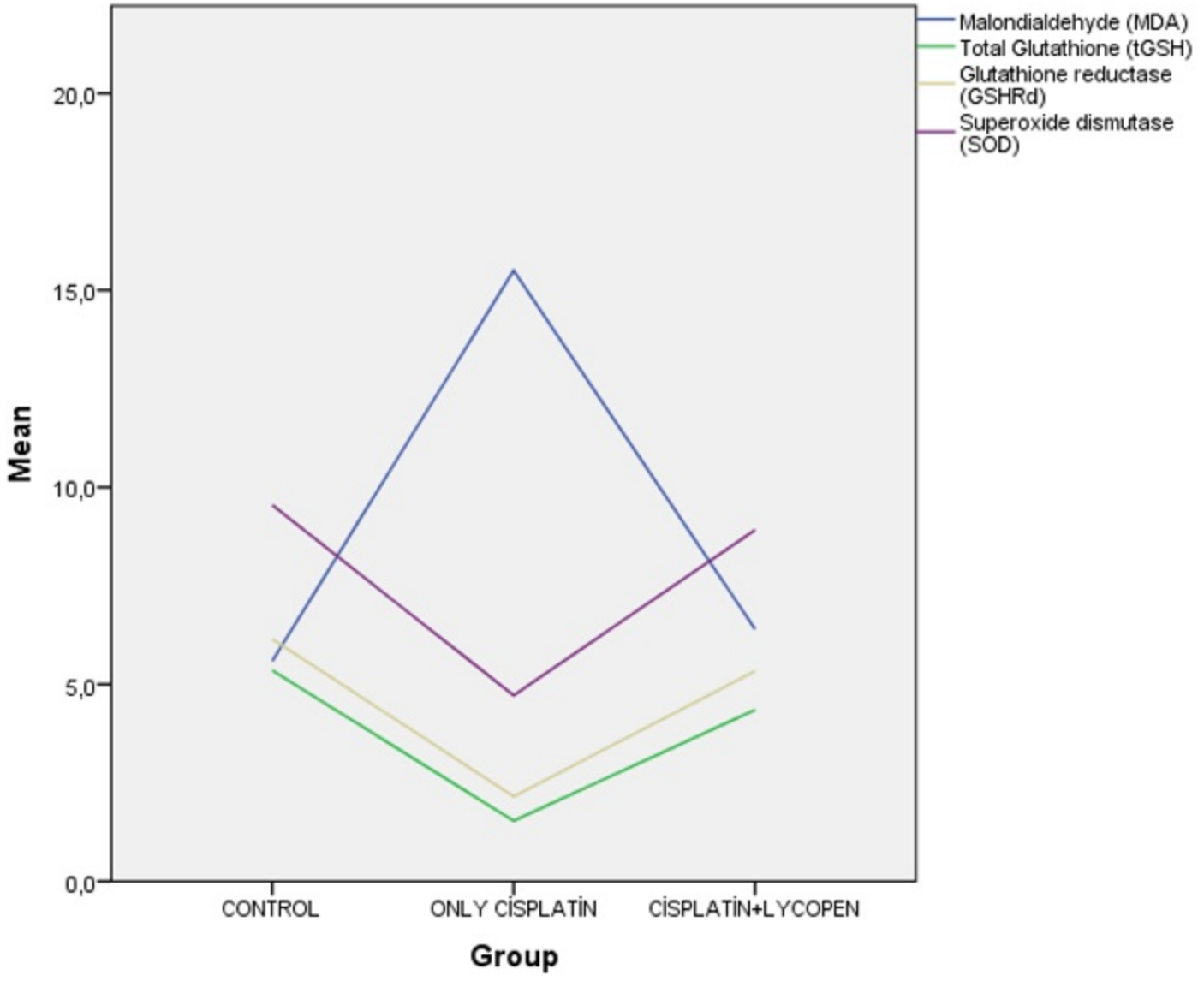
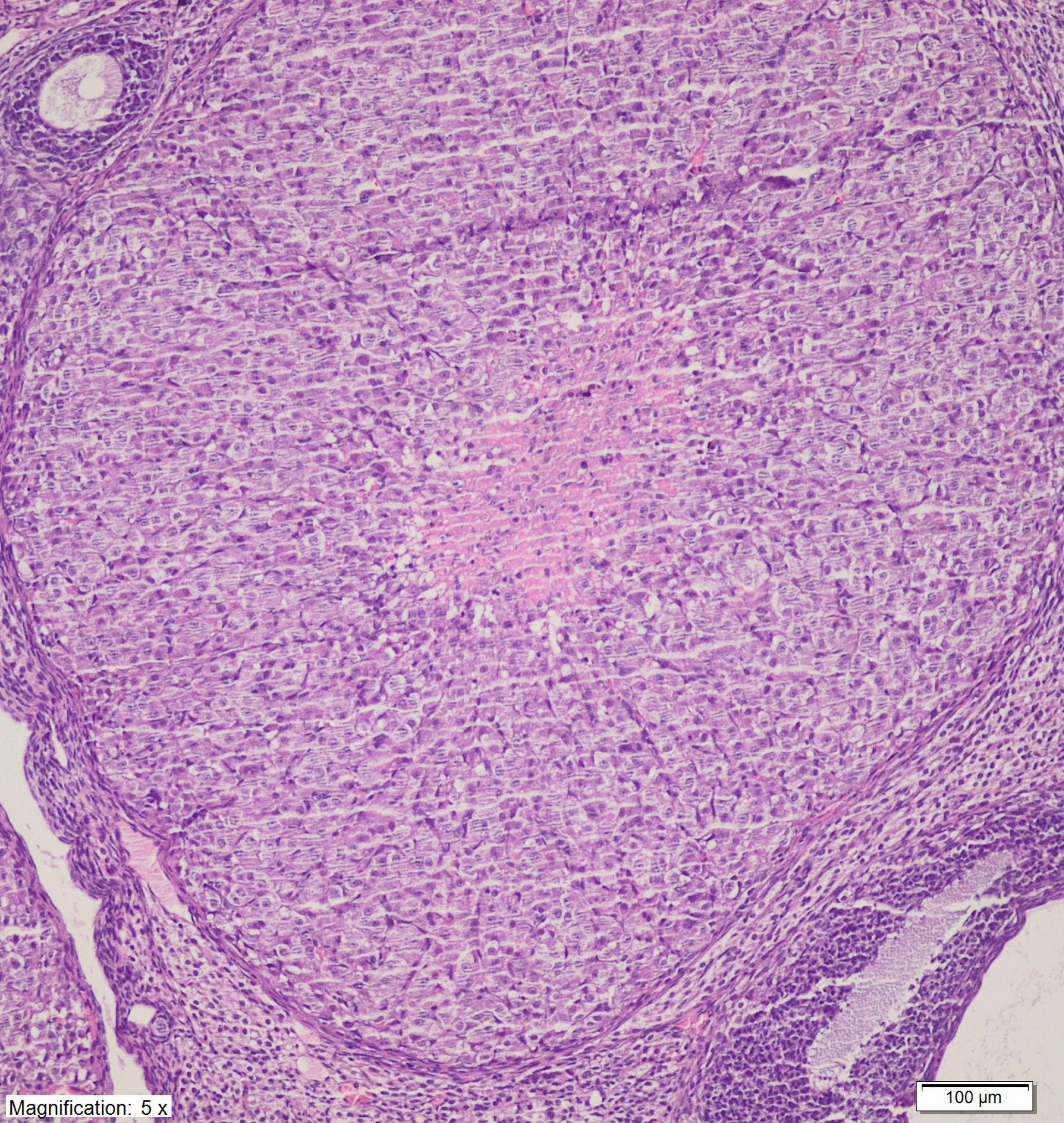
Figure 1: MDA, GSH, GR, and SOD levels between groups

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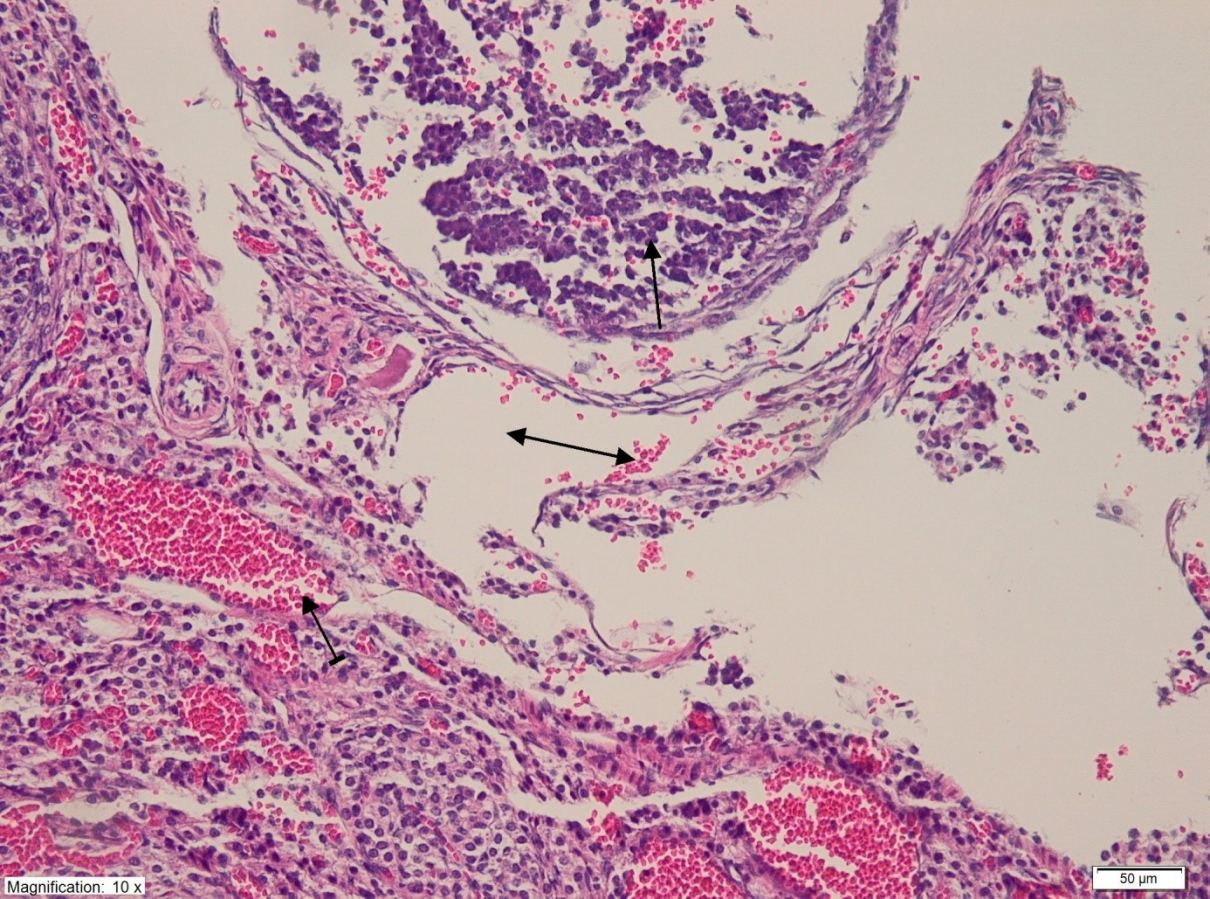
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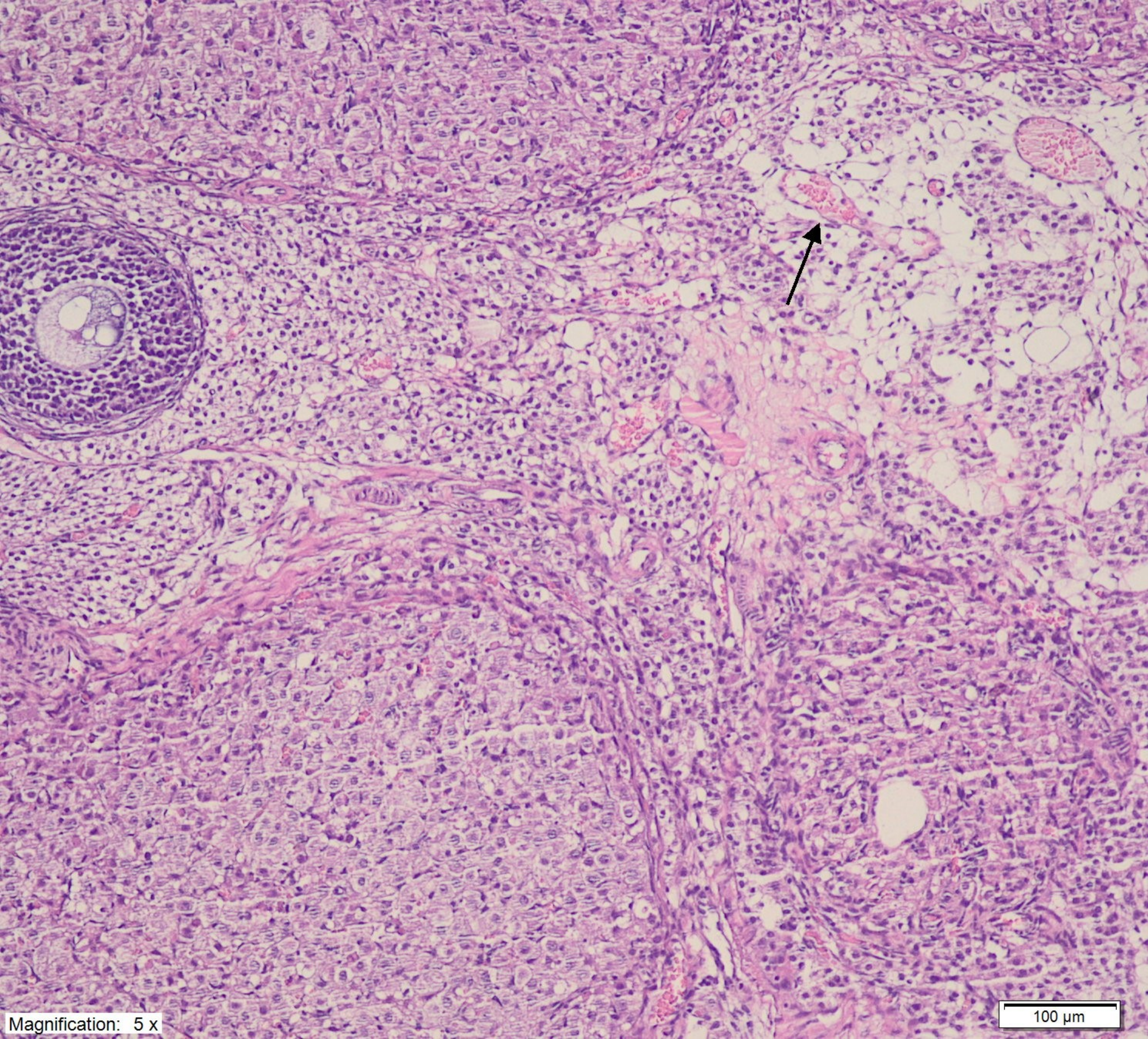
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