Doctoral School in Medical Sciences

As a manuscript
CZU: 618.11-008.9:618.11-007.59-005.4-092.9(043.2)

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Metabolic feature of ischemia/reperfusion caused by ovarian torsion/detorsion

315.01. Medical biochemistry

Summary of doctoral thesis in medical sciences

Chișinău, 2021

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Doctoral thesis defense will take place on February 17, 2021, at 2:00 p.m., in the online meeting (meet.google.com/mjj-voan-tuj) of the Commission for public defense of the doctoral thesis, approved by the decision of the Scientific Council of the Consortium no.12 from December 14, 2020.

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SUMMARY

THE RESEARCH CONCEPTUAL FRAMEWORK

The topicality and importance of the researched problem. Ovarian torsion (OT) is a rare but severe condition, with a prevalence of 2,7% [1] - 3% [2], up to 6% [3]. It is currently ranked fifth in gynecological surgical emergencies [1] and can be found at any age, the majority of cases occur in women of reproductive age [4]. It is a medical emergency that affects 4,9/100.000 of the female population aged 1-20 years [5]. Approximately 80% of cases occur during the reproductive age period [2] and 20% of cases occur during pregnancy [6]. The incidence of the disease during pregnancy in the literature data varies from about 1-10 to 1000 [7], to 1 to 5000 pregnancies [2] and occurs more frequently in the first trimester, in patients undergoing infertility treatment for in vitro fertilization (IVF) or ovarian stimulation [8], this being a cause of increased incidence of the disease [9].

Ovarian torsion is defined as the partial or total rotation around its vascular axis of the fallopian tube, ovary or both [5], with obstruction of venous, lymphatic and arterial flow, resulting in ischemic changes that can cause ovarian necrosis [5, 8]. Although the main risk factor for OT is an ovarian mass, it has been observed that healthy ovaries may also be involved in the pathological process [4] and this is probably due to excessive ligament laxity, tubal spasm or changes in intra-abdominal pressure in neonatal and prepubertal age [10]. It has been found that in patients under the age of 15 in more than 50% of cases the twisted ovaries are normal [4, 5] while in adult women the condition is frequently determined by the presence of ovarian abnormalities (teratoma, endometrioma, cysts, ovarian hyperstimulation syndrome, etc.) [11].

The correct diagnosis is very important for women of reproductive age, especially since the clinical manifestations of the disease are nonspecific [12] and the doctor's intervention may be delayed because of this [5]. Postponed treatment due to misdiagnosis can lead to complications such as ovarian loss with negative effects on fertility [5, 12]. If surgery is not performed, thrombophlebitis, bleeding, infection [13], peritonitis [12, 13] and death [5, 12] may occur. Traditional management in ovarian torsion involves ovarectomy while conservative management includes detorsion of the twisted segment. If the ovarian torsion is diagnosed and treated early by the detorsion maneuver, the prospects of maintaining fertility are favorable [14], the restoration of adequate blood flow being the purpose of the surgery performed [15]. Thus, OT being a surgical emergency requires immediate diagnosis and treatment to avoid various serious complications [5] and to reduce the incidence of infertility [12, 13, 15] which is an important public health problem for which the diagnosis and treatment are invasive, expensive and stressful [16].

According to previous research [14, 15, 17, 18] in the ovarian torsion ROS (reactive oxygen species) play a major role in tissue damage caused by I/R (ischemia/reperfusion). So, despite the organ preservation advantage, the detorsion of the twisted adnexa can have negative consequences caused by the reperfusion phenomenon. In various studies it has been shown that during the detorsion process an excessive amount of molecular oxygen is supplied to the tissues and large amounts of ROS are produced, which are involved in tissue damage [15, 17, 18]. According to the literature, the reperfusion lesion has been well documented in other organs than the ovary. However, there are publications that indicate that exposure of the ovaries to I/R is accompanied by oxidative stress and that the administration of certain substances reduces the degree of injury and helps a faster recovery [15, 17, 18]. Thus, lesions through the I/R phenomenon in OT require good knowledge, monitoring and a therapeutic strategy that would reduce the negative effects of ROS on the ovaries, ensuring their proper functioning after the detorsion maneuver and preventing the occurrence of complications.

The aim of the research was to study the metabolic changes caused by ischemia/reperfusion in experimental ovarian torsion/detorsion to highlight specific diagnostic indices of ovarian tissue lesions and to argue treatment strategies that would reduce reperfusion lesions.

Research objectives:

1. Ascertainment of metabolic features of lesions caused by ischemia/reperfusion in the ovaries in experimental ovarian torsion/detorsion.

2. Study of blood biochemical expression of ischemia/reperfusion lesions caused by ovarian torsion/detorsion.

3. Assessment of correlations of blood and tissue biochemical changes in experimental ovarian torsion/detorsion.

4. Evaluation of the controlled reperfusion method on tissue and blood biochemical indices in experimental ovarian torsion/detorsion.

Scientific research methodology

A preclinical, experimental study was performed, respecting all scientific rigors and ethical principles of institutional, national and international research. The study was performed on white laboratory rats (*Rattus albicans*), modeling the ovarian torsion, with subsequent detorsion/reperfusion, also applying the controlled reperfusion technique (*on-off* model). To achieve the objectives of the thesis, the homogenates of ovarian tissue and blood serum were investigated to assess the metabolic indices of ischemia, oxidative stress and antioxidant system. The Research Ethics Committee positive decision was obtained on 08.02.2016, approval number 27, at number 24.

The novelty and scientific originality of the obtained results

The research revealed important data including metabolic indices that ensure the functioning of ovarian cells in conditions of induced ischemia. The biochemical changes that take place in the experimental ovarian torsion/detorsion, the depth of the disorders and their blood reflection were appreciated. The indices of ischemic damage, oxidative stress and antioxidant protection were measured both at the tissue and blood level, with the determination of the presence of blood-tissue correlations of the evaluated parameters.

Hypotheses have been made about the conditions that favor the resistance of the ovaries to ischemia, the connection between tissue and blood metabolic changes. A spectrum of laboratory indices from the blood serum with diagnostic value in OT was identified, but also for monitoring after detorsion. Certain therapeutic interventions have been proposed, for a more effective result of the surgical treatment of OT with preservation of the ovaries.

The study deepened the knowledge regarding ovarian metabolism in ovarian torsion after simple and controlled reperfusion.

The theoretical significance of the work. Our research revealed important data regarding ovarian metabolism in physiological conditions and in ischemia/reperfusion syndrome within ovarian torsion, with the assessment of the effects of classical and controlled reperfusion.

The applicative value of the research. In our study, laboratory investigation methods were applied that ensured the obtaining of high precision results that allowed us to argue the possibility of using laboratory indices (lactate, ischemia modified albumin, lactate dehydrogenase-pyruvate) in the differential diagnosis of ovarian torsion and in monitoring of treatment outcomes (malondialdehyde, advanced oxidation protein products, advanced glycation end products). The comparative evaluation of metabolic changes in ovarian tissue and blood serum after classical and *on-off* detorsion highlighted the advantage of controlled reperfusion in preventing the worsening

of tissue lesions induced by oxidative stress in the surgical treatment of OT with preservation of ovaries, the application of which is easy and no additional costs required.

Implementation in practice – in the university and postgraduate teaching process at the Department of Biochemistry and Clinical Biochemistry of "Nicolae Testemițanu" State University of Medicine and Pharmacy.

Approval of scientific results. The research results were presented, analyzed, discussed and approved at several national and international scientific forums: *6th International Medical Congress for Students and Young Doctors MedEspera,* SUMPh "Nicolae Testemiţanu", Chişinău, Moldova, May 12-14, 2016; IMU Annual Scientific Conference of young specialists „*Performanţe şi perspective în urgenţe medico-chirurgicale*", Institute of Emergency Medicine, Chişinău, Moldova, May 20, 2016; *Zilele Universității și Conferința științifică anuală consacrată aniversării a 90-a de la naşterea ilustrului medic şi savant Nicolae Testemiţanu,* SUMPh "Nicolae Testemiţanu", Chișinău, Moldova, October 18-20, 2017; *Annual Young Medical Scientists' Conference 2017*, Bogomolets National Medical University, Kiev, Ukraine, October 27-29, 2017; *IBC – SOFIA 2017, 2nd INTERNATIONAL BIOMEDICAL CONGRESS of SOFIA 2017,* Medical University – Sofia, Bulgaria, November 17-19, 2017; Conference „*Biological markers in fundamental and applied biology. From theory to practice*", European Scientific Center "Biomarker", Brno, Czech Republic, January 10, 2018; Conference „*Modern technologies of diagnostics and monitoring of therapy in experimental, clinical medicine and pharmacy*", European Scientific Center "Biomarker", Brno, Czech Republic, January 15, 2018; Scientific and practical conference „*Theoretical and practical aspects of the use of biological markers in fundamental and applied medicine and biology*", European Scientific Center "Biomarker", Prague, Czech Republic, March 27-29, 2018 (**was awarded a grant for publication of the results: https://escbm.org/page/view/grants**); *7th International Medical Congress for Students and Young Doctors MedEspera*, SUMPh "Nicolae Testemiţanu", Chișinău, Moldova, May 3-5, 2018; *Zilele Universității și Conferința științifică anuală a cadrelor științifico-didactice, doctoranzilor, masteranzilor, rezidenților și studenților*, SUMPh "Nicolae Testemiţanu", Chișinău, Moldova, October 18, 2018 and October 17, 2019; *VI Bukovinian International Medical Congress, BIMCO 2019*, Bukovinian State Medical University, Chernivtsi, Ukraine, April 2-5, 2019; *VII Bukovinian International Medical Congress, BIMCO 2020,* Bukovinian State Medical University, Chernivtsi, Ukraine, April 7-10, 2020.

Publications related to doctoral thesis. 19 scientific papers have been published, including 7 articles, of which 2 in journals from international databases (SCOPUS, **IF$_{\text{CiteScore2019}}$**: **0,6** and Web of Science, **IF$_{\text{ISI}}$**: **0,8**), 3 articles in journals from the National Register of profile journals, B category, including 1 article in *Moldovan Journal of Health Sciences (Revista de Științe ale Sănătății din Moldova)*, 2 articles in international collections and 12 theses in the proceedings of national and international scientific conferences and congresses.

The structure of the thesis. 143 pages which include: list of abbreviations, list of figures, list of tables, introduction, 3 chapters, general conclusions, practical recommendations, bibliography, information regarding the valorization of research results, 3 annexes, declaration on accountability.

Keywords: experimental ovarian torsion, ischemia/reperfusion injury, controlled reperfusion, oxidative stress, reactive oxygen species, antioxidant system

THESIS CONTENT

1. OXIDATIVE METABOLISM IN OVARIAN TORSION/DETORSION

1st chapter presents a synthesis of specialized literature, with the aim of highlighting those aspects that underline the topicality of the conducted research. 1st subchapter reflects the mechanisms of occurrence and exacerbation of oxidative stress, its influence on the female reproductive system, pathological changes induced by reactive oxygen species, biochemical markers that are suggestive for oxidative damage, and antioxidant protection mechanisms. 2nd subchapter describes the biochemical mechanisms of ischemia/reperfusion injury development, which can lead to irreversible cell and tissue damages. 3rd subchapter presents data on the phenomenon of ischemic postconditioning (PcI), which would have a beneficial effect, lowering ROS production and thus reducing reperfusion lesions.

2. MATERIALS AND METHODS OF STUDY IN EXPERIMENTAL OVARIAN TORSION

2.1. The experimental model. The research is one preclinical, experimental, which included 70 white laboratory rats (*Rattus albicans*) from the vivarium of SUMPh „Nicolae Testemițanu", females, with a body weight of 180-265 grams, of reproductive age (6-12 months), randomly divided into 7 groups: control, sham and 5 groups with ovarian torsion, according to research protocol. The surgeries were performed under sterile conditions and under general anesthesia (intraperitoneal) with 10% solution of ketamine hydrochloride (Calypsol®, Gedeon Richter, Hungary), 50 mg/kg. The convenient time to perform the surgery was considered the moment when the rats remained motionless in a supine position. The abdominal skin was shaved and disinfected with 100 mg/mL solution of Povidone iodine (Betadine®, Egis Pharmaceuticals PLC, Hungary). The surgical procedure took about 15 minutes. All rats underwent a 2-2,5 cm laparotomy – a vertical incision in the lower abdomen, allowing access to the ovaries.

Rats from the control and sham groups were not subject to ovarian ischemia. Those from the control group a blood sample from the abdominal aorta and their ovaries were taken. Those from the sham group were left with an open abdominal cavity for a period of 15 minutes, after which it was closed, followed after 3 hours by subjects' reanesthesia, repeated laparotomy to collect the ovaries and blood from the abdominal aorta.

In the other research groups the animals were exposed to ovarian torsion for 3 hours. Ovarian ischemia was caused by twisting the ovarian pedicle 3 times clockwise, being fixed to the abdominal wall by applying 3/0 silk suture, followed by closing the abdominal wall by applying 3/0 silk sutures. Subsequently, after 3 hours, the animals were reanesthetized and subjected to the following maneuvers, depending on the group to which they belonged, as follows: 3rd group – laparotomy and collection of biological material; 4th group – laparotomy with ovarian detorsion and closing of the abdominal wall by applying 3/0 silk sutures, and after 1 hour reanesthesia of the animals, laparotomy with sampling of biological material; 5th group – laparotomy to remove the torsion applying the controlled reperfusion method, then, closing of the abdominal wall by applying 3/0 silk sutures, and after 1 hour reanesthesia of the animals, laparotomy and collection of biological material; 6th group – laparotomy with ovarian detorsion and closing of the abdominal wall by applying 3/0 silk sutures, and after 24 hours reanesthesia of the animals, laparotomy with sampling of biological material; 7th group – laparotomy to remove the torsion with the restoration of blood flow by applying the *on-off* method, then, closing of the abdominal wall by applying 3/0 silk sutures, and after 24 hours reanesthesia of the animals, laparotomy and biological material taken.

The controlled reperfusion method (*on-off*) was performed by applying an atraumatic clip on ovarian pedicle, after which the ovary was subject to detorsion. In the first 2 minutes, until the complete restoration of blood flow, this clip was opened and closed (*on-off*), with an interval of 10 seconds for each maneuver, to allow the gradual restoration of blood circulation. Subsequently, the clip was removed, allowing complete resumption of the blood flow.

All animals were euthanized under anesthesia after sampling the biological material.

2.2. Preparation of biological material and biochemical investigations

2.2.1. Preparation of biological material for study

To confirm the chosen ovarian torsion model, one ovary from each group was randomly selected for histopathological evaluation, which was placed in 10% formalin solution and sent for investigation within the Department of Morphopathology of SUMPh "Nicolae Testemițanu". The presence of interstitial edema, vascular congestion, hemorrhage and leukocyte infiltration was examined.

The blood samples were placed in test tubes for 30 minutes to allow them to coagulate, then centrifuged at 1500 rpm for 10 minutes and the serum was stored in Eppendorf tubes at -40°C until biochemical examination. Ovarian tissue was homogenized in 0,1 M phosphate buffer (pH=7,4), so that the final dilution of the homogenate was 1:10, then centrifuged for 15 minutes at 4°C, 3000 rpm. The supernatant was placed in Eppendorf tubes and stored at -40°C until investigation. In order to evaluate the amount of glutathione, ovarian tissue was homogenized in 5% sulfosalicylic acid solution in a ratio of 1:5. Ovarian homogenates were prepared under specific conditions (using ice) to allow further assessment of enzymatic activity.

2.2.2. Biochemical tests. All biochemical investigations were performed according to modified methods for Synergy H1 (Hydrid Reader) (BioTek Instruments, SUA) microplate spectrofluorometer and Power Wave HT (BioTek Instruments, SUA) spectrophotometer of Biochemistry Laboratory of SUMPh "Nicolae Testemițanu". There were evaluated **ischemia markers**: *lactate* and *lactate dehydrogenase-lactate (LDH-L)* according to the instructions of the ELITechGroup kits (France), *lactate dehydrogenase-pyruvate (LDH-P)* according to the instructions of the standard kits from DAC-SpectroMed (Moldova), *ischemia modified albumin (IMA)* [19]; **oxidative stress indices**: *malondialdehyde (MDA)* [20], *nitric oxide derivatives (NOD)* [21], *advanced oxidation protein products (AOPP)* [22], *advanced glycation end products (AGE)* [23]; **antioxidant system markers**: *superoxide dismutase (SOD)* [24], *catalase (CAT)* [25], *glutathione peroxidase (GPO)* [26], *glutathione-S-transferase (GST)* [27], *glutathione reductase (GR)* [28], *total glutathione (GT)* [29], *reduced glutathione (GSH)* [30], *oxidized glutathione (GSSG)* – according to the formula *GSSG=GT-GSH*, *ceruloplasmin (CP)* [21]; **other metabolic indices**: *glucose-6-phosphate dehydrogenase (G6PDH)* [21], *cytosolic $NADP^+$-dependent isocitrate dehydrogenase (ICDH)* [21], *total protein* – according to the instructions of the ELITechGroup kit (France).

2.3. Statistical analysis of results was performed using the SPSS software (Statistical Package for the Social Sciences, version 23.0), applying: Kolmogorov-Smirnov, Shapiro-Wilk, Levene, One-Way Anova with Tukey, Welch's Anova with Games-Howell tests and Spearman's rank correlation coefficient.

3. BIOCHEMICAL CHANGES IN OVARIAN ISCHEMIA/REPERFUSION

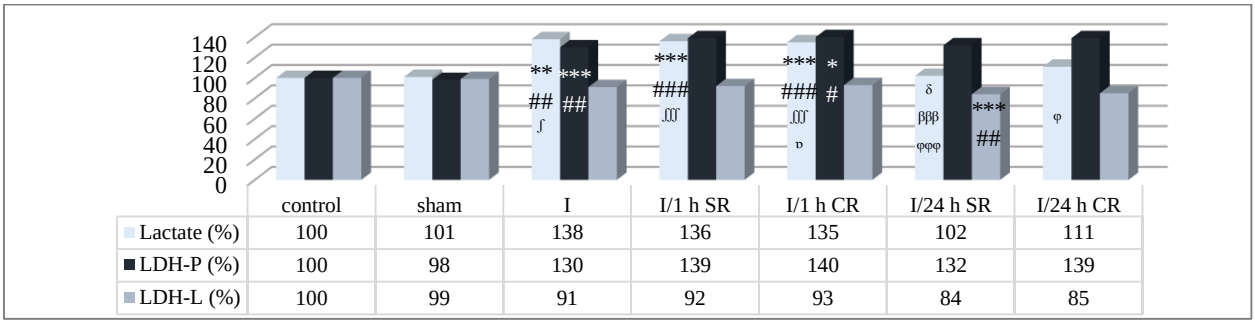
3.1. The impact of surgery on the studied biochemical markers

The assessment of the impact of surgery on biochemical parameters did not reveal statistically significant differences between the values measured in blood serum and ovarian homogenate between control (intact animals) and sham (animals subjected only to surgery) groups. Exceptions were IMA and CP levels in the blood serum. An increase in IMA (figure 3) by approximately 4% (p=0,003) and CP by almost 9% (p=0,028) (figure 12) was identified. Thus, the results of the study of changes in biochemical markers can be analyzed without considering the impact of surgery, except for the analysis of changes in IMA and CP values in blood serum.

3.2. Modification of metabolic indices in ovarian ischemia

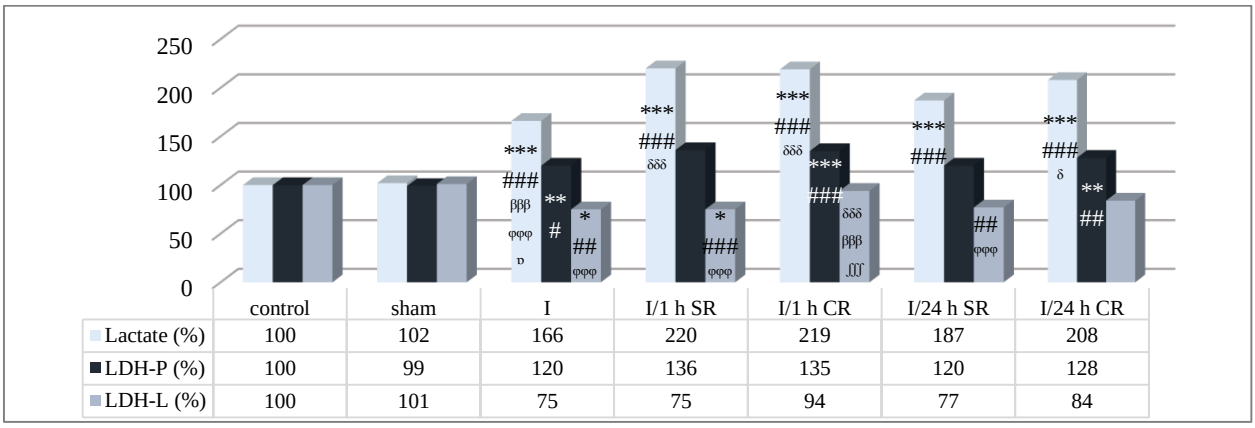
In our research we aimed to identify the metabolic changes of the structural-functional impairment of the twisted ovaries and the compensation mechanisms, as well as the elucidation of the preventive potential of the *on-off* technique for restoring the blood flow to the organ.

We established that ovarian torsion, with complete blockage of the blood flow, intensified anaerobic metabolism, increased the production of ROS, induced oxidative modification of various molecules and reduced the antioxidant protective activity of enzymes in ovarian tissue.



	control	sham	I	I/1 h SR	I/1 h CR	I/24 h SR	I/24 h CR
Lactate (%)	100	101	138	136	135	102	111
LDH-P (%)	100	98	130	139	140	132	139
LDH-L (%)	100	99	91	92	93	84	85

Figure 1. **Changes in lactate level and LDH-P and LDH-L activities in homogenate in experimental ovarian torsion**



	control	sham	I	I/1 h SR	I/1 h CR	I/24 h SR	I/24 h CR
Lactate (%)	100	102	166	220	219	187	208
LDH-P (%)	100	99	120	136	135	120	128
LDH-L (%)	100	101	75	75	94	77	84

Figure 2. **Changes in lactate level and LDH-P and LDH-L activities in blood serum in experimental ovarian torsion**

Note (for 1st and 2nd figure): I – ischemia; SR – simple reperfusion; CR – controlled reperfusion (*on-off*); LDH-P – lactate dehydrogenase-pyruvate; LDH-L – lactate dehydrogenase-lactate; h – hour. Statistically significant difference compared to: control (*), sham ($^{\#}$), I ($^{\delta}$), I/1 h SR ($^{\beta}$), I/1 h CR ($^{\varphi}$), I/24 h SR ($^{\int}$), I/24 h CR ($^{\upsilon}$): *, $^{\#}$, $^{\delta}$, $^{\beta}$, $^{\varphi}$, $^{\int}$, $^{\upsilon}$ – p<0,05; **, $^{\#\#}$, $^{\delta\delta}$, $^{\beta\beta}$, $^{\varphi\varphi}$, $^{\int\int}$, $^{\upsilon\upsilon}$ – p<0,01; ***, $^{\#\#\#}$, $^{\delta\delta\delta}$, $^{\beta\beta\beta}$, $^{\varphi\varphi\varphi}$, $^{\int\int\int}$, $^{\upsilon\upsilon\upsilon}$ – p<0,001.

Thus, in the animals from **the experimental group exposed to ovarian ischemia**, the increase in homogenate of the amount of lactate by almost 38%, p=0,006, and of the LDH-P activity by 30%, p<0,001, was found (figure 1). So, it is obvious that the adaptation mechanisms

in the conditions of oxygen supply interruption are triggered in the cells, the anaerobic oxidation of glucose ensuring the necessary energy for survival. Similar changes in ischemia markers were also reported in the blood serum, where an increase in lactate values by almost 66%, p<0,001 (figure 2), LDH-P activity by 20%, p=0,002 (figure 2), was found, and the amount of IMA detected was approximately 19% higher, p<0,001 (figure 3), compared to the control group.

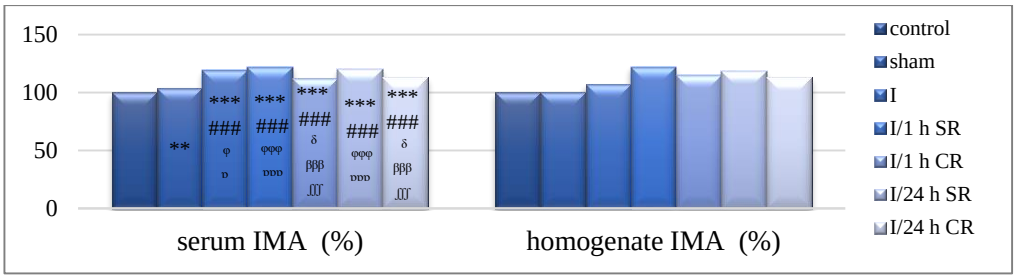


Figure 3. **Ischemia modified albumin (IMA) in experimental ovarian torsion**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham (#), I (δ), I/1 h SR (β), I/1 h CR (φ), I/24 h SR (ʃ), I/24 h CR (ν): *, #, δ, β, φ, ʃ, ν – p<0,05; **, ##, δδ, ββ, φφ, ʃʃ, νν – p<0,01; ***, ###, δδδ, βββ, φφφ, ʃʃʃ, ννν – p<0,001.

The recorded data suggest the need for careful evaluation of a patient with lower abdominal pain complaints to rule out ovarian torsion when lactate, LDH-P and/or serum IMA are high.

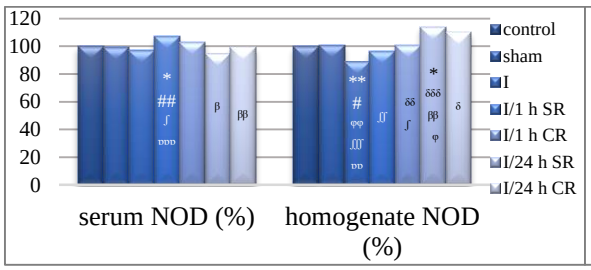


Figure 4. **Nitric oxide derivatives (NOD) in experimental ovarian torsion**

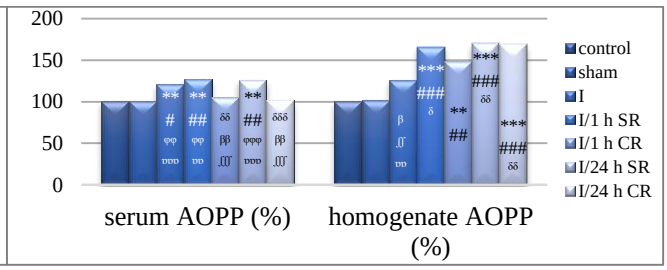


Figure 5. **Advanced oxidation protein products (AOPP) in experimental ovarian torsion**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham (#), I (δ), I/1 h SR (β), I/1 h CR (φ), I/24 h SR (ʃ), I/24 h CR (ν): *, #, δ, β, φ, ʃ, ν – p<0,05; **, ##, δδ, ββ, φφ, ʃʃ, νν – p<0,01; ***, ###, δδδ, βββ, φφφ, ʃʃʃ, ννν – p<0,001.

At the same time, in the homogenate of the rats from the group only with OT, a consumption of NOD was noticed, being determined their values by almost 11% lower than in the control group, p=0,003 (figure 4). We assume that NOD were involved in the production of nitric oxide.

The results of our study suggest that the resistance of the ovaries to ischemia could be due to both the intense activity of the LDH-P enzyme and the efficient use of NOD by the ovarian tissue.

There was observed that ischemia of the ovaries without reperfusion intensified the formation of MDA, AGE and AOPP, which are known as markers of oxidative lesions. Thus, in the homogenate were determined higher levels of MDA – by 23%, p<0,001 (figure 7), of AGE – by 19%, p=0,031 (figure 6), and a tendency of AOPP to increase, marked by larger levels by about 26%, p>0,05 (figure 5), compared to the control group. In the blood serum of animals only with OT were recorded increased values of DAM – by 8%, p<0,001 (figure 7), of AOPP – by almost 21%, p=0,007 (figure 5), the AGE remaining at levels close to those in the control group, p>0,05 (figure 6).

The obtained results confirm that the condition is accompanied by an increase in oxidative stress and one of the therapeutic approaches should be focused on reducing the formation of free radicals, even until the detorsion intervention.

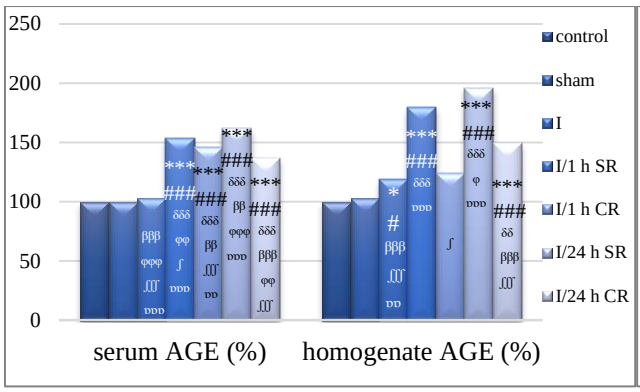


Figure 6. **The level of advanced glycation end products (AGE) in research groups**

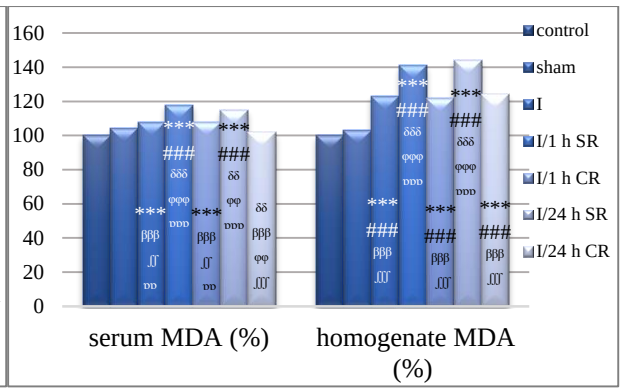
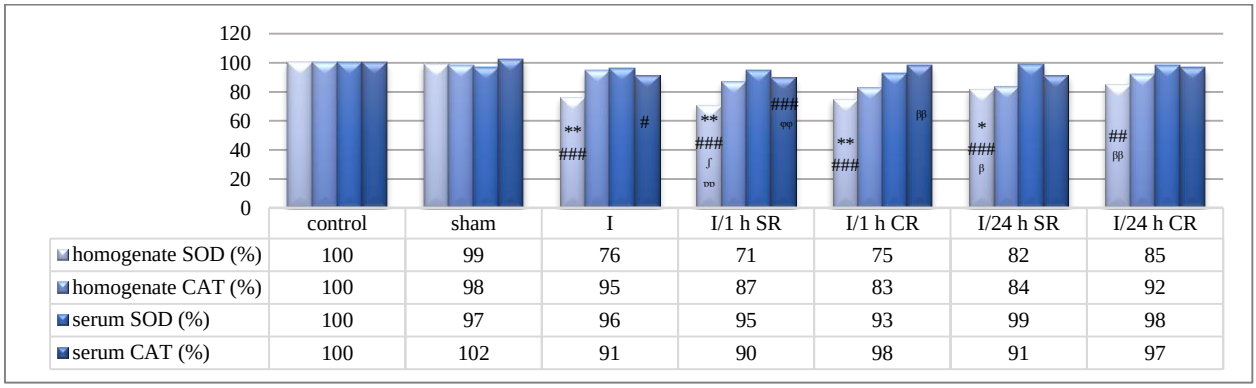


Figure 7. **The level of malondialdehyde (MDA) in experimental ovarian torsion**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham (#), I (δ), I/1 h SR (β), I/1 h CR (φ), I/24 h SR (ʃ), I/24 h CR (ν): *, #, δ, β, φ, ʃ, ν – p<0,05; **, ##, δδ, ββ, φφ, ʃʃ, νν – p<0,01; ***, ###, δδδ, βββ, φφφ, ʃʃʃ, ννν – p<0,001.

We did not identify statistically significant changes (p>0,05) in homogenate in the ovarian torsion group of CAT (figure 8), GR and GPO (figure 9), G6PDH and ICDH activity (figure 11), except for the statistically significant decrease of SOD – by 24%, p=0,002 (figure 8) and of GST – by 36%, p<0,001 (figure 9). It seems that the decrease of superoxide anion dismutation reaction during the ischemia period is one of the pathogenic links that induces tissue oxidative lesions recorded in our research. At the same time, the tissue antioxidant protection during this period is supported by CAT, GR, GPO, G6PDH and ICDH, which maintained their activity similar to that established in the control group. In addition, we noticed a tendency to increase the consumption of total glutathione and reduced glutathione by ovarian cells, their values being lower by about 13% and 23%, respectively, p>0,05 (figure 10), after OT compared to the group control, which would attest to the compensatory, protective response of ovarian cells exposed to high concentrations of free radicals, by amplifying glutathione-dependent neutralization mechanisms.



	control	sham	I	I/1 h SR	I/1 h CR	I/24 h SR	I/24 h CR
homogenate SOD (%)	100	99	76	71	75	82	85
homogenate CAT (%)	100	98	95	87	83	84	92
serum SOD (%)	100	97	96	95	93	99	98
serum CAT (%)	100	102	91	90	98	91	97

Figure 8. **Superoxide dismutase (SOD) and catalase (CAT) activity in blood serum and ovarian homogenate in experimental ovarian torsion/detorsion**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham (#), I (δ), I/1 h SR (β), I/1 h CR (φ), I/24 h SR (ʃ), I/24 h CR (ν): *, #, δ, β, φ, ʃ, ν – p<0,05; **, ##, δδ, ββ, φφ, ʃʃ, νν – p<0,01; ***, ###, δδδ, βββ, φφφ, ʃʃʃ, ννν – p<0,001.

After ovarian torsion modeling, GR, an important enzyme in glutathione recycling, increased its serum activity by almost 25%, p=0,045 (figure 9), and CP increased by approximately 7%, p<0,001 (figure 12), which suggests their involvement in maintaining of a balanced redox state of the body in induced ischemia conditions.

So, the antioxidant activity is relatively adapted in OT, but the deficiencies in the SOD activity in the tissue require the evaluation of treatment strategies with antioxidants that are able to capture ROS, possibly thus reducing oxidative damage.

3.3. The influence of the type of reperfusion on the biomarkers evaluated in ovarian torsion

As the conservative treatment of OT involves the detorsion of the ovarian pedicle to restore the blood flow, a comparative analysis of the results obtained in the **reperfusion groups** was performed to assess the usefulness of the proposed method of controlled reperfusion in reducing oxidative damage.

It was found that the type of reperfusion did not have a significant impact on the intensity of anaerobic processes in the ovarian tissue, as 1 hour after the restoration of blood flow were measured amounts of lactate and LDH-P activity similar to those during the ischemia period, $p>0,05$. However, after 24 hours from the detorsion maneuver, the decrease of the lactate level in the homogenate was observed, recording values being closed to those from the control group, $p>0,05$ (figure 1). The uptake of the lactate from the ovaries after their revascularization could explain the recorded serum increases (figure 2) and the corresponding tissue changes in our research (figure 1). Over time (after 24 hours) there was a tendency to decrease serum lactate level (figure 2), while maintaining the activity of the enzyme LDH-P in ovarian tissue at a level close to that in the group exposed only to ovarian torsion, $p>0,05$ (figure 1), which can attest to the restoration of the ovaries' ability to use lactate and other energy substrates due to the reactivation of aerobic oxidation processes.

The type and the term of reperfusion did not have a significant influence on IMA (figure 3) from the ovarian tissue, highlighting only a tendency to increase, more expressed in the groups with simple reperfusion *vs*. groups with controlled reperfusion: by 22% *vs*. 15% after 1 hour and by 18% *vs.* 13% after 24 hours compared to the control group, $p>0,05$. However, the values of this biomarker increased statistically significant by almost 22% *vs.* 12% after 1 hour, respectively by 20% *vs.* 13% after 24 hours in the blood serum compared to the control group, $p<0,001$. At the same time, recording a lower serum level of IMA in the *on-off* groups compared to the group exposed only to OT, $p<0,05$, and maintaining the values of this parameter in both groups with simple detorsion close to those in the group only with ovarian ischemia, $p>0,05$, is the proof that the *on-off* maneuver reduces the albumin damage and ensures it a higher ability to fix Co^{2+} than in simple reperfusion conditions.

The results of the NOD study (figure 4) in the reperfusion groups suggested the dual role of nitric oxide ($NO^{\cdot}$), its effects possible being dependent on both its concentration and other radicals, such as the superoxide anion. When large amounts of superoxide anion are produced exceeding the capacities of first-line antioxidant enzymes to provide effective protection, $NO^{\cdot}$ would have an increased lesional potential due to involvement in the formation of peroxynitrite and not NOD. The NOD level in the homogenate of the animals in the groups with 1 hour reperfusion changed not statistically significant compared to the control group, $p>0,05$, possibly due to the involvement of $NO^{\cdot}$ more in the production of peroxynitrite than in NOD. However, in these groups the NOD increased compared to the group exposed only to ischemia, but without any statistical significance in the group with simple reperfusion, $p>0,05$, and statistically significant in the group with controlled reperfusion, $p=0,001$. This fact highlights once again the protective effect of the *on-off* reperfusion, confirmed by a more expressed consumption of $NO^{\cdot}$ for the formation of NOD, probably reducing in this way the amount of peroxynitrite generated. Once the functionality of

antioxidant enzymes was restored, after 24 hours, the amount of NOD in the tissue increased, statistically significant compared to the group with ovarian ischemia, both after simple reperfusion, $p<0,001$ and after *on-off* reperfusion, $p=0,006$, which can be appreciated as a lower use of nitric oxide for the production of peroxynitrite compared to the initial period (after 1 hour). One of the therapeutic strategies that should be developed in the future should be aimed to reduce the level of superoxide anion. A low level of this radical would also mean a lower amount of peroxynitrite generated, respectively with a decrease of tissue damage.

Controlled reperfusion method *vs.* **simple reperfusion** had a favorable effect on reducing the oxidative changes of different molecules, revealing the advantage of the *on-off* detorsion technique. Nevertheless, it should be noted that the application of the *on-off* maneuver alone cannot prevent all oxidative events that are related to the time of restoration of blood flow in the ovarian tissue and manifested especially at a distance (after 24 hours).

Thus, after 1 hour from the controlled reperfusion we registered a tendency to increase the AGE by 24%, $p>0,05$, which passed the threshold of statistical significance after 24 hours, being measured larger quantities by 49%, $p<0,001$, compared to the control group. At the same time, the level of AGE recorded after 24 hours was significantly higher compared also to the group only with OT, $p<0,01$ (figure 6). Statistically significant increases in homogenate were also noted for AOPP – by approximately 48%, $p=0,001$, after 1 hour, respectively by 69% – after 24 hours, $p<0,001$, above the values recorded in the control group, which, however, after 1 hour were close to those in the group with ovarian ischemia, observing only a tendency to increase – by 17%, $p>0,05$, while after 24 hours, the increase was almost 34%, $p=0,009$, above the level of the group only with ovarian ischemia (figure 5). Also, the amount of MDA measured in the ovarian tissue after applying the *on-off* technique was approximately 22%, after 1 hour, and 24%, after 24 hours, $p<0,001$, above the values determined in the control group, but close to those in the ischemia group, $p>0,05$ (figure 7), which proves the ability of controlled reperfusion to prevent the enhancement of lipid peroxidation after the restoration of blood flow in the ischemic ovary. Moreover, the simple reperfusion of ovarian tissue, exposed to ischemia for a period of 3 hours, demonstrated the exacerbation of oxidative damage, a fact confirmed by increased levels of MDA – by 41% after 1 hour and by 44% after 24 hours, $p<0,001$ (figure 7), of AOPP – by 66% after 1 hour, respectively by 70% after 24 hours, $p<0,001$ (figure 5), of AGE – by 80% after 1 hour and by 96% after 24 hours, $p<0,001$ (figure 6), above the levels of the control group, exceeding statistically significant including the values established in the group of animals exposed only to ovarian ischemia, $p<0,05$.

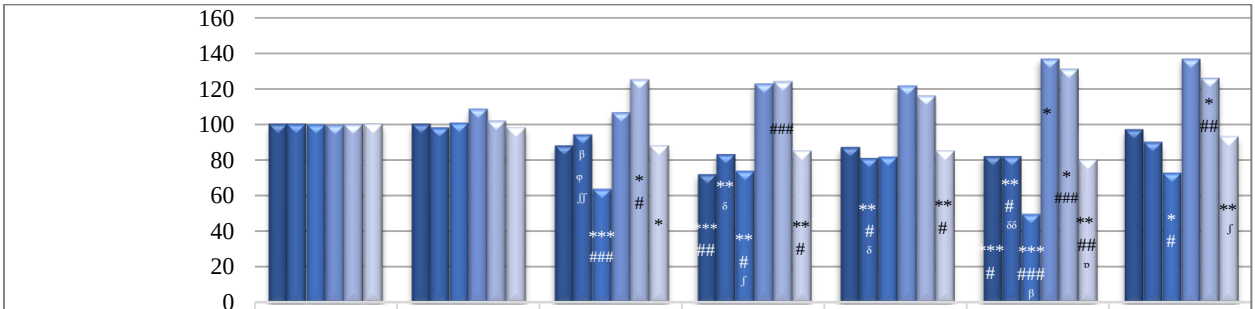
In both groups with controlled detorsion in blood serum we observed a lower level of AOPP than in the group of animals only with OT, $p<0,01$, and very close to that of the control group, $p>0,05$ (figure 5). In addition, there was a tendency to increase serum GSSG by 29% after 1 hour and by almost 40% after 24 hours from the application of ischemic postconditioning, $p>0,05$, compared to the control group, but not exceeding the values noted in the group only with ovarian ischemia, $p>0,05$ (figure 10). Furthermore, the amount of serum MDA after 1 hour from the *on-off* detorsion was close to that of the group only with OT, $p>0,05$, in both groups the lipid peroxidation index being approximately 8% higher, $p<0,001$, compared to the values recorded in the control group, and reaching a level close to that of the reference group (control) after 24 hours, when the differences were insignificant, $p>0,05$ (figure 7).

Even if the amount of serum AGE was statistically significant higher in the groups with controlled reperfusion compared to both the control group (by 46% after 1 hour and by 37% after

24 hours) and the one only with ovarian ischemia (by 42 % after 1 hour, respectively by 33% after 24 hours), p<0,001, without any obvious improvement, however, compared to the groups exposed to simple reperfusion, the AGE values were decreasing after the *on-off* technique, p<0,01 (figure 6), which reveals the advantage of PcI.

After simple reperfusion, these changes characterized by increases in serum levels of the biochemical indices of oxidative lesions were much more expressed than after the *on-off* technique. Thus, the AOPP level rise by more than 26% in both groups, p<0,01 (figure 5), the AGE – by 54% after 1 hour and by 62% after 24 hours, p<0,001 (figure 6), and the MDA – by 18% after 1 hour, respectively by 15% after 24 hours, p<0,001 (figure 7), compared to the control group, being above the values noted in the group only with OT. Therefore, we can emphasize that in the treatment of OT, detorsion with the restoration of blood flow is absolutely necessary when it is desired to preserve the organ, but it is not effective to prevent the oxidative damage of various molecules.

At the moment, we do not have enough data to make any recommendation to use the biomarkers of oxidative stress independently for the diagnosis of ovarian torsion. Nevertheless, they could complete the spectrum of laboratory markers used in the diagnosis of OT and the monitoring of the evolution of the pathological condition, as well as the efficiency of the applied treatment, the reference values for the respective laboratory indices have to be determined in further studies.



	control	sham	I	I/1 h SR	I/1 h CR	I/24 h SR	I/24 h CR
homogenate GPO (%)	100	100	88	72	87	82	97
homogenate GR (%)	100	98	94	83	81	82	90
homogenate GST (%)	100	101	64	74	82	50	73
serum GPO (%)	100	109	107	123	122	137	137
serum GR (%)	100	102	125	124	116	131	126
serum GST (%)	100	98	88	85	85	80	93

Figure 9. **Glutathione peroxidase (GPO), glutathione reductase (GR) and glutathione-S-transferase (GST) activity in experimental ovarian torsion**

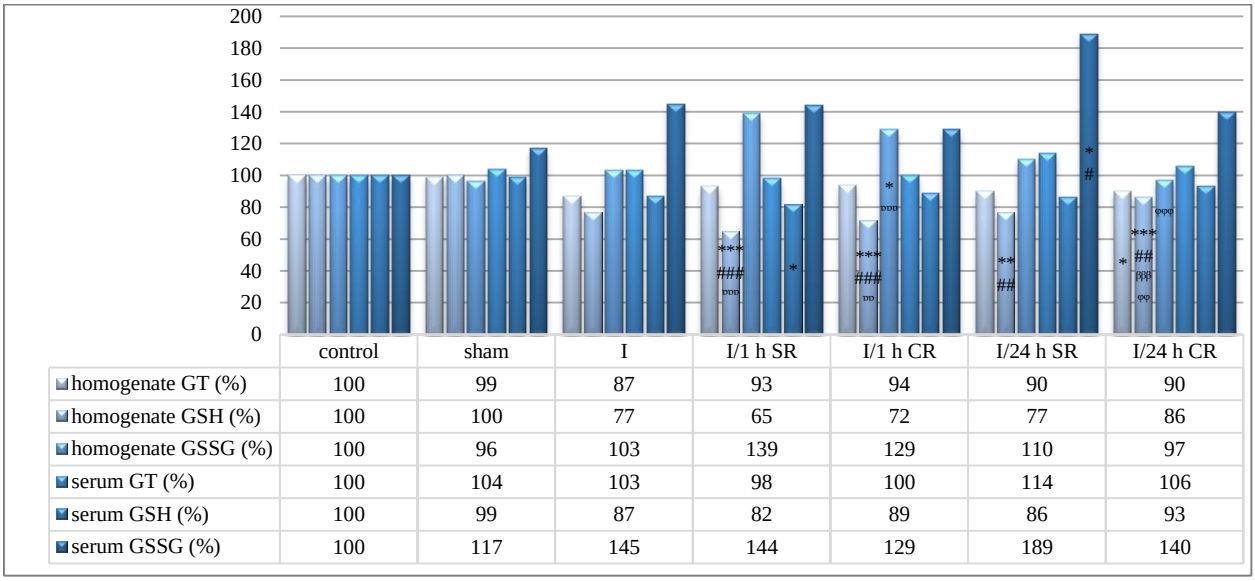
Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham ($^{\#}$), I ($^{\delta}$), I/1 h SR ($^{\beta}$), I/1 h CR ($^{\varphi}$), I/24 h SR ($^{\int}$), I/24 h CR ($^{\nu}$): *, $^{\#}$, $^{\delta}$, $^{\beta}$, $^{\varphi}$, $^{\int}$, $^{\nu}$ – p<0,05; **, $^{\#\#}$, $^{\delta\delta}$, $^{\beta\beta}$, $^{\varphi\varphi}$, $^{\int\int}$, $^{\nu\nu}$ – p<0,01; ***, $^{\#\#\#}$, $^{\delta\delta\delta}$, $^{\beta\beta\beta}$, $^{\varphi\varphi\varphi}$, $^{\int\int\int}$, $^{\nu\nu\nu}$ – p<0,001.

Our study identified changes in the activity of the antioxidant system as a result of detorsion of the twisted ovaries. **Controlled reperfusion** *vs.* **simple reperfusion** was distinguished by the fact that it maintained the activity of antioxidant enzymes in the ovaries at a higher level, as well as the amount of total glutathione and reduced glutathione, which ensured a greater protection of the ovarian tissue against oxidative stress. Thus, 1 hour after the usage of the *on-off* technique, in homogenate it was found that CAT (figure 8), GPO and GST (figure 9), GT (figure 10) and ICDH (figure 11) were maintained at a level close to that of the group control, p>0,05, recording only a tendency to decrease, while the activity of G6PDH (figure 11) had a tendency to increase, but without exceeding the threshold of statistical significance, p>0,05. At the same time, there was a statistically significant decrease in SOD activity by almost 25%, p=0,002, compared to the control

group, which, however, remained close to that observed in the group only with ovarian ischemia, p>0,05 (figure 8), and a reduction of GR activity by 19%, p=0,006, compared to the control group, the values being also lower than those in the group only with OT – by 13%, p=0,036 (figure 9). Moreover, a consumption of GSH was noted, its level being below 28%, p<0,001, compared to that measured in the control group, but which was not different compared to that in the group only with ovarian ischemia, p>0,05 (figure 10), which is an adaptive change in the activity of the antioxidant system to ensure the protection of cells.

After 24 hours of controlled reperfusion, the activity of CAT (figure 8), GPO (figure 9), G6PDH and ICDH (figure 11) remained approximately at the level of the control group, p>0,05, and there was observed the restoration of SOD (figure 8) and GR activity (figure 9), the registered values being close to those in the control group, p>0,05, however, with the maintenance of a consumption of GT and GSH, and a GST activity similar to that from the group of animals exposed to only ovarian ischemia, p>0,05, the values noted being for GT by 10%, p=0,012, for GSH by 14%, p<0,001 (figure 10), and for GST by 27%, p=0,04 (figure 9), smaller compared to the control group.

It was established that the reduction of GR activity in the initial period of controlled reperfusion (after 1 hour) had unfavorable consequences on the process of glutathione recycling in the ovaries, increasing the GSSG level by almost 29%, p=0,011 (figure 10) above the values of the control group, and as once with the restoration of GR activity, after 24 hours, the amount of GSSG to decrease also to a level close to that in the control group and below the values of the group only with OT, p>0,05.



	control	sham	I	I/1 h SR	I/1 h CR	I/24 h SR	I/24 h CR
homogenate GT (%)	100	99	87	93	94	90	90
homogenate GSH (%)	100	100	77	65	72	77	86
homogenate GSSG (%)	100	96	103	139	129	110	97
serum GT (%)	100	104	103	98	100	114	106
serum GSH (%)	100	99	87	82	89	86	93
serum GSSG (%)	100	117	145	144	129	189	140

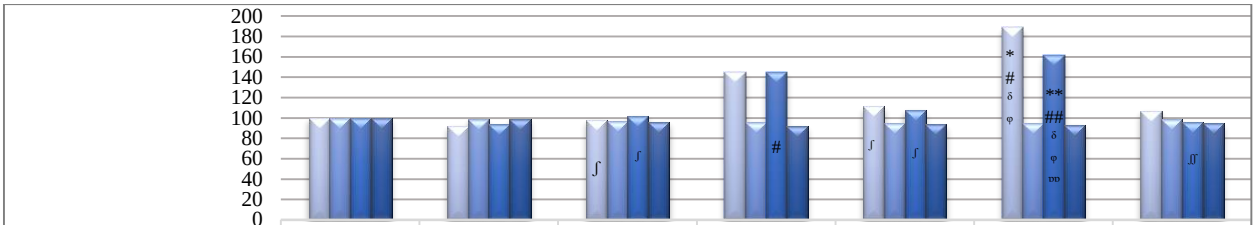
Figure 10. **The influence of ovarian torsion/detorsion on total glutathione (GT), reduced glutathione (GSH) and oxidized glutathione (GSSG) levels**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham ($^{\#}$), I ($^{\delta}$), I/1 h SR ($^{\beta}$), I/1 h CR ($^{\varphi}$), I/24 h SR ($^{\int}$), I/24 h CR ($^{\nu}$): *, $^{\#}$, $^{\delta}$, $^{\beta}$, $^{\varphi}$, $^{\int}$, $^{\nu}$ – p<0,05; **, $^{\#\#}$, $^{\delta\delta}$, $^{\beta\beta}$, $^{\varphi\varphi}$, $^{\int\int}$, $^{\nu\nu}$ – p<0,01; ***, $^{\#\#\#}$, $^{\delta\delta\delta}$, $^{\beta\beta\beta}$, $^{\varphi\varphi\varphi}$, $^{\int\int\int}$, $^{\nu\nu\nu}$ – p<0,001.

There was not observed a restoration of SOD and GR activity at the level considered as a reference (control group) in our research, after simple reperfusion of ovarian tissue, as we found after applying the *on-off* technique, most of the parameters evaluated after simple detorsion keeping their values close to those in the group only with ovarian ischemia even after 24 hours from the restoration of blood flow (SOD, GPO, GST, GT, GSH). Thus, compared to the reference group (control), in the homogenate of the animals from the groups with simple reperfusion,

significant reductions of SOD activity were observed both after 1 hour, by 29%, p=0,001, and after 24 hours, by 18%, p=0,03 (figure 8), of GPO – by 28% after 1 hour and 18% after 24 hours, p<0,001 (figure 9), of GR – by 17% after 1 hour, p=0,006, respectively by 18% after 24 hours, p=0,003 (figure 9), of GST – by 26% after 1 hour, p=0,008, and 50% after 24 hours, p<0,001 (figure 9), and the amount of GSH – by 35% after 1 hour, p<0,001, respectively 23% after 24 hours, p=0,002 (figure 10), with a concomitant tendency to increase of GSSG level by 39% after 1 hour and by 10% after 24 hours, p>0,05 (figure 10), recording also an increase of G6PDH activity by 45% after 1 hour, p>0,05, respectively by 89% after 24 hours, p=0,023 (figure 11).

The activity of CAT (figure 8) and ICDH (figure 11) did not register significant changes in the ovarian tissue in our study, in the groups with simple reperfusion, there being observed only a tendency to decrease, compared to both the control group and the one only with ovarian ischemia, p>0,05.



	control	sham	I	I/1 h SR	I/1 h CR	I/24 h SR	I/24 h CR
homogenate G6PDH (%)	100	92	98	145	111	189	107
homogenate ICDH (%)	100	99	97	96	95	95	99
serum G6PDH (%)	100	94	102	145	108	162	96
serum ICDH (%)	100	99	96	92	94	93	95

Figure 11. **Glucose-6-phosphate dehydrogenase (G6PDH) and cytosolic NADP$^+$-dependent isocitrate dehydrogenase (ICDH) activity in experimental ovarian torsion**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham ($^{\#}$), I ($^{\delta}$), I/1 h SR ($^{\beta}$), I/1 h CR ($^{\varphi}$), I/24 h SR ($^{\int}$), I/24 h CR ($^{\nu}$): *, $^{\#}$, $^{\delta}$, $^{\beta}$, $^{\varphi}$, $^{\int}$, $^{\nu}$ – p<0,05; **, $^{\#\#}$, $^{\delta\delta}$, $^{\beta\beta}$, $^{\varphi\varphi}$, $^{\int\int}$, $^{\nu\nu}$ – p<0,01; ***, $^{\#\#\#}$, $^{\delta\delta\delta}$, $^{\beta\beta\beta}$, $^{\varphi\varphi\varphi}$, $^{\int\int\int}$, $^{\nu\nu\nu}$ – p<0,001.

The extend of ovarian oxidative lesions found after simple detorsion in our research could be due to a significant reduction in GR enzyme activity, recorded by statistically significant lower values in simple reperfusion groups compared to the group only with ovarian ischemia, p<0,05 (figure 9). As the antioxidant efficacy of this enzyme depends on NADPH, the synthesis of which does not seem to suffer when the blood flow was restored, being even observed an enhance of G6PDH activity after 24 hours from detorsion by over 93%, p=0,018, above the level of ovarian ischemia group (figure 11), we assume a preferential tissue consumption of NADPH by prooxidant enzymes.

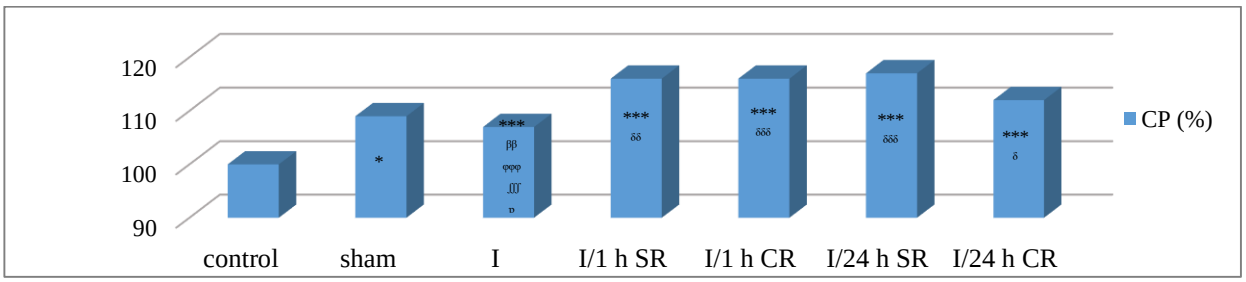


Figure 12. **Serum ceruloplasmin (CP) level in experimental ovarian torsion**

Note: I – ischemia; SR – simple reperfusion; CR – controlled reperfusion *(on-off)*; h – hour. Statistically significant difference compared to: control (*), sham ($^{\#}$), I ($^{\delta}$), I/1 h SR ($^{\beta}$), I/1 h CR ($^{\varphi}$), I/24 h SR ($^{\int}$), I/24 h CR ($^{\nu}$): *, $^{\#}$, $^{\delta}$, $^{\beta}$, $^{\varphi}$, $^{\int}$, $^{\nu}$ – p<0,05; **, $^{\#\#}$, $^{\delta\delta}$, $^{\beta\beta}$, $^{\varphi\varphi}$, $^{\int\int}$, $^{\nu\nu}$ – p<0,01; ***, $^{\#\#\#}$, $^{\delta\delta\delta}$, $^{\beta\beta\beta}$, $^{\varphi\varphi\varphi}$, $^{\int\int\int}$, $^{\nu\nu\nu}$ – p<0,001.

At the same time, it seems that CP has an important role in OT by influencing the pathological process after the restoration of blood flow, and its statistically significant increase in all groups with reperfusion above the level recorded in the group only with ovarian ischemia, $p<0,05$ (figure 12), support the antioxidant protection of the body.

The treatment of ovarian torsion by *on-off* reperfusion detorsion reduced tissue oxidative damage caused by the I/R phenomenon. However, additional assessments are needed to identify the exact mechanism by which the *on-off* method provides protection of ovarian tissue after revascularization.

Moreover, it was found that not in all experimental groups was a blood-tissue correlation of the examined parameters, which does not allow us to draw favorable conclusions about the presence of an absolute interdependence of phenomena occurring in the ovaries with their blood biochemical expression.

It should be noted that the results of our study are from the exposure of laboratory animals to OT and some findings may not be valid for human subjects. Thus, it is essential to continue research on women suspected of OT with verification of the hypotheses already formulated in this work regarding the usefulness of biochemical markers studied in the diagnosis and/or monitoring of the evolution of this pathological condition with severe repercussions on fertility.

GENERAL CONCLUSIONS

1. In ovarian torsion the metabolism is adapted to the conditions of interruption of blood flow, a fact reflected by an increased tissue level of lactate (by 38%, $p=0,006$) and an intense activity of LDH-P (by 30%, $p<0,001$), anaerobic energy production supporting vital functions and cell survival. Increased tissue consumption of NOD (by 11%, $p=0,003$) could confer resistance of the ovaries to ischemia.

2. Ovarian torsion induces amplification of pathological oxidative processes in the ovaries and structural-functional damage of cells, expressed by increased MDA (by 23%, $p<0,001$) and AGE (by 19%, $p=0,031$), suggestive for an exacerbation of free radicals production.

3. Decreased superoxide anion dismutation reaction related to SOD enzyme (by 24%, $p=0,002$) and reduction of GST activity (by 36%, $p<0,001$) in the twisted ovaries could be factors responsible for creating the imbalance characteristic for oxidative stress. However, the tissue antioxidant protection during this period is supported by CAT, GR, GPO, G6PDH and ICDH, which maintained their activity similar to that of the control group.

4. Serum changes of the studied biochemical indices are not the expression of tissue modifications, given the presence of a small number of blood-tissue correlations of the evaluated parameters only in certain research groups, which is not an argument for their use to assess the OT tissue alterations based on serum measurements.

5. Controlled reperfusion compared to simple one has shown its advantage through its ability to prevent the increase in lipid peroxidation in the ovaries after the restoration of the blood flow (MDA, $p>0,05$), and partially the damage to cellular structures (AGE), by restoring the activity of SOD and GR enzymes, and the amount of GSSG from the homogenate at the level considered as the reference (control), after 24 hours after detorsion, and the prevention of exacerbation of CAT, GPO and GST function disorders.

PRACTICAL RECOMMENDATIONS

1. Our work may be a basis for expanding research on ovarian torsion and the experimental model of ovarian torsion applied in our study is a true one and can be used in similar experimental research.

2. It is recommended to use in clinical practice the evaluation of LDH-P activity, the amount of lactate and serum IMA when the ischemic cause of lower abdominal pain is suspected, in order to complete the indices that substantiate the application of diagnostic laparoscopy as a "gold standard" for confirming the diagnosis of ovarian torsion.

3. It is proposed to use MDA, AOPP and AGE to monitor the effects of reperfusion and/or drug treatment on the evolution of ovarian torsion.

4. The controlled reperfusion technique, proposed and tested in this study, is recommended for use in the surgical treatment of ovarian preservation in patients with ovarian torsion, as its application has shown a decrease in oxidative damage and a higher degree of antioxidant protection, having the advantage of being easy to apply and no additional costs required.

5. When making the therapeutic decision, it is recommended to take into account the fact that alone, the *on-off* method of organ detorsion does not completely prevent the negative effects of reperfusion, requiring other interventions to reduce oxidative stress and its consequences.

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INFORMATION REGARDING THE VALORIZATION OF RESEARCH RESULTS

LIST OF SCIENTIFIC PUBLICATIONS AND EVENTS at which the results of the researches for the doctoral thesis in medical sciences with the topic „Metabolic feature of ischemia/reperfusion caused by ovarian torsion/detorsion" were presented

- **Articles in ISI, SCOPUS journals and other international databases:**

1. **Lazăr C**., Vozian M., Pantea V., Mișina A., Tagadiuc O. Ischemia modified albumin in experimental ovarian torsion with and without controlled reperfusion. *Rev Romana Med Lab.* 2019; 27(1): p. 43-50. ISSN online: 2284-5623; ISSN-L: 1841-6624; Available at: http://www.rrml.ro/articole/articol.php?year=2019&vol=1&poz=5 **(Web of Science, IF$_{ISI}$: 0,8)**, published with the support of European Scientific Center „Biomarkers" https://escbm.org/page/view/grants
2. **Lazăr C**., Vozian M., Pantea V., Svet I., Mishina A., Tagadiuc O. The effect of controlled reperfusion on experimental ovarian torsion. *Russian Open Medical Journal*. 2019; 8(4): e0404. Available at: https://romj.org/2019-0404 **(Web of Science**, **SCOPUS, IF$_{CiteScore2019}$: 0,6)**

- **Articles in accredited national scientific journals:**

✓ **articles in B category journals:**

3. **Lazăr C**., Tagadiuc O., Protopop S., Mişina A., Pantea V. Aspecte ale metabolismului în ţesutul ovarian. *Buletin de Perinatologie*. 2016; 4(72): p. 52-58. ISSN 1810-5289. Available at: https://ibn.idsi.md/ro/vizualizare_articol/49646

4. **Lazăr C**., Protopop S., Mişina A., Tagadiuc O. Efectele speciilor reactive de oxigen asupra sistemului de reproducere feminin. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale.* 2017; 2(54): p. 83-90. ISSN 1857-0011. Available at: https://ibn.idsi.md/ro/vizualizare_articol/54131
5. **Lazăr C**., Mişina A., Tagadiuc O. Rolul indicilor de laborator în diagnosticul torsiunii ovariene (revista literaturii). *Moldovan Journal of Health Sciences (Revista de Ştiinţe ale Sănătăţii din Moldova).* 2018; 16(2): p. 52-61. ISSN 2345-1467. Available at: https://ibn.idsi.md/ro/vizualizare_articol/65758

- **Articles in international scientific collections:**

6. Mișina A., Madan D., Tagadiuc O., **Lazăr C**., Fuior L. Diagnosticul și tratamentul torsiunii ovariene la copii și adolescente. *Archives of the Balkan Medical Union.* 2015; 50(2), supl. 1: p. 84-89. ISSN 0041-6940.
7. **Lazăr C**., Mișina A., Cuţescu I., Tagadiuc O. Mechanisms of reactive oxygen species production in the lesions caused by ischemia/reperfusion. *Archives of the Balkan Medical Union.* 2016; 51(1), supl. 1: p. 199-204. ISSN 0041-6940.

- **Abstracts/theses submitted at national or international scientific conferences:**

8. **Lazăr C**. The main paths of reactive oxygen species production in disorders caused by ischemia/repefusion. In: *Abstract book of 6th International Medical Congress for Students and Young Doctors MedEspera*. Chişinău, Moldova, May 12-14, 2016, p. 251. ISBN 978-9975-3028-3-8.
9. **Лазэр К**., Тагадюк О., Мишина А., Возиан М., Пантя В. Эффекты контролируемой реперфузии на уровень малонового диальдегида в сыворотке крови при ишемии-реперфузии яичников. *XXX Юбилейный международный конгрессс курсом эндоскопии. Новые технологии в диагностике и лечениигинекологических заболеваний*. Moscow, Russia, June 6–9, 2017, p. 92-93. ISBN 978-5-906484-36-9.
10. **Lazăr C**., Pantea V., Vozian M., Misina A., Tagadiuc O. Simple reperfusion increases the AOPP in ovarian torsion. In Abstract book: *IBC - SOFIA 2017, 2nd INTERNATIONAL BIOMEDICAL CONGRESS of SOFIA 2017.* Sofia, Bulgaria, November 17-19, 2017, p. 38.
11. **Lazăr C**. Ceruloplasmin in experimental ovarian torsion/detorsion. In: *Biological markers in fundamental and clinical medicine*, vol. 1, no. 3, 2017, p. 24-25. ISSN 2570-5911 (PRINT), ISSN 2570-5903 (ON-LINE).
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16. **Lazăr C**. Este dialdehida malonică un marker util în torsiunea ovariană? In: *Culegere de rezumate ştiinţifice ale studenţilor, rezidenţilor şi tinerilor cercetători a USMF "Nicolae Testemiţanu"*, Chişinău, Moldova, 2018, p. 14. ISBN 978-9975-82-103-2.
17. **Lazăr C**. Are nowadays laboratory indices useful in ovarian torsion? In: *"BIMCO Journal" - Abstract book of the Bukovinian International Medical Congress 2019,* Chernivtsi, Ukraine, p. 3. ISSN 2616-5392.
18. **Lazăr C**. Modificări oxidative ale proteinelor în torsiunea ovariană. In: *Culegere de rezumate ştiinţifice ale studenţilor, rezidenţilor şi tinerilor cercetători a USMF "Nicolae Testemiţanu"*, Chişinău, Moldova, 2019, p. 20. ISBN 978-9975-82-148-3.
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- **Oral communications at scientific forums:**

✓ **international**

20. **Lazăr C**. The main paths of reactive oxygen species production in disorders caused by ischemia/repefusion. *6th International Medical Congress for Students and Young Doctors MedEspera.* SUMPh „Nicolae Testemițanu", Chişinău, Moldova, May 12-14, 2016.
21. **Lazăr C**., Tagadiuc O., Misina A., Vozian M., Pantea V. Controlled reperfusion and oxidative stress-induced lesions in experimental ovarian torsion/detorsion. *Annual Young Medical Scientists' Conference 2017,* Bogomolets National Medical University, Kiev, Ukraine, October 27-29, 2017 (***3rd place***).
22. **Lazăr C**. Ceruloplasmin in experimental ovarian torsion/detorsion. Conference „*Biological markers in fundamental and applied biology. From theory to practice*", European Scientific Center „Biomarkers", Brno, Czech Republic, January 10, 2018.
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24. **Lazăr C**. Ischemia-modified albumin in experimental ovarian torsion. Scientific and practical conference „*Theoretical and practical aspects of the use of biological markers in fundamental and applied medicine and biology*", European Scientific Center „Biomarkers", Prague, Czech Republic, March 27-29, 2018 (***1st place***).
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- **Posters at scientific forums:**

✓ **international**

31. **Lazăr C**. Simple reperfusion increases the AOPP in ovarian torsion. *IBC - SOFIA 2017, 2nd INTERNATIONAL BIOMEDICAL CONGRESS of SOFIA 2017,* Medical University – Sofia, Sofia, Bulgaria, November 17-19, 2017.

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32. **Lazăr C**., Tagadiuc O. Rolul mitocondriilor în declanşarea stresului oxidativ în patologii determinate de ischemie/reperfuzie. IMU Annual Scientific Conference of young specialists „*Performanţe şi perspective în urgenţe medico-chirurgicale*." Institute of Emergency Medicine, Chişinău, Moldova, May 20, 2016.

LAZĂR CORNELIA

METABOLIC FEATURE OF ISCHEMIA/REPERFUSION CAUSED BY OVARIAN TORSION/DETORSION

315.01. MEDICAL BIOCHEMISTRY

Summary of doctoral thesis in medical sciences

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