Biol. Stud. 2024; 18(3): 25–36 • doi: https://doi.org/10.30970/sbi.1803.788
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 612.616.2:612.015.1]-07

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF INDICATORS OF GLUTATHIONE ANTIOXIDANT SYSTEM IN SPERMATOZOA OF INFERTILE MEN WITH DIFFERENT FORMS OF PATHOSPERMIA

Zoryana Fedorovych*, *Mykola Vorobets*, *Olena Onufrovych*, *Oksana Melnyk*, *Natalia Gromnatska*, *Nataliya Lychkovska*, *Anna Besedina*, *Zinoviy Vorobets*, *Roman Fafula

Danylo Halytsky Lviv National Medical University, 69 Pekarska St., Lviv 79010, Ukraine

Fedorovych, Z., Vorobets, M., Onufrovych, O., Melnyk, O., Gromnatska, N., Lychkovska, N., Besedina, A., Vorobets, Z. & Fafula, R. (2024). Diagnostic sensitivity and specificity of indicators of glutathione antioxidant system in spermatozoa of infertile men with different forms of pathospermia. *Studia Biologica*, 18(3), 25–36. doi:10.30970/sbi.1803.788

Background. One of the most important antioxidant defence mechanism in spermatozoa is the gluthatione system which includes glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and reduced glutathione (GSH). It is promising to use ROC analysis, which allows to assessing the diagnostic sensitivity and specificity of indicators.

Materials and methods. Infertile men were divided into 3 groups: patients with oligozoospermia (n = 30), asthenozoospermia (n = 34), and oligoasthenozoospermia (n = 22). To assess the diagnostic sensitivity and specificity of indicators, the values of the biomarkers were tested using the receiver operating characteristic (ROC) curve, and the area under it (AUC), the standard error (SE) and the 95% confidence interval (CI 95%) were calculated.

Results. The ROC analysis showed that GP activity was characterized by excellent diagnostic significance for diagnosing both oligozoospermia and asthenozoospermia (the sensitivity was 100%, and specificity – 100%). The GR activity has moderate diagnostic significance, since the AUC is 0.654 (95% CI from 0.503 to 0.785, P = 0.0645) for oligozoospermic, the AUC is 0.612 (95% CI from 0.454 to 0.7555, P = 0.1979) for asthenozoospermic men. The analysis of the ROC curve revealed a good diagnostic value of GsT activity in sperm samples for the diagnosis of pathospermia (sensitivity of 75% and specificity of 80%). Simultaneously, it was shown that GSH content could not serve as valuable biomarkers for distinguishing patients with pathospermia from healthy controls, with an AUC of 0.615, corresponding to moderate diagnostic significance for oligozoospermia.

Conclusion. The results of this study show that the cut-off points for the biomarkers glutathione peroxidase and glutathione-S-transferase can be used to distinguish between patients with pathospermia and normozoospermia, and the parameters themselves can serve as valuable diagnostic biomarkers to distinguish patients with pathospermia from healthy controls, regardless of the causes of pathospermia. The value of these indicators below the cut-off point indicates the probable presence of pathology. Indicators of GR activity and GSH content have a low diagnostic value, which makes them unsuitable for use as laboratory tests for the diagnosis of male infertility.

Keywords: male infertility, antioxidant enzymes, glutathione system, ROC analysis

INTRODUCTION

It is known that free radical processes and an excess of reactive oxygen species are associated with the development of male infertility. The ratio between free radical processes and activity of antioxidant defense systems can change depending on the functional state of the organism and under the influence of various environmental factors. Under physiological conditions, a balanced ratio of the pro- and antioxidant system is maintained. There is a shift towards the increase of pro-oxidant processes under the development of oxidative stress (Pizzino *et al.*, 2017). The antioxidant system is multicomponent and includes both enzymatic and non-enzymatic links. The body's reaction to stress can be accompanied by a short-term increase in the concentration of reactive oxygen species, which play the role of secondary messengers and are involved in signal transduction and in the expression of a number of genes (Hong *et al.*, 2024). The mobilization of antioxidant protection is noted simultaneously, which ensures a decrease in the level of reactive compounds, thus preventing manifestations of their toxic effects. Under pathophysiological conditions, the toxic effect of active forms is manifested, which is accompanied by a sharp intensification of free radical processes and a decrease in the activity of antioxidant protection (Agarwal & Bui, 2017; Mannucci *et al.*, 2022).

Our previous studies have demonstrated that free radical processes are significantly activated, which is confirmed by the increase in the content of TBA-active products in spermatozoa and seminal plasma of infertile men with various forms of pathospermia. The inhibition of the activities of the enzymes of the glutathione antioxidant system (glutathione peroxidase (GPx, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2), glutathione-S-transferase (GST, EC 2.5.1.18)), as well as a decrease in the content and redox index of glutathione in the spermatozoa of infertile men were revealed (Fafula *et al.*, 2017; Vorobets *et al.*, 2018; Fafula *et al.*, 2023). The obtained results indicate only the direction of changes in the indicators of men's spermatozoa with various forms of pathospermia, however, the question of their diagnostic value often arises.

The conventional methods of diagnosing infertility do not always indicate the cause of cell morphology and function disorders, reveal the mechanism of metabolic changes, or accurately and fully reflect them. It is promising to use the method called ROC analysis that allows to assessing the diagnostic sensitivity and specificity of indicators. Sensitivity is the probability of a positive result in the patient; it characterizes the ability of the sample to detect disease (pathology). Specificity is the probability of a negative result in a healthy person; it characterizes the ability of the sample to detect the absence of the disease (Trevethan, 2017). This analysis is based on the construction of the so-called ROC curve, which is built in the coordinates "sensitivity – 1-specificity" and shows the dependence of the number of correctly classified positive examples on the number of incorrectly classified negative examples. The ROC analysis allows an objective determination of the diagnostic significance of a separate test based on AUC (Area Under Curve) values. AUC is an integral indicator of the prognostic efficiency of a marker, which determines the probability that the value of a trait-marker, or biomarker, in a randomly selected patient is greater than in a randomly selected healthy person (Goncalves *et al.*, 2014; Xu *et al.*, 2014).

The aim of present work is to analyze the diagnostic sensitivity and specificity of indicators of the glutathione antioxidant systems of spermatozoa of men with different forms of pathospermia.

MATERIALS AND METHODS

The material for analysis was data of previous studies performed on spermatozoa of infertile men with various forms of pathospermia. The data of 86 men who underwent primary examination due to infertility in the consultative polyclinic of the Lviv Regional Clinical Hospital were analyzed. Inclusion criteria: age 21-39 years, infertility in marriage 1–10 years, male infertility factor, under the conditions of oligozoospermia and/or asthenozoospermia. Exclusion criteria: infertility in marriage for more than 10 years, azoospermia, excessive alcohol consumption and exposure to any harmful physical and chemical factors during diagnostic and therapeutic procedures. All patients were divided into 3 groups: patients with oligozoospermia (n = 30), asthenozoospermia (n = 34), oligoasthenozoospermia (n = 22). The control group included 40 somatically healthy men of similar age with preserved fertility, normozoospermia and confirmed paternity. Indicators of spermograms (concentration of spermatozoa, their motility, morphology and percentage of live forms) were assessed using light microscopy, according to the guidelines for their implementation (WHO, 2010). Approval for the study was taken from the ethics committee of Danylo Halytsky Lviv National Medical University (protocol No 4 from 26 April 2021; protocol No 7 from 26 June 2023).

To assess the diagnostic sensitivity and specificity of indicators, the values of the biomarkers were tested using the receiver operating characteristic (ROC) curve. The area under it (AUC), the standard error (SE) and the 95% confidence interval (CI 95%) were calculated. The diagnostic significance of the area under the ROC curve is assessed as follows: 0.9–1.0 – excellent diagnostic significance of the studied indicator, >0.8–0.9 – good diagnostic significance, >0.7–0.8 – satisfactory diagnostic significance, >0.6–0.7 – moderate diagnostic significance, 0.5–0.6 – insufficient diagnostic significance. ROC curves are often used to determine the "optimal" cutoff point at which subjects will be classified as positive or negative (Kumar *et al.*, 2011). Software used for analysis was MedCalc® Statistical Software version 22.018 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024).

RESULTS AND DISCUSSION

The receiver operating curve (ROC) analysis for glutathione peroxidase (GP), glutathione reductase (GR), glutathione-S-transferase (GsT) and reduced glutathione (GSH) was performed to evaluate the utility of these biomarkers in distinguishing infertile patients from healthy controls. The results of the evaluation of the ROC curve for GP activity as a biomarker for diagnosing pathospermia are shown in **Fig. 1** and **Table 1**.

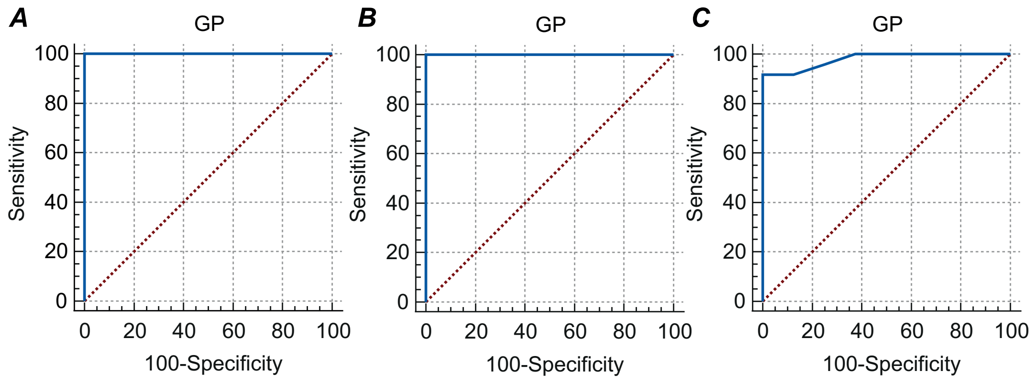

Fig. 1. Receiver operating characteristic (ROC) curves of glutathione peroxidase activity as a marker in the diagnosis of oligozoospermia (***A***), asthenozoospermia (***B***) and oligoasthenozoospermia (***C***)

Table 1. **The diagnostic value of GP activity in the diagnosis of different forms of pathospermia**

Indicators / Pathospermia	AUC	Standard error (for AUC)	95%CI (for AUC)	Sensitivity (%)	Specificity (%)	Associated criterion (cut-off point), μmol of GSH per minute per 1 mg of protein
Oligozoospermia	1.000 P <0.0001	0.000	0.920 to 1.000	100.00	100.00	≤4.2
Asthenozoospermia	1.000 P <0.0001	0.000	0.920 to 1.000	100.00	100.00	≤4.2
Oligoasthenospermia	0.979 P <0.0001	0.0163	0.889 to 0.999	91.67	100.00	≤4.25

When the parameter of GP activity was used as a biomarker, the optimal cut-off point was 4.2 μmol of GSH per minute per 1 mg of protein, which indicates that patients with GP activity lower than 4.2 μmol of GSH per minute per 1 mg of protein probably have pathospermia. At the indicated cut-off point in the study of the diagnostic significance GP activity, the ROC curve analysis showed the area under the curve equal to1 (95% CI from 0.920 to 1.000, P <0.0001), which is characterized by excellent diagnostic significance for predicting both oligozoospermia and asthenozoospermia (the sensitivity was 100%, and specificity – 100%). When examining the ROC curves for the GP activity as a biomarker for diagnosing oligoasthenospermia, the AUC area was 0.979 (95% CI 0.889 to 0.999, P <0.0001), which corresponds to excellent diagnostic significance. The cut-off point for glutathione peroxidase was 4.25 μmol of GSH per minute per 1 mg of protein, the sensitivity of the test was 91.67%, the specificity was 100%, which indicates that, according to the ROC analysis, the model is excellent.

Graphs of the ROC curve for establishing the diagnostic significance of GR activity in men with different forms of pathospermia are shown in **Fig. 2**. The quantitative assessment of ROC curve using the calculation of the AUC area is given in **Table 2**.

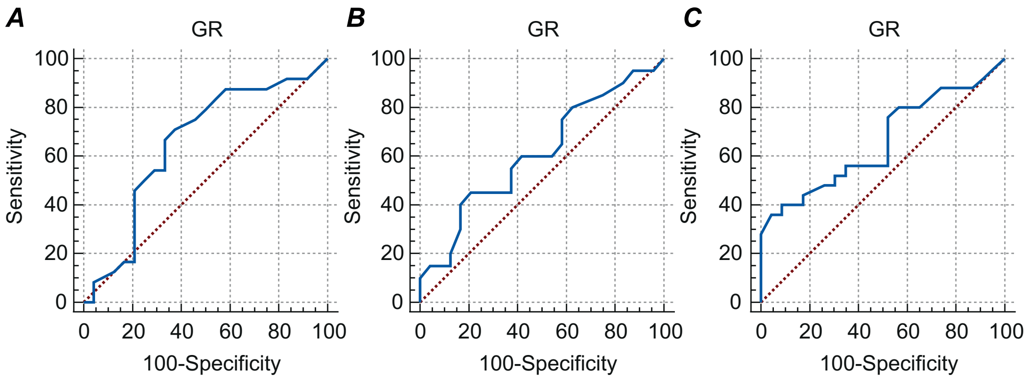

Fig. 2. ROC curves of GR activity as a marker in the diagnosis of oligozoospermia (***A***), asthenospermia (***B***) and oligoasthenozoospermia (***C***)

Table 2. The diagnostic value of GR activity in the diagnosis of different forms of pathospermia

Indicators / Pathospermia	AUC	Standard error (for AUC)	95%CI (for AUC)	Sensitivity (%)	Specificity (%)	Associated criterion (cut-off point), nmol NADPH per minute per 1 mg of protein
Oligozoospermia	0.654 P = 0.0645	0.0831	0.503 to 0.785	70.83	62.5	≤0.44
Asthenozoospermia	0.612 P = 0.1979	0.0874	0.454 to 0.755	45	79.17	≤0.32
Oligoasthenospermia	0.659 P = 0.0469	0.0801	0.508 to 0.790	36	95.65	≤0.26

The GR activity has moderate diagnostic significance, since the AUC is 0.654 (95% CI from 0.503 to 0.785, P = 0.0645) for oligozoospermic men the AUC is 0.612 (95% CI from 0.454 to 0.7555, P = 0.1979) for asthenospermic men. At a cut-off point of 0.44 nmol of NADPH per minute per 1 mg of protein, the sensitivity is sufficient and is 70.83%, while the specificity is 62.5% for patients with oligozoospermia. While the cut-off point for asthenospermic men is 0.32 nmol of NADPH per minute per 1 mg of protein, the sensitivity is 45% and the predictive value of a positive result – the specificity is sufficient and is 79.17%. GR activity is characterized by a significant diagnostic value, with an AUC of 0.659 (95% CI 0.508 to 0.790, P = 0.0469), high specificity (95.65%) and low sensitivity (36%) at a cutoff point ≤0.26 nmol of NADPH per minute per 1 mg of protein for patients with oligoasthenospermia.

The results of testing the GsT activity in men for pathosermia are presented in **Fig. 3** and **Table 3**.

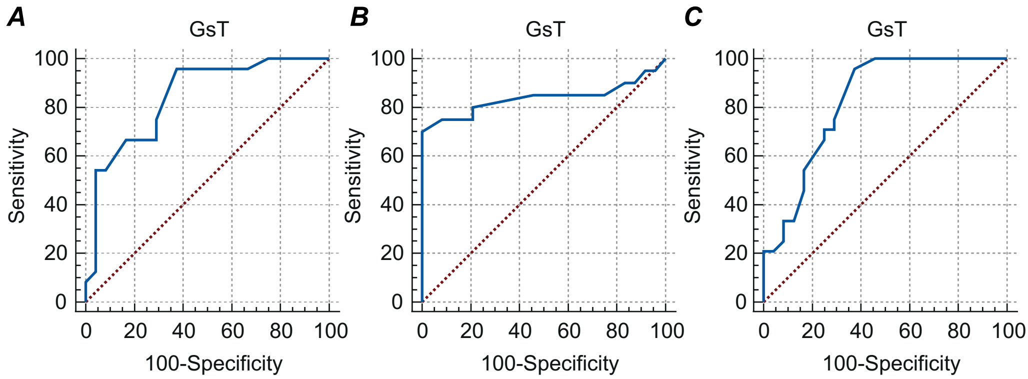

Fig. 3. ROC curves of GsT activity as a marker in the diagnosis of oligozoospermia (***A***), asthenospermia (***B***) and oligoasthenozoospermia (***C***)

Table 3. **The diagnostic value of GsT activity in the diagnosis of different forms of pathospermia**

Indicators / Pathospermia	AUC	Standard error (for AUC)	95%CI (for AUC)	Sensitivity (%)	Specificity (%)	Associated criterion (cut-off point), μmol of GSH per minute per 1 mg of protein
Oligozoospermia	0.843 P <0.0001	0.0575	0.709 to 0.932	95.83	62.5	≤0.48
Asthenozoospermia	0.838 P <0.0001	0.0740	0.695 to 0.931	70	100	≤0.31
Oligoasthenospermia	0.850 P <0.0001	0.0546	0.717 to 0.937	75	80	≤0.46

The analysis of the ROC curve revealed a good diagnostic value of GsT activity in sperm samples for the diagnosis of pathospermia. For patients with oligozoospermia, the AUC was 0.843 (95% CI from 0.709 to 0.932, P <0.0001). The optimal cut-off point for oligozoospermia was 0.48 μmol of GSH per minute per 1 mg of protein. GsT as a biomarker is characterized by high sensitivity (95.83%) and moderate specificity (62.5%). The ROC curve analysis for patients with asthenospermia showed that the AUC was 0.838 (95% CI 0.695 to 0.931, P <0.0001). The cut-off value was 0.31 μmol of GSH per minute per 1 mg showing the sensitivity of 70% and the specificity of 100%. For patients with oligoasthenospermia, AUC was equal to 0.85 (95% CI from 0.717 to 0.937, P <0.0001). The optimal cut-off point was 0.46 μmol of GSH per minute per 1 mg of protein and was characterized by good sensitivity of 75% and specificity of 80%.

The results of the evaluation of the ROC curve for GSH as a biomarker for diagnosing pathospermia are shown in **Fig. 4** and **Table 4**.

The determination of GSH content as a biomarker to distinguish between samples with normal sperm characteristics according to the WHO criteria and pathozoospermic samples was found to be unsuitable. The ROC curves analysis showed that GSH could not serve as valuable biomarkers for differentiating patients with pathospermia from healthy controls, with an AUC of 0.615, corresponding to moderate diagnostic significance for oligozoospermia. For asthenospermia and oligoasthenospermia, the AUC for the ROC curves were 0.598 and 0.529, respectively, which corresponds to an insufficient prognostic value.

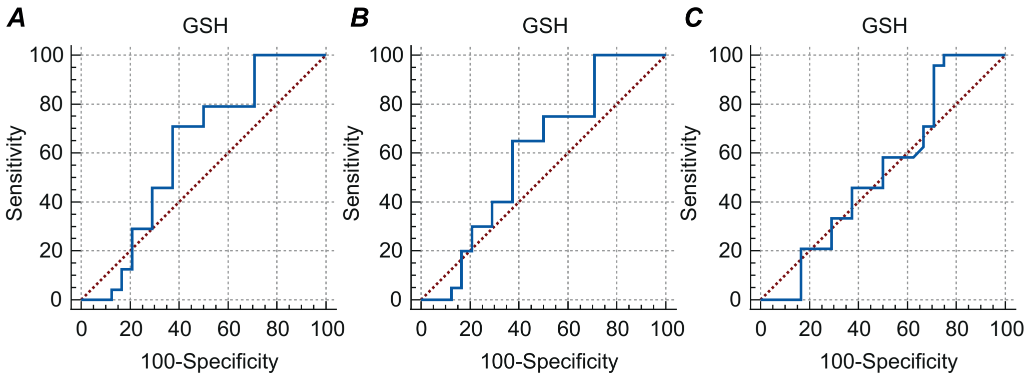

Fig. 4. ROC curves of GSH as a marker in the diagnosis of oligozoospermia (***A***), asthenospermia (***B***) and oligoasthenozoospermia (***C***)

Table 4. **The diagnostic value of GSH content in the diagnosis of different forms of pathospermia**

Indicators / Pathospermia	AUC	Standard error (for AUC)	95%CI (for AUC)	Sensitivity (%)	Specificity (%)	Associated criterion (cut-off point), pmol of GSH per 10^6 cells
Oligozoospermia	0.615 P <0.1812	0.0857	0.463 to 0.751	70.83	62.5	≤32
Asthenozoospermia	0.598 P <0.2647	0.0878	0.439 to 0.743	100.00	29.17	≤38
Oligoasthenospermia	0.529 P <0.7424	0.0872	0.379 to 0.674	95.83	29.17	≤38

It is beyond doubt that oxidative stress is a major contributor to male and female infertility (Barati *et al.*, 2020; Aitken *et al.*, 2022). The changes in the activity of glutathione antioxidant enzymes and GSH content might either result from a reduction in glutathione synthesis or the utilisation of glutathione in the detoxification process against induced oxidative stress. A decrease in the activity of antioxidant enzymes can be related both to the oxidative destruction of the corresponding DNA regions and the direct damage to enzyme molecules resulting from the effects of reactive oxygen species (Hamilton *et al.*, 2016). In addition, studies indicate that oxidative stress is also involved in impaired sperm DNA packaging (Hammadeh *et al.*, 2010; Rashki Ghaleno *et al.*, 2021).

It is important note that each form of pathospermia can be characterized by a certain level of oxidative stress markers (TBARS) and activity of enzymatic antioxidant systems (GPx, GR, GsT). These changes indicate a depletion of compensatory mechanisms in sperm cells. Zhang *et al.* (2014) reported a significantly lower GPx activity, with the extent of reduction revealing a disorder-associated trend (asthenospermia < oligospermia ≈ oligoasthenozoospermia).

The analysis of ROC curves is often used to highlight the diagnostic significance of individual indicators, which makes it possible to predict pathological changes in the spermogram (Akobeng *et al.*, 2007; Wei *et al.*, 2019). According to ROC analysis, it was established that the activities of glutathione peroxidase and glutathione-S-transferase have the highest diagnostic significance for oligozoospermia, asthenozoospermia and oligoasthenozoospermia. The AUC areas are maximal or close to the maximal values for the studied biomarkers and were statistically significant. The test results are characterized by high values of specificity and sensitivity. It is possible that other indicators related to the glutathione antioxidant protection may have an important prognostic value.

Other findings about the diagnostic significance of glutathione peroxidase are controversial. Our results are consistent with the study of Otasevic *et al*. who show that glutathione peroxidase activity was decreased in all groups with sperm pathologies and was a very good indicator of aberrations in functional sperm parameters, explaining up to 94.6% of infertility cases where functional sperm parameters were affected (Otasevic *et al.*, 2019). However, conflicting data were obtained in another study. N'Guessan et al. showed that glutathione peroxidase enzymatic activity is not related to sperm quality (N'Guessan *et al.*, 2023). Regarding glutathione-S-transferase activity, a decrease in enzyme activity in asthenozoospermic/asthenoteratozoospermic samples was noted in other studies (Lopes *et al.*, 2021; Zhang *et al.*, 2021).

There are still some limitations in our study. Firstly, the determination of indicators of the glutathione antioxidant system in routine use is a complex and costly process. Secondly, the sample size included may have affected the results to a certain extent. Thirdly, the time span is different which may be a factor of heterogeneity.

The ROC analysis established the parameters that have the greatest diagnostic significance for enzyme indicators, which can be used as additional criteria for establishing pathospermia. This study is important for further research on the activities of enzymes of the glutathione antioxidant system and for the development of tests for screening male fertility.

CONCLUSIONS

The results of this study show that the cut-off points for the biomarkers glutathione peroxidase and glutathione-S-transferase can be used to distinguish between patients with pathospermia and normozoospermia, and the parameters themselves can serve as valuable diagnostic biomarkers to differentiate patients with pathospermia from healthy controls, regardless of the causes of pathospermia. The value of these indicators below the cut-off point indicates the probable presence of pathology. Indicators of GR activity and GSH content have a low diagnostic value, which renders them unsuitable for use as laboratory tests for the diagnosis of male infertility.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The article is published with the support of a named scholarship of the Verkhovna Rada of Ukraine for young scientists – doctors of science to carry out scientific work: „Biophysical and chemical mechanisms of reducing the fertilization potential of spermatozoa and the development of new prognostic markers for the diagnosis of male infertility" (Decree of the Verkhovna Rada of Ukraine "On appointment in 2023 of named scholarships of the Verkhovna Rada of Ukraine for young scientists – doctors of science").

The publication presents the results of the research project "Ionic, molecular and membrane mechanisms of reduction of the fertilization potential of male spermatozoa and its correction" financed by the state budget (the Ministry of Health of Ukraine), 2023–2025.

COMPLIANCE WITH ETHICAL STANDARTS

Conflict of interest: the authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Animal rights: this article does not include animal studies.

Human rights: all studies were conducted in accordance with the Declaration of Helsinki guidelines.

AUTHOR CONTRIBUTIONS

Conceptualization, [F.Z.; V.Z.; F.R.]; methodology, [F.Z.; V.M., O.O.; M.O.]; validation, [F.Z.; L.N.]; formal analysis, [F.Z.; V.M.; L.N.; G.N.].; investigation, [V.M.; M.O.; G.N.; B.A.]; resources, [O.O.; F.R.]; data curation, [O.M., L.N.; G.N.; B.A.]; writing – review and editing, [F.Z.; V.M., V.Z., F.R.]; visualization, [F.Z., V.M.] supervision, [F.R.; V.Z.]; project administration, [V.Z.; F.R.]; funding acquisition, [F.R.; V.Z.].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Agarwal, A., & Bui, A. D. (2017). Oxidation-reduction potential as a new marker for oxidative stress: correlation to male infertility. *Investigative and Clinical Urology,* 58(6), 385–399. doi:10.4111/icu.2017.58.6.385
Crossref • PubMed • PMC • Google Scholar

Aitken, R. J., Drevet, J. R., Moazamian, A., & Gharagozloo, P. (2022). Male infertility and oxidative stress: a focus on the underlying mechanisms. *Antioxidants*, 11(2), 306. doi:10.3390/antiox11020306
Crossref • PubMed • PMC • Google Scholar

Akobeng, A. K. (2007). Understanding diagnostic tests 3: receiver operating characteristic curves. *Acta Paediatrica*, 96(5), 644–647. doi:10.1111/j.1651-2227.2006.00178.x
Crossref • PubMed • Google Scholar

Barati, E., Nikzad, H., & Karimian, M. (2019). Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cellular and Molecular Life Sciences*, 77(1), 93–113. doi:10.1007/s00018-019-03253-8
Crossref • PubMed • PMC • Google Scholar

Fafula, R. V., Onufrovych, O. K., Iefremova, U. P., Melnyk, O. V., Nakonechnyi, I. A., Vorobets, D. Z., & Vorobets, Z. D. (2017). Glutathione content in sperm cells of infertile men. *Regulatory Mechanisms in Biosystems*, 8(2), 157–161. doi:10.15421/021725
Crossref • Google Scholar

Fafula, R., Melnyk, O., Gromnatska, N., Vorobets, D., Fedorovych, Z., Besedina, A., & Vorobets, Z. (2023). Prooxidant-antioxidant balance in seminal and blood plasma of men with idiopathic infertility and infertile men in combination with rheumatoid arthritis. *Studia Biologica*, 17(2), 15–26. doi:10.30970/sbi.1702.719
Crossref • Google Scholar

Gonçalves, L., Subtil, A., Oliveira, M. R., & de Zea Bermudez, P. (2014). ROC curve estimation: an overview. *Revstat – Statistical Journal*, 12, 1–20. doi:10.57805/revstat.v12i1.141
Crossref • Google Scholar
Hamilton, T. R., de Castro, L. S., Delgado, J. de C., de Assis, P. M., Siqueira, A. F., Mendes, C. M., Goissis, M. D., Muiño-Blanco, T., Cebrián-Pérez, J. Á., Nichi, M., Visintin, J. A., & D'Ávila Assumpção, M. E. (2016). Induced lipid peroxidation in ram sperm: semen profile, DNA fragmentation and antioxidant status. *Reproduction*, 151(4), 379–390. doi:10.1530/rep-15-0403
Crossref • PubMed • Google Scholar
Hammadeh, M., Hamad, M., Montenarh, M., & Fischer-Hammadeh, C. (2010). Protamine contents and P1/P2 ratio in human spermatozoa from smokers and non-smokers. *Human Reproduction*, 25(11), 2708–2720. doi:10.1093/humrep/deq226
Crossref • PubMed • Google Scholar
Hong, Y., Boiti, A., Vallone, D., & Foulkes, N. S. (2024). Reactive oxygen species signaling and oxidative stress: transcriptional regulation and evolution. *Antioxidants*, 13(3), 312. doi:10.3390/antiox13030312
Crossref • PubMed • PMC • Google Scholar
Kumar, R., & Indrayan, A. (2011). Receiver operating characteristic (ROC) curve for medical researchers. *Indian Pediatrics*, 48(4), 277–287. doi:10.1007/s13312-011-0055-4
Crossref • PubMed • Google Scholar
Lopes, F., Pinto-Pinho, P., Gaivão, I., Martins-Bessa, A., Gomes, Z., Moutinho, O., Oliveira, M. M., Peixoto, F., & Pinto-Leite, R. (2021). Sperm DNA damage and seminal antioxidant activity in subfertile men. *Andrologia*, 53(5), e14027. doi:10.1111/and.14027
Crossref • Google Scholar
Mannucci, A., Argento, F. R., Fini, E., Coccia, M. E., Taddei, N., Becatti, M., & Fiorillo, C. (2022). The impact of oxidative stress in male infertility. *Frontiers in Molecular Biosciences*, 8, 799294. doi:10.3389/fmolb.2021.799294
Crossref • PubMed • PMC • Google Scholar
N'Guessan, M. F., Sery, B. B., Bi, F. J. V., Ekissi, N. A., Djohan, Y. F., Coulibaly, F. A., & Djaman, A. J. (2023). Evaluation of glutathione peroxidase enzymatic activity in seminal plasma of patients treated at the institute Pasteur in Cote d'Ivoire. *Advances in Reproductive Sciences*, 11(04), 116–126. doi:10.4236/arsci.2023.114011
Crossref • Google Scholar
Otasevic, V., Kalezic, A., Macanovic, B., Jankovic, A., Stancic, A., Garalejic, E., Korac, A., & Korac, B. (2019). Evaluation of the antioxidative enzymes in the seminal plasma of infertile men: contribution to classic semen quality analysis. *Systems Biology in Reproductive Medicine*, 65(5), 343–349. doi:10.1080/19396368.2019.1600171
Crossref • PubMed • Google Scholar
Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763. doi: 10.1155/2017/8416763
Crossref • PubMed • PMC • Google Scholar
Rashki Ghaleno, L., Alizadeh, A., Drevet, J. R., Shahverdi, A., & Valojerdi, M. R. (2021). Oxidation of sperm DNA and male infertility. *Antioxidants*, 10(1), 97. doi:10.3390/antiox10010097
Crossref • PubMed • PMC • Google Scholar
Trevethan, R. (2017). Sensitivity, specificity, and predictive values: foundations, pliabilities, and pitfalls in research and practice. *Frontiers in Public Health*, 5, 307. doi: 10.3389/fpubh.2017.00307
Crossref • PubMed • PMC • Google Scholar
Vorobets, M. Z., Fafula, R. V., Besedina, A. S., Onufrovych, O. K., & Vorobets, D. Z. (2018). Glutathione s-transferase as a marker of oxidative stress in human ejaculated spermatozoa from patients with pathospermia. *Regulatory Mechanisms in Biosystems*, 9(2), 287–292. doi:10.15421/021842
Crossref • Google Scholar

Wei, J., Liu, X., Xue, H., Wang, Y., & Shi, Z. (2019). Comparisons of visceral adiposity index, body shape index, body mass index and waist circumference and their associations with diabetes mellitus in adults. *Nutrients*, 11(7), 1580. doi:10.3390/nu11071580
Crossref ● PubMed ● PMC ● Google Scholar

World Health Organization. (2021). WHO laboratory manual for the examination and processing of human semen (6th ed.). World Health Organization. Retrieved from https://iris.who.int/bitstream/handle/10665/343208/9789240030787-eng.pdf?sequence=1&isAllowed=y
Google Scholar

Xu, P., Liu, X., Hadley, D., Huang, S., Krischer, J., & Beam, C. (2014). Feature selection using bootstrapped ROC curves. *Journal of Proteomics & Bioinformatics*, S9. doi:10.4172/jpb.S9-006
Crossref ● Google Scholar

Zhang, R., Zuo, Y., & Cao, S. (2021). Upregulated microRNA-423-5p promotes oxidative stress through targeting glutathione S-transferase mu 1 in asthenozoospermia. *Molecular Reproduction and Development*, 88(2), 158–166. doi:10.1002/mrd.23454
Crossref ● PubMed ● Google Scholar

Zhang, W. D., Zhang, Z., Jia, L. T., Zhang, L. L., Fu, T., Li, Y. S., Wang, P., Sun, L., Shi, Y., & Zhang, H. Z. (2014). Oxygen free radicals and mitochondrial signaling in oligospermia and asthenospermia. *Molecular Medicine Reports*, 10(4), 1875–1880. doi:10.3892/mmr.2014.2428
Crossref ● PubMed ● Google Scholar

ДІАГНОСТИЧНА ЧУТЛИВІСТЬ ТА СПЕЦИФІЧНІСТЬ ПОКАЗНИКІВ ГЛУТАТІОНОВОЇ АНТИОКСИДАНТНОЇ СИСТЕМИ СПЕРМАТОЗОЇДІВ НЕПЛІДНИХ ЧОЛОВІКІВ ІЗ РІЗНИМИ ФОРМАМИ ПАТОСПЕРМІЇ

Зоряна Федорович, Микола Воробець, Олена Онуфрович, Оксана Мельник, Наталія Громнацька, Наталія Личковська, Анна Беседіна, Зіновій Воробець, Роман Фафула

Львівський національний медичний університет імені Данила Галицького
вул. Пекарська, 69, Львів 79010, Україна

Вступ. Однією із найважливіших систем антиоксидантного захисту сперматозоїдів є глутатіонова антиоксидантна система, яка включає глутатіонпероксидазу (GPx), глутатіонредуктазу (GR), глутатіон S-трансферазу (GsT) і відновлений глутатіон (GSH). Перспективним є використання ROC аналізу, який дає змогу встановити діагностичну чутливість і специфічність показників.

Матеріали та методи. Усі пацієнти були розподілені на 3 групи: неплідні чоловіки з олігозооспермією (n = 30), астенозооспермією (n = 34) та олігоастенозооспермією (n = 22). Для аналізу діагностичної чутливості та специфічності значення біомаркерів перевіряли за допомогою кривої робочої характеристики приймача, тобто кривої ROC, та розраховували площу під нею (AUC) з урахуванням стандартної похибки (SE) 95 % і довірчого інтервалу (CI) 95 %.

Результати. За допомогою ROC-аналізу з'ясовано, що активність GP характеризується високою діагностичною значущістю для діагностування як олігозооспермії, так і астенозооспермії (чутливість 100 %, специфічність 100 %). Активність GR має помірне діагностичне значення, оскільки AUC становить 0,654 (95 % CI від 0,503 до 0,785, P = 0,0645) для олігозооспермії та 0,612 (95 % CI від 0,454 до 0,7555, P = 0,1979) для астенозооспермії. Аналіз кривих ROC виявив хорошу діагностичну цінність активності GsT для діагностики патоспермії (чутливість 75 %

і специфічність 80 %). Водночас підтверджено, що вміст GSH не може слугувати біомаркером для відрізнення пацієнтів з патоспермією від фертильних чоловіків, з AUC 0,615, що відповідає помірній діагностичній значущості для олігозооспермії.

Висновки. Результати цього дослідження доводять, що граничні точки для біомаркерів GP та GsT можна використовувати для розрізнення пацієнтів з патоспермією та нормозооспермією, а самі параметри можуть слугувати цінними діагностичними біомаркерами для розрізнення пацієнтів із патоспермією від фертильних осіб групи контролю, незалежно від причин патоспермії. Значення цих показників нижче граничної точки вказує на ймовірну наявність патології. Показники активності GR і вмісту GSH мають низьку діагностичну цінність, що не дає змоги вважати їх лабораторними тестами для діагностики чоловічого непліддя.

Ключові слова: чоловіче непліддя, антиоксидантні ензими, система глутатіону, ROC аналіз

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
13 May, 2024	18 July, 2024	30 August, 2024	25 September, 2024