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Correlation Between Selenium Protein-1 Levels and the Quality of Oocytes and Embryos in Females Undergoing *In-Vitro* Fertilization

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ABSTRACT

Evaluation of oocyte quality is one of the most important goals of embryologists in assisted reproductive technologies, due to its importance in successful fertilization and embryonic development. Females undergoing in vitro fertilization are exposed to massive systemic oxidative stress due to the ovarian stimulation protocols used, which negatively affects oocyte maturation and embryonic development. Glutathione peroxidase is one of the most important endogenous antioxidants purported to defend against oxidative stress by modifying free radical electrons, and therefore it is necessary to investigate the effect of its blood levels on oocyte maturation and the embryo quality in women undergoing in vitro fertilization treatment. An observational, cross-sectional study was conducted on 60 females undergoing IVF treatment by pituitary down-regulation with GnRH agonist. Glutathione peroxidase-1 levels in serum and follicular fluid were determined using an ELISA kit. The maturity of the oocyte nucleus as well as the development of the resulting embryos after sperm injection are evaluated by IVF specialists. Grade I embryos resulting from the injection of high-quality oocytes are what the doctor seeks to obtain to return these embryos to the uterus. We investigated the relationship between serum glutathione peroxidase levels with the number and quality of MII oocytes and Grade I embryos using Statistical Package for Social Sciences version 23. We found that the relationship between GPX activity in the blood (measured in µU/ml) and the number of mature oocytes (MII), maturation rate, fertilization rate, and the number of Grade I embryos were found to be statistically non-significant, with a p-value of 0.709, 0.327,0.720, 0.385, respectively. The GPX activity measured in the blood was not associated with any IVF/ICSI indicators. This suggests that further comprehensive research is needed in this area.

1. Introduction

Glutathione peroxidase (GPX), with enzyme code EC:9.1.11.1, is a general term for a family of eight isoforms that exhibit peroxidase or peroxide reductase activity (1.11.1). These enzymes primarily act on hydrogen peroxide but also function on organic hydroperoxides, such as lipid hydroperoxides. GPX utilizes glutathione (GSH) as an electron donor. GPX is a homotetrameric protein with a molecular weight ranging from 83 to 95 kDa and was first discovered in 1957 as an enzyme protecting red blood cells from oxidative damage [6]. GPX contains selenocysteine, with selenium (Se^{+2}) playing a crucial enzymatic role. This is why it is sometimes referred to as selenium glutathione peroxidase [3]. The fundamental biochemical function of all peroxidase enzymes is to protect organisms from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols. Different genes encode several isoenzymes, varying in location and preferred substrate. In humans, eight isoforms have been identified, with GPX1 being the most abundant in the cytoplasm of nearly all mammalian tissues [8]. Oxidative stress continuously generates reactive oxygen species (ROS) within living organisms under aerobic conditions. Maintaining a balance between ROS production and elimination through the antioxidant defense system is crucial for preserving physiological functions in the body [7]. Oxidative stress affects oocytes, sperm, embryos, and their environments, impacting oocyte quality, sperm-egg interaction, embryo development, and subsequent implantation [1]. This, in turn, affects the success rates of assisted reproductive technologies. Women undergoing ovarian stimulation as part of the IVF cycle experience high systemic concentrations of estrogen (E2) and progesterone (P4) due to external gonadotropin administration [2]. This induces systemic oxidative stress due to the high doses applied. These elevated levels can damage oocytes and embryonic cells, negatively affecting pregnancy outcomes and presenting additional challenges for embryologists in achieving oocyte competency in this highly oxidative environment. The goal is to inject sperm into high-quality oocytes (MII oocytes) to produce Grade I embryos for transfer into the uterus.

2. Materials and Methods

This study included 60 females scheduled for IVF/ICSI treatment using Gn-RH agonist protocol stimulation. Blood and follicular fluid samples were collected from each participant after obtaining their consent and that of their partners. The participants were healthy, with normal reproductive function, natural uterus, and ovaries, as confirmed by ultrasound examinations conducted at the hospital's clinic before IVF cycle preparation. The participants' ages ranged from 20 to 40 years. Cases with female factors such as PCOS, uterine fibroids, cervicitis, endometritis, intrauterine adhesion, and endometriosis, which can affect IVF outcomes, were excluded. Women who had taken selenium-containing dietary supplements for at least two to three months before oocyte retrieval were also excluded. Blood samples were collected using heparinized tubes immediately before anesthesia on the same day as oocyte retrieval. Follicular fluid samples (4 ml) were obtained from each participant during oocyte retrieval using dry tubes. Whole blood and follicular fluid samples were centrifuged within a maximum of 30 minutes and stored at -75 to -80 degrees Celsius until analysis. GPX activity in both plasma and follicular fluid was measured using a CUSABIO ELISA kit (Cat. No. CSB-EL009866HU) with a sandwich ELISA technique, employing an incubator and BioTek ELISA reader (EPSON model) available in the immunology section of Al-Assad University Hospital in Damascus.

2.1 Ethical considerations

This study adhered to the ethical guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee and the Scientific Research and Postgraduate Board of Damascus University (Approval No. 9- 2016/2017 academic year).

3. Results

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The blood's average GPX activity (measured in µU/ml) was 416.5±142.7, while in the follicular fluid, it was 175.8±78.5, as shown in Figure 1. A strong positive correlation (r=0.80) between GPX activity in the blood and follicular fluid was statistically significant (p=0.0001), as depicted in Figure 2.

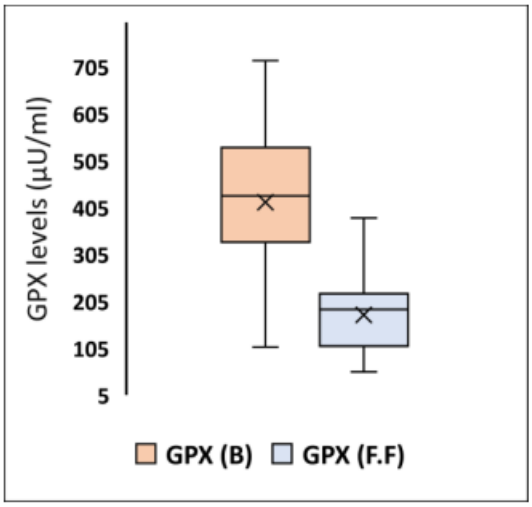

Figure 1: Glutathione Peroxidase Activity in Blood and Follicular Fluid

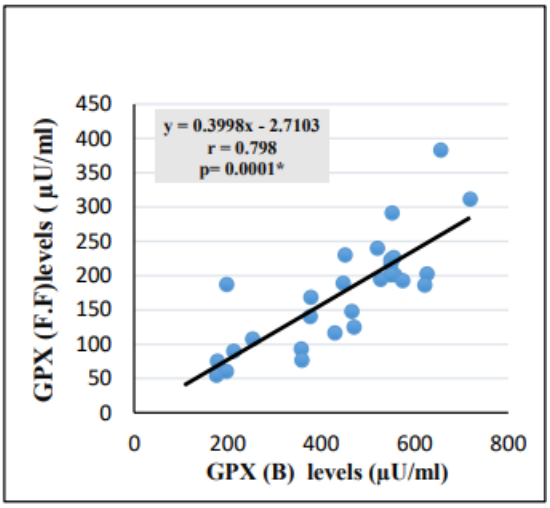

Figure 2: Relationship between GPX Activity in Blood and Follicular Fluid

The relationship between GPX activity in the blood (measured in µU/ml) and the number of mature oocytes (MII) was found to be statistically non-significant (Figure 3), with a p-value of 0.709.

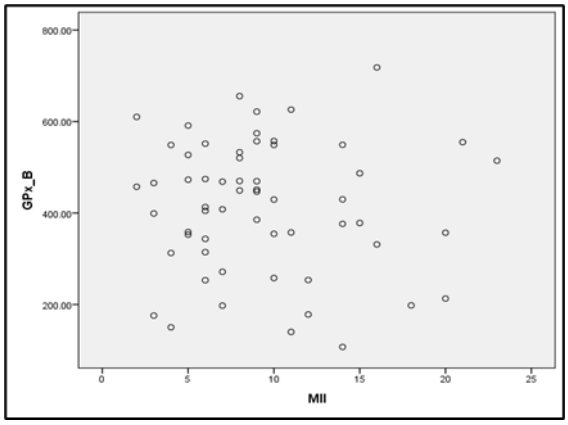

Figure 3: Relationship between GPX Activity in Blood and Number of Mature Oocytes (MII)

The average GPX blood levels (measured in µU/ml) did not differ significantly based on the variation in oocyte maturation rate (MR%) (Figure 4), with a p-value of 0.327.

Similarly, the average GPX blood levels (measured in µU/ml) did not differ significantly based on the variation in fertilization rate (FR%) (Figure 5), with a p-value of 0.720.

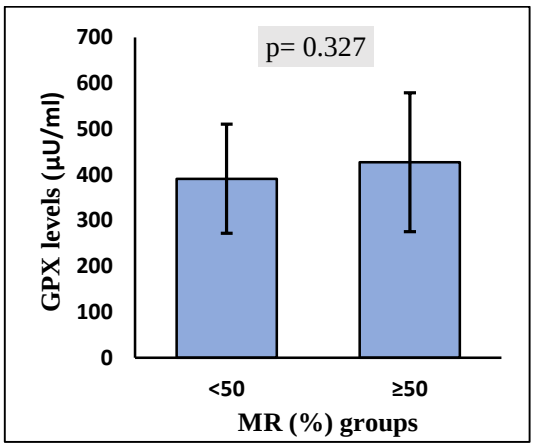

Figure 1: Average Blood GPX Levels Based on Oocyte Maturation Rate

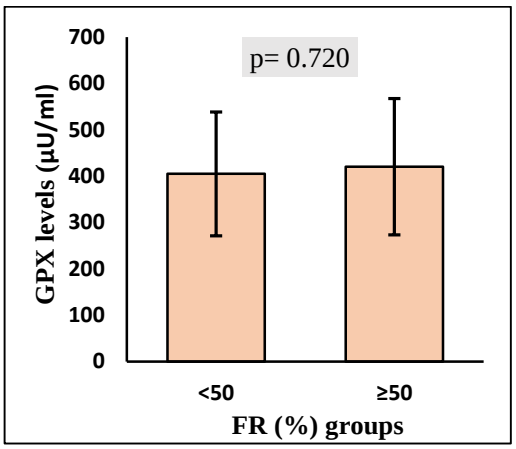

Figure 2: Average Blood GPX Levels Based on Fertilization Rate

The relationship between GPX activity in the blood (measured in µU/ml) and the number of Grade I embryos was found to be statistically non-significant (Figure 6), with a p-value of 0.385.

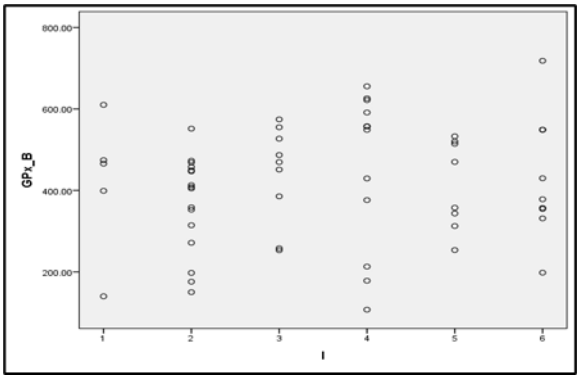

Figure 6: Relationship between GPX Activity in Blood and Number of Grade I Embryos

4. Discussion

The significant relationship found between GPX levels in the blood and follicular fluid indicates that the levels in bodily fluids, including follicular fluid, reflect the overall state of GPX in the blood. The observed decrease in levels in the follicular fluid compared to blood is due to the selective permeability of the Blood-Follicle Barrier (BFB) to compounds from blood vessels based on their size and charge, as studied in mouse ovaries [4]. Additionally, the Tight Junction (TJ)-permeability barrier of the granulosa cells surrounding the growing follicles [9] plays a role in this. Therefore, GPX levels in the blood can be reliably used to compare results related to oocytes and embryos resulting from IVF in the female study group.

Our choice of Selenium Glutathione Peroxidase among endogenous antioxidants was motivated by its greater efficiency in modifying lipid hydroperoxides compared to catalase [5]. However, our study did not find any association between GPX blood levels and IVF/ICSI indicators such as the number and quality of oocytes, their maturation rate, fertilization rate, and the number and quality of resulting embryos. GPX is considered an endogenous antioxidant that may not be sufficient to modify oxidative stress levels in bodily fluids, especially in critical and non-standard conditions such as ovarian stimulation and the growth of oocytes and embryonic cells during IVF in females. Additionally, GPX activity depends on dietary selenium intake and the overall activity of the entire glutathione system (glutathione, peroxidases, and

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reductases). Nevertheless, we were intrigued by the weak, non-linear relationship (r=0.25) found between blood GPX levels and oocyte maturation rate, which became significant (p=0.049). This prompted us to study the relationship between follicular fluid GPX levels and maturation rate, which revealed a stronger, statistically significant (p=0.009) positive correlation (r=0.5). The weak relationship in blood may not have been evident due to the relatively small sample size, and the fact that GPX is an endogenous antioxidant with multiple enzyme isoforms, some of which are intracellular, while others are extracellular plasma isoforms [10]. It is worth noting that our results are largely consistent with a recent Iranian study conducted in 2020 [11], which found that glutathione-based antioxidant functions were more effective in the follicular fluid of oocytes and high-quality embryos in cases of PCOS, a condition associated with high oxidative stress levels. The logical explanation for the relationship we found becomes evident when we consider the biochemical composition of the follicular fluid, which directly influences oocytes due to their direct interaction with the constituents of the follicular fluid, particularly those of endogenous origin. From this perspective, the quality of oocytes and the levels of antioxidants in the follicular fluid are closely interconnected. In contrast, blood levels of antioxidants reflect the overall metabolic status of the body. This result can be considered a cornerstone for launching more comprehensive research in this field. The aforementioned result did not affect the relationship between GPX levels and fertilization rates due to the use of the ICSI technique, which generally enhances fertilization rates regardless of factors related to oocytes and sperm.

5. Conclusions

The reliability of GPX blood levels of can be confidently relied upon to compare results related to oocytes and embryos resulting from IVF in females, as demonstrated by their significant correlation with their levels in the follicular fluid.

The GPX activity measured in the blood was not associated with any IVF/ICSI indicators. However, the positive correlation between blood GPX levels and oocyte maturation rate was significant. This suggests that further comprehensive research is needed in this area.

6. References

[1] Adeoye, O., Olawumi, J., Opeyemi, A., & Christiania, O. (2018). Review on the role of glutathione on oxidative stress and infertility. Jornal Brasileiro de Reproducao Assistida, 22(1), 61–66. https://doi.org/10.5935/1518-0557.20180003

[2] Aurrekoetxea, I., Ruiz-Sanz, J. I., Del Agua, A. R., Navarro, R., Hernández, M. L., Matorras, R., Prieto, B., & Ruiz-Larrea, M. B. (2010). Serum oxidizability and antioxidant status in patients undergoing in vitro fertilization. Fertility and Sterility, 94(4), 1279–1286. https://doi.org/10.1016/j.fertnstert.2009.05.028

[3] Chaudiere, J., & Tappel, A. L. (1983). Purification and characterization of selenium-glutathione peroxidase from hamster liver. Archives of Biochemistry and Biophysics, 226(2), 448–457. https://doi.org/10.1016/0003-9861(83)90314-4

[4] Hess, K. A., Chen, L., & Larsen, W. J. (1998). The ovarian blood follicle barrier is both charge- and size-selective in mice. Biology of Reproduction, 58(3), 705–711. https://doi.org/10.1095/biolreprod58.3.705

[5] Lubos, E., Loscalzo, J., & Handy, D. E. (2011). Glutathione peroxidase-1 in health and disease: From molecular mechanisms to therapeutic opportunities. Antioxidants and Redox Signaling, 15(7), 1957–1997.

https://doi.org/10.1089/ars.2010.3586

[6] MILLS, G. C. (1957). Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. The Journal of Biological Chemistry, 229(1), 189–197.

[7] Mohd Mutalip, S. S., Ab-Rahim, S., & Rajikin, M. H. (2018). Vitamin E as an antioxidant in female reproductive health. Antioxidants, 7(2), 22. https://doi.org/10.3390/antiox7020022

[8] Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., & Van Remmen, H. (2007). Trends in oxidative aging theories. Free Radical Biology and Medicine, 43(4), 477–503. https://doi.org/10.1016/j.freeradbiomed.2007.03.034

[9] Siu, M. K. Y., & Cheng, C. Y. (2013). The Blood-Follicle Barrier (BFB) in disease and in ovarian function. Advances in Experimental Medicine and Biology, 763, 186–192. https://doi.org/10.1007/978-1-4614-4711-5_9

[10] Ufer. (2011). The roles of glutathione peroxidases during embryo development. Frontiers in Molecular Neuroscience, 4. https://doi.org/10.3389/fnmol.2011.00012

[11] Zal, F., Ahmadi, P., Davari, M., Khademi, F., Akbarzadeh Jahromi, M., Anvar, Z., & Namavar Jahromi, B. (2020). Glutathione-dependent enzymes in the follicular fluid of the first-retrieved oocyte and their impact on oocyte and embryos in polycystic ovary syndrome: A cross-sectional study. International Journal of Reproductive BioMedicine, 18(6), 415–424. https://doi.org/10.18502/ijrm.v13i6.7283