


# Oxidative Stress is Associated with Reduced Sperm Motility in Normal Semen

American Journal of Men's Health  
September–October 1–8  
© The Author(s) 2020  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1557988320939731  
journals.sagepub.com/home/jmh  


Wiktoria Kurkowska<sup>1</sup>, Agnieszka Bogacz<sup>1</sup>, Marta Janiszewska<sup>1</sup>,  
Ewa Gabryś<sup>1</sup>, Michał Tiszler<sup>1</sup>, Francesco Bellanti<sup>2</sup>,  
Sławomir Kasperczyk<sup>1</sup>, Anna Machoń-Grecka<sup>1</sup> ,  
Michał Dobrakowski<sup>1</sup>, and Aleksandra Kasperczyk<sup>1</sup>

## Abstract

Infertility is among the most serious medical problems worldwide. Male factors contribute to 40%–50% of all infertility cases, and approximately 7% of men worldwide are affected by infertility. Spermatozoa are extremely vulnerable to oxidative insult. Oxidative stress results in axonemal damage and increased midpiece sperm morphological defects, which lead to reduced sperm motility. The aim of the study is to evaluate the association between sperm motility and the levels of selected antioxidants, cytokines, and markers of oxidative damage in the seminal plasma.

The study group included 107 healthy males, who were split into two subgroups based on the percentage of motile spermatozoa after 1 hr: low motility (LM,  $n = 51$ ) and high motility (HM,  $n = 56$ ).

The glucose-6-phosphate dehydrogenase (G6PD) activity was 52% lower in the LM group compared to that in the HM group. The level of malondialdehyde (MDA) was 12% higher in the LM group compared to that in the HM group. Similarly, the median values of interleukin (IL)-1 $\beta$ , IL-10, IL-12, and tumor necrosis factor alpha (TNF- $\alpha$ ) were higher in the LM group than those in the HM group. Results of the present study revealed that the percentage of motile spermatozoa after 1 hr correlated positively with the levels of IL-1 $\beta$ , IL-10, IL-12, and TNF- $\alpha$ .

The lower motility of spermatozoa in healthy men is associated with a decreased activity of G6PD and increased levels of cytokines, which may be related to increased oxidative stress in seminal plasma that manifests as an increased level of MDA.

## Keywords

Sperm, oxidative stress, immunology, fertility

Received February 5, 2020; revised June 3, 2020; accepted June 12, 2020

Infertility is usually defined in the biomedical context as the inability to conceive after 12 months of regular unprotected intercourse (Greil et al., 2010). Infertility is among the most serious medical problems worldwide (Dobrakowski et al., 2018; Mascarenhas et al., 2012). Male factors contribute to 40%–50% of all infertility cases, and approximately 7% of men worldwide are affected by infertility (Agarwal and Bui, 2017). Male infertility could be a result of anatomical abnormalities, such as semen outflow tract obstruction, neurological disorders of ejaculation, and urogenital trauma; however, most of the cases are caused by impaired spermatogenesis and failure in sperm function. As male fertility can be influenced by a variety of factors, one possible

explanation for the declining trend would be that there are environmental and/or occupational factors along with lifestyle practices that contribute to the deterioration of semen quality. The lifestyle factors that are associated with male infertility are as follows: smoking cigarettes,

<sup>1</sup>Department of Biochemistry, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Zabrze, Poland

<sup>2</sup>Department of Medical and Surgical Sciences, University of Foggia, Foggia, Puglia, Italy

## Corresponding Author:

Anna Machoń-Grecka, Department of Biochemistry, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, ul. Jordana 19, Zabrze, 41-808, Poland.

Email: machongrecka.a@gmail.com



alcohol intake, use of illicit drugs, obesity, psychological stress, advanced paternal age (APA), dietary practices, and coffee consumption (Durairajanayagam, 2018).

Semen analysis is routinely performed to assess the reproductive potential of the male partner in infertile couples, even though there are no standard parameters to diagnose infertility (Guzick et al., 2001). Sperm motility is considered the most important parameter of a semen specimen during the evaluation of fertility (Amelar et al., 1980).

Sperm quality is particularly altered by oxidative stress (Walczak-Jedrzejowska et al., 2013), which is defined as an imbalance between the generation of reactive oxygen species (ROS) and the protective action of antioxidant systems responsible for their neutralization and removal (Sikka, 2001). Imbalance between production and utilization of ROS leads to damage of many cell structures, especially phospholipids of cellular membranes. Oxidative damage to lipids, called lipid peroxidation, triggers, in turn, signaling cascades of the inflammatory processes which promote peroxidation of lipids, resulting in intracellular oxidative burden. The sequence of events involves lipid peroxidation, loss of membrane integrity with increased permeability, reduced sperm motility, structural DNA damage, and apoptosis (Alahmar, 2019). ROS as by-products are released during the physiological metabolic processes of cells in semen (Ko et al., 2014). Results of earlier studies indicate that the cytokine levels in seminal plasma have been correlated with semen quality parameters (Kopa et al., 2005). A number of studies have reported that ROS and cytokines possess a complex and exquisite interplay (Chyra-Jach et al., 2018). Although ROS can promote the expression and production of cytokines, some cytokines can modulate prooxidant and antioxidant systems and the generation of ROS (Jiang et al., 2016; Seshadri et al., 2009).

Spermatozoa are extremely vulnerable to oxidative insult because they lack the necessary repair systems and are unable to restore the oxidative damages. Besides, spermatozoa membranes are rich in polyunsaturated fatty acids (PUFAs), which makes them highly susceptible to lipid peroxidation. Oxidative stress results in axonemal damage, decreased sperm viability, and increased mid-piece sperm morphological defects. These dysfunctions may contribute to decreased sperm motility (Agarwal et al., 2014). In light of this, the present study was undertaken to examine the associations between sperm motility and the levels of selected antioxidants, cytokines, and oxidative damage markers.

## Materials

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/

KB1/I/13/09). A total of 107 fertile males living in Upper Silesia (Poland), who attended the andrology clinic for the diagnosis of infertility, were enrolled as the study group. All patients were healthy and fertile with normal sperm parameters according to the World Health Organization (WHO) standards. Written informed consent was obtained from all the study subjects. A questionnaire about the participants' medical history was also collected. Subjects who reported drug consumption, smoking habits, alcohol abuse, and a history of any chronic disease were excluded from the study. Also, any diseases that may cause secondary infertility were included in the exclusion criteria.

Semen specimens were collected via masturbation after 3 days of abstinence, in the morning before the first meal. According to the percentage of motile spermatozoa after 1 hr, the patients were divided into two groups based on the median: low motility group with less than or equal to 57% motile spermatozoa (LM group,  $n = 51$ ) and high motility group with more than 57% motile spermatozoa (HM group,  $n = 56$ ).

## Methods

Semen specimens were analyzed according to the WHO standards (WHO, 2010). The seminal plasma and spermatozoa lysate were prepared according to Kasperczyk et al. (2015).

The activity of antioxidant enzymes was measured in the seminal plasma according to the methods described in Kasperczyk et al. (2015). The method of Oyanagui (1984) was used to measure the activity of superoxide dismutase (SOD) in seminal plasma. The enzymatic activity of SOD was expressed in nitric units (NU). The activity of SOD is equal to 1 NU when it inhibits nitric ion production by 50%. Activity of SOD was expressed in NU/mg of protein. The glutathione peroxidase (GPx) activity was measured by the kinetic method of Paglia and Valentine (1967). The GPx activity was expressed as U/g of protein. The activity of glutathione-S-transferase (GST) was measured according to the kinetic method of Habig and Jakoby (1981). The GST activity was expressed as the  $\mu$ moles of thioether produced per minute per gram of protein (mU/g protein). The activities of glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GR) were measured according to Richterich (1971). G6PD and GR activity were expressed as  $\mu$ moles of NADPH produced and utilized, respectively, per minute per gram of protein (U/min/g protein). Catalase (CAT) activity was measured by the method of Johansson and Borg (1988). The activity of catalase was expressed as U/g of protein.

Malondialdehyde (MDA) was measured fluorometrically as a 2-thiobarbituric acid-reactive substance (TBARS) in seminal plasma according to Ohkawa et al.

**Table 1.** Age, Semen Analysis, and Oxidative Stress Intensity and Antioxidant Reserves in Seminal Plasma.

	LM (N = 51)	HM (N = 56)	p value
Age (years)	34 ± 6	32 ± 5	<b>.013</b>
Sperm volume (ml)	3.72 ± 1.90	3.69 ± 1.53	.943
pH value	7.57 ± 0.08	7.56 ± 0.07	.727
Sperm cell count in 1 ml (mln/ml)	80.0 ± 53.7	78.9 ± 66.9	.920
Total sperm cell count (mln)	274 ± 191	264 ± 190	.770
Motile sperm cells after 1 hr (%)	51.0 ± 3.52	66.0 ± 7.54	<b>&lt;.001</b>
Progressively motile sperm cells after 1 hr (%)	22.0 ± 5.8	30.5 ± 11.6	<b>&lt;.001</b>
Motile spermatozoa after 24 hr (%)	16.7 ± 12.6	21.5 ± 18.4	.124
Progressively motile spermatozoa after 24 hr (%)	4.84 ± 5.42	8.06 ± 9.53	<b>.037</b>
Normal morphology (%)	50.3 ± 7.34	53.2 ± 8.43	.056
SOD activity (NU/mg protein)	4.13 ± 1.45	4.53 ± 1.80	.333
CAT activity (U/g protein)	13.7 ± 13.97	17.7 ± 17.7	.123
GR activity (U/g protein)	1.76 ± 1.77	1.73 ± 1.92	.447
GPx activity (U/g protein)	3.00 ± 4.59	1.51 ± 2.70	.222
GST activity (mU/g protein)	85.5 ± 61.3	82.2 ± 53.3	.974

Note. Data are expressed as mean ± SD. Statistical differences were analyzed by Student's *t* test. CAT = catalase; GPx = glutathione peroxidase; GR = glutathione reductase; GST = glutathione-S-transferase; HM = high motility; LM = low motility; SOD = superoxide dismutase. *p* < .05.

(1979) with modifications according to Kasperczyk et al. (2015). Concentrations were given in μmol/L.

The levels of interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) were measured in seminal plasma using a Bio-Plex 200 System (Bio-Rad Laboratories Inc., USA) according to the manufacturer's instructions. Data were presented in pg/ml.

The results are expressed as median values and interquartile ranges (IQRs) or mean and standard deviation (SD). The statistical analyses were performed using non-parametric tests for data without a normal distribution (Mann–Whitney *U* test) or parametric tests for data with a normal distribution (Student's *t* test). Normality and homogeneity of variances were verified using Shapiro–Wilk's and Levene's test, respectively. Statistical analysis of the impact of age in the examined groups was done using the one-way analysis of variance (ANOVA). Additionally, regression analysis was performed. The level of statistical significance was assumed to be *p* < .05. The statistical analyses were carried out using Stat-Soft software (Statistica 10.0 PL).

## Results

Table 1 reports the age, semen parameters, and antioxidant enzyme activities in the enrolled patients, according to the study groups. Patients in the LM group were older than those in the HM group. Semen volume, sperm concentration in 1 ml, total sperm count, normal morphology (%), and motile spermatozoa after 24 hr (%) were similar

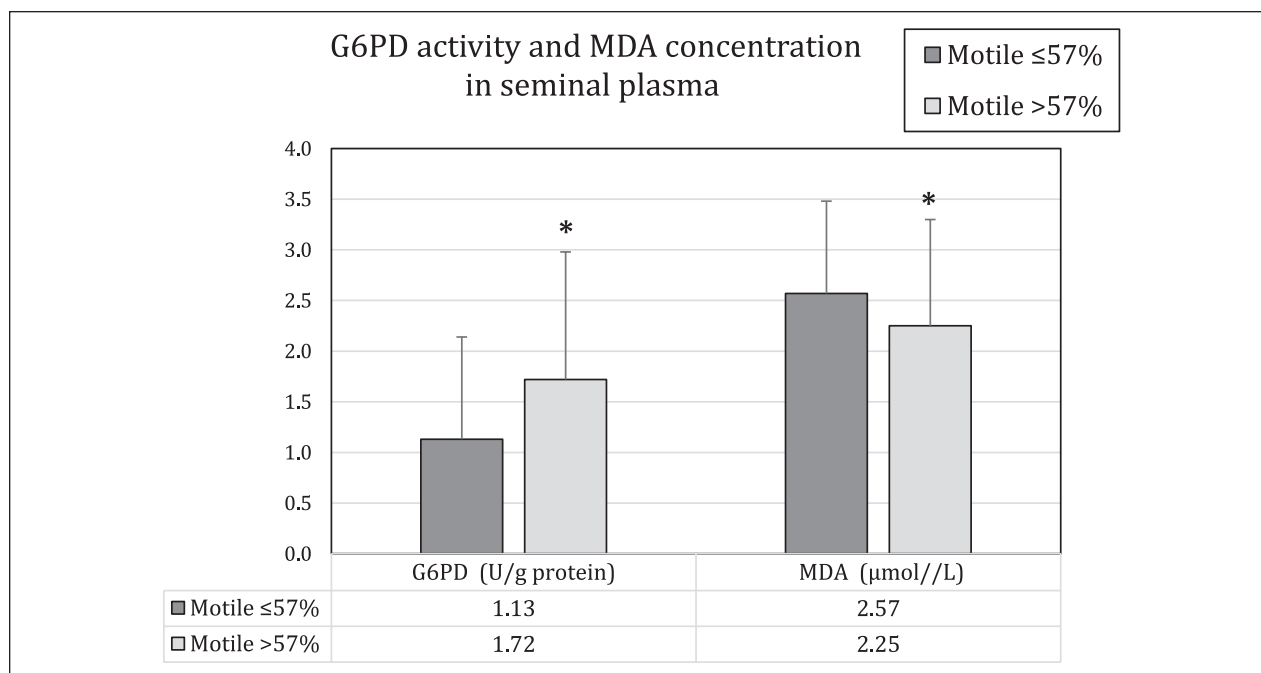
between the examined groups. Other parameters characterizing motility differed between the examined groups due to the criterion of study group division.

The activity of G6PD was 52% lower in the LM group compared to that in the HM group, while the level of MDA was 12% higher in LM group compared to that in the HM group (Figure 1). Similarly, the median values of IL-1β, IL-10, IL-12 (p70), and TNF-α were higher in the LM group than those in the HM group (Figures 2 and 3). The level of other antioxidant enzyme activities and interleukins was similar in both groups (Tables 1 and 2.)

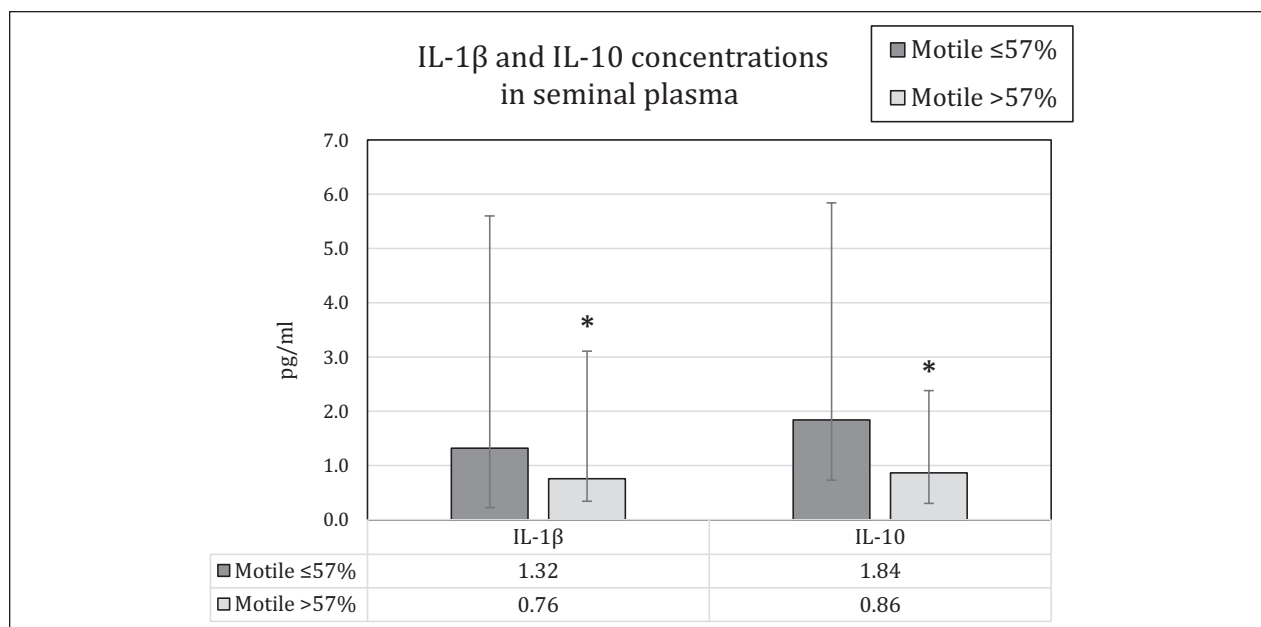
The percentage of motile spermatozoa after 1 hr was negatively correlated to the levels of IL-1β (*R* = −0.37, *p* = 0.015), IL-10 (*R* = −0.31, *p* = .040), IL-12 (*R* = −0.30, *p* = .047), and TNFα (*R* = −0.32, *p* = .042; data not reported). The activity of G6PD had a tendency toward positive correlation to the percentage of motile spermatozoa after 1 hr (*R* = 0.26 *p* = .057). There was no significant correlation between age and motile sperm (*p* = .248). Moreover, the analysis of the impacts of age in the examined groups did not obtain any significant differences (data not shown).

## Discussion

The effect of age on fertility in men is not as explicit as that in women. Since the average age of paternity has been rising in Western countries, the issue of defining APA is becoming an important issue. The semen analysis seems to be crucial for assessing the fertility potential of males. The comparisons of semen parameters of younger



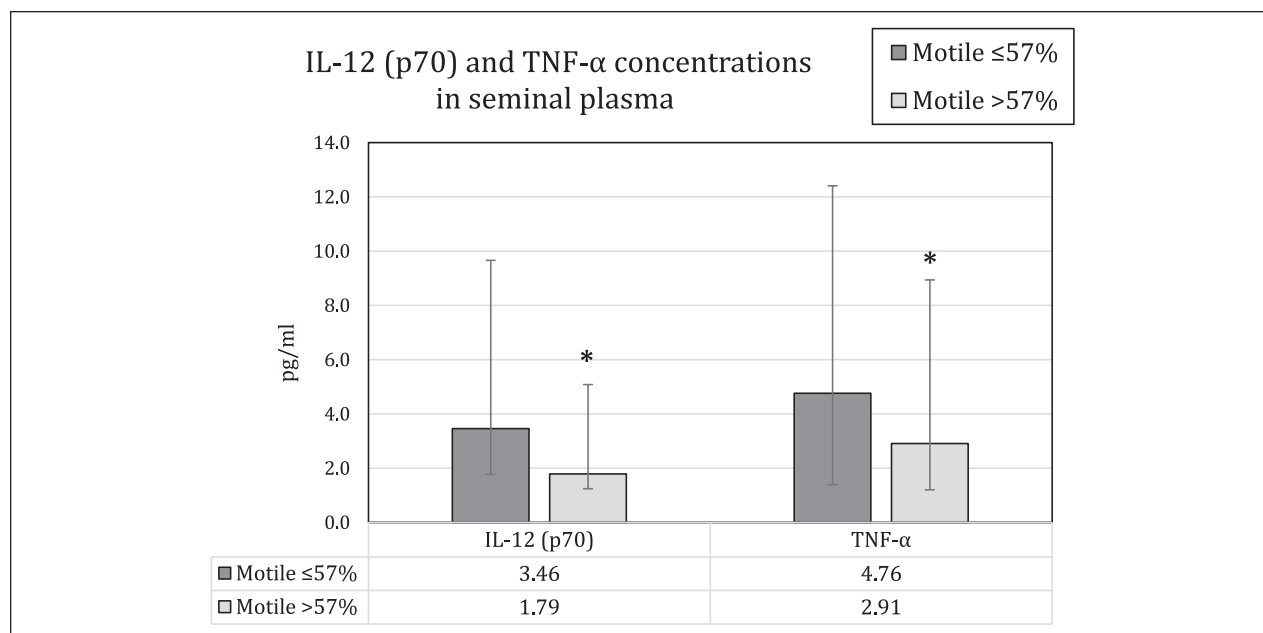
**Figure 1.** Glucose-6-phosphate dehydrogenase (G6PD) activity and malondialdehyde (MDA) concentration in seminal plasma. Data are shown as mean and SD. \*  $p < .05$ .



**Figure 2.** Concentrations of cytokines IL-1 $\beta$  and IL-10 in seminal plasma. Data are shown as median, lower and upper quartiles. \*  $p < .05$ .

and older men in individual studies demonstrated inconsistent findings, while meta-analyses proved that age has a negative impact on semen quality, including volume, morphology, and motility (Mazur and Lipshultz, 2018). The present study showed that study participants with

higher motility were significantly younger than those with lower motility. These findings are in concordance with the study of Beguería et al. (2014) who reported that the percentage of motile spermatozoa significantly decreased with age by 1.2% every 5 years.



**Figure 3.** Concentrations of cytokines IL-12 (p70) and TNF-α in seminal plasma. Data are shown as median, lower and upper quartiles. \*  $p < .05$ .

**Table 2.** Concentrations of Cytokines in Seminal Plasma.

	Motile Sperm Cells After 1 hr ≤57% <i>n</i> = 51		Motile Sperm Cells After 1 hr >57% <i>n</i> = 56		<i>p</i> value
	Median	IQR	Median	IQR	
IL-1β (pg/ml)	1.32	3.18	0.76	1.9	.036
IL-2 (pg/ml)	0.00	0.54	0.00	4.36	.521
IL-4 (pg/ml)	0.08	0.03	0.10	0.16	.205
IL-5 (pg/ml)	22.3	63.1	49.8	115	.725
IL-6 (pg/ml)	11.5	14.3	8.4	12.8	.689
IL-7 (pg/ml)	560	372	615	405	.248
IL-8 (pg/ml)	184	132	145	132	.618
IL-10 (pg/ml)	1.84	2.89	0.86	0.95	.020
IL-12 (p70; pg/ml)	3.46	4.5	1.79	2.75	.030
IL-13 (pg/ml)	0.60	1.34	0.37	0.27	.331
IL-17 (pg/ml)	8.07	8.18	7.01	9.11	.627
G-CSF (pg/ml)	16.1	26.3	16.2	27	.780
GM-CSF (pg/ml)	187	39.71	210	62.53	.568
IFN-γ (pg/ml)	51.8	64.2	46.3	93.0	.644
MCP-1 (MCAF; pg/ml)	1084	1457	848	772	.416
MIP-1β (pg/ml)	61.4	52.6	44	25	.056
TNF-α (pg/ml)	4.76	4.28	2.91	4.32	.049

*Note.* Data are expressed as median ± IQR. Statistical differences were analyzed by Mann–Whitney *U* test. HM = high motility; IFN-γ = interferon gamma; IL = interleukin; LM = low motility; TNF-α = tumor necrosis factor gamma; G-CSF = granulocyte colony – stimulating factor; GM-CSF = granulocyte macrophage colony – stimulating factor; MCP-1 = monocyte chemoattractant protein-1; MIP-1β = macrophage inflammatory protein 1 beta.  $p < .05$ .

The enzymatic and nonenzymatic compounds that interact with each other to protect spermatozoa against ROS build up the protective antioxidant system in semen.

The triad of main antioxidant enzymes includes SOD, CAT, and GPx. SOD neutralizes the superoxide anion, while CAT and GPx remove the hydrogen peroxide

produced by SOD. GPx catalyzes the reduction of organic peroxides (Walczak-Jedrzejowska et al., 2013). The bio-availability of the reduced glutathione (GSH) is indispensable for GPx, which oxidizes GSH to its disulfide form (GSSG). The recycling of GSH from GSSG depends on GR, which needs NADPH. One of the major sources of NADPH is glucose-6-phosphate dehydrogenase (G6PD), the enzyme of the pentose phosphate pathway that transforms glucose-6-phosphate into phospho-6-glucono-lactone (Griveau et al., 1995).

An *in vitro* study by Williams and Ford (2004) reported that the pentose phosphate pathway in human spermatozoa responds dynamically to oxidative stress and, along with GPx and GR, serves as a functional effective antioxidant defense. In a study on goat semen, Qiu et al. (2016) reported that the pentose phosphate pathway is more important than glycolysis to maintain sperm motility. The present study did not identify any association between sperm motility and the activities of SOD, CAT, GPx, and GR. Nevertheless, the activity of G6PD was significantly lower in the LM group than that in the HM group. The level of MDA was significantly higher in the LM group. Such data suggest the existence of additional mechanisms that connect the activity of G6PD, oxidative stress and motility. According to the study of Fernandez and O'Flaherty (2018), peroxiredoxins (PRDXs) may serve as key components of such mechanism. This family of antioxidant enzymes is present in all subcellular compartments of human spermatozoa and can scavenge a wide variety of ROS, thanks to the cysteine (Cys) residue at their active sites. Oxidation of the Cys thiol group inactivates PRDXs by forming a disulfide bond. Similarly to glutathione, the reduction of oxidized PRDXs is catalyzed by the thioredoxin- (TRX) thioredoxin reductase (TRD) that needs NADPH produced by G6PD. This cascade of reactions indicates how decreased G6PD activity may lead to an elevated MDA level, even though the activity of GPx and GR remains unchanged. On the contrary, Roshankhah et al. (2016) reported that G6PD deficiency does not increase the susceptibility of sperm to oxidative stress induced by hydrogen peroxide; however, the small sample group represents a substantial limitation of this study. The authors suggest an existence of alternative mechanisms for the generation of NADPH, such as NADPH-linked malic enzyme, NADPH-linked isocitrate dehydrogenase, isocitrate dehydrogenase and glutamate dehydrogenase, nicotinamide nucleotide transhydrogenase, and NADH kinase.

MDA is widely measured to assess the oxidative damage to lipids in spermatozoa, even though the results of the association between the seminal MDA level and sperm quality are controversial (Ammar et al., 2019). Tomar et al. (2017) reported that MDA levels in normospermic men were significantly lower than those in

asthenoteratospermic and oligoasthenoteratospermic men and had a negative correlation with sperm count, motility, and morphology. A negative correlation between MDA and motility was also identified in studies by Subramanian et al. (2018) and Atig et al. (2012). In contrast, Ammar et al. (2019) and Suleiman et al. (1996) reported no correlation between MDA and motility. In the present study, despite a significant difference between MDA levels in the studied groups, there was no correlation between MDA level and motility, which suggests a possible nonlinear dependence between these parameters in the semen of fertile males. Our previous study demonstrated that the level of MDA in seminal plasma was higher in males with asthenospermia than in controls; however, the level of MDA in spermatozoa lysate was significantly lower in males with oligospermia, asthenospermia, and oligoasthenospermia than that in the fertile controls (Dobrakowski et al., 2018).

The proliferation of germ cells and differentiation of mesenchymal cells are regulated by cytokines produced by testes. These polypeptide regulatory factors in seminal plasma are correlated with semen quality parameters. Genital infection is related to elevated levels of several cytokines, which could play a role in the immune defense of the male genital tract. Besides, many reports indicate that there are complex interactions between ROS and cytokines. ROS can induce the expression and production of cytokines, while cytokines can modulate the generation and utilization of ROS (Jiang et al., 2016). It has been proposed that poor sperm quality related to oxidative stress and lipid peroxidation may be partially due to the action of proinflammatory cytokines such as IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  (Martínez et al., 2007); however, studies on this topic are not conclusive. The present study reports that IL-1 $\beta$  and TNF- $\alpha$  levels in the seminal plasma are higher in males with low sperm mobility, with negative correlation between the percentage of motile spermatozoa after 1 hr and the levels of these cytokines. Furthermore, we showed similar results for IL-10 and IL-12. Both cytokines are associated with T-cell function and are detected at low concentration in physiological semen. Increased levels of these cytokines were detected in the semen of men with genital infections (Politch et al., 2007). Naz and Evans (1998) reported a positive correlation between IL-12 level and the number and normal morphology of sperm. A positive association between IL-6 and lipid peroxidation, as well as infertility, was identified by Camejo et al. (2011); however, the authors of this study did not find such association for TNF- $\alpha$ . Nevertheless, Fraczek et al. (2007) reported that proinflammatory cytokines *per se* are unable to induce lipid peroxidation due to oxidative stress. Such results must be considered with caution, because the levels of proinflammatory cytokines vary between fertile men and fluctuate



over time in seminal fluid, in response to duration of sexual abstinence and other unidentified factors.

The results of the conducted tests may indicate that in normal semen, factors that increase sperm motility are high G6PD activity and low level of oxidative stress as well as low values of some cytokines. Impact on the above-mentioned factors, for example, through the use of drugs and supplements can contribute to the improvement of sperm motility.

The results of the present research should be evaluated in the context of its limitations. The possible confounding role of lifestyle and additional disease states linked with ROS. Moreover, it is possible that the study was underpowered to detect significant differences in measured parameters.

## Conclusions

Lower motility of spermatozoa of healthy men is associated with decreased activity of G6PD and increased levels of IL-1 $\beta$ , IL-10, IL-12, and TNF- $\alpha$ , which may be related to elevated oxidative stress in seminal plasma.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Medical University of Silesia KNW-1-167/N/9/K.

## ORCID iD

Anna Machoń-Grecka  <https://orcid.org/0000-0002-2395-0477>

## 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.
- Agarwal, A., Virk, G., Ong, C., & du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *World Journal of Mens Health*, 32(1), 1–17.
- Alahmar, A. T. (2019). Role of oxidative stress in male infertility: An updated review. *Journal of Human Reproductive Sciences*, 12(1), 4–18.
- Amelar, R. D., Dubin, L., Schoenfeld, C. (1980, September). Sperm motility. *Fertility and Sterility*, 34(3), 197–215. [https://doi.org/10.1016/s0015-0282\(16\)44949-6](https://doi.org/10.1016/s0015-0282(16)44949-6)
- Ammar, O., Houas, Z., & Mehdi, M. (2019). The association between iron, calcium, and oxidative stress in seminal plasma and sperm quality. *Environmental Science and Pollution Research International*, 26(14), 14097–14105.
- Atig, F., Raffa, M., Ali, H. B., Abdelhamid, K., Saad, A., & Ajina, M. (2012). Altered antioxidant status and increased lipid per-oxidation in seminal plasma of Tunisian infertile men. *International Journal of Biological Sciences*, 8(1), 139–149.
- Beguería, R., García, D., Obradors, A., Poisot, F., Vassena, R., & Vernaev, V. (2014). Paternal age and assisted reproductive outcomes in ICSI donor oocytes: Is there an effect of older fathers? *Human Reproduction*, 29(10), 2114–2122.
- Camejo, M. I., Abdala, L., Vivas-Acevedo, G., Lozano-Hernández, R., Angeli-Greaves, M., & Greaves, E. D. (2011). Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters. *Biological Trace Element Research*, 143(3), 1247–1254.
- Chyra-Jach, D., Kaletka, Z., Dobrakowski, M., Machoń-Grecka, A., Kasperczyk, S., Birkner, E., & Kasperczyk, A. (2018). The associations between infertility and antioxidants, proinflammatory cytokines, and chemokines. *Oxidative Medicine and Cellular Longevity*, 2018(2018), 8354747.
- Dobrakowski, M., Kaletka, Z., Machoń-Grecka, A., Kasperczyk, S., Horak, S., Birkner, E., Zalejska-Fiolka, J., & Kasperczyk, A. (2018). The role of oxidative stress, selected metals, and parameters of the immune system in male fertility. *Oxidative Medicine and Cellular Longevity*, 2018(2018), 6249536.
- Durairajanayagam, D. (2018). Lifestyle causes of male infertility. *Arab Journal of Urology*, 16(1), 10–20.
- Fernandez, M. C., & O'Flaherty, C. (2018, June 15). Peroxiredoxin 6 is the primary antioxidant enzyme for the maintenance of viability and DNA integrity in human spermatozoa. *Human Reproduction*, 33(8), 1394–1407.
- Fraczek, M., Sanocka, D., Kamieniczna, M., & Kurpisz, M. (2007). Proinflammatory cytokines as an intermediate factor enhancing lipid sperm membrane peroxidation in vitro conditions. *Journal of Andrology*, 29(1), 85–92.
- Greil, A. L., Slauson-Blevins, K., & McQuillan, J. (2010). The experience of infertility: A review of recent literature. *Sociology of Health and Illness*, 32(1), 140–162.
- Griveau, J. F., Dumont, E., Renard, P., Callegari, J. P., & Le Lannou, D. (1995). Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. *Journal of Reproduction and Fertility*, 103(1), 17–26.
- Guzick, D. S., Overstreet, J. W., Factor-Litvak, P., Brazil C. K., Nakajima, S. T., Coutifaris, C., Carson, S. A., Cisneros, P., Steinkampf, M. P., Hill, J. A., Xu, D., Vogel, D. L.; National Cooperative Reproductive Medicine Network. (2001, November 8). Sperm morphology, motility, and concentration in fertile and infertile men. *The New England Journal of Medicine*, 345(19), 1388–1393. <https://doi.org/10.1056/NEJMoa003005>
- Habig, W. H., & Jakoby, W. B. (1981). Assays for differentiation of glutathione S-transferases. *Methods Enzymol*, 77, 398–405.
- Jiang, L., Zheng, T., Huang, J., Mo, J., Zhou, H., Liu, M., Gao, X., & Yu, B. (2016). Association of semen cytokines with reactive oxygen species and histone transition abnormalities.

- Journal of Assisted Reproduction and Genetics*, 33(9), 1239–1246.
- Johansson, L. H., & Borg, L. A. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. *Analytical Biochemistry*, 74, 31–36.
- Kasperczyk, A., Dobrakowski, M., Horak, S., Zalejska-Fiolka, J., & Birkner, E. (2015). The influence of macro and trace elements on sperm quality. *Journal of Trace Elements in Medicine and Biology*, 30, 153–159.
- Ko, E. Y., Sabanegh, E. S., & Agarwal, A. (2014). Male infertility testing: Reactive oxygen species and antioxidant capacity. *Fertility and Sterility*, 102(6), 1518–1527.
- Kopa, Z., Wenzel, J., Papp, G. K., & Haidl, G. (2005). Role of granulocyte elastase and interleukin-6 in the diagnosis of male genital tract inflammation. *Andrologia*, 37(5), 188–194.
- Martínez, P., Proverbio, F., & Camejo, M. I. (2007). Sperm lipid peroxidation and pro-inflammatory cytokines. *Asian Journal of Andrology*, 9, 102–127.
- Mascarenhas, M. N., Flaxman, S. R., Boerma, T., Vanderpoel, S., & Stevens, G. A. (2012). National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. *PLoS Medicine*, 9(12), 1–12.
- Mazur, D. J., & Lipshultz, L. I. (2018). Infertility in the aging male. *Current Urology Reports*, 19(7), 54.
- Naz, R. K., & Evans, L. (1998). Presence and modulation of interleukin-12 in seminal plasma of fertile and infertile men. *Journal of Andrology*, 19(3), 302–307.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95, 351–358.
- Oyanagui, Y. (1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Analytical Biochemistry*, 142, 290–296.
- Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70, 158–169.
- Politch, J. A., Tucker, L., Bowman, F. P., & Anderson, D. J. (2007). Concentrations and significance of cytokines and other immunologic factors in semen of healthy fertile men. *Human Reproduction*, 22(11), 2928–2935.
- Qiu, J. H., Li, Y. W., Xie, H. L., Li, Q., Dong, H. B., Sun, M. J., Gao, W. Q., & Tan, J. H. (2016). Effects of glucose metabolism pathways on sperm motility and oxidative status during long-term liquid storage of goat semen. *Theriogenology*, 86(3), 839–849.
- Richterich, R. (1971). *Chemia kliniczna*. PZWL.
- Roshankhah, S., Rostami-Far, Z., Shaveisi-Zadeh, F., Movafagh, A., Bakhtiari, M., & Shaveisi-Zadeh, J. (2016). Glucose-6-phosphate dehydrogenase deficiency does not increase the susceptibility of sperm to oxidative stress induced by H<sub>2</sub>O<sub>2</sub>. *Clinical and Experimental Reproductive Medicine*, 43(4), 193–198.
- Seshadri, S., Bates, M., Vince, G., & Jones, D. I. (2009). The role of cytokine expression in different subgroups of subfertile men. *American Journal of Reproductive Immunology*, 62, 275–282.
- Sikka, S. (2001). Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*, 8(7), 851–862.
- Subramanian, V., Ravichandran, A., Thiagarajan, N., Govindarajan, M., Dhandayuthapani, S., & Suresh, S. (2018). Seminal reactive oxygen species and total antioxidant capacity: Correlations with sperm parameters and impact on male infertility. *Clinical and Experimental Reproductive Medicine*, 45(2), 88–93.
- Suleiman, S. A., Ali, M. E., Zaki, Z. M. S., El-Malik, E. M., & Nasr, M. A. (1996). Lipid peroxidation and human sperm motility: Protective role of vitamin. *Journal of Andrology*, 17(5), 530–537.
- Tomar, G., Joshi, T., Varghes, A., Sasidharan, S., & Kural, M. R. (2017). Relationship of antioxidant system and reactive oxygen species with clinical semen parameters in infertile men. *Journal of Family Medicine and Primary Care*, 6(3), 574–577.
- Walczak-Jedrzejowska, R., Wolski, J. K., & Slowikowska-Hilczer, J. (2013). The role of oxidative stress and antioxidants in male fertility. *Central European Journal of Urology*, 66(1), 60–67.
- WHO. (2010). *Laboratory manual for the examination of human semen* (5th ed.). Cambridge University Press.
- Williams, A. C., & Ford, W. C. (2004). Functional significance of the pentose phosphate pathway and glutathione reductase in the antioxidant defenses of human sperm. *Biology of Reproduction*, 71(4), 1309–1316.