



CrossMark

# Glutathione administration reduces the DNA fragmentation index in sperm preparation with the Swim-Up (SU) and mini-Density Gradient Centrifugation (mini-DGC) methods at Doctor Soetomo General Hospital, Surabaya, Indonesia

Lutfi Hardiyanto<sup>1\*</sup>, Amarudin<sup>2</sup>, Indra Gusti Mansur<sup>2</sup>, Supardi<sup>1</sup>,  
Wahyuning Ramelan<sup>2</sup>, Hamdani Lunardhi<sup>1</sup>

## ABSTRACT

**Background:** Spermatozoa requires Reactive Oxygen Species (ROS) in physiological quantities for capacitation for fertilization purposes. However, if the balance between ROS production and antioxidant capacity is disrupted, it can result in oxidative stress, negatively affecting chromatin spermatozoa, leading to male infertility. This study evaluates the glutathione administration reduces the DNA fragmentation index in sperm preparation with the Swim-Up (SU) and mini-Density Gradient Centrifugation (mini-DGC) methods at Doctor Soetomo General Hospital, Surabaya, Indonesia.

**Methods:** A true experimental study by post-test only control group was conducted using ejaculate fluid of 9 infertile patients with normozoospermic at the Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta in January - February 2019 period. The spermatozoa DNA fragmentation was examined by following the instructions for using Spermfunc<sup>®</sup> DNAf Kit for Determination of the DNA Fragmentation Level in Spermatozoa by

Sperm Chromatin Dispersion (SCD) for both preparation methods. Data were analyzed using SPSS version 16.0 for Windows.

**Results:** SU vs. SU+glutathione method gave a lower but insignificant DNA fragmentation effect ( $p > 0.05$ ). The mini-DGC vs. mini-DGC+glutathione method showed significant results ( $p < 0.05$ ). SU+glutathione vs mini-DGC+glutathione showed no significant results ( $p > 0.05$ ). Meanwhile, the SU vs. mini-DGC method showed significant results ( $p < 0.05$ ). The recovery rate (RR) of the mini-DGC method has a higher value and is even better if glutathione is given compared to the SU method.

**Conclusion:** Spermatozoa preparation in the SU method gives a lower DNA fragmentation effect than the mini-DGC method. The administration of glutathione can be a particular consideration in carrying out spermatozoa preparation to protect spermatozoa from damage during preparation.

**Keywords:** Glutathione, DNA Fragmentation, Swim-Up, Mini-DGC.

**Cite This Article:** Hardiyanto, L., Amarudin., Mansur, I.G., Supardi., Ramelan, W., Lunardhi, H. 2020. Clinical profile of steroid-induced glaucoma in Bali Mandara Eye Hospital year 2019. *Intisari Sains Medis* 11(3): 1527-1532. DOI: 10.15562/ism.v11i3.739

<sup>1</sup>Department of Andrology,  
Doctor Soetomo General Hospital,  
Surabaya, Indonesia

<sup>2</sup>Department of Medical Biology,  
Faculty of Medicine, Universitas  
Indonesia, Jakarta, Indonesia

\*Corresponding to:  
Lutfi Hardiyanto;  
Department of Andrology,  
Doctor Soetomo General  
Hospital, Surabaya, Indonesia;  
[hardiyanto1975@gmail.com](mailto:hardiyanto1975@gmail.com)

Received: 2020-03-19  
Accepted: 2020-10-03  
Published: 2020-12-01

## INTRODUCTION

The incidence of infertility around the world is increasing.<sup>1</sup> Some of the infertility causes include hormonal problems, genetic diseases, infections, surgery on the gonads/genitals, autoimmune diseases, systemic diseases, heavy metal intoxication, smoking, radiation, side effects of drugs, and varicoceles.<sup>1</sup> Therapy in idiopathic infertility cases is still empirical, and oxidative stress is closely related to male infertility.<sup>1,2</sup>

Spermatozoa require physiological amounts of Reactive Oxygen Species (ROS) for capacitation,

hyperactivation, acrosome reactions, and spermatozoa-oocyte fusion for fertilization purposes.<sup>3</sup> However, if the balance between ROS production and antioxidant capacity is disturbed, it can result in the formation of oxidative stress, which can negatively affect spermatozoa chromatin by inducing Deoxyribonucleic Acid (DNA) strand break, resulting in male infertility which is characterized by abnormal motility and decreased spermatozoa survival.<sup>4-8</sup> ROS can be produced by high temperatures resulting in increased mitochondrial activity.<sup>7</sup> Obesity, aging, and even

some drugs such as chemotherapy drugs can increase ROS.<sup>9</sup>

Antioxidants can play a role in ROS inactivation to stop cell damage.<sup>7,10</sup> Some of the antioxidants that have been widely used today include glutathione.<sup>11,12</sup> The addition of glutathione can protect spermatozoa against ROS. The integrity of spermatozoa DNA is important for the occurrence of pregnancy and transmission of genetic information. Spermatozoa DNA damage is associated with decreased fertilization rates, embryo quality, and pregnancy rates, as well as increased rates of spontaneous abortion.<sup>9</sup>

Spermatozoa preparation is a method of performing Intrauterine Insemination (IUI), which aims to separate spermatozoa cells from seminal plasma and debris from all spermatozoa cells present in semen.<sup>13</sup> According to the American Pregnancy Association, the IUI success rate is only 10-20%.<sup>13</sup> Several studies have shown an association of increased abortion rates, pregnancy failure, and Assisted Reproduction Technology (ART) failure with DNA fragmentation or low DNA integrity in spermatozoa.<sup>14</sup> Men with a normal spermogram may still experience infertility and the problem may be related to abnormalities in the spermatozoa DNA. Therefore spermatozoa with normal DNA are required for fertilization and embryo development.

Swim-Up (SU) and Density Gradient Centrifugation (DGC) are spermatozoa preparations that are often carried out to increase the quality, concentration, and motility of spermatozoa, thereby increasing the capacity of spermatozoa in fertilization.<sup>15</sup> Some studies suggest that SU and DGC improve normal morphology and intact DNA in patients with teratozoospermia.<sup>16</sup>

Researchers have obtained several studies that show that glutathione can provide improved sperm parameters. Still, until now, there have not been too many studies on glutathione's effect in reducing the DNA Fragmentation Index of spermatozoa. Likewise, the Recovery Rate (RR) from the harvest using the existing methodology after glutathione administration. Based on the above explanation, this study evaluates the glutathione administration reduces the DNA fragmentation index in sperm preparation with the Swim-Up (SU) and Mini-Density Gradient Centrifugation (Mini-DGC) methods at Doctor Soetomo General Hospital, Surabaya, Indonesia.

## METHODS

This study was a true experimental clinical study with a post-test only control group design. The study was conducted at the Department of Medical Biology, Faculty of Medicine, Universitas Indonesia,

Jakarta, in January - February 2019. The inclusion criteria were men who had idiopathic infertility, aged 20-45 years, abstinence 48 hours - 7 days, normozoospermic spermogram, free of previous antioxidant therapy (more than 7 days), ejaculate volume 2 ml or more, minimum concentration 15 million / ml and signed informed consent and informations for consent. In contrast, the exclusion criteria were significant urogenital abnormalities, such as anorchia, a volume of one or each testicle less than 10 cc, grade III varicocele, obstruction of the spermatozoa transport channel, leukospermia, hematospermia, a history of organic disorders, such as impaired kidney function, kidney stones, impaired liver function, chronic indigestion, and dyspepsia syndrome, active and chronic inflammatory diseases, smoking and occupational diseases that result in exposure to ROS. The dependent variable was the Spermatozoa DNA Fragmentation Index, while the independent variables were glutathione, the SU and the mini-DGC method.

The research materials were fresh human semen samples, semen analysis morphological staining reagent, Spermfunc® DNAf Kit, 70% alcohol, 90% alcohol, 100% alcohol, aquadest, normal saline (NS), Glutathione vial 600 mg Water Soluble, preparation medium, Sil-Select Plus™. The research tools used were gloves, ejaculate collection glass, object-glass, cover glass, manual differential counter, light microscope, improved Neubauer counting room, timer, micropipette, disposable tip, 5 ml syringe, Eppendorf tube, water bath, floating cork, incubator. 37°C, chemical thermometer, bulb pipette pump, refrigerator, filter paper, tissue, slide immersion tray in liquids, drying tray, tweezers.

Each sample was then examined for the DNA fragmentation index for the two methods, namely SU and mini-DGC. The SU method is try to medium temperature and sample at 37°C, transfer 1 ml of the medium into the tube, transfer the liquefaction of sperm (up to 0.5 ml) into the medium with the help of a pipette or syringe, then mix until homogeneous (add glutathione in the treatment group) then tilt for 30 minutes. For the mini-DGC method is by applying samle to medium temperatur at 37°C, transfer 0.5 ml Sil-Select Plus (top layer) into the centrifuge tube, transfer 0.5 ml Sil-Select Plus 90% (bottom layer) in the top layer with a 21-G syringe and needle, try to separate the two layers with clear boundaries, put the liquefaction of sperm (0.5 ml) to the top layer with the help of a pipette or syringe (add glutathione to the treatment group), centrifuge for 10 minutes at 350-450 grams, remove the supernatant until it is near the sediment, add 2 ml of washing medium with a syringe and

**Table 1. Characteristics of research samples before preparation and based on the DNA Fragmentation Index**

Variable	Mean±SD
Age (Years)	36.5±5.9
Ejaculate Volume (ml)	3.3±0.4
Spermatozoa Concentration (million/ml)	58.7± 22.68
Spermatozoa Motility (%)	
Progressive	34.7±4.0
Non-Progressive	23.2±1.9
Immotile	42.1±4.9
Spermatozoa Morphology (%)	29.9±5.9
Total Motile Count (million sperm/ejaculate)	77.6±49.2

**Table 2. The comparison of DNA fragmentation index with SU, mini-DGC, and SU with DGC methods related to the glutathione administration**

Variables	DNA Fragmentation Index	p
SU (mean±SD)		
Without Glutathione	22.09±7.15	0.501
With Glutathione	20.18±5.13	
Mini-DGC (mean±SD)		
Without Glutathione	31.47 ± 6.59	0.017*
With Glutathione	24.42 ± 4.61	
Glutathione Administration (mean±SD)		
SU	20.18 ± 5.13	0.141
Mini-DGC	24.42 ± 4.61	
Spermatozoa Preparation Comparison (mean±SD)		
SU	22.09 ± 7.15	0.002*
Mini-DGC	31.47 ± 6.59	

SU: Swim-Up; mini-DGC: mini-Density Gradient Centrifugation

**Table 3. The results of the Recovery Rate (RR) evaluation**

Recovery Rate	Percentage (%)
Swim-Up only	15±0.03
Swim-Up + glutathione	18.6±0.02
Mini-DGC only	37.7±0.04
Mini-DGC + glutathione	48.9±0.03

resuspend the precipitate, centrifuge for 5 minutes at 300-400 grams, discard the supernatant and add sufficient amount of medium (according to sperm concentration and requirement, 0.3-0.6 ml) and resuspend the spermatozoa in the medium. Data analysis using Statistical Package for the Social Sciences (SPSS) version 16.0 for windows with a p-value <0.05 was considered statistically meaningful.

## RESULTS

The results of this study were obtained from 9 samples. In one part, the preparation was carried out using the SU method directly. In contrast, in the other part, the preparation was carried out using the mini-DGC method. For both methods,

one part acts as a control and one part is treated with glutathione. The characteristics of the research sample before preparation and the DNA fragmentation index are depicted in Table 1. The average age of samples was 36.5±5.9 years old, followed by 3.3±0.4 ml of ejaculate volume, 58.7± 22.68 million/ml of spermatozoa concentration, and 77.6±49.2 million sperm/ejaculate of Total Motile Count (TMC) (Table 1).

The results showed that the SU group had a mean DNA fragmentation index of 22.09±7.15, while the SU group with glutathione was given a mean DNA fragmentation index of 20.18±5.13 (Table 2). It can be seen that the mean DNA fragmentation index in the Swim-Up group with glutathione administration was not statistically significant lower than the control group (p=0.501) (Table 2).

From the data above, it can be seen that the results in the Mini-DGC group had a mean DNA fragmentation index of 31.47±6.59, while the Mini-DGC group with glutathione had a mean DNA fragmentation index around 24.42±4.61 (Table 2). It can be seen that the mean DNA fragmentation index in the Mini-DGC group with glutathione administration was lower than that of the Mini-DGC group, with statistical test results showing significant difference with p=0.017 (Table 2).

The Swim-Up group results with glutathione administration had a mean DNA fragmentation index of 20.18±5.13, while the Mini-DGC group with glutathione had a mean DNA fragmentation index of 24.42±4.61 (Table 2). It can be seen that the mean DNA fragmentation index in the mini-DGC group with glutathione was higher than that in the Swim-Up group with glutathione, but the statistical test results showed no significant difference with p=0.141 (Table 2).

From the data above, it can be seen that the results in the SU group had a mean DNA fragmentation index of 22.09±7.15, while the Mini-DGC group had a mean DNA fragmentation index of 31.47±6.59. It can be seen that the mean DNA fragmentation index in the SU group was lower than that in the Mini-DGC group, with statistical test results showing a significant difference (p = 0.002) (Table 2).

In the study, the Recovery Rate assessment was also assessed where the samples were divided into four groups and each used 0.5 ml of cement for the preparation process. The results of the RR assessment can be seen in Table 3.

## DISCUSSION

It is known that infertility cases in a person are learned through semen analysis, but several other tests that can sharpen the accuracy of the diagnosis

are being enforced. ROS is one of the causes of infertility by producing DNA fragmentation in spermatozoa. Some researchers also reported that the abnormalities of routine semen analyses were associated with defects in spermatozoa's genetic material.<sup>17</sup>

Smoking can increase DNA fragmentation in spermatozoa so that the resulting offspring are susceptible to cancer.<sup>18</sup> Several investigations have shown that increased DNA fragmentation is more common in the infertile group and a prolonged time to pregnancy.<sup>19</sup> Decreased IVF success rates and increased missed abortion were also reported in men with spermatozoa with high DNA fragmentation.<sup>20</sup>

Centrifugation, which is often carried out in the multilevel column preparation process, has been known to have a detrimental effect and cause the emergence of ROS, which continues in the lipid peroxidation process resulting in decreased motility.<sup>21</sup> Glutathione protects against cell damage caused by oxidants and free radicals.<sup>22</sup>

The average quality of semen parameters used in this study was normozoospermic and met the study's requirements. Our study represents the characteristics of the samples included in the study. The mean age was  $36.5 \pm 5.9$  years, including in the reproductive age category. Thus it can be expected that metabolic processes and various reactions in the body can still be expected to be within normal limits so that the spermatogenesis process can also be expected to be within normal limits.<sup>23</sup> The mean volume was  $3.3 \pm 0.4$  ml, where this mean was included in the normal category according to WHO 2010 laboratory guidelines, including the progressive category of motility ( $34.7 \pm 4\%$ ) and sperm morphology ( $29.9 \pm 5.9\%$ ).<sup>23</sup>

Table 1 shows that the Swim-Up spermatozoa preparation method with glutathione administration has a lower DNA fragmentation index than the DNA fragmentation index using the Swim-Up method without glutathione control. Although it does not have a significant difference in statistical tests, it can be concluded that glutathione in the preparation can provide a more protective effect against spermatozoa from DNA damage during preparation. The insignificant statistical test in this group was also possible because the samples used were within normal criteria so that not much DNA damage occurred during the preparation. In addition, the small sample size allows the protective effect of glutathione to be less pronounced.

Our study also shows that the mini-DGC method with glutathione administration has a much lower DNA fragmentation index value than

the Mini-DGC method alone, with statistical test results showing significant. It is known that the Mini-DGC method inflicts greater damage to the sperm membrane, so that DNA damage is much more significant when compared to the Swim-Up method, with a higher ROS increase.<sup>24</sup> The Mini-DGC group with glutathione administration had a better DNA fragmentation index, so it was believed that glutathione had significantly better protective effects against spermatozoa DNA damage, mainly when the preparation was carried out. This can be a consideration in the future that sperm preparation with a small amount can use the mini-DGC method with glutathione before the preparation is carried out so that it is hoped that more sperm with better quality will be used so that the success of ART will be higher.

Our study found no significant results for both methods, even though glutathione has been added. However, the SU method, with the application of glutathione, has a lower DNA fragmentation index than the Mini-DGC method. This further confirms that the centrifugation process in the Mini-DGC method seems to contribute more to DNA damage compared to other preparation methods, so that the preparation of large numbers of sperm is better with the Swim-Up method with the addition of glutathione before preparation is carried out.

Our study shows that the Swim-Up method's DNA fragmentation index has a statistic significantly lower value than the Mini-DGC method. These data indicate that spermatozoa have a higher susceptibility to DNA damage in the Mini-DGC method resulting in more significant DNA fragmentation so that it can be a hope of choice for the preparation method to minimize the occurrence of spermatozoa DNA damage, which is marked by the occurrence of DNA fragmentation so that the success of ART can be expected.

The centrifugation process carried out in multilevel preparations seems to have contributed a lot to spermatozoa's DNA damage, which ended with increased DNA fragmentation.<sup>25</sup> Even more prolonged periods of time and large rotational forces can have a much more significant effect on DNA damage.

The recovery rate (RR) in the mini-DGC method has a higher value and is even better when glutathione is applied. This is consistent with a previous study, where the RR with the Mini-DGC method gave higher values compared to the SU method.<sup>26</sup> Glutathione showed a better RR value when compared without glutathione with the same method. In this case, glutathione can provide protection and improve sperm analysis parameters.

## Conclusion

This study's results indicate that the application of glutathione in sperm preparation using the Swim-Up and mini-DGC methods showed significantly better tilapia DNA Fragmentation Index compared to the same method without glutathione. Meanwhile, the Swim-Up method of sperm preparation had a very low DNA Fragmentation Index value compared to the mini-DGC method. This indicates that centrifugation contributed more to DNA damage when the preparation was carried out. In the future, a larger number of samples is needed so that the effect of glutathione administration can be more real and visible.

## DISCLOSURE

### Conflict of Interest

The authors declare that there is no competing interest regarding publication.

### Ethics Consideration

This study has obtained the ethical review number 1236/UN2.F1/ETIK/2018 from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

### Funding

The authors are responsible for the study's funding without the involvement of grants, scholarships, or other funding resources.

### Author Contributions

All authors are responsible for the study from the conceptual framework, data gathering, data analysis until reporting the results of study through publication.

## REFERENCES

- Vander Borgh M, Wyns C. Fertility and infertility: Definition and epidemiology. *Clin Biochem*. 2018;62:2-10.
- Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility: an update and review of practice. *Reprod Biomed Online*. 2012;24(6):591-602.
- Evenson DP. The Sperm Chromatin Structure Assay (SCSA<sup>®</sup>) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. *Anim Reprod Sci*. 2016;169:56-75.
- Esteves SC, Agarwal A, Majzoub A. An evidence-based perspective on the role of sperm chromatin integrity and sperm DNA fragmentation testing in male infertility. *Transl Androl Urol*. 2017;6(Suppl 4):S665-S672.
- Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Effects of the reduced form of coenzyme Q10 (ubiquinol) on semen parameters in men with idiopathic infertility: a double-blind, placebo controlled, randomized study. *J Urol*. 2012;188(2):526-531.
- Zhang XD, Chen MY, Gao Y, Han W, Liu DY, Huang GN. The effects of different sperm preparation methods and incubation time on the sperm DNA fragmentation. *Hum Fertil (Camb)*. 2011;14(3):187-191.
- Lavranos G, Balla M, Tzortzopoulou A, Syriou V, Angelopoulou R. Investigating ROS sources in male infertility: a common end for numerous pathways. *Reprod Toxicol*. 2012;34(3):298-307.
- Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. *Indian J Med Res*. 2009;129(4):357-367.
- Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod Biomed Online*. 2014;28(6):684-703.
- Cakiroglu B, Eyyupoglu SE, Gozukucuk R, Uyanik BS. Ubiquinol effect on sperm parameters in subfertile men who have astheno-teratozoospermia with normal sperm concentration. *Nephrourol Mon*. 2014;6(3):e16870.
- Sheweita SA, Tilmisany AM, Al-Sawaf H. Mechanisms of male infertility: role of antioxidants. *Curr Drug Metab*. 2005;6(5):495-501.
- Lanzafame FM, La Vignera S, Vicari E, Calogero AE. Oxidative stress and medical antioxidant treatment in male infertility. *Reprod Biomed Online*. 2009;19(5):638-659.
- Kumar S, Azgaonkar H, Awasthi RT, Kapoor A, Srinivas S. Role of intra-uterine insemination and sperm preparation techniques in treatment of unexplained infertility and male infertility. *Med J Armed Forces India*. 2000;56(3):209-212.
- Evgeni E, Charalabopoulos K, Asimakopoulos B. Human sperm DNA fragmentation and its correlation with conventional semen parameters. *J Reprod Infertil*. 2014;15(1):2-14.
- Mahfouz R, Sharma R, Sharma D, Sabanegh E, Agarwal A. Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. *Fertil Steril*. 2009;91(3):805-811.
- Xue X, Wang WS, Shi JZ, Zhang SL, Zhao WQ, Shi WH, et al. Efficacy of swim-up versus density gradient centrifugation in improving sperm deformity rate and DNA fragmentation index in semen samples from teratozoospermic patients. *J Assist Reprod Genet*. 2014;31(9):1161-1166.
- Sheikh N, Amiri I, Fariman M, Najavi R, Hadeie J. Correlation between sperm parameters and sperm DNA fragmentation fertile in fertile and infertile men. *Iranian Journal of Reproductive Medicine*. 2008;6(1):13-18.
- Ranganathan B, Rao KA, Thalaivarasai Balasundaram S. Deterioration of semen quality and sperm-DNA integrity as influenced by cigarette smoking in fertile and infertile human male smokers-A prospective study. *J Cell Biochem*. 2019;10.1002/jcb.28458.
- Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod*. 1999;14(4):1039-1049.
- Zequiraj A, Beadini S, Beadini N, Aliu H, Gashi Z, Elezaj S, et al. Male Infertility and Sperm DNA Fragmentation. *Open Access Maced J Med Sci*. 2018;6(8):1342-1345.
- Henkel RR, Schill WB. Sperm preparation for ART. *Reprod Biol Endocrinol*. 2003;1:108.
- Gadea J, Molla M, Selles E, Marco MA, Garcia-Vazquez FA, Gardon JC. Reduced glutathione content in human sperm is decreased after cryopreservation: Effect of the addition of reduced glutathione to the freezing and thawing extenders. *Cryobiology*. 2011;62(1):40-46.
- Riddell D, Pacey A, Whittington K. Lack of compliance by UK andrology laboratories with World Health Organization recommendations for sperm morphology assessment. *Hum Reprod*. 2005;20(12):3441-3445.
- Yamanaka M, Tomita K, Hashimoto S, Matsumoto H, Satoch M, Kato H, et al. Combination of density gradient centrifugation and swim-up methods effectively decreases

- morphologically abnormal sperms. *J Reprod Dev.* 2016;62(6):599-606.
25. Alvarez JG, Lasso JL, Blasco L, Nunez RC, Heyner S, Caballero PP, et al. Centrifugation of human spermatozoa induces sublethal damage; separation of human spermatozoa from seminal plasma by a dextran swim-up procedure without centrifugation extends their motile lifetime. *Hum Reprod.* 1993;8(7):1087-1092.
  26. Muratori M, Tarozzi N, Carpentiero F, Danti S, Perrone FM, Cambi M, et al. Sperm selection with density gradient

centrifugation and swim up: effect on DNA fragmentation in viable spermatozoa. *Sci Rep.* 2019;9(1):7492.



This work is licensed under a Creative Commons Attribution