Salud, Ciencia y Tecnología. 2025; 5:1104
doi: 10.56294/saludcyt20251104

ORIGINAL

Impacts of UC-MSCS on the Quality of Sperm in Asthenoteratozoospermia

Impactos de UC-MSCS en la calidad del esperma en la astenoteratozoospermia

Ria Margiana[1,2], Rajendra Falah Adriansyah[3], Mohammad Ayodhia Soebadi[4], Reny I'tishom[5], Tania Graciana[1], Silvia W. Lestari[6]

[1]Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[2]Master's Programme Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[3]International Undergraduate Programme, Faculty of Medicine, Univeritas Airlangga.
[4]Department of Urology, Faculty of Medicine, Universitas Airlangga, Universitas Airlangga Teaching Hospital, Surabaya, East Java, Indonesia.
[5]Department of Biomedical Science , Faculty of Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.
[6]Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

Cite as: Margiana R, Falah Adriansyah R, Ayodhia Soebadi M, I'tishom R, Graciana T, Lestari SW. Impacts of UC-MSCS on the quality of sperm in asthenoteratozoospermia. Salud, Ciencia y Tecnología. 2025; 5:1104. https://doi.org/10.56294/saludcyt20251104

Submitted: 19-04-2024 **Revised:** 20-07-2024 **Accepted:** 30-11-2024 **Published:** 01-01-2025

Editor: Prof. Dr. William Castillo-González

Corresponding author: Ria Margiana

ABSTRACT

Introduction: male infertility is caused by the condition of male partners, characterized by abnormal sperm shape and decreased motility, called asthenoteratozoospermia. Since active ingredients can regulate biological activity, secretory products from mesenchymal stem cells (MSCs) have recently become objects of interest as drugs.
Method: the study hypothesis is that UC-MSC secretome impacts sperm quality in asthenoteratozoospermia-affected males depending on the parameters of sperm quality, antioxidant enzyme activity, and ROS concentration. The following sperm quality parameters were assessed, viability, intermotility, ultrastructure, SOD, 8OHdG, and catalase.
Results: altogether, findings highlight that the stem MSC secretome isolated from the umbilical cord enhances the quality of sperm and will enhance fertility when applied in the process. It also decreases the concentration of biochemical markers of oxidative stress during the sperm preparation process. For example, it leads to a decrease in OHdG and an increase in SOD.
Discussion: the results imply that MSC secretome may be an important therapeutic factor in reproductive health and reduced oxidative stress. According to the data, MSC secretome is likely an anti-oxidative treatment for enhancing the reproductive system.

Keywords: Mesenchymal Stem Cells; Secretome; Sperm Qualityinfertility; Asthenoteratozoospermia; Oxidative Stress.

RESUMEN

Introducción: la infertilidad masculina es causada por la condición de las parejas masculinas, caracterizada por una forma anormal de los espermatozoides y una motilidad reducida, llamada astenoteratozoospermia. Dado que los ingredientes activos pueden regular la actividad biológica, los productos secretores de las células madre mesenquimales (MSC) se han convertido recientemente en objetos de interés como fármacos.
Método: la hipótesis del estudio es que el secretoma de las UC-MSC afecta la calidad de los espermatozoides en los varones afectados por astenoteratozoospermia dependiendo de los parámetros de calidad de los espermatozoides, actividad enzimática antioxidante y concentración de ROS. Se evaluaron los siguientes parámetros de calidad de los espermatozoides: viabilidad, intermotilidad, ultraestructura, SOD, 8OHdG y

catalasa.
Resultados: en conjunto, los hallazgos destacan que el secretoma de las MSC madre aislado del cordón umbilical mejora la calidad de los espermatozoides y mejorará la fertilidad cuando se aplique en el proceso. También disminuye la concentración de marcadores bioquímicos de estrés oxidativo durante el proceso de preparación de los espermatozoides. Por ejemplo, conduce a una disminución de OHdG y un aumento de SOD.
Discusión: los resultados indican que el secretoma de las MSC puede ser un factor terapéutico importante para la salud reproductiva y la reducción del estrés oxidativo. Según los datos, es probable que el secretoma de las MSC sea un tratamiento antioxidante para mejorar el sistema reproductivo.

Palabras clave: Células Madre Mesenquimales Células Madre Mesenquimales; Secretoma; Infertilidad; Astenoteratozoospermia; Estrés Oxidativo.

INTRODUCTION

Couples who have difficulties in conception are estimated to be approximately 15 % of couples in the whole wide world, which is a significant challenge in reproductive medicine.[1,2] Asthenoteratozoospermia is a critical factor in infertility in males due to the low motility of sperm and its structurally aberrant morphology.[3] Because of the secretome that provides trophic factors, cytokines, and growth factors, mesenchymal stem cells (MSCs) have a significant therapeutic promise in regenerative medicine.[4] The secretome includes a range of bioactive molecules contributing to tissue remodeling, immune modulation, and angiogenesis.[5] Bioactive molecules conveyed through a secretome regulate various phases of the cell.[6] Therefore, the research aims to characterize the effects of Umbilical Cord-MSC (UC-MSC) secretomes on sperm quality in male asthenoteratozoospermia via measures of Reactive Oxygen Species (ROS) levels, anti-oxidant enzyme activity, and sperm parameters cell by transferring bioactive molecules.[6] Thus, the research goal is to investigate the impact of UC-MSC secretomes on sperm quality in male asthenoterazoospermia using indicators of ROS levels, activities of anti-oxidant enzymes, and sperm parameters.

This research evaluates the effects of UC-MSC-derived secretomes on sperm motility, concentration, and morphology. Secondary objectives involve determining ROS levels and anti-oxidant enzyme activity changes in sperm after exposure to secretomes, characterization of the molecular composition of UCMSC-derived secretomes, defining mechanisms of interaction with sperm cells, and understanding how UC-MSC-derived secretomes affect sperm function. The study hypothesizes that UC-MSC-derived secretomes will reduce ROS levels, enhance anti-oxidant enzyme activity, and improve sperm quality parameters in individuals with asthenoteratozoospermia.

METHOD

Study Design

The KET-1862/UN2.F1/ETIK/PPM.00.02/2023 study was previously authorized by the Universitas Indonesia Faculty of Medicine ethics committee. In this interventional investigation, individuals with asthenoteratozoospermia are studied to see how UC-MSC-derived secretomes affect sperm quality metrics. The study was conducted collaboratively at Universitas Indonesia and Permata Hospital, esteemed institutions in Jakarta, Indonesia. The sample calculation was based on this formula

$$n = \left[\frac{(Z\alpha+Z\beta)S}{x1-x2}\right]^2 = \left[\frac{(1.64+0.84)22}{10}\right]^2 = 5.49^2 = 29$$

The study included the remaining semen samples from 30 participants diagnosed with asthenoteratozoospermia, divided into two groups: secretome Treatment (n=15) and Placebo (n=15). The sampling used was purposive sampling.

The inclusion criteria were men aged 20-45 diagnosed with asthenoteratozoospermia who were seeking infertility evaluation and treatment at Permata Hospital, no current use of anti-oxidant or fertility medications, average hormonal profile (testosterone, FSH, LH), and non-smokers or mild smokers. The exclusion criteria included the presence of other significant male factor infertility issues (e.g., azoospermia), history of testicular trauma, surgery, or infection, and patients with systemic illnesses (e.g., diabetes, hypertension). All participants would be randomly assigned to receive either UC-MSC-derived secretomes or Placebo. Both participants and researchers conducting analyses would be blinded to group assignments.

Intervention

Secretome Isolation and Characterization

UC-MSCs were cultured under standard conditions and secretomes were isolated from conditioned media using ultracentrifugation. The secretome characterization assessed the size distribution using nanoparticle tracking analysis (NTA).[7] The characterization and isolation of umbilical cord MSCs were using ELISA to quantify important trophic and cytokines factors on the secretome of MSCs.[8,9]

Examination of Secretome

The secretome of the UC-MSC is compared to the semen samples in the oxidative stress status and the content of various trophic factors, such as VEGF and NGF, pre- and post-secretome treatment.[10]

ROS Levels in Secretome

Specific activities, including the Dichlorofluorescein diacetate (DCF-DA) assay were used to quantify the levels of ROS in the secretome.[11,12]

Measurement of Anti-oxidant Enzyme Activity in Secretome :

The amount of Superoxide dismutase(SOD), Catalase (CAT), and glutathione peroxidase(GPx) in the secretome has been measured by specific enzymatic methods.[13]

Preparation of Sperm Samples and Examination of Samples

Sperm samples were collected from healthy donors and processed using a standard sperm preparation kit, following the manufacturer's instructions. One set of processed sperm samples was an experimental group, while the other was a control group.

Measurement of ROS levels and anti-oxidant enzyme activity in semen

The tests described above can be used to test segments can be tested for ROS and anti-oxidant enzyme levels described above. This comparison technique evaluates oxidative stress and anti-oxidant capacity changes after secretome treatment. Measurement of SOD in semen was measured by WST-1 method to quantify total superoxide dismutase (T-SOD) in sperm. Catalase and 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured by using ELISA method.

Addition of MSC Secretome and Evaluation of Sperm Parameters

The secretome of MSCs originating from the umbilical cord was obtained by collecting the conditioned medium from cultivated cells. Subsequently, the secretome was introduced to the experimental group of produced sperm samples at different concentrations, whereas the control group was administered a placebo. Then the quality of sperm was assessed before and after treatment with the MSC secretome using CASA and microscopy techniques.[14]

Data Collection and Statistical Analysis

Details of the participants, more specifically demographic data, medical history, sample SEM, and hormonal profiles, were collected. Seminal fluid samples were retrieved before and after adding the MSC secretome to fully evaluate the sperm characteristics, including motility, morphology, and viability. A case-control study was conducted to analyze sperm characteristics before and after treatment and investigate the effects of MSC secretome on sperm recovery. Statistically processed data was used to answer how MSC secretome addition affects sperm quality parameters.The normality of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. [15] The critical p value is 0,05.

RESULTS

Analyses of VEGF and NGF using ELISA assays

Before and after treatment by secretome, an enzyme-linked immunosorbent assay for VEGF was performed to identify the variations in the concentration of VEGF. With increased VEGF delivered after grafting, there is an increased angiogenesis potential and improved body tissue healing and regeneration.

We also examined the effect secretome addition had on changes in NGF levels before and after treatment . Higher levels of NGF after addition match NGF concentrations and represent a prospect for promoting neuron outgrowth and operation by neurotrophic effects with greater capacity.

Table 1. VEGF and NGF Levels Before and After Secretome Addition

Parameter	Pre-Secretome (Mean ± SD)	Post-Secretome (Mean ± SD)	p-value
VEGF (pg/mL)	150 ± 12	350 ± 25	0,001
NGF (pg/mL)	120 ± 10	300 ± 20	0,001

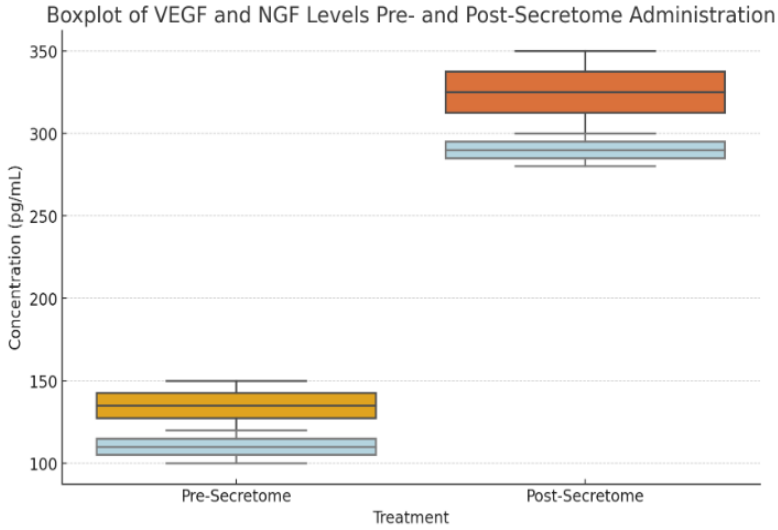


Figure 1. Box plot showing VEGF and NGF levels

Comparison of ROS and anti-oxidant enzyme as the impact from the addition of secretome

Levels of ROS

Following the secretome treatments, semen samples were used to assess ROS levels and examine oxidative stress changes. The ROS levels in the post-secretome delivery samples were lower than in the initial investigation. This also implies that the treatment with MSC secretome resulted in anti-oxidant properties on sperm cells.

Anti-oxidant Enzyme Activity

When reused post-secretome semen samples were introduced, there were possibilities for changes in the activities of the anti-oxidant enzymes, which in turn raised the level of SOD, CAT, and GPx. The changes detected stemmed from treatments designed to modulate anti-oxidant protection through secretome therapy, improving ROS clearance and restoring redox status.

Sperm Quality

Sperm Motility and Morphology

The results indicated that the secretome sample's progressive motility was significantly high. Spheroid motility in the Placebo group was 30 %, while in the secretome sample, motility was 53 %. The impact on sperm motility, depending on the dose, shows that only the increased concentration of MSC secretome contributes to the enhancement of movement and progressive motility of sperm. Spermatesis, or the ability of sperm to swim and reach the egg, must be improved in this case due to fertility problems associated with asthenoteratozoospermia.

Similarly, the improvement of the sperm morphology with the increase of the dosage reveals that exposure to a higher concentration of MSC secretome yielded a higher percentage of sperm with normal morphology. Sperm abnormalities in asthenoteratozoospermia include elevated abnormal size, shape, and structure rates. These can hinder the normal functioning of sperm and their ability to cause fertilization.

Table 2. Summary of Sperm Motility and Morphology

	Medium/Placebo sample	Secretome sample	
Concentration	20	15	≥ 15 million/ml
Motility			
Progressive	30	53	32 - 72 %
Non-progressive	5	7	1 – 18 %
Immotile	65	40	5 - 22 %
Vitality	66	73	58 – 91 %
Morphology	3	1	4 – 44 %

https://doi.org/10.56294/saludcyt20251104

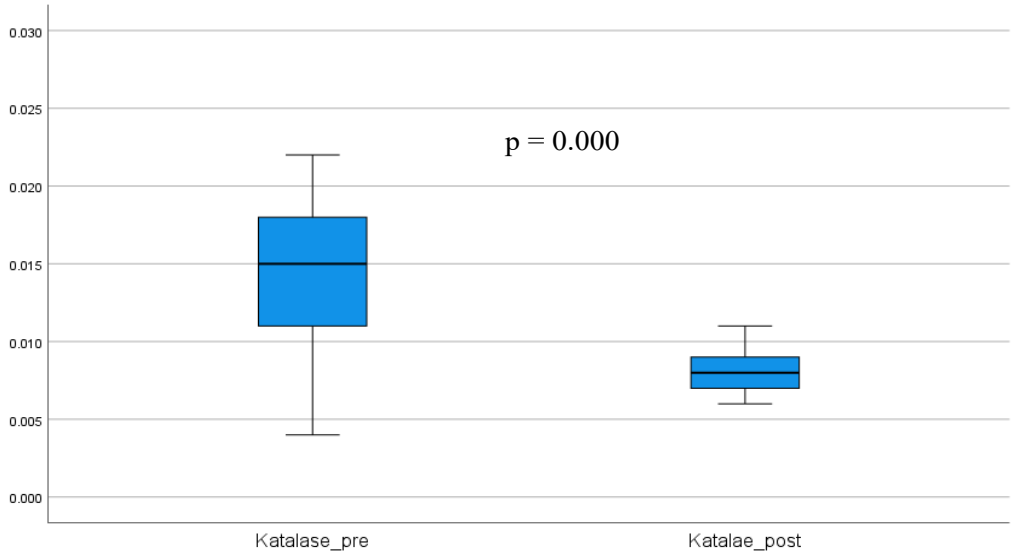
Sperm Viability

With the increased concentration in the MSC secretome, sperm viability improves so that a larger population of sperm cells can fertilize. Ensuring that sperm is viable is essential in maintaining sperm functionality in the female body and its duration of fertility, thereby improving the chances of fertilization and pregnancies.

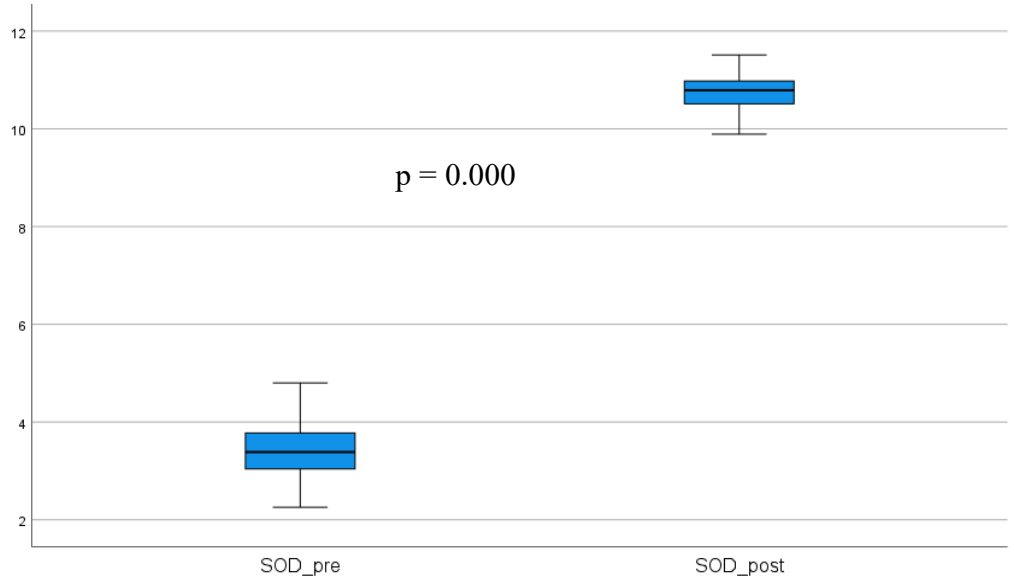
Catalase, SOP, and OHdG

The normality of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. [15] The levels of SOD, catalase, and OHdG were compared using paired t-tests. A p-value equal to 0,05 was used to set the significance level. Since all p-values were determined to be greater than 0,05, the normality tests performed on Delta catalase, Delta SOD, and Delta OHdG shows data is a normal distribution. The mean SOD level before the experiment was 3,4329 ± 0,54062, while the mean SOD level after the experiment was 10,7629 ± 0,44112. The difference between the two means was 7,33007 ± 0,65959. The p-value obtained from the analysis was 0,000, indicating a significant difference between the two SOD levels.

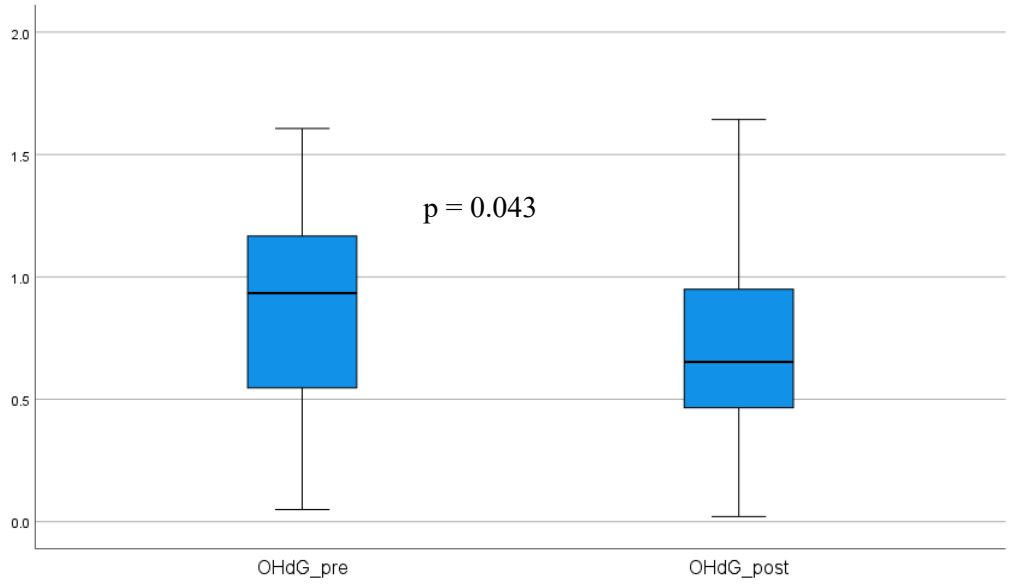
The mean catalase level before the experiment was 0,0151 ± 0,00505, whereas the mean catalase level after the experiment was 0,0089 ± 0,00416. The difference found here was 0,00617 ± 0,00693 between the two means. The calculated p-value from the analysis was 0,000, suggesting a significant difference between the two data sets. Paired T-tests were also done to compare the OHdG level. OHdG_pre was 0,8392 ± 0,40616, and OHdG_post was 0,7054 ± 0,40426 on average. The overall mean of the experimental treatment was 6. The difference between these two means was 0,13377 ± 0,34674. The analysis yielded a p-value of 0,043, less than 0,05, inferring that the difference is statistically significant.



(a)



(b)



(c)

Figure 2. (a) Boxplot Catalase (b) Boxplot SOD (c) Boxplot 8-OHdG

DISCUSSION

Changes in lifestyle, poor diet, pollution, and severe sickness are among the many variables that may play role in its etiology.[16] Fertilization of eggs depends on active sperm motility.[17] Spermatozoa with morphological defects cannot fertilize an egg because they cannot penetrate an ovum.[18] Thus, sperm morphology, motility, and the likelihood of a natural conception are related.[17]

Extensive studies have shown that chemotherapeutic drugs often harm reproductive system, spermatogenesis, and male fertility. Past studies have shown that paclitaxel (PTX)-induced therapeutic effects lie in the microtubule aggregation. Additionally, PTX may induce germ cell death with spermatogenesis abnormalities such as decreased sperm count and motility . The increase in levels of ROS caused by the PTX is a significant source of germ cell harm.

VEGF and NGF are necessary for identifying the secretome since they yield essential results. Secretome composition indicators: VEGF and NGF represent an extensive array of bioactive chemicals in the secretome of MSC, as depicted in Table 2.Characterizing the secretome is crucial to deciphering its diverse molecular structure and potential remedies concerning the deterioration of sperm quality.

Indicators of the secretome's effectiveness: We hypothesize that VEGF and NGF can be used as biomarkers to determine the efficacy and biological activity of the secretome treatment. Monitoring variations in these nurturing factors will help relate variations in secretome therapy to clinical trial results, which have been found to improve treatment techniques and boost healing value.

The results of enhanced sperm motility and morphology following secretome treatment correlate with other research showing the need for motile spermatozoa in fertilization. [17] The increase in sperm parameters following the addition of secretome also confirms that secretome therapy could improve sperm quality, which aligns with the findings of the relationship between sperm motility/ morphological structure and natural conception. [8,17] Moreover, any morphological abnormality in the sperms is directly responsible for the reduced chances of fertility because the unhealthy sperms do not go through the zonal penetration to reach the ovum. This fact substantiates the role of secretome therapy in enhancing sperms' shape and fertility capacity positively.[18]

One of the most reported infertility issues in men is low sperm concentration, which can make the sperm low motility. The motility parameters of spermatozoa are increased upon secretome addition. The improvement also reveals that MSC secretome might improve sperm mobility.

Higher ROS concentrations in the secretome may indicate intracellular oxidative stress in MSCs during preparation.[23] Since an increase in ROS generation can be harmful and oppose the secretome's therapeutic potential on the targeted cells, ROS regulation is crucial. Modifying the culture environment and providing anti-oxidants is necessary to reduce oxidative injury and preserve the integrity of secretome.[24]

The study's outcomes regarding the decrease in oxidative stress by administering secretome also parallel the findings in the literature that increased ROS levels harm sperm quality. Increased levels of ROS may caused by chemotherapeutic drugs like PTX, which have the effect of reducing the sperm count and the motility of the sperm.[26] These results, whereupon treatment with secretome, ROS levels are reduced, and antioxidant enzyme activity is enhanced, also support research studies that show how PTX-induced oxidative damage may

be reduced by antioxidants.[22,27]

Prior studies revealed that MSCs have regenerative and anti-inflammatory properties to manage testicular damage using various sources such as BM-MSCs and UC-MSCs.[28,29] This work also supports our previous findings that MSCs can improve sperm quality and treat male infertility. MSC secretome could prevent oxidative stress-induced damage to the sperm cells by decreasing the ROS level and increasing the SOD, which is consistent with the previous studies that demonstrated the beneficial application of MSCs in male reproductive health.[30]

MSCs can restore damaged cells, prevent cellular aging, and fight inflammation and oxidative damage.[30] Researchers have shown that MSCs derived from human amniotic (hA-MSCs), bone marrow (BM-MSCs), and UC-MSCs may effectively reduce the severity of testicular injury caused by busulfan.[28,29,32] BM-MSCs protect rat testes by regulating oxidative stress and working through anti-inflammatory and immune-modulatory mechanisms.[33] These studies lay a solid foundation for future research on MSCs' potential benefits to men's reproductive health.

Sperm Morphology

Before the addition of secretome, semen was shown to have altered morphology, exhibiting distortions in size, shape, and viability, which are indicators of sperm health. These conditions, known as teratozoospermia, can impair sperm function and reduce fertility.[34]

After the secretome addition, there is noticeable improvement in sperm form and size. Sperm Cell Enumeration (SCE) is elevated, the proportion of sperm standard forms is higher, and teratozoospermia decreases due to therapy. The enhancement means that adding the MSC secretome may promote the generation of normal-sized sperms.[35]

Sperm viability

After the secretome is administered, the sperm viability are enhanced. Research conducted after the treatment shows a higher proportion of viable sperm cells, indicating improved viability and survival ability. The enhancement implies that MSC secretome may safeguard sperm against oxidative harm.

SOD

An increase in superoxide dismutase (SOD) levels following the treatment is indicative of UC-MSC secretome potential to enhance anti-oxidant defenses in the body. While statistically significant, the observed decline in catalase levels indicates an intricate interplay among many anti-oxidant enzymes that necessitates additional exploration. The drop in OHdG levels suggests a reduction in oxidative DNA damage, demonstrating the potential of MSC secretome in protecting against oxidative stress.[36]

Figure 6 strengthens the anti-oxidative potentials of MSC secretome by revealing a significant decrease in ROS content and a concomitant increase in SOD activity after treatment. These changes, in support of the information provided in Table 3, indicate that the secretome could lessen oxidative damage in semen since the ability of ROS to harm sperm is well-established; increasing natural anti-oxidant mechanisms would benefit semen quality.

CONCLUSION

Determination of ROS levels and anti-oxidant enzyme activity in the secretome and semen samples provided valuable data about the treatment system's oxidative stress state and anti-oxidant potential. This study found that ROS and anti-oxidant enzyme activity levels were lower in the MSC secretome group.

The changes in sperm motility, morphology, and viability before and after MSC secretome treatment confirm the positive effect of MSC secretome therapy on sperm quality in infertile patients with asthenoteratozoospermia. Secretome addition is likely to positively impact sperm quality, fertility rates, and the chance of conception with male factor infertility.

UC-MSC substantially alters levels of oxidative stress markers implicated in sperm preparation. In particular, it reduces OHdG concentration while increasing SOD concentration. The findings suggest that the MSC secretome can be used as a therapy to improve reproductive health by countering oxidations. Further quantitative and qualitative research is needed to corroborate these findings and improve the approaches to treatment for the best outcome.

REFERENCES

1. Pallotti F, Barbonetti A, Rastrelli G, Santi D, Corona G, Lombardo F. The impact of male factors and their correct and early diagnosis in the infertile couple's pathway: 2021 perspectives. Journal of Endocrinological Investigation. 2022 Oct;45(10):1807-22. https://link.springer.com/article/10.1007/s40618-022-01778-7

2. Barnes A, Riche D, Mena L, Sison T, Barry L, Reddy R, Shwayder J, Parry JP. Efficacy and safety of intrauterine

insemination and assisted reproductive technology in populations serodiscordant for human immunodeficiency virus: a systematic review and meta-analysis. Fertility and sterility. 2014 Aug 1;102(2):424-34. https://doi.org/10.1016/j.fertnstert.2014.05.001

3. Ferreux L, Bourdon M, Chargui A, Schmitt A, Stouvenel L, Lorès P, Ray P, Lousqui J, Pocate-Cheriet K, Santulli P, Dulioust E. Genetic diagnosis, sperm phenotype and ICSI outcome in case of severe asthenozoospermia with multiple morphological abnormalities of the flagellum. Human Reproduction. 2021 Nov 1;36(11):2848-60. https://doi.org/10.1093/humrep/deab200

4. Hofer HR, Tuan RS. Secreted trophic factors of mesenchymal stem cells support neurovascular and musculoskeletal therapies. Stem cell research & therapy. 2016 Dec;7:1-4. https://doi.org/10.1186/s13287-016-0394-0

5. Fadilah NI, Jailani MS, Hisham MA, Raj NS, Shamsuddin SA, Ng MH, Fauzi MB, Maarof M. Cell secretomes for wound healing and tissue regeneration: Next generation acellular based tissue engineered products. Journal of Tissue Engineering. 2022 Jan;13. 10.1177/20417314221114273

6. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity. 2017;2017(1):8416763. https://doi.org/10.1155/2017/8416763

7. Banche Niclot AG, Marini E, Ferrero I, Barbero F, Rosso E, Fenoglio I, Barge A, Pessina A, Coccè V, Paino F, Mareschi K. A New Paclitaxel Formulation Based on Secretome Isolated from Mesenchymal Stem Cells Shows a Significant Cytotoxic Effect on Osteosarcoma Cell Lines. Pharmaceutics. 2023 Sep 19;15(9):2340. https://doi.org/10.3390/pharmaceutics15092340

8. Kalinina N, Kharlampieva D, Loguinova M, Butenko I, Pobeguts O, Efimenko A, Ageeva L, Sharonov G, Ischenko D, Alekseev D, Grigorieva O. Characterization of secretomes provides evidence for adipose-derived mesenchymal stromal cells subtypes. Stem cell research & therapy. 2015 Dec;6:1-2. https://doi.org/10.1186/s13287-015-0209-8

9. Idrisova KF, Zeinalova AK, Masgutova GA, Bogov Jr AA, Allegrucci C, Syromiatnikova VY, Salafutdinov II, Garanina EE, Andreeva DI, Kadyrov AA, Rizvanov AA. Application of neurotrophic and proangiogenic factors as therapy after peripheral nervous system injury. Neural Regeneration Research. 2022 Jun 1;17(6):1240-7. https://journals.lww.com/nrronline/fulltext/2022/06000/Application_of_neurotrophic_and_proangiogenic.10.aspx

10. Kaltsas A. Oxidative stress and male infertility: the protective role of anti-oxidants. Medicina. 2023 Oct 4;59(10):1769. https://doi.org/10.3390/medicina59101769

11. Pasciu V, Nieddu M, Sotgiu FD, Baralla E, Berlinguer F. An Overview on Assay Methods to Quantify ROS and Enzymatic Antioxidants in Erythrocytes and Spermatozoa of Small Domestic Ruminants. Animals. 2023 Jul 13;13(14):2300. https://doi.org/10.3390/ani13142300

12. Bader R, Ibrahim JN, Mourad A, Moussa M, Azoury J, Azoury J, Alaaeddine N. Improvement of human sperm vacuolization and DNA fragmentation co-cultured with adipose-derived mesenchymal stem cell secretome: in vitro effect. International journal of stem cells. 2019 Nov 30;12(3):388-99. DOI: https://doi.org/10.15283/ijsc19047

13. Xu L, Yang J, Xu M, Shan D, Wu Z, Yuan D. Speciation and adaptive evolution reshape anti-oxidant enzymatic system diversity across the phylum Nematoda. BMC biology. 2020 Dec;18:1-8. https://doi.org/10.1186/s12915-020-00896-z

14. Singh AK, Kumar A, Bisla A. Computer-assisted sperm analysis (CASA) in veterinary science: A review. The Indian Journal of Animal Sciences. 2021 Jun 1;91(6):419-29. https://doi.org/10.56093/ijans.v91i6.115435

15. Ahmad F, Khan RA. A power comparison of various normality tests. Pakistan Journal of Statistics and Operation Research. 2015 Sep 8:331-45. https://doi.org/10.18187/pjsor.v11i3.845

16. Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, Goulis DG, Krausz C, Giwercman A. European

Academy of Andrology guideline Management of oligo-astheno-teratozoospermia. Andrology. 2018 Jul;6(4):513-24. https://doi.org/10.1111/andr.12502

17. Hasan MM, Kabir MF, Khandaker MA, Kamruzzaman AH, Akter M, Nuri A. Management of Severe Asthenoteratozoospermia with Traditional Medicine: A Case Study. European Journal of Medical and Health Research. 2024 Jan 1;2(1):91-4. https://doi.org/10.59324/ejmhr.2024.2(1).13

18. Chemes HE, Rawe VY. Sperm pathology: a step beyond descriptive morphology. Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. Human Reproduction Update. 2003 Sep 1;9(5):405-28. https://doi.org/10.1093/humupd/dmg034

19. EJACULATION WT. Prasad V Kulkarni1, Krushndev L Shende2. Int. J. Ayur. Pharma Research. 2013;1(3):47-53. https://core.ac.uk/download/pdf/333819227.pdf

20. Zi T, Liu Y, Zhang Y, Wang Z, Wang Z, Zhan S, Peng Z, Li N, Liu X, Liu F. Protective effect of melatonin on alleviating early oxidative stress induced by DOX in mice spermatogenesis and sperm quality maintaining. Reproductive Biology and Endocrinology. 2022 Jul 18;20(1):105. https://doi.org/10.1186/s12958-022-00977-4

21. Ghafouri-Fard S, Shoorei H, Abak A, Seify M, Mohaqiq M, Keshmir F, Taheri M, Ayatollahi SA. Effects of chemotherapeutic agents on male germ cells and possible ameliorating impact of anti-oxidants. Biomedicine & Pharmacotherapy. 2021 Oct 1;142:112040. https://doi.org/10.1016/j.biopha.2021.112040

22. Wang Y, Zhou Y, Zheng Z, Li J, Yan Y, Wu W. Sulforaphane metabolites reduce resistance to paclitaxel via microtubule disruption. Cell death & disease. 2018 Nov 14;9(11):1134. https://doi.org/10.1038/s41419-018-1174-9

23. González-González A, García-Sánchez D, Dotta M, Rodríguez-Rey JC, Pérez-Campo FM. Mesenchymal stem cells secretome: The cornerstone of cell-free regenerative medicine. World journal of stem cells. 2020 Dec 12;12(12):1529. https://doi.org/10.4252/wjsc.v12.i12.1529

24. Bari E, Perteghella S, Di Silvestre D, Sorlini M, Catenacci L, Sorrenti M, Marrubini G, Rossi R, Tripodo G, Mauri P, Marazzi M. Pilot production of mesenchymal stem/stromal freeze-dried secretome for cell-free regenerative nanomedicine: a validated GMP-compliant process. Cells. 2018 Oct 30;7(11):190. https://doi.org/10.3390/cells7110190

25. Panahi M, Rahimi B, Rahimi G, Yew Low T, Saraygord-Afshari N, Alizadeh E. Cytoprotective effects of anti-oxidant supplementation on mesenchymal stem cell therapy. Journal of cellular physiology. 2020 Oct;235(10):6462-95. https://doi.org/10.1002/jcp.29660

26. Ajayi AF, Akhigbe RE. The physiology of male reproduction: Impact of drugs and their abuse on male fertility. Andrologia. 2020 Oct;52(9):e13672. https://doi.org/10.1111/and.13672

27. Wang Z, Teng Z, Wang Z, Song Z, Zhu P, Li N, Zhang Y, Liu X, Liu F. Melatonin ameliorates paclitaxel-induced mice spermatogenesis and fertility defects. Journal of Cellular and Molecular Medicine. 2022 Feb;26(4):1219-28. https://doi.org/10.1111/jcmm.17177

28. Qian C, Meng Q, Lu J, Zhang L, Li H, Huang B. Human amnion mesenchymal stem cells restore spermatogenesis in mice with busulfan-induced testis toxicity by inhibiting apoptosis and oxidative stress. Stem cell research & therapy. 2020 Dec;11:1-2. https://doi.org/10.1186/s13287-020-01803-7

29. Ghasemzadeh-Hasankolaei M, Batavani R, Eslaminejad MB, Sayahpour F. Transplantation of autologous bone marrow mesenchymal stem cells into the testes of infertile male rats and new germ cell formation. International journal of stem cells. 2016 Nov 30;9(2):250-63. DOI: https://doi.org/10.15283/ijsc16010

30. Zhang Y, Liu Y, Teng Z, Wang Z, Zhu P, Wang Z, Liu F, Liu X. Human umbilical cord mesenchymal stem cells (hUC-MSCs) alleviate paclitaxel-induced spermatogenesis defects and maintain male fertility. Biological Research. 2023 Aug 13;56(1):47. DOI:10.1186/s40659-023-00459-w

31. Merzenich H, Zeeb H, Blettner M. Decreasing sperm quality: a global problem?. BMC public health. 2010

Dec;10:1-5. https://doi.org/10.1186/1471-2458-10-24

32. Yang RF, Liu TH, Zhao K, Xiong CL. Enhance mouse germ cell-associated gene expression by injecting human umbilical cord mesenchymal stem cells into the testis of chemical-induced azoospermic mice. Asian Journal of Andrology. 2014 Sep 1;16(5):698-704. DOI: 10.4103/1008-682X.129209

33. Ahmed EA, Ahmed OM, Fahim HI, Mahdi EA, Ali TM, Elesawy BH, Ashour MB. Combinatory Effects of Bone Marrow-Derived Mesenchymal Stem Cells and Indomethacin on Adjuvant-Induced Arthritis in Wistar Rats: Roles of IL-1β, IL-4, Nrf-2, and Oxidative Stress. Evidence-Based Complementary and Alternative Medicine. 2021;2021(1):8899143. https://doi.org/10.1155/2021/8899143

34. Milardi D, Grande G, Sacchini D, Astorri AL, Pompa G, Giampietro A, De Marinis L, Pontecorvi A, Spagnolo AG, Marana R. Male fertility and reduction in semen parameters: a single tertiary-care center experience. International journal of endocrinology. 2012;2012(1):649149. https://doi.org/10.1155/2012/649149

35. Sagaradze G, Monakova A, Efimenko A. Potency Assays for Mesenchymal Stromal Cell Secretome-Based Products for Tissue Regeneration. International Journal of Molecular Sciences. 2023 May 27;24(11):9379. https://doi.org/10.3390/ijms24119379

36. Chen S, He Z, Xu J. Application of adipose-derived stem cells in photoaging: basic science and literature review. Stem Cell Research & Therapy. 2020 Dec;11:1-5. https://doi.org/10.1186/s13287-020-01994-z

37. LEWIS B, AITKEN RJ. A redox-regulated tyrosine phosphorylation cascade in rat spermatozoa. Journal of Andrology. 2001 Jul 8;22(4):611-22. https://doi.org/10.1002/j.1939-4640.2001.tb02221.x

ACKNOWLEDGMENT

We sincerely thank the PUTI (Program Unggulan Terpadu Indonesia) for the generosity granted in 2023. On behalf of PUTI , we are grateful for their efforts and support towards pursuing research quality and innovation.

FINANCING

Funding by PUTI (Program Unggulan Terpadu Indonesia) in 2023 (number NKB-404/UN2/RST/HKP.05.00/2023).

CONFLICT OF INTEREST

None.

AUTHORSHIP CONTRIBUTION

Conceptualization: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Data curation: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Formal analysis: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Research: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Methodology: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Project management: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Resources: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Software: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Supervision: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Validation: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Display: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

Drafting - original draft: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny

I'tishom, Tania Graciana, Silvia W. Lestari.

Writing - proofreading and editing: Ria Margiana, Rajendra Falah Adriansyah, Mohammad Ayodhia Soebadi, Reny I'tishom, Tania Graciana, Silvia W. Lestari.

https://doi.org/10.56294/saludcyt20251104