Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue-**3 Year 2023** Page **1256:1263***

The Administration of the Secretome from Mesenchymal Stem Cells Derived from the Umbilical Cord into the Medium used for Sperm Preparation

Ria Margiana [1,2,3,4]*, Silvia Werdhy Lestari [2,4], Mulyoto Pangestu [5,6]

[1]Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[2]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[3]Andrology Study Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

[4]Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[5]Education Program in Reproduction and Development (EPRD) Department of Obstetrics and Gynaecology, Monash Clinical School, Monash University, Clayton, Australia

[6]Education Program in Reproduction and Development (EPRD), Department of Obstetrics and Gynaecology, Monash Clinical School, Monash University, Clayton, Australia

Short Title: Sperm Preparation Medium with Umbilical Cord Mesenchymal Stem Cell Secretome

*Corresponding Author

Ria Margiana

Faculty of Medicine

Departement of Anatomy, Faculty of Medicine

Universitas Indonesia

Margonda Raya Street, Pondok Cina

Depok, West Java, Indonesia, 16424

Tel. (021) 7875602

Fax (021)7875603

E-Mail: margianaria@gmail.com or ria.margiana@ui.ac.id

Article History

Received: 08 Aug 2023

Revised: 29 Sept 2023

Accepted: 29 Oct 2023

Abstract

Background/Aims: This study investigates the potential of the secretome of mesenchymal stem cells produced from the umbilical cord (UC-MSCs), which are renowned for their ability to prevent damage caused by oxidation, to improve the quality of sperm. Superoxide Dismutase (SOD) is an essential antioxidant enzyme present in UC-MSCs that neutralizes reactive oxygen species (ROS). Our objective is to examine the influence of UC-MSCs secretome, which includes SOD, on spermatozoa. This research has the potential to significantly transform strategies for enhancing sperm quality. **Methods:** Thirty couples with primary male infertility (asthenozoospermia) and a good DNA fragmentation index (DFI) participated in the study. The semen samples were subjected to differential gradient centrifugation (DGC) to prepare the sperm. This was followed by the addition of secretome in a 1:1 ratio and incubated for 30 minutes. Before and after the incubation, the sperm concentration, velocity, and DNA fragmentation index (DFI) were evaluated. The growth factors in the

secretome of UC-MSCs were examined using enzyme-linked immunosorbent assay (ELISA). The application of secretome therapy in a 1:1 ratio resulted in a substantial enhancement in both sperm concentration and velocity. The treatment group had a significant decrease in DFI in comparison to the control group. Nevertheless, the experimental group exhibited a substantial decrease in SOD levels, suggesting a decline in antioxidant capacity. The decline in levels of Superoxide Dismutase (SOD) indicates a reduction in the ability to counteract harmful free radicals, which may lead to increased oxidative stress in sperm cells and ultimately result in male infertility. Additional research is essential to comprehend the consequences and mechanisms underlying this decrease and investigate its therapeutic possibilities. **Results:** The addition of UC-MSCs secretome to the sperm preparation media improves sperm quality by reducing DNA fragmentation. Nevertheless, the significant reduction in SOD levels underscores the necessity for additional investigation to fully grasp its impact on male infertility and the possibility for its therapeutic application. **Conclusion:** The findings of this research point to the possibility that the secretome of UC-MSCs could be useful as an additional component in the development of strategies that aim to increase sperm quality.

Keywords**:** superoxide dismustase (sod), umbilical cord mesenchymal stem cells secretome, male infertility, sperm quality, antioxidant capability

Introduction

This study aims to offer a comprehensive chronicle of the research endeavor's history, along with its intended objectives.

The secretome of umbilical cord-derived mesenchymal stem cells (UC-MSCs) has been found to have a considerable amount of potential. UC-MSCs are known for their antioxidant capabilities, which enable them to reduce oxidative and nitrosative stress. The present study is significant as it aims to investigate the impact of the secretome of umbilical cord-derived mesenchymal stem cells (UC-MSCs) on spermatozoa. If this inquiry proves successful, it has the potential to yield significant advancements in methodologies for enhancing sperm quality. The objective of this experiment is to determine if the inclusion of uc-mscs secretome in the conventional method of sperm preparation leads to alterations in the sperm's concentration and velocity. The purpose of this study is to offer a thorough examination of the research, its goals, and the potential impact of superoxide dismutase (SOD) on improving sperm quality. Furthermore, the objective of the study is to ascertain the impact of different time durations and concentration ratios on the alterations.

Materials and Methods

Semen samples were collected from a group of 30 married couples who were divided into two distinct groups based on whether they had received secretome administration. The groups were categorized based on whether the subjects had been subjected to secretome administration. It was determined that the couples experienced primary male infertility, specifically asthenozoospermia, and had a favorable DNA fragmentation index (DFI) of 15%. The sperm preparation procedure was conducted using the differential gradient centrifugation (DGC) method. Subsequently, the samples were fortified with secretome in a 1:1 ratio with the washing media. The ejaculate samples underwent a 30-minute incubation treatment. The researchers assessed the sperm concentration, velocity, and DNA

fragmentation index (DFI) before and after the incubation process. This was done after their completion of the incubation and sperm washing techniques.

The secreted proteins of mesenchymal stem cells generated from the umbilical cord (UC-MSCs) have demonstrated significant promise. UC-MSCs are renowned for their antioxidant properties, namely their ability to diminish oxidative and nitrosative damage. Superoxide dismutase (SOD) is an important antioxidant enzyme found in UC-MSCs. Its primary function is to counteract and neutralize damaging reactive oxygen species (ROS). The inclusion of Superoxide Dismutase (SOD) in the secretome of Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) may play a crucial role in their ability to effectively reduce oxidative stress.

This study is highly important since it aims to examine the influence of UC-MSCs secretome, which includes SOD, on spermatozoa. If this research is successful, it could potentially change techniques for improving sperm quality by utilizing the antioxidant characteristics of SOD. The aim of this experiment is to assess if the addition of UC-MSCs secretome, which has a high concentration of SOD, to the standard sperm preparation process results in notable changes in sperm concentration and velocity. In addition, the study seeks to evaluate the impact of various time durations and concentration ratios of UC-MSCs secretome, together with its SOD content, on these changes.

This research introduces the use of SOD in the examination, which has the potential to improve sperm quality and provide insights into the specific function of this antioxidant enzyme in the treatment of male infertility. Comprehending the role of SOD in the secretome of UC-MSCs could lead to the development of more focused and efficient treatments for male infertility caused by oxidative stress.

Measurement of Growth Factors This part of the study involves the analysis of the secretome (a collection of biologically active chemicals) produced by umbilical cord mesenchymal stem cells (UC-MSCs) to determine the levels of certain growth factors. Growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and nerve growth factor (NGF) are among those that have been measured. The enzyme-linked immunosorbent assay, also known as the ELISA method, is utilized to determine the relative amounts of various growth factors. Utilizing antibodies that are specific to each growth factor and then binding them to a substrate in a microplate is how this procedure is carried out. After that, the samples of the secretome are added to the plate. A reaction will take place, which will result in a change of color if the growth factors are present in the samples. Using a microplate reader, this variation in color is quantified as optical density (OD) at a certain wavelength, which is commonly 450 nm. The results of the ELISA are measured in terms of picograms per milliliter (pg/mL), which express the amount of growth factors present in the secretome samples.

The interpretation of data and the determination of whether differences or associations seen have statistical significance are both essential parts of research, and one of the most important parts of research is statistical analysis. To this investigation, the SPSS software, version 24, was utilized for the statistical analysis. The chosen level of confidence for statistical significance is 95% (p 0.05), which indicates that results are deemed to be statistically significant if the p-value is less than 0.05. This level of confidence is referred to as the significance level.

Results

Both the concentration of sperm and their velocity were shown to be significantly different as a direct outcome of the 1:1 (v/v) secretome treatment. The group that was watched had the highest concentration of spermatozoa; the number that was recorded for it was 68 million per milliliter. During the same duration of 45 minutes of incubation, it demonstrated the greatest recorded velocity of spermatozoa, which was measured at 30.51 micrometers per second. Significantly, the group that was used as a control did not get any administration of uc-mscs secretome, and as a result, their spermatozoa moved at a velocity that was measured to be 22.91 micrometers per second. When compared to the levels of DNA fragmentation index (DFI) in the control group, the levels of DNA

fragmentation index (DFI) in the treatment group, in which the secretome was delivered in a volume-to-volume ratio of 1:1, displayed a considerable reduction. This discovery lends credence to the idea that the level of DNA fragmentation is decreasing.

The growth factor evaluation revealed a notable elevation in the levels of growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and nerve growth factor (NGF), in the experimental group that underwent secretome treatment. The observed rise in these growth factors in the experimental group suggests that the injection of the UC-MSCs secretome significantly enhanced their expression, potentially leading to an improvement in sperm quality. Additional investigation is required to comprehend the precise consequences of these heightened growth factor levels on male fertility.

The experimental group exhibited a notable reduction in Superoxide Dismutase (SOD) levels compared to the control group, indicating a diminished antioxidant capability in the experimental group. The statistical method consisted of doing an analysis of variance (anova) in a single direction, followed by post hoc analyses of pairwise relationships. The value of p less than 0.05 was decided upon as the level of significance to use.

Discussion/Conclusion

The experimental group exhibited a notable reduction in Superoxide Dismutase (SOD) levels compared to the control group, indicating a diminished antioxidant capability in the experimental group. Oxidative stress is mitigated by the crucial antioxidant enzyme, superoxide dismutase, which effectively counteracts detrimental reactive oxygen species (ROS), particularly superoxide radicals. A decline in SOD levels may suggest a diminished capacity to mitigate oxidative damage.

Impact on Sperm Quality: In the context of your research on sperm parameters and male infertility, a reduced amount of Superoxide Dismutase (SOD) in the experimental group may lead to heightened oxidative stress in sperm cells. Oxidative stress can adversely impact the quality of sperm, perhaps resulting in reduced motility, DNA damage, and other problems linked to male infertility.

Significance of the Study: Within the scope of your research, the observation of reduced SOD levels in the experimental group carries important implications. It indicates that the introduction of the UC-MSCs secretome to the experimental group may not have adequately maintained or improved the antioxidant capability of the sperm cells. Further investigation is necessary to fully comprehend the consequences of this outcome on male fertility and the possible healing advantages of the UC-MSCs secretome.

Clinical Significance: The reduction in SOD levels may have wider significance in the treatment of male infertility. If it is established that SOD plays a critical role in preserving sperm quality and minimizing oxidative stress, then it may be worth considering ways to augment SOD levels or other antioxidant defenses in infertility therapies.

Additional Research: This outcome underscores the significance of doing more inquiry to ascertain the reasons behind the drop in SOD levels observed in the experimental group. It is crucial to investigate the mechanisms responsible for this decline and see if it may be alleviated to attain the required enhancements in sperm quality.

The findings of the growth factor analysis in this study demonstrated a notable elevation in the concentrations of crucial growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and nerve growth factor (NGF), in the experimental group that underwent treatment with UC-MSCs secretome. The increase in growth factors has significant implications for comprehending the potential of UC-MSCs secretome in enhancing sperm quality. In order to provide a comprehensive understanding of these findings, it is crucial to juxtapose them with other research studies conducted in the domain of male infertility and stem cell therapy.

Multiple studies have investigated the impact of growth factors on male fertility and have consistently returned comparable results. Chen et al. (2021) conducted a study to examine the therapeutic efficacy

of mesenchymal stem cells in the treatment of osteoporosis. While the study primarily examined a distinct facet of mesenchymal stem cell therapy, it emphasized the significance of growth factors released by these cells in facilitating tissue regeneration. While the environment changes, the notion of growth factor-mediated amplification coincides with our findings, where UC-MSCs secretome led to a rise in growth factors.

In addition, Bader et al. (2019) investigated the enhancement of human sperm quality through the co-cultivation with secretome of adipose-derived mesenchymal stem cells. Although the study did not analyze the particular growth factors, our findings align with their conclusions, indicating that the secretome of mesenchymal stem cells has a beneficial effect on sperm quality. Their research provides evidence that secretome derived from different types of mesenchymal stem cells, such as UC-MSCs, can have a significant impact on the treatment of male infertility.

Aitken et al. (2022) examined the correlation between oxidative stress and male infertility within the setting of male reproductive issues. The study primarily examined oxidative stress pathways and highlighted the importance of antioxidants, such as superoxide dismutase, in safeguarding sperm against oxidative harm. Our investigation revealed a decline in superoxide dismutase (SOD) concentrations in the experimental group, indicating a potential decrease in antioxidant capability. This is consistent with Aitken's focus on the significance of antioxidants in male reproductive ability. The results of our research can enhance the overall comprehension of the significance of antioxidants and growth factors in the management of male infertility.

Nevertheless, it is important to highlight that the experimental group exhibited a decrease in SOD levels, as mentioned in the "Results" section, which is in direct opposition to the elevated levels of growth factors reported in the same group. The observed disparity indicates an intricate correlation between antioxidants, growth factors, and the quality of sperm. Further work is necessary to discover how these contradicting findings may overlap and impact male fertility.

Overall, the experimental group that received UC-MSCs secretome showed higher amounts of growth factors. This indicates that this strategy has the potential to improve sperm quality. Through a comparative analysis of these findings with previously published study reports, we get significant insights into the function of secretome in the treatment of male infertility. However, the differential decline in SOD levels in the same experimental group underlines the need for more thorough research to clarify the complicated interplay between antioxidants, growth hormones, and male fertility. These findings highlight the significance of further investigation into the mechanisms that underlie the secretome of UC-MSCs and its clinical relevance in treating male infertility. To summarize, the experimental group showed a substantial reduction in SOD levels compared to the control group. This data is significant and has potential implications for male infertility and the efficiency of the UC-MSCs secretome in addressing oxidative stress. Additional investigation is required to further explore the underlying mechanisms and their therapeutic importance.

In conclusion, the incorporation of uc-mscs secretome into the medium that was used for the preparation of sperm led to substantial enhancements in sperm concentration and velocity, as well as a reduction in the amount of dna fragmentation that occurred after incubation. The findings of this research point to the possibility that the secretome of uc-mscs could be useful as an additional component in the development of strategies that aim to increase sperm quality. On the other hand, greater in-depth study is required to adequately justify its use in therapeutic settings.

Author Contributions

Conceptualization: RM and SW. Data acquisition: RM and MP. Data analysis orang interpretation: RM, SW and MP. Drafting of the manuscript: RM. Critical revision of the manuscript: RM. Approval of the final version of the manuscript: all authors.

Funding Sources

This research was made possible by the Regular Fundamental Research by Kemeristek DIKTI Fund 2023, which can be referenced using the following number: NKB-1116/UN2.RST/HKP.05.00/2023.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

Aitken RJ, Drevet JR, Moazamian A, Gharagozloo P. Male infertility and oxidative stress: A focus on the underlying mechanisms. Antioxidants. 2022;11(2):306. doi:10.3390/antiox11020306

Amidi F, Ataie NN, Agha HM, Nayernia K, Mazaheri Z, Yamini N, & Saeednia S. In vitro differentiation process of human Wharton's jelly mesenchymal stem cells to male germ cells in the presence of gonadal and non-gonadal conditioned media with retinoic acid. In Vitro Cellular & Developmental Biology-Animal. 2015;51:1093-101.

Ardıç CM, Ilgın S, Baysal M, Karaduman AB, Kılıç V, Aydoğan-Kılıç G, et al. Olanzapine induced reproductive toxicity in male rats. Scientific Reports. 2021;11(1). doi:10.1038/s41598-021-84235-4

Bader R, Ibrahim J-N, Mourad A, Moussa M, Azoury J, Azoury J, et al. 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;12(3):388–99. doi:10.15283/ijsc19047

Chen T, Yang T, Zhang W, Shao J. The therapeutic potential of mesenchymal stem cells in treating osteoporosis. Biological Research. 2021;54(1). doi:10.1186/s40659-021-00366-y

Douglas C, Parekh N, Kahn LG, Henkel R, Agarwal A. A novel approach to improving the reliability of manual semen analysis: A paradigm shift in the workup of infertile men. The World Journal of Men's Health. 2021;39(2):172. doi:10.5534/wjmh.190088

Ding DC, Chang YH, Shyu WC, & Lin SZ. Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. Cell transplantation, 2015;24(3):339-47.

Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells, 2006;(24):1294-301. https://doi.org/10.1634/stemcells.2005-0342 PMid:16410387

Kuroda Y, Kitada M, Wakao S, Dezawa M. Mesenchymal stem cells and umbilical cord as sources for schwann cell differentiation: their potential in peripheral nerve repair. The Open Tissue Engineering and Regenerative Medicine Journal, 2011;4:54-63. https://doi.org/10.2174/1875043501104010054

Margiana R, Jusuf AA, Lestari SW. Immunohistochemistry Detection Method of Rejection Reaction of Human Umbilical Cord Derived Mesenchymal Stem Cell on Rat Sciatic Nerve Tissue. Garda Rujukan Digital, 2018;10(7).

Lestari SW, Lestari SH, Margiana R. An Update of Sperm Preparation : A review of supplementation substances to improve sperm quality. Biomed Pharmacol J 2018;11(1).

Li G, Zhao D, Wang Q, Zhou M, Kong L, Fang M, et al. Infertility-related stress and quality of life among infertile women with polycystic ovary syndrome: Does body mass index matter? Journal of Psychosomatic Research. 2022;158:110908. doi:10.1016/j.jpsychores.2022.110908

Li T, Xia M, Gao Y, Chen Y, & Xu Y. Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy. Expert opinion on biological therapy. 2015;15(9): 1293-306.
Marofi F, Alexandrovna KI, Margiana R, Bahramali M, Suksatan W, Abdelbasset WK, Chupradit S, Nasimi M, Maashi MS. MSCs and their exosomes: a rapidly evolving approach in the context of cutaneous wounds therapy. Stem Cell Research & Therapy. 2021 Dec;12(1):1-20.
Matsuse D, Kitada M, Kohama M, Nishikawa K, Makinoshima H, Wakao S, et al. Human umbilical cord-derived mesenchymal stromal cells differentiate into functional schwann cells that sustain peripheral nerve regeneration. J Neuropathol Exp Neurol, 2010;69(9):973-85. https://doi.org/10.1097/NEN.0b013e3181eff6dc PMid:20720501
Mishra PJ, Mishra PJ, Banerjee D. Cell-free derivatives from mesenchymal stem cells are effective in wound therapy. World J Stem Cells. 2012;4(5):35-43.
Mortimer D. Sperm preparation methods. J. Androl. 2000;21(3):357-66.
Pawitan JA, Liem IK, Budiyanti E, Fasha I, Feroniasanti L, Jamaan T, et al. Umbilical cord derived stem cell culture: multiple-harvest explant method. International Journal of PharmTech Research, 2014;6(4):1202-1208. Available at: https://www.researchgate.net/publication/267026055_Umbilical_cord_derived_stem_cell_culture_Multipleharvest_explant_method
Pereira WC, Khushnooma I, Madkaikar M, Ghosh K. Reproducible methodology for the isolation of mesenchymal stem cells from human umbilical cord and its potential for cardiomyocyte generation. Journal of Tissue Engineering and Regenerative Medicine, 2008;2:394-9. https://doi.org/10.1002/term.107 PMid:18615777
Shojaeian A, Mehri GA, Rahmati DS, & Banitalebi DM. Evaluation of isolation method in remaining of differentiation potential of perivascular human umbilical cord mesenchymal stem cells toward male germ cell-like. Journal of Shahrekord University of Medical Sciences. 2021;23(2): 81-86.
Yang RF, Liu TH, Zhao K, & Xiong CL.Enhancement of mouse germ cell-associated genes expression by injection of human umbilical cord mesenchymal stem cells into the testis of chemical-induced azoospermic mice. Asian Journal of Andrology. 2014;16(5): 698.
Zarbakhsh S, Goudarzi N, Shirmohammadi M, Safari M. Histological study of bone marrow and umbilical cord stromal cell transplantation in regenerating rat peripheral nerve. Cell J, 2016;17(4):668-77. https://doi.org/10.22074/cellj.2016.3839

Zhang Y, Pan Y, Liu Y, Li X, Tang L, Duan M, Li J, Zhang G. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-β receptor inhibition. Stem Cell Res Ther. 2021;12:434.
Zhang, YuSheng, et al. Human umbilical cord mesenchymal stem cells (hUC-MSCs) alleviate paclitaxel-induced spermatogenesis defects and maintain male fertility. Biological Research 2023;56(1): 1-18.
Zhang ZY, Xing XY, Ju GQ, Zhong L, & Sun J. Mesenchymal stem cells from human umbilical cord ameliorate testicular dysfunction in a male rat hypogonadism model. Asian journal of andrology. 2017;19(5): 543.
Zhong L, Yang M, Zou X, Du T, Xu H, & Sun J. Human umbilical cord multipotent mesenchymal stromal cells alleviate acute ischemia-reperfusion injury of spermatogenic cells

via reducing inflammatory response and oxidative stress. Stem Cell Research & Therapy. 2020;11:1-12.