Multiple Injection of Human Wharton's Jelly Mesenchymal Stem Cells Ameliorate Spermatogenic Cells on Physiological Aging Male Rats

Alif Iman Fitrianto[1], Wining Astini[1], Adisti Dwijayanti[2], Frans Dhyanagiri Suyatna[2], I Ketut Mudite Adnyane[1], Arief Boediono[1*]

[1]Faculty of Veterinary Medicine, Bogor Agricultural University
Jl. Agatis Kampus IPB Dramaga, Bogor, West Java, Indonesia, 16680
[2]Faculty of Medicine, University of Indonesia
Jl. Salemba Raya No 6, Jakarta, Indonesia, 10430
*Corresponding author: ab@apps.ipb.ac.id

ABSTRACT

Recently, the most common therapy on men who suffered fertility decline due to aging was testosterone replacement, but now it is known that this therapy has a long-term risk of damage to the cardiovascular system and prostate. Stem cells are an alternative therapy that has a potency to improve the fertility of aging men that less causing side effects. The aim of this study was to evaluate the injection of Human Wharton's Jelly Mesenchymal Stem Cells (hWJ-MSC) in physiologic aging male rats on spermatogenic cells. This study used 3 young male rats (8-12 weeks) and 6 physiological aging rats (22-24 months) which divided into 3 groups, the young rat group did not give any treatment, physiological aging male rats received NaCl (0.9%) 0.4 mL, and physiological aging male rats received $1x10^6$ cells/kg BW of hWJ-MSCs. The observations were performed on histological analysis. The results indicate that the hWJ-MSCs injections increased the number of spermatogonia and Leydig cells significantly ($P<0.05$), and improve the tubules circumference and interstitial area significantly ($P<0.05$). The mechanism of spermatogenic cells repairs suspected due to various bioactive molecules that secreted by hWJ-MSCs which can affect the surrounding cells.

Keywords: aging, Leydig cells, spermatogonia, stem cells

INTRODUCTION

There are changes in the male reproductive system due to aging, changes on morphology and function of the testicular organs can lead to hypogonadism (Hermann *et al.,* 2019). The changes include decrease in the number of spermatogonia and a narrowing of the seminiferous tubules (Takano and Abe,1987).

Recently, the most common therapy on men who suffered fertility decline due to aging was testosterone replacement, but now it is known that this therapy has a long-term risk of damage to the cardiovascular system and prostate (Zirkin and Tenover, 2012).

Therefore, alternative therapy is needed that can improve fertility problems in older men without causing risk and other side effects. Stem cells are an alternative therapy that has potency to improve the fertility of aging men. Stem cells are cells that have the potential to differentiate into various cell types. This study will evaluate hWJ-MSCs xenotransplantation on the aging male rats.

METHODS

Materials

This study used 9 male rats Spraque Dawley (SD), consist of 3 rats aged 8-12 weeks and 6 rats aged 22-24 months. The source of hWH-MSCs from Stem Cell and Cancer Institute, PT Kalbe Farma, Jakarta.

Experimental Animals

During the study, male rats were kept in individual cages, at 25-26 ^{o}C temperature with 70-73% humidity, and 12 hours light and 12 hours dark. Feed used standard feed for rodensia and used commercial drinking water. Feed and drinking were given ad libitum.

Preparation and administration of hWJ-MSCs

Fresh human umbilical cords (UC) were collected from women aged 25-40 years. The preparation, cell culture of hWJ-MSCs, identification of biological properties of hWJ-MSCs and the experimental protocols followed with the same procedures which was previously describe (Widowati et al., 2015). The young rats group did not give any treatment, aging male rats received 0.4 mL of NaCl 0.9%, and the aging male rats received 0.4 mL of hWJ-MSCs with a dose 1x106 cells/kg BW and injected 4 times with 3 months intervals. The injection via intravenously in V. lateral caudalis in the tail.

Histology

Histological analysis of testes was performed to evaluate the internal structure of the testes in aging male rat and compared with after transplantation of hWJ-MSCs. This examination was done by histology staining method, the sample fixed with paraformaldehide 4%, then tissue processing used paraffin embedding method and used Hematoxylin-Eosin.

The number of spermatogonia, tubules circumference, the number of Leydig cells, and the interstitial area analysis

Histological analysis was using ImageJ software and by Olympus CX31 light microscope (Olympus, Tokyo, Japan).

Statistical data

The observations analyzed using SPSS 13 computer program. The data presented descriptively by observing the relationship between the changes in the control and treatment by using randomized block design.

RESULT

Histological analysis was performed include the number of spermatogonia, tubules circumference, the number of Leydig cells, and the interstitial area (Figures 1 and 2). The result indicate that the number of spermatogonia and Leydig cells significantly decreased ($P<0.05$) in physiological aging male rats compared to young rats, the injections of hWJ-MSCs significantly increased the number of spermatogonia and Leydig cells ($P<0.05$).

The tubules circumference obtained significantly decreased ($P<0.05$) in physiological aging male rats compared to young rats, injections of hWJ-MSCs significantly increased the tubules circumference ($P<0.05$). The interstitial area increased significantly ($P<0.05$) in physiological aging male rats compared to young rats, injections of hWJ-MSCs made the interstitial area significantly narrower ($P<0.05$).

Table 1. The number of spermatogonia, Leydig cells, tubules circumferences, and interstitial area

Groups	Number of spermatogonia (cells)	Tubules Circumference (μm^2)	Number of Leydig Cells (cells)	Interstitial area (μm^2)
Young rats	72.0 ± 9.3^{a}	409.20 ± 31.75^{b}	17.8 ± 5.1^{a}	536.2 ± 13.4^{c}
Physiological aging rats	30.6 ± 6.5^{c}	339.87 ± 50.07^{c}	10.0 ± 3.5^{c}	1117.1 ± 49.4^{a}
Physiological aging rats + hWJMSCs	68.1 ± 8.6^{ab}	474.38 ± 63.20^{a}	17.8 ± 6.0^{a}	787.4 ± 28.8^{b}

Noted: The difference superscript on the same column shows a significant different ($P<0.05$).

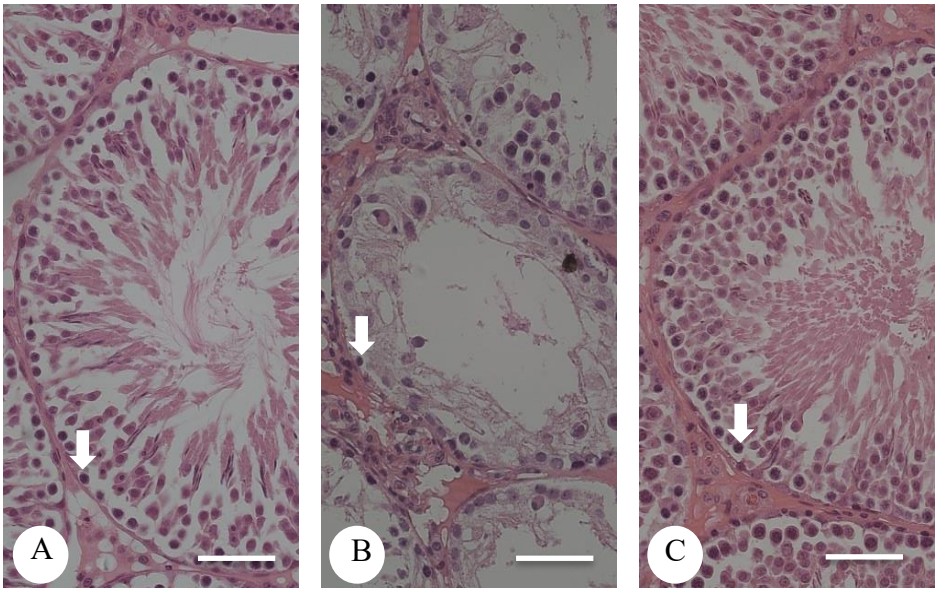

Figure 1. Comparison of the number of spermatogonia and tubules circumferences. A: Young rats, B: Physiological aging rats, C: Physiological aging rats + hWJMSCs. Arrow: spermatogonia. (Bar: 25 µm).

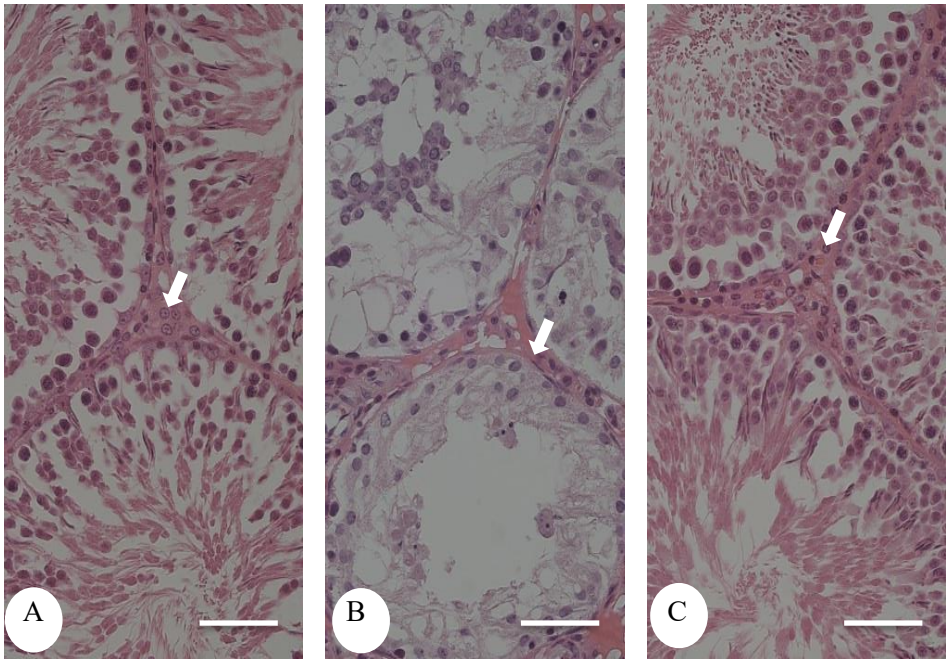

Figure 2. Comparison of the number of Leydig cells and interstitial area. A: Young rats, B: Physiological aging rats, C: Physiological aging rats + hWJMSCs. Arrow: Leydig cells. (Bar: 25 µm).

DISCUSSION

The seminiferous tubules became atrophy and narrowing of the diameter in aging rats (17 months to 2 years) (Takano and Abe,1987). This is because in young rats (aged less than 12 months), all of the tubules are still filled with active spermatogenic cells, whereas at the age of rats over 18 months the tubules became degeneration which is characterized by decreasing in the number of spermatogenic cells and the size of the tubules getting smaller (Wright *et al*., 1993). This process begins with hypospermatogenesis, which then proceeds to the loss of all spematogenic cells in aging rats (24 months). The occurrence of hypospermatogenesis begins when rats at the early 12 months, and continues to reach the peak of loss of spermatogenic cells at the 24 months (Mahmood, 2008).

After transplantation of hWJ-MSCs, it was found that there was an improvement in the number of spermatogonia and Leydig cells. The repair process is due to the ability of differentiation of hWJ-MSCs to become spermatogoanial stem cells (SSC) and then undergo spermatogenesis to become spermatozoa. MSCs are able to survive in the testes, able to migrate into the basal membrane of the seminiferous tubules, even able to differentiate into spermatogonia cells (Ghasemzadeh-Hasankolaei *et al*., 2016). Derived SSCs from MSCs then proliferate and undergo spermatogenesis to become primary spermatocytes, spermatids, and then spermatozoa. These results made seminiferous tubule contain active spermatogenic cells, and made tubules circumference in the group of rats transplanted with hWJ-MSCs wider than physiological aging rats.

MSCs that transplanted into the testes are not only able to differentiate into spermatogenic cells in the tubules, but also detected to be able to differentiate into morphology that resembling Leydig cells in the interstitial (Leydig-like cells) (Monsefi *et al.*, 2013). Hou *et al*. prove that human bone marrow mesenchymal stem cells (hBM-MSCs) are able to differentiate into Leydig cells *in vitro* (Hou *et al.*, 2016).

The mechanism of repair on spermatogenic cells in this study is not clear, the process can occur through direct differentiation of MSCs into spermatogonia or through various cytokines secreted by MSCs, which affect SSCs through the Niche in the tubuli.

MSCs are able to secrete various cytokines, such as growth factors, proinflmmatory and atiinflammatory cytokines and other cytokines which play an important role in the regeneration of surrounding cells (Pawitan, 2014). The type of cell transplantation in this study is xenogenic, the mechanism of repair of spermatogenic cells that occurs is suspected by "signaling" process, meaning that hWJ-MSCs will secrete various bioactive molecules in the form of growth factors, cytokines, and chemokines which affect the surrounding cells (Meirelles *et al.*, 2009). Beside of the niche, the intrinsic factor of MSCs itself is also a supporting factor for the regeneration process or differentiation into spermatogonia both directly and indirectly.

CONCLUSION

There are various changes in aging rats, include decreased numbers of spermatogonia and Leydig cells. Xenogenic transplants of hWJ-MSCs have been shown to improve the number of spermatogonia and the number of Leydig cells.

REFERENCES

Ghasemzadeh-Hasankolaei M, Batavani R, Eslaminejad MB, Sayahpour F. 2016. 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* 9(2): 250-263.

Hermann M, Untergasser G, Rumpold H, Berger P. 2000. Ageing of the male reproductive system. *Experimental Gerontology*. 35: 1267-1279.

Hou L, Dong Q, Wu Y-J, Sun YX, Guo Y-Y, Huo Y-H. 2016. Gonadotropins facilitate potential differentiation of human bone marrow mesenchymal stem cells into Leydig cells in vitro. *Kaohsiung Journal of Medical Science*. 32: 1-9.

Mahmood IM. 2008. Histological changes in testicular tissue with age. *Tikrit Journal of Pure Science*. 3(3): 1-4.

Meirelles LS, Fontes AM, Covas DT, Caplan AI. 2009. Mechanism involved in therapeutic properties of mesenchymal stem cells. *Cytokines and Growth Factor Review*. 20: 419- 427.

Monsefi M, Fereydouni B, Rohani L, Talaei T. 2013. Mesenchymal stem cells repair germinal cells of seminiferous tubules of sterile rats. *Iran Journal of Reproductive Medicine*. 11(7): 537-544.

Pawitan JA. 2014. Prospect of Stem Cell Conditioned Medium in Regenerative Medicine. *BioMed Research International*. 10: 1-14.

Takano H, Abe K. 1987. Age-related histologic changes in the adult mouse testis. Archives of Histology and Cytology. 50: 533-544.

Widowati W, Wijaya L, Murti H, Widyastuti H, Agustina D, Laksmiwati DR, Fauziah N, Sumitro SB, Widodo MA, Bachtiar I. 2015. Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation. *Biomarkers and Genomic Medicine*. 7: 8-17.

Wright WW, Fiore C, Zirkin BR. 1993. The effect of aging on the seminiferous epithelium of the brown Norway rat. *Journal of Andrology*. 14(2): 110-117.

Zirkin BR, Tenover JL. 2012. Ageing and declining testosterone: past, present, and hope for the future. *Journal of Andrology*. 33(6): 1111-1118.

ACKNOWLEDGEMENT

Thanks to Stem Cell and Cancer Institute who provided hWJ-MSCs and Dr Boenjamin Setiawan PhD for supporting this research fund.