KOMISI ETIK PENELITIAN
FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA
Animal Care and Use Committee (ACUC)

KETERANGAN KELAIKAN ETIK

" ETHICAL CLEARENCE "

No : 239-KE

KOMISI ETIK PENELITIAN (*ANIMAL CARE AND USE COMMITTEE*) FAKULTAS KEDOKTERAN HEWAN UNIVERSITAS AIRLANGGA SURABAYA, TELAH MEMPELAJARI SECARA SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN, MAKA DENGAN INI MENYATAKAN BAHWA :

PENELITIAN BERJUDUL	: Quiscence Bone Marrow Mesenchymal Stem Cells Melalui Hypoxia Precondition Sebagai Upaya Mempertahankan Viabilitas Kultur Stem Cells
PENELITI UTAMA	: Erma Safitri
UNIT/LEMBAGA/TEMPAT PENELITIAN	: Program Studi Ilmu Kedokteran Fakultas Kedokteran Universitas Airlangga
DINYATAKAN	: LAIK ETIK

Surabaya, 14 Januari 2013

Mengetahui,
Dekan FKH-Unair,

Prof. Romziah Sidik, Ph.D.,drh.
NIP. 195312161978062001

Ketua,

Dr. E. Bimo Aksono, M.Kes.,Drh.
NIP. 196609201992031003

CMR2014
INTERNATIONAL CONFERENCE ON MULTIDISCIPLINARY RESEARCH
Islamic University of North Sumatra
16 - 18 October 2014

E20

Certificate of Appreciation

Presented to

ERMA SAFITRI

in recognition and appreciation of being a

PRESENTER

at the

The 3rd International Conference on Multidisciplinary Research 2014
(ICMR 2014)

in 16th -18th October 2014

Universitas Islam Sumatera Utara (UISU) Medan,

The Conference is jointly organized by
Universitas Islam Sumatera Utara (UISU) Medan,
the School of Distance Education,
Universiti Sains Malaysia (USM) Penang Malaysia.
and Universitas Syiah Kuala (UNSYIAH) Banda Aceh

Prof. Jumino Suhadi, M.A., Ph.D
Chairman

Dr. Ir. Mhd. Asaad, M.Si.
Rector

ISBN 978-602-18851-9-2

PROCEEDING

The 3rd International Conference on Multidisciplinary Research 2014

BIOSCIENCES CHAPTER

ICMR2014
INTERNATIONAL CONFERENCE ON MULTIDISCIPLINARY RESEARCH

The Conference is jointly organized by
Universitas Islam Sumatera Utara (UISU) Medan,
Universitas Syiah Kuala (UNSYIAH) Banda Aceh
and the School of Distance Education,
Universiti Sains Malaysia (USM) Penang Malaysia.

Universitas Islam Sumatera Utara (UISU) Medan
October, 16 - 18, 2014

EDITORIAL BOARD

INTERNATIONAL STEERING COMMITTEE

TABLE OF CONTENT

EDITORIAL BOARD

INTERNATIONAL STEERING COMMITTEE

Marrow Mesenchymal Stem Cell (MMSCs) Transplantation for the Improvement of Reproductive Function in Rat Testis Degeneration

Erma Safitri[1,2], Suzanita Utama[1] and Thomas V. Wdiyatno[3]

[1]Department of Veterinary Reproduction, Fac. of Veterinary Medicine, Airlangga University;
[2]Stem Cells Research Division, Institute of Tropical Disease (ITD), Airlangga University;
[3]Department of Veterinary Pathology, Fac. of Veterinary Medicine, Airlangga University
Corresponding Author : rma_fispro@yahoo.com

Abstract. The purpose of this research was to obtain adaptive MMSCs for the therapy of testicular degeneration in rat. Adaptive MMSCs can be achieved by hypoxia precondition during in vitro culture. Hypoxia precondition in this research was conducted by applying 3% O_2 which was then compared with those cultured in normoxic 21% O_2. The specific target of this research was devided into two experiments, Experiment 1 was the isolation and identification of mesenchymal stem cell from rat bone marrow. Identification was confirmed by the expression of CD 105^+ and $CD45^-$ cells. In Experiment 2 cellular MMSCs testiculomyoplasty were performed on male rats that have been preconditioned with testicle degeneration. Next, the success was identified in vivo based on the expression of spermatogonial stem cell (SSCs) and the improvement of testicle tissue. Transplantation results showed that under 3% O2 concentration SSCs were expressed and testis tissue improvement occured. Meanwhile at 21% O_2 concentration testis tissue improvement did not occur. The conclusion of this research showed that hypoxia precondition using 3% O_2 concentration very support MMSCs to remain adaptive after being transplanted to patient with spermatogenesis impairment, in addition to the testicle tissue improvement.
Keywords: *MMSCs, Testicular Degeneration, Testicle Tissue Improvement*

Introduction

Testicular degeneration that lead to permanent damage of male main reproductive organ is normally followed by pathological remodelling (Schlatt *et al.*, 2002). Regeneration is merely possible to occur when normal spermatogonia and Sertoli cells still remaining in seminiferous tubules in a long period of time.Therefore, regeneration capacity to replace the degenerated cells is very limited (Hafez, 2000).

Transplantation of Marrow Mesenchymal Stem Cells (MMSCs) from bone marrow gave very promising solution in regenerating and returning the degenerating testicle tissue (Blanchard *et al.*, 1998). However, the minimum viability of the transplanted MMSCs in degenerated testicle caused a limited efficacies of this therapy (Kenichiro *et al.*, 2005; Tang *et al.*, 2005).

Hypoxia condition was needed during culture in order the stem cells remained adaptive and viable before and after transplantation (Safitri *et al.*, 2014). This condition was adjusted to mimick in vivo condition where the stem cells were produced. In testicle tissue, stem cells for spermatogenesis were in hypoxiacondition, i.e. under 1-6 % O2 concentration (Wenger and Katschinski, 2005). In this research theconcentration of O2 applied was 3 % which was compared with conventional condition 21 % O2.

Research Methods

Procedure of RAT MSCs isolation and culture:

Rat was premedicated and general anesthetized. MSCs from bone marrow was harvested by an aspiration at the middle of rat femur bone below the condylus. Aspirate contained rat MSCs from bone marrow was placed in heparinized tubes. Sample in tube was placed in thermos maintained at 4°C during transportation to be processed.

Sample was transferred into 15 ml sterile blue capped tubes and then the tube was rinsed twice with 5 ml sterile PBS and added up to a total volume of 10 ml. The diluted sample

was loaded over a same volume of Ficoll in a separate 15 ml tube. Centrifugation at 1600 rpm was performed for 15 minutes at room temperature. After centrifugation, the cells were collected from Ficoll-PBS interface using sterile pasteur pipette and transferred into a 15 ml tube. The cells were then resuspended in PBS up to a total volume of 15 mL. The tube was inverted gently 5 times to homogenize the suspension.

The suspension was then centrifuged again at 1600 rpm for 10 minutes. Supernatant and floating cells were discarded and cell pellet was resuspended in 6 ml of α MEM media. Mononucleated cells were plated in 10 cm2 plates at 2 x 10^7 and incubated at 37°C in a humidified atmosphere containing 5% CO2 for 24 hours to let the cells adhere. After 24 hours, media and non-adherent cells were discarded. Adherent cells were rinsed twice using 5 ml of PBS. Ten ml of fresh α MEM media was then added into dish and the dish was returned into the incubator. Culture was observed daily under an inverted microscope. Every 4 days medium was changed, preceeded by a rinse using 10 ml PBS then 10 ml of fresh α MEM media were replaced. Culture was continued until approximately 75-80% confluence was attained. After confluence, cells were passaged into several dishes for subculture (Rantam *et al.*, 2009). Passage was conducted 3 times, then cells were divided into two hypoxic precondition treatments of 3% in hypoxic chamber inside a 5% CO2 incubator while another treatment was the use of 21% oxygen (normoxia).

At the second day after hypoxic precondition treatments cells were analysed for the expression of surface marker CD105 and CD45 by flowcytometry. Flow cytometric analysis was performed using a flowcytometer (FACSCalibur) and CellQuest software. Antibodies used were monoclonal *APC anti-mouse CD105 (Biolegend),* dan *PerCP-Cy5.5 anti-rabbit cross anti-rat CD45 (BD).*

Two days after hypoxic preconditioning MMSCs were transplanted into rats that had been induced for testicular degeneration. The following ten days surgery was performed to collect testicle tissue. Testicle tissue improvement was observed through HE stained histopathological preparation.

Results and Discussion

Flowcytometric analysis showed that under 3% O2 concentration, the level of CD105 and CD44 cells in MMSCs culture were not altered (still undifferentiated). Meanwhile under 21% O2 concentration, cells have experienced alteration (became differentiated), that was indicated by the decrease of CD105 and CD44 cells (Figure 1 - 3).

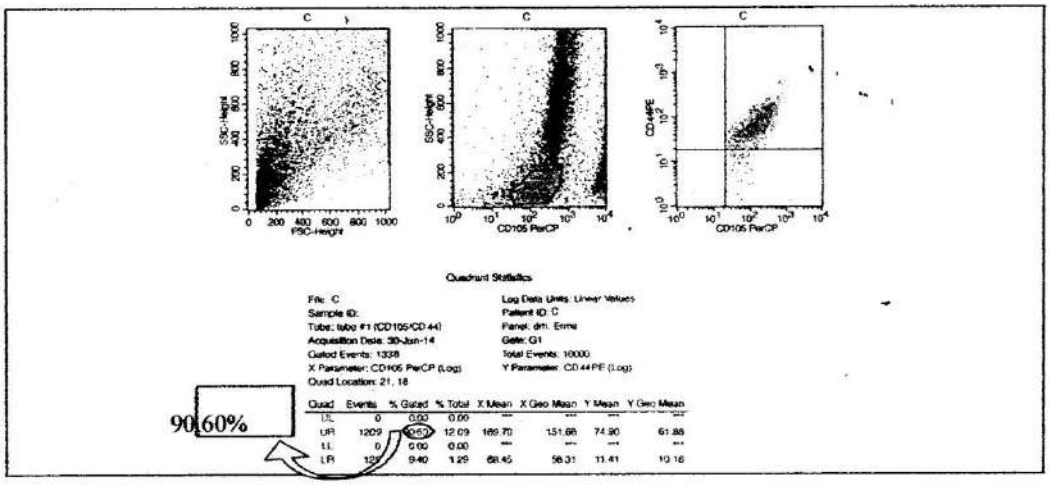

Fig. 1: Flowcytometric analysis of MSCs culture before hypoxic preconditioning (Control) showing positive expression of CD105 and CD44 (90,60.%).

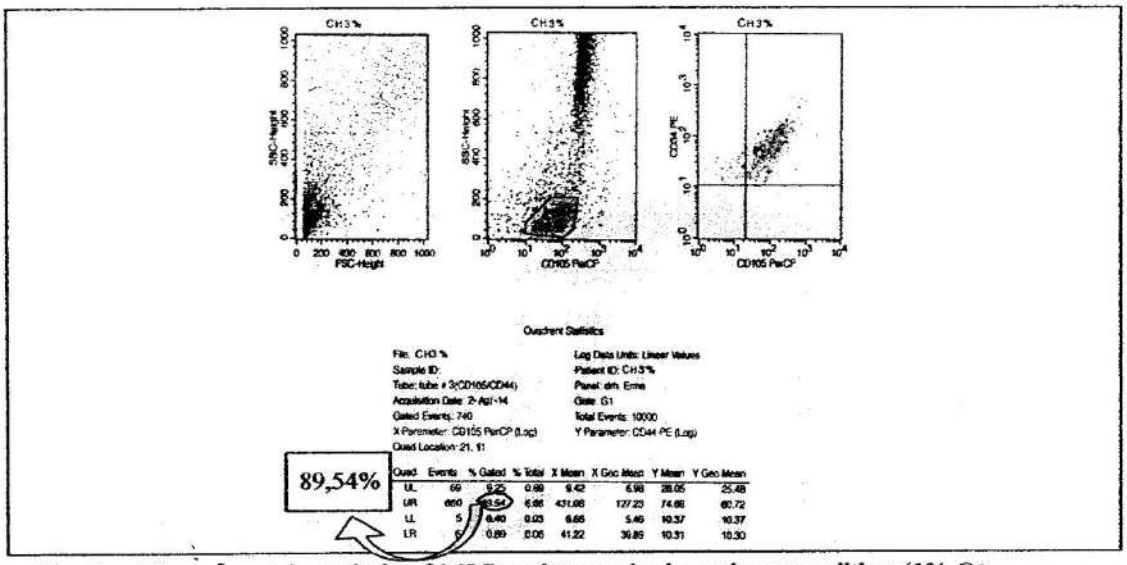

Fig. 2: Flowcytometric analysis of MSCs culture under hypoxic precondition (1% O2 concentration) showing positive expression of CD105 and CD44 (89,54%).

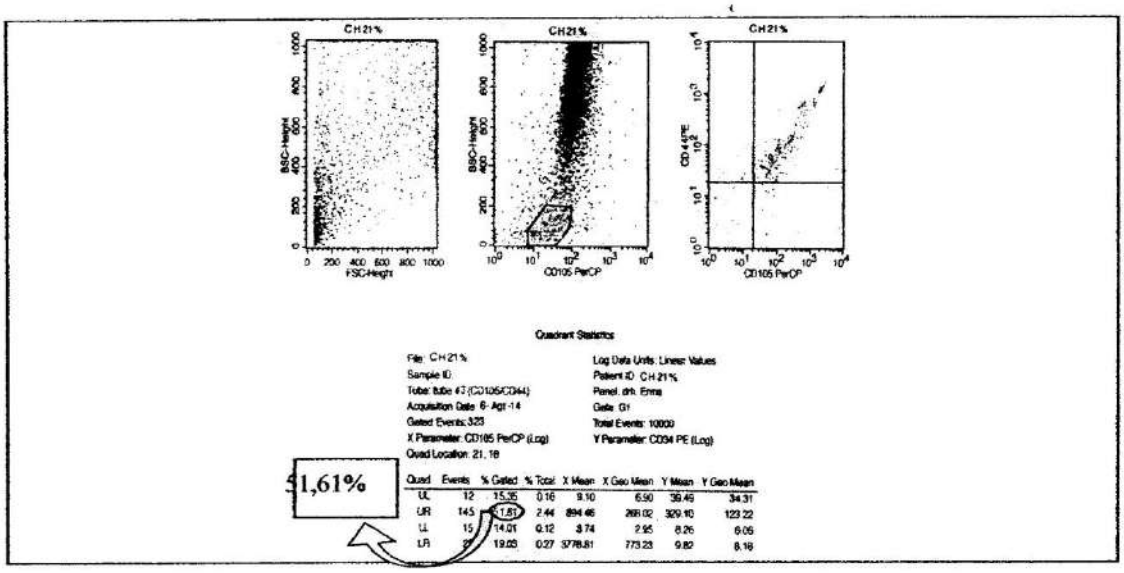

Fig. 3: Flowcytometric analysis of MSCs culture under 21% O2 concentration (normoxia) showing positive expression of CD105 and CD44 (51,61%).

Histopathological observation showed that testicular tissue transplanted with MMSCs cultured under 3 % O2 tension was regenerated, meanwhile testicular tissue transplanted with MMSCs cultured under 21 % O2 tension was not regenerated.

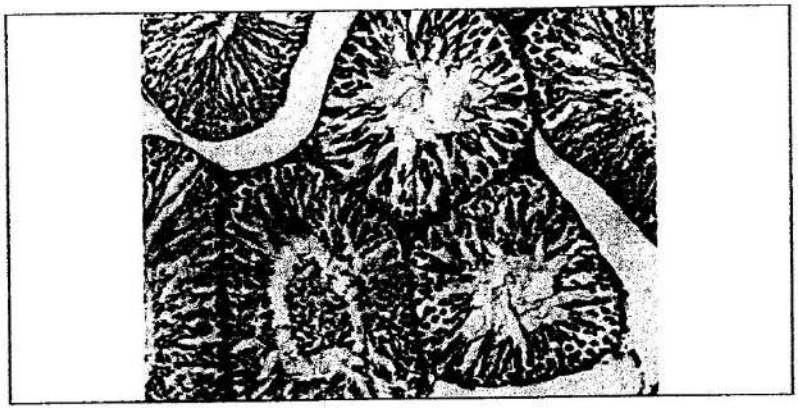

Fig. 4: Testicular tissue transplanted with MMSCs cultured under 3 % O2 tension shows regeneration (200X)

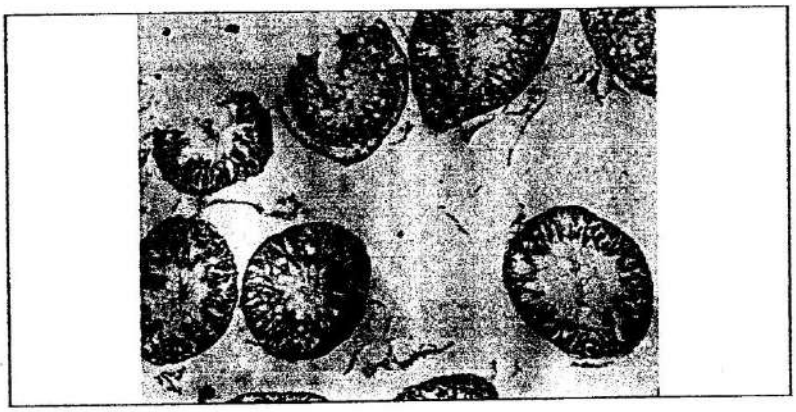

Fig. 5: Testicular tissue transplanted with MMSCs cultured under 21% O2 tension shows no regeneration (200X)

Undifferentiated state of the stem cells was one of the main purposes in this research. Halim and co-workers (2010) stated that undifferentiation is one of the unique characteristics of stem cells that distinguish them from other cells of body organ. The existence of this unique undifferentiation made in vitro cultured stem cells capable to be viable longer compared to their progenitor or matured stem cells. This instance was based on researches conducted by Elliason et al. (2010) and Takubo (2011) revealed that for the transplanted stem cells viability to be maintained, the cultured stem cells have to be undifferentiated.

Undifferentiated state of stem cells, if fulfilled, further on will cause stem cells to be predicted to be able to differentiate into whatever cells needed when transplanted. This instance became the specialty of stem cells in vivo, that in the undifferentiated state, those cells can differentiated into more than one type of cells (multipotent/pluripotent). Therefore if undifferentiated state could be maintained in vitro, the expected capacity to differentiate into whatever cells needed upon transplantation will be fulfilled.

It can be said that stem cells inside the body are cells that have not had specific shapes and functions as in other cells of body organ (Halim *et al*., 2010) such as myocardium cells to pulsate, neuron cells as the transmitter of nerve impulses and langerhans cells of pancreas to produce insulin (Guz et al., 2001).

According to Lin *et al*. (2008), stem cells are different from the three cell types, stem cells have not had specific functions such as to pulsate, to transmit nerve impulses, producing hormones or other functions. Based on various researches it is said that stem cells population in a mature tissue inside the body showed an inactive cell population that start functioning in certain time and condition.

The existence of MMSCs *in vitro* in this research as inactive undifferentiated cells was aimed to keep regeneration continuity of tissue or organ composer. This instance can be done according to the undifferentiated stem cells capacity for later differentiation into body cells which are needed (Jones and Wagers, 2008). As the example ectoderm cells can differentiate into neuron cells (Halim dkk., 2010) or the endodermcells such as hepatocyte cells (Schwartz *et al*., 2002) and germline cells (spermatogonia, sertoli and Leydig cells) and germline tissues (seminiferous tubules) (Kilani, 2009).

Conclusion

The conclusion of this research showed that hypoxia precondition using 3% O_2 concentration very support MMSCs to remain adaptive after being transplanted to patient with spermatogenesis impairment, in addition to the testicle tissue improvement.

References

Elliason P, M Rehn, P Hammar, P Larsson, O Sirenko, LA Flippin, J Cammenga, and JI Johnson. 2010. Hypoxia Mediates Low Cell-Cycle Activity and Increases The Proportion of Long-Term-Reconstituting Hematopoietic Stem Cells During in Vitro Culture. *Exp.Hematol*. 38. 301-310

Guz Y, I Nazir and, G Teitelman. 2001. Regeneration of Pancreatic β Cells from Intra-Islet

Precursor Cells in Experimental Model of Diabetes. Endocrinology142(11):4956-4968 Halim DH, F Murty, A Sandra, T Boediono, B Djuwantono, dan B Setiawan. 2010. Stem Cell

Dasar Teori dan Aplikasi Klinis. Penerbit Erlangga. Jakarta.

Jones DL and AJ Wagers. 2008. No place like home: anatomy and function of the Stem Cell Niche. Nature Reviews. Molecular Cell Biology. Januari (9):11-21

Kilani Z. 2009. Stem cell transplantation in human testis for the treatment of male infertility. ISRCTN75604065. http://www.farah-hospital.com

Kenichiro H, N Noritoshi, I Takashi, T Itoh, S Murakami, Y Shimizu, W Taki, K Miyatake, K Kangawa. 2005. Adrenomedullin Enhances Therapeutic Potency of Mesenchymal Stem Cells after Experimental Stroke in Rats. Stroke. 36:853-858.

Lin JL, MJ Wang, D Lee, CC Liang, and S Lin. 2008. Hypoxia Inducible Factor-1α

Regulates Matrix Metalloproteinase-1 Activity in Human Bone Marrow-Derived Mesenchymal Stem Cells. FEBS Letters 582: 2615-2619.

Rantam FA, Ferdiansyah, Nasronudin and Purwati. 2009. Stem Cell Exploration. Methods of Isolation and Culture. First Ed. Airlangga University Press. Surabaya

Safitri E, S. Utama, C. Bumi, Aulani'am and FA. Rantam. 2014. Hypoxic Preconditioning for Viable and Self Renewing Mesenchymal Stem Cells (MSCs) as The Regeneration of

Spermatogenesis Process. Accepted International Journal May 2014. Advances in Natural and Applied Sciences (18 ANAS May 2014). Scientific Journal Impact Factor (SJIF 2012= 2,698).

Schlatt S, L Foppiani, C Rolf, GF Weinbauer and Nieschlag. 2002. Germ Cell Transplantation into X-irradiated monkey testes. Human Reproduction. 17(1): 55-62.

Schwartz RE, M Reyes, L Koodie, Y Jiang, and M Blackstad. 2002. Multipotent Adult

Progenitor Cells from Bone Marrow Differentiate into Functional Hepatocyte-Like Cells. J Clin Invest. 109: 1291-1302.

Takubo K. 2011. The Hypoxia Regulatory System in Hematopoietic Stem Cells. Advances in Hematopoietic Stem Cell Research. 133-146.

Wenger RH and DM Katschinki. 2005. Review. The Hypoxic Testis and Post-Meiotic Expression of PAS Domain Proteins. Seminar in Cell & Developmental Biology. 1084-9521/S – see front matter @2005 Elsevier Ltd.All rights reserved. DOI: 10.1016/j.semedb.2005.03.008. 547-553.