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# Utilization of viable bone marrow derivat stem cells through an adaption in low oxigen tension as an attempt to increase cellular transplantation efficacy for spermatogenesis process

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**Abstract.** Cellular transplantation using stem cells provides very promising solutions in the regeneration and repairment of cells that have experienced degeneration where recovery through medical or surgical intervension is impossible. However, the very low viability of transplanted stem cells limits the transplantation efficacy. The aim of this research was to obtain viable bone marrow derived stem cells by an adaptation treatment in a low oxygen tensioned in vitro culture. Low oxygen tension adaptation was adjusted to the niche of the stem cells in vivo. In this study, in vitro culture of stem cells in 1% oxygen was compared to those of the conventional culture in 21 % oxygen.Results showed that under 1% oxygen tension cell proliferation was slower with larger or rounded triangle shaped cells, and senescence or dead cells was low. Meanwhile under 21 % oxygen tension cell proliferation was two fold faster with flattened and slender cells, and senescence or dead cells was higher. In conclusion, conventional in vitro culture under 21 % oxygen caused cell aging (senescence) and rapid cell death, therefore the transplanted cells were not viable.

**Key words:** Stem cells, bone marrow, low oxygen tension, viability.

## Introduction

Infertility case particularly oligospermy is the most frequent case in male reproductive disorder. In the most severe stage, this case could be followed with mono- as well as bilateral pathological changes of both testis (Hafez, 2000; Schlatt et al., 2002). Unfortunately regeneration that is possible to occur, even though in a long period of time, could only succeed if there are remaining normal spermatogonia cells in the seminiferous tubules. However the regeneration ability becomes very limited to replace the degenerating cells.

Cell therapy using bone marrow derived stem cells provides a very promising solution in the case of regenerating and restoring germ cells and testis tissues undergoing degeneration where recovering through medical or surgical intervention is impossible (Blanchard et al., 2008). However, the low viability of the transplanted stem cell in degenerative testis to differentiate into spermatogonia cells causes the limitation of the efficacy of this therapy (Kenichiro et al., 2005; Tang et al., 2005). A study on stem cell by Geng (2003) revealed that 99 % of the stem cells injected died four days after injection.

This indicates that microenviroment in the degenerative tissue or body organs is not conducive for the stem cells' survival. The estimated mechanism accounted for the decreasing function of stem cells are the high amount of the stem cells undergoing senescence and death along with the decreasing capacity to adhere and differentiate. Therefore the increase of the implanted stem cells' survival became important to increase the efficacy of stem cell therapy.

Therefore adaptation of the stem cells during in vitro culture adjusted to the in vivo niche of the stem cell need to be conducted in an attempt to increase viability at the time of transplantation to oligospemy patient through the low oxygen tension preconditioning during culture.

## Material and Methods

Rabbit was anesthetized at the coxygea area. Stem cell was harvested by an aspiration of bone marrow at the middle of femur bone below the condylus. Aspirate was placed in hepaninized tubes. Sample in tube was placed in thermos maintained at 4°C during transportation to laboratory to be processed.

Aspirate was transferred into 15 ml sterile blue capped tubes then tube was rinsed twice with 5 ml sterile Phosphate Buffered Saline (PBS). PBS was topped up to a total volume of 8 ml. Diluted sample was loaded over a same volume of Ficoll in a separate 15 ml tube. Centrifugation was performed at 1600 rpm for 15 minutes at room temperature. After centrifugation cells were collected from Ficoll-PBS interface using sterile pasteur pipette and transferred into 15 ml tube. Cells were then resuspended in PBS up to a total volume of 15 ml. Tube was inverted gently 5 times to homogenize the suspension. Tube 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 prior to incubation. Cells were plated in 10 $cm^2$ plates and incubated at 37°C in a humidified atmosphere containing 5% $CO_2$ 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 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 were replaced. Culture was continued until approximately 75-80% confluence was attained. After confluence, cells were passaged into several dishes for subculture. Passage was conducted 3 times, then cells were divided into two low oxygen tension treatments of 1% in hypoxia chamber inside 5% $CO_2$ incubator while another treatment was the use of 21% oxygen (conventional culture).

## Results and discussions

Results of this research showed that under low oxygen tension of 1%, cells proliferated slower, cells looked bigger and round, formed triangle shape with fewer cells undergoing senescence or death; meanwhile under 21% oxygen tension cells proliferated twice as fast with flat and slender shaped cells with more cells undergoing senescence or even dead (Figure 1-6).

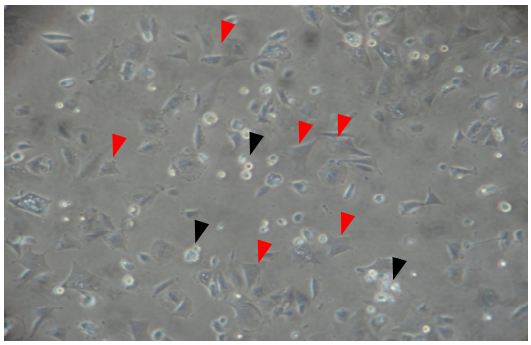

Figure 1. Two hours after incubation under 1% $O_2$. Profileration was slower, cells were enlarged, rounded and formed triangle shapes (red arrow head) with fewer cells undergoing senescence and dead (black arrow head); (400 x magnification).

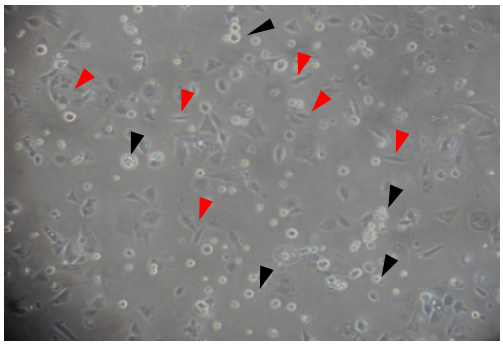

Figure 2. Two hours after incubation under conventional 21% $O_2$ tension. Profileration was faster, cells were flat and slender (red arrow head) with more cells undergoing senescence and dead (black arrow head)'; (400 x magnification).

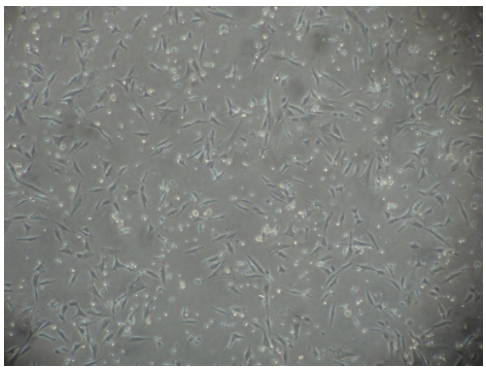

Figure 3. One day after incubation under low 1% $O_{2;}$. Proliferation was slower and cells were larger; (400 x magnification).

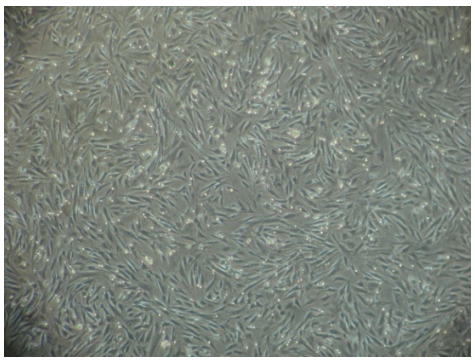

Figure 4. One day after incubation under conventional 21 % $O_{2;}$ Proliferation was faster, cells were flat, slender and elongated; (400x magnification).

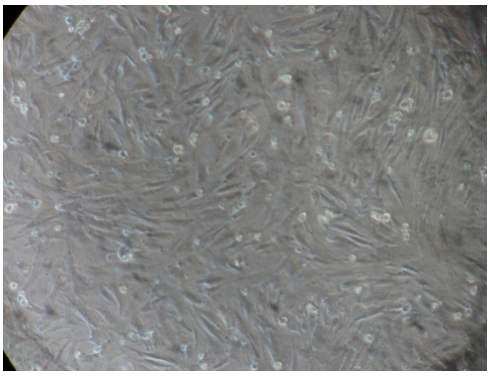

Figure 5. Two days after incubation under low 1 % $O_{2;}$. Proliferation was slower and cells were larger; (1000 x magnification).

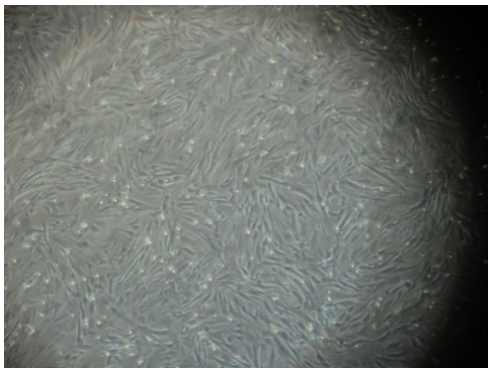

Figure 6. Two days after incubation under conventional 21 % $O_{2;}$. Proliferation was faster, cells were small, flat and confluency was rapidly attained ; (1000 x magnification).

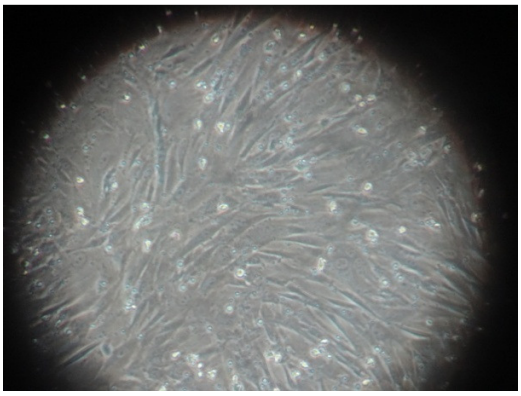

Figure 7. Three days after incubation under low 1 % $O_2$;. Proliferation was slower and cells were larger; (1000 x magnification).

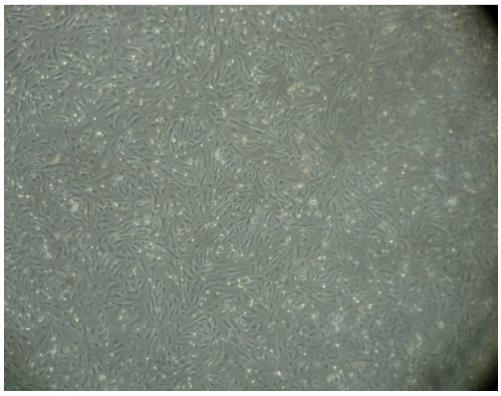

Figure 8. Three days after incubation under conventional 21 % $O_2$;. Proliferation was faster, cells were small, flat and confluency was rapidly attained; (1000 x magnification).

From the result of this research it could be explained as follows: that low 1% oxygen tension applied affected HIF-1α release from van HoupelLindau (vPL) and accumulated in nucleus. The high level of HIF-1α would inhibit Reactive Oxygen Species (ROS) that acted as free radical. The inhibition of ROS would inhibit the expression of protein genes P53 and P21. Therefore, cell cycle arrest genes were sensitized, which ended up with slow proliferation and maintenance of stem cells. This maintenance was also supported by the reduced ROS by the role of HIF-1α therefore p53 gene expression was inhibited. The inhibition of p53 gene expression caused an inhibition of the opening of mitochondrial membrane pt pore. Therefore, cytochrome C that acted as apoptotic protease activing factor-1 (APAF-1) caused inhibition of the release of various caspases (Caspase 9 and Caspase 3) as apoptotic cascade. The inhibition of P53, cytochrome C and caspases would cause the inhibition of cell death of the cultured stem cells. Meanwhile, the decreased P21 caused an inhibition of the active cycling cell which prevented cell senescence process from happening. The inhibition of cell death and senescence caused the stem cell proliferation to become slower and also making the speed of cell culture reaching confluency slower.

## Conclusions

From the research results, it could be concluded that conventional in vitro culture using 21% $O_2$ caused cells to undergo senescence and die faster. Therefore, the transplanted stem cells were not viable. Meanwhile, in the use of low oxygen tension at 1% concentration cells were more viable because of the slower senescence and did not die.

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