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by Erma Safitri

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HYPOXIC PRECONDITION FOR INDUCE PLURIPOTENCY OF RABBIT'S BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Erma Safitri[1,2*], R. Heru Prasetyo[3], Mas'ud Hariadi[1] and Pudji Srianto[1]

[1]Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia.
[2]Stem Cells Research Division of Institute Tropical Disease (ITD), Universitas Airlangga, Indonesia.
[3]Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Indonesia.
*e-mail: rma_fispro@yahoo.com, erma-s@fkh.unair.ac.id

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ABSTRACT : Hypoxic precondition *in vitro* culture can be induced of pluripotency from MSCs like hypoxia niche 1-3% in rabbit's bone marrow reside. This study was done in two phases: First phase, hypoxia precondition treatment on the stem cells culture with several O_2 concentration (21, 1 and 3%) and several cultivation time (1, 2, 4, 8 days); Second phase, analysis of pluripotency based on genotype expression of OCT4 and SOX2 genes after hypoxia precondition treatment. The analysis of pluripotency: expression of genotype OCT4 and SOX2 genes from the third groups sample, revealed cDNA chain at 114 bp (OCT4) and 319 bp (SOX2). But based on analyses in the same line, only 2 groups sample was found result gene sequenz of OCT4 and SOX2 code similarity with whole genom that found in gene bank was on sample that cultured with hypoxia (1% and 3% O_2). The matching of OCT4 gene sequenz with the data based on similarity significance value cut off that must ≥ 60% (73% in culture 1% O_2 and 65% in culture 3% O_2), eventhough in normoxia culture (21% O_2) similarity significance value cut off < 60% (only 43%). The matching of SOX2 gene sequenz with the data based on similarity significance value cut-off ≥ 60% (85% in cultur 1% O_2 and 3% O_2), eventhough in normoxia culture (21% O_2) similarity significance value cut off < 60% (only 50%). Conclusions of this study : Hypoxic precondition (1% and 3% O_2) and cultivation time (2, 4, 8 days) in vitro culture can be induced of pluripotency from rabbit's MSCs based on genotype expression of OCT4 and SOX2 genes with similarity significance value cut off that must ≥ 60%.

Key words : Hypoxia precondition, induce pluripotency, rabbit's BMSCs, genotype OCT4 & SOX2.

INTRODUCTION

Several studies have reported that *in vitro* conventional culture of rabbit's bone marrow mesenchymal stem cells (BMSCs) with high oxygen (O_2) tension (>20%), which have been considered as normoxia, frequently leads to the formation of senescence cells (Tsai *et al*, 2011), apoptosis (Wang *et al*, 2008) and a gene mutation (G: C to T: A) (Szablowska-Gadomska *et al*, 2011). This causes loss of stem cells viability before transplantation. After being transplanted, stem cells die between 93-99% on day 3 to 7 post-transplantation (Toma *et al*, 2002; Geng, 2003; Suzuki *et al*, 2004; Freyman *et al*, 2006; Sadek *et al*, 2009), even death may reach 99% on the first day post-transplantation (Wang *et al*, 2008).

Therefore, low O_2 tension (hypoxia) is required to support conducive microenvironment during *in vitro* culture so that the stem cells remain viable during transplantation, even may become pluripotent. In this study, hypoxia was adjusted to normal physiological conditions needed in the place of the stem cells inside the body. The mesenchymal stem cells physiology requires integral components in the form of low O_2 tension of 1-3% in bone marrow (Chow *et al*, 2001), 10-15% in adipose tissue (Bizzari *et al*, 2006) and 2-9% in almost all tissues (Gruber *et al*, 2010). Therefore, we need a conducive environment for stem cells during culture process through the provision of hypoxic precondition in order to keep its form as pluripoten cells. However, until now concentration and the duration of optimal hypoxia preconditions that can be applied to BMSCs in vitro culture to make pluripotential cells have not been defined.

Studies on hypoxic conditioning to support in vitro microenvironment (niche) in several sources of stem cells have been done, such as hematopoietic stem cells (HSCs) with O_2 concentration of 0-5% (Arai and Suda, 2017; Suda *et al*, 2011; Simsek *et al*, 2011), later in adipose stem cells (ASCs) by 5% (Mantymaa, 2010), neural stem cells (NSCs) of 1-5% (Mantymaa, 2010) and Human

Cord Blood (HCB) of 3% for 7 days (Ivanovic *et al*, 2004). At mesenchymal stem cells (MSCs), it is done in O_2 concentration of 0.5-3% (Tsai *et al*, 2011; Wang *et al*, 2008; Rosova *et al*, 2008; Hu *et al*, 2008). Until now, studies are still being conducted in the search for absolute factor of in vitro niche that is able to control stem cells proliferation to remain viable and undifferentiated (Halim *et al*, 2010), in addition to prevent apoptosis process, the formation of senescence cells or gene mutations. If it can be materialized, even with in vitro pluripotency, the availability of stem cells may be produced easily by researchers and clinicians as a key ingredient in cell transplantation therapy (Masarani *et al*, 2006; Kilani, 2009).

However, the lack of viability in the form of short term maintenance conditions (Takubo, 2011) of the stem cells to be transplanted to patients with degenerative disease or injury causes the effectiveness of this therapy be limited (Masarani *et al*, 2006). This can be explained as follows: physiological environment of stem cells in the body requires low O_2 levels (hypoxia), whereas *in vitro* culture has been carried out under normoxia. Therefore, a hypoxic environment needs to be created during in vitro culture of the stem cells to adjust to the physiological microenvironment where the stem cells stay.

Target genes *in vitro* culture are expected to lead to the expression of pluripotency genes (Grskovic and Santos, 2008), such as OCT4, SOX2, NANOG (Szablowska-Gadomska *et al*, 2011; Neganova *et al*, 2016), REX1 (Kolf *et al*, 2007), KLF-4 and c-MYC (Neganova *et al*, 2016; Takahashi *et al*, 2007). However, it remains unclear how to create pluripotency cells in vitro hypoxic precondition culture before the stem cells are transplanted. Therefore, this study was conducted to determine O_2 concentration (whether 21, 1, or 3%) and time (whether 1, 2, 4 or 8 days) of hypoxic preconditioning needed to achieve pluripotency stem cells.

MATERIALS AND METHODS

Hypoxic preconditioning in Rabbit's BMSCs culture

Isolated cells from Rabbit's BMSCs (Rabbit strain New Zealland) have been isolated, grown up to the third passage. The cells were taken aseptically and then placed on a culture disk with a density of 2×10^7 cells/cm^2 at 10 dishes with a diameter of 5 cm^2. The medium culture with IMDM, this medium was containing 15% FBS, BMSCs simulator supplements and antibiotics (100 U penicillin/100 ug/mL streptomycin) at a temperature of 37°C, 5% CO_2 and 95% air (Rantam and Ferdiansyah, 2014). Treatment at BMSCs cells was done by providing hypoxic conditions in several doses of O_2 (21, 1 and 3%) concentrations by integrating culture flask into a specialized incubator for hypoxic conditions (Modular Incubator Chamber) cultured for 1, 2, 4, 8 days (cultivation time).

At this stage the samples MSCs were divided into three treatment groups each of 10 replications, namely: T0 group (Control): The third passage of MSCs, in normoksia precondition (O_2 = 21%) for 1, 2, 4, and 8 days; Treatment group 1 (T1): the third passage of MSCs were given hypoxya precondition (O_2 = 1%) for 1, 2, 4, and 8 days; and the treatment group 2 (T2): the third passage of MSCs were given hypoxya precondion (O_2 = 3%) for 1, 2, 4, and 8 days

Analyses of pluripotency induction

Analysis of pluripotency induction was based on the expression of genes such as OCT4 and SOX2 genotypically by PCR and DNA sequencing.

OCT4 & SOX2 coding gene expression using PCR and DNA sequencing

Polymerase Chain Reaction (PCR) of OCT4 dan SOX2

After hypoxic precondition treatment, PCR stages of OCT4 and SOX2 was done through several procedures, such as RNA extraction, spectrophotometer, cDNA, PCR, PCR product analysis through electrophoresis process and DNA visualization (Rantam and Ferdiansyah, 2014; Martin *et al*, 2013).

RNA extraction of from cultured cells. Cultured cells as many as 10^3 cells in Eppendorf stored in minus 20°C were removed and left at room temperature until the condition was not frozen. Centrifugation was done at 12,000 rpm for 10 minutes, then the supernatant was discarded. Furthermore, pellet resuspension was made with 200 PBS and 400 µL of lysis buffer was added and vortexed for 15 seconds. Then, samples were transferred to the filter by pipetting, then centrifugated 8000 g for 1 minute. The next process, 90 µL DNase was taken with a pipette and the buffer was incubated in sterile Eppendorf, then 10 µL DNAse was added, mixed and put in the filter, and then incubated for 15 minutes at room temperature and added with 500 mL wash buffer I and centrifuged 8000 g for 15 seconds. Then, 500 mL wash buffer II was added again and centrifuged 8000 g for 15 seconds as well. As much as 200 mL wash buffer II was added again but centrifuged 13,000 g for 2 minutes. The next stage, the filter was transferred to a new Eppendorf tube and RNA elution was carried out with 50 µL elution buffer. Furthermore, it was centrifuged 8000 g for 1 minute and the RNA was stored at -80°C (Rantam and

Ferdiansyah, 2014).

Spectrophotometer was done to observe RNA purity and RNA levels to be amplified by PCR. A total of 10 mL samples, which have been added with 690 mL distilled water, was prepared and then vortexed. Subsequently the samples were transferred to the corresponding cuvettes of the spectrophotometer and the results were observed at wavelengths ë = 260 and ë = 280 (Martin *et al*, 2013).

cDNA is DNA synthesis stage that must be passed before PCR amplification. At this stage the obtained RNA was included in 0.5 ml eppendorf tube and stored in a heating block 65°C for 10 minutes to denature the RNA. The next process to obtain cDNA was that each sample was added with 10 µl MMX, which consists of tube 11 = 4.4 µl tubes; tube 1 = 4 µl; tube 2 = 0.2 µl; tube 3 = 0.4 µl; tube 4 = 0.4 µl; tube 5 = 0.6 µl, mixed up down, not vortexed (Rantam and Ferdiansyah, 2014).

PCR amplification : PCR principle consists of three phases, denaturation of double-stranded DNA, primer annealing to DNA targets and primer extension by the presence of DNA polymerase. Resulted DNA is the exponential accumulation of specific target DNA. Three stages in the amplification are: 1. cDNA denaturation stage, performed by incubating on a heating block at 37°C for 60 minutes, followed by 65°C for 10 minutes and keeping in the fridge overnight for further PCR process. Synthesized cDNA results were added with PCR mix each 12.5 µl. PCR mix consists of tag polymerase, ion buffers MgCl2+ and dNTP; 2. Annealing stage is the attachment of the primer to the template, primers OCT4 (Oligo-Macrogen) with 5'-AGC OCT4f = AAA ACC CGG AGG AGT-3 '(18mer) and OCT4r =' 5-CCA CAT TAT GTG CGG CCT ATC- 3 '(21mer) and SOX2 primer (Oligo-Macrogen) with SOX2f = 5'-TTG CTG CCT CTT GAC TAA TAG GA-3' (23mer) and SOX2r = '5-CTG GGG AAA CTT CTC CTC TC-3' (20mer) respectively of 2.5 µl; 3. The final stage is the primer extension, the lengthening process of nucleotide base strand. This process is done by adding annealing results on phase 2 with a sample of OCT-4 and SOX2 each 7.5 µl. This process is carried out at a temperature of 65°C in a heating block for 2 hours with the aim to form two new DNA double strands. The amplification process can be seen in Table 1 (Safitri *et al*, 2014).

Analysis of PCR products. cDNA PCR products that have been amplified through several phases above were then visualized using UV rays to obtain cDNA strand at specific base pair. To obtain the base pair, we previously performed electrophoresis on 2% agarose gel + 0.5 TBE using ethidium bromide staining. Furthermore, the PCR product is undergoing sequencing a process to obtain nucleotide sequences.

DNA sequencing

Stages of DNA sequencing of OCT4 and SOX2 after hypoxic preconditioning is done through several procedures, *ie*. PCR product purification, labeling, precipitation and sequensing as well as software analysis of the sequencing results.

In the purification of PCR products, one volume produced by PCR was added with 5 volumes of PBS, then added with 10 v of sodium acetate 3 M pH 5. Furthermore, as DNA binding, the sample was put on QIA quick column and centrifuged at 13,000 rpm for 1 minute. Supernatant resulting from centrifugation was then discarded. Thereafter it was added with 75 µl PE buffer and centrifugated at 13,000 rpm for 1 minute. The supernatant was discarded and then centrifugated again for 1 minute at a speed of 14,000 rpm. QIA quick column was placed in 1.5 ml Eppendorf tube, then added with 30 µl EB buffer right in the middle of the column and centrifugated for 1 minute. The supernatant was then removed and transferred to a new tube.

In labeling, we made reagents mixture consisting of 4 ml of Ampli Tag RNA polymerase, sequencing buffer, dNTPs, dye labeled terminators, 21 mL of purified PCR product, 1.5 mL primers and coupled with ddH_2O up to volume 15 µl, then vortexed.

During precipitation, sequencing product of 15 µl each was added with 1.5 mL EDTA, 125 Mm pH 8, 1.5 µl of 3M sodium acetate pH 2.5 and 37.5 mL of absolute ethanol then vortexed and incubated at 4°C. Samples were wrapped in aluminum foil to avoid light and stored in a refrigerator at a temperature of -20°C.

Sequencing : DNA base sequence can be determined by sorting appearing fragments starting from the bottom (the shortest). The instrument used was ABI 3110 Capillary Sequencer XI. DNA fragments can be visualized as primers labeled with fluorescence. The result of such sequencing is in the form of electropherogram.

Sequensing results were analyzed using software Bioedit and BLAST (Basic Local Alignment Search Tool). Analysis of Bioedit results used Sequence Alignment Editor program that aims to analyze bioinformatics sequence of DNA, RNA and protein. One of the stages is the procedures for doing sequence alignment with readings in both directions (forward-reverse) to sequence a DNA (Schwartz and Pachter, 2007).

The sequence obtained from this study can be analyzed by comparing available data with the already published data in Gene Bank database. One form of analysis is the sequence alignment. Sequence alignment may be used to compare two or more sequences to assist in differentiating bases. The program used for sequence alignment is Bioedit and BLAST. The function of the programs is to analyze sequence data, comparing the sequence studied with databases of various different strains from various countries and to see the matches of a sample from its base sequence (query confidence) (Thomsen *et al*, 2017).

Data analysis

Genotype expression results were analyzed by PCR and DNA sequencing, which in this study was observed descriptively. Phenotype expressions analyzed were those on OCT4 and SOX2 as two markers of pluripotency induction of stem cells, whereas genotype observations was done on the OCT4 and SOX2.

RESULTS

Genotype expression of genes OCT4 and SOX2 (PCR and DNA sequencing)

After cultured with hypoxic preconditioning treatment, OCT4 and SOX2 genotype characterization of BMSCs underwent PCR-DNA sequencing method. The aim of this characterization was to obtain the expression of OCT4 and SOX2-encoding genes, which were expressed in imunofluorescence at 48 hours of treatment (1% and 3% O_2), but were not expressed at 21% O_2. In this study the expression of OCT4 and SOX2 appeared after 2 days of treatment of hypoxic preconditioning. This indicated that the cultivation time of hypoxia preconditioning was capable of causing the expression of OCT4 and SOX2 as a transcription factor and that MSCs were pluripotent and self-renewal. PCR results of OCT4 with PCR product was 114 base pair (bp) (Fig. 1) and SOX2 was up to 319 base pair (bp) (Fig. 2).

cDNA strand shown in both figures indicate MSCs cell count concentration sufficient to express the OCT4 and SOX2 encoding gene. Subsequently, we performed purification of PCR products, then labelled and sequenced to obtain the nucleotide composition of the OCT4 and SOX2 encoding gene.

Characterization OCT4 and SOX2 by DNA Sequencing

Sequencing DNA will produce DNA sequence as described through a string of letters symbolizing nucleotides as DNA elements, such as ACGT. This sequencing technique uses a method known as the Sanger method, a method that uses the termination or end of the DNA synthesis reactions that are specific to particular sequences by using nucleotides. DNA chain elongation at the Sanger method begins in a specific area of DNA template by using primers. The primer will be complementary to the DNA from the sample as the target of observation. Sequencing is done for the characterization of MSCs genotypically after MSCs culture with hypoxic preconditioning with the aim of obtaining OCT4 and SOX2 coding genes nucleotide base arrangement expressed by MSCs.

The results of the visualization of raw sequencing electropherogram results of sequencing using the primer OCT4 (Oligo-Macrogen) with 5'-AGC OCT4f = AAA ACC CGG AGG AGT-3 '(18mer) and OCT4r =' 5-CCA GTG CCT TAT CAT CGG ATC-3 '(21mer) and SOX2 primer (Oligo-Macrogen) with 5'-GCT SOX2f = CTC CAA AGT GCG ACG AA-3' (20mer) and SOX2r = '5-AAG AAA AGT GGC TTT CTG AGA TA-3' (23mer).

Nucleotide bases arrangement shown in electropherogram of sequencing results of the samples after hypoxic preconditioning revealed that the primary base encoded by OCT4 and SOX2 shows the amplified DNA had a good purity. Based on the results of the amplification, analysis of the sequencing results can be followed with Bioedit using Sequence Alignment Editor for analyzing bioinformatics of DNA sequence, RNA and protein.

The series of sequencing analysis was carried out in several stages, beginning with a sequence alignment (alignment analysis) in order to compare two or more sequences that different bases can be easily seen. This alignment analysis phase uses special program that the Bioedit and BLAST (Basic Local Alignment Search Tool). The program analyzes data from sequences, comparing studied sequences with database of various types of different transcription factors and to find matches sample a from its base sequence (query confidence). The results of sequencing analysis, alignment analysis and OCT4 whole genome in this study can be seen in Fig. 3.

Based on the analysis of OCT4 encoding gene by PCR after hypoxic preconditioning (1% and 3% O_2), which was compared to normoxia (21% O_2) and visualized using electrophoresis on 2% agarose gel by with ethidium bromide, three samples showed cDNA strand of 114 bp (Fig. 1). However, alignment analysis showed only 2 matches between the results of OCT4 encoding gene sequences with whole genome samples in gene banks (Fig. 3). Both matched data were on samples cultured with hypoxia (1% O_2 and 3% O_2).

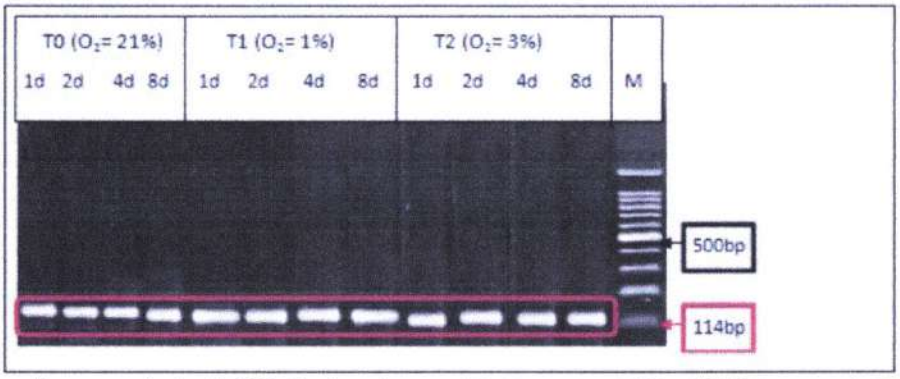

Fig. 1 : OCT4 encoding gene analysis with PCR after hypoxic preconditioning treatment (1% and 3% O_2) compared with normoxia (21% O_2) visualized electrophoresically in 2% agarose gel with ethidium bromide staining. The result reveals cDNA strand of 114 bp. M = marker.

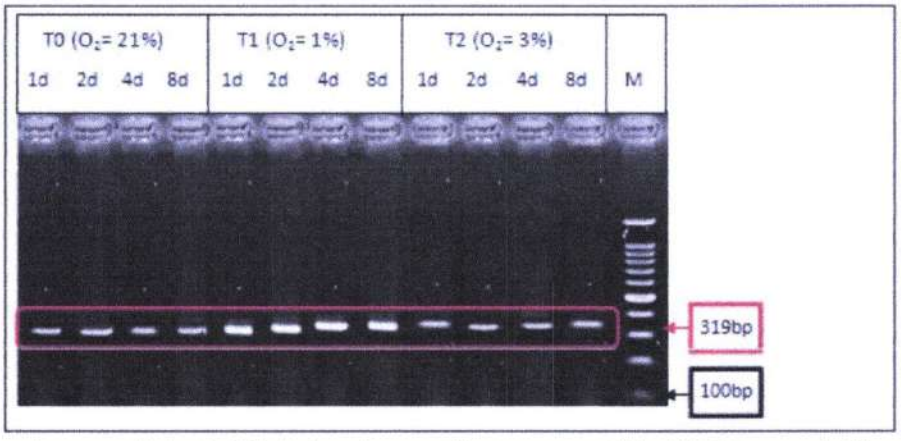

Fig. 2 : SOX2 encoding gene analysis with PCR after hypoxic preconditioning treatment (1% and 3% O_2) compared with normoxia (21% O_2) visualized electrophoresically in 2% agarose gel with ethidium bromide staining. The result reveals cDNA strand of 319 bp. M = marker.

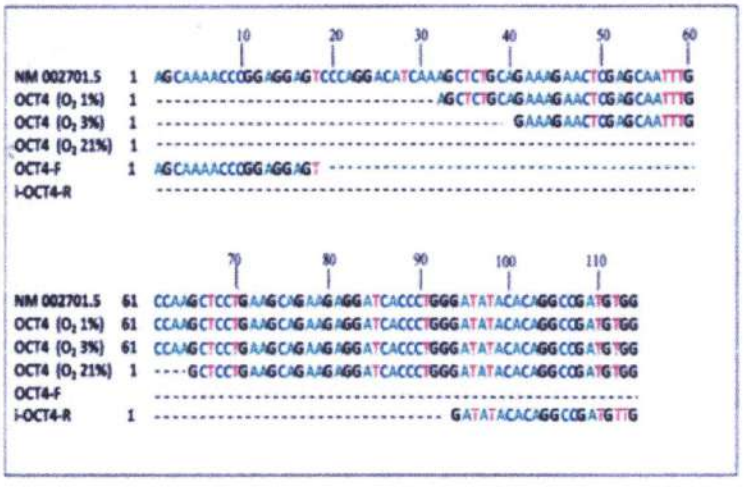

Fig. 3 : OCT4 sequencing analysis using multiple alignment. NM 002701.5 → OCT4 whole genome.

Compatibility data was based on similarity significance of the cut-off value that should be ≥60% (73% in cultures of 1% O_2 and 65% at 3% O_2 culture), whereas in normoxia culture (21% O_2) similarity significance cut-off value <60% (only 43%). The results of sequencing analysis, alignment analysis, and whole genome OCT4 in this study can be seen in Fig. 3.

Based on the analysis of SOX2 encoding gene by PCR after hypoxic preconditioning (1% and 3% O_2), compared to normoxia (21% O_2) and visualized in electrophoresically on 2% agarose gel by ethidium bromide staining, three samples showed cDNA strand of 319 bp (Fig. 2). However, based on the analysis of the data alignment there were 2 data resulting from the sample's SOX2 encoding gene sequences that match with the whole genome in the gene bank (figure 4). Both data were on hypoxic cultured samples (1% O_2 and 3% O_2). Data matching was based on similarity significance cut-off value that should be ≥60% (85% in cultures of 1% O_2 and 85% at 3% O_2 culture), whereas in normoxic culture (21% O_2) similarity significance cut-off value was < 60% (only 50%) (Fig. 4).

In this study, the expression of OCT4 and SOX2 in hypoxic preconditioning 1% and 3% O_2, respectively occurred on day 2 and 4, while that in normoxia did not appear. This indicated that the cultivation time (number of days) of hypoxic preconditioning may cause the expression of OCT4 and SOX2 as a transcription factor so that MSCs became pluripotential. PCR result of OCT4 revealed 114 base pair (bp) (Fig. 1) and SOX2 as many as 319 base pair (bp) (Fig. 2).

Based on the analysis of the OCT4 and SOX2 encoding gene by PCR after hypoxic preconditioning (1% and 3% O_2) compared to normoxia (21% O_2) and visualized in electrophoresis on 2% agarose gel with ethidium bromide staining, three samples showed the cDNA strand of 114 bp (Fig. 1) and 319 bp (Fig. 2). However, based on the analysis of the data alignment revealed only 2 results of OCT4 and SOX2 encoding gene sequences samples matched with whole genome in the gene bank (Fig. 3). Both matches are on samples with hypoxic culture (1% O_2 and 3% O_2). Compatibility data was based on significance value similarity that must have cut-off value ≥ 60%. In OCT4 sample, 73% in 1% O_2 culture and 65% at 3% O_2 culture, in normoxia culture (21% O_2) similarity significance cut off value was < 60% (only 43%). In SOX2 sample, 85% in 1% O_2 culture and 3% O_2, whereas in normoxia culture (21% O_2) similarity significance cut-off value was < 60% (only 50%).

DISCUSSION AND CONCLUSSION

The results of pluripotency analysis based on genotype expression of OCT4 and SOX2 genes by PCR and DNA sequencing and phenotypically with imunofluorescence in this study were in line with the results of the study of Covello *et al* (2006) and Forristal *et al* (2010), who found that after 48 hours of hypoxic administration in cultured stem cells HIF2-α expression will be produced directly to become upstream regulator of OCT4 transcription factors, which is essential for maintaining pluripotency. Likewise, another transcription factor, such as SOX2 and Nanog, are also regulated by HIF2-α (Forristal *et al*, 2010). Furthermore, Yu *et al* (2007) wrote that these three transcription factors, OCT4, SOX2 and Nanog, maintain stemness and suppress genes that cause differentiation. OCT4 and SOX2, two of the four transcription factors introduced by Takahashi *et al* (2006), were firstly found in iPS (induced Pluripotent Stem) cells program.

According to Yoshida *et al* (2009), the relationship between hypoxia, HIF2-α and pluripotency genes such as OCT4, SOX and Nanog are crucial to induce pluripotent stem cells, because it has been proved that hypoxia increases efficiency and reprogramming. The study by Zadori *et al* (2009) in NSCs transplantation indicates that preconditioning of hypoxic environment was also necessary for the process of *in vitro* culture. Replecement therapeutic effectiveness of stem cells requires knowledge on the mechanisms that affect stem cells early development, such as migration, proliferation and stem cells commitment (Wu *et al*, 2008) and the dependence on oxygen levels in the appropriate environmental conditions. There is a clear functional relationship between hypoxia inducible factor and stemness with transcription factors altogether *in vivo*. The niche of NSCs is hypoxic conditions that create high reliance on early development process at oxygen level.

The results of this study, that pluripotency activity after hypoxic treatment on days 2 and 4 through OCT4 and SOX2 expression, genetically support one of the main focuses in the research of adult stem cells *in vitro*. This is because *in vivo* we need balance between differentiation, apoptosis and self renewal of stem cells. The balance is regulated by one of microenvironment niche where stem cells are located. According to Barria *et al* (2004) in feeder-free in vitro culture, the fate of stem cells in influencing balance between self-renewal, differentiation and apoptosis, in addition of being affected by growth factor, interleukins or serum, also influenced by the conditions given during the culture process, which in this study was hypoxic conditions 1 and 3%.

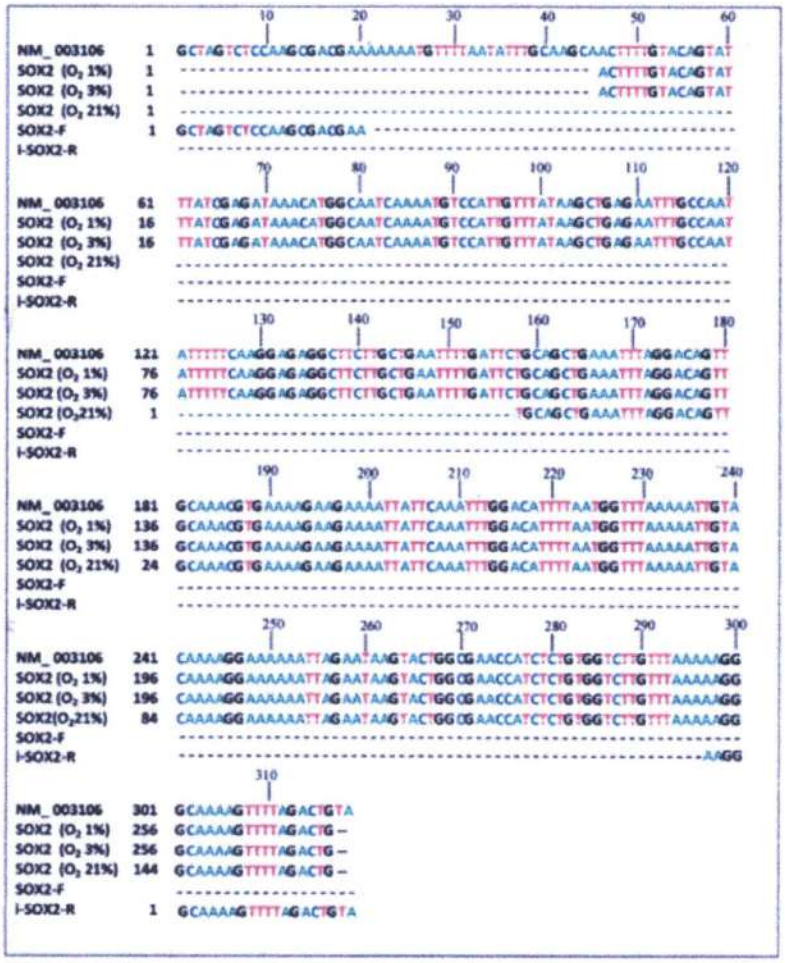

Fig. 4 : SOX2 sequencing analysis using multiple alignment. NM 003106 → SOX2 whole genome.

Table 1 : BMSCs PCR cycle in the heating block (Safitri *et al*, 2014).

No	Temperature	Time	Notes
1.	Room	15 minutes	RNA Predenaturation
2.	65°C	10 minutes	RNA Denaturation
3.	37°C	60 minutes	DNA Denaturation
4.	65°C	10 minutes	Advanced DNA Denaturation
5.	65°C	2 hours	Extension
6.	4°C (refrigerator)	10 minutes	Soaking

Pluripotency is the potential to differentiate into any cell of the body of the three embryonic layers (ectoderm, mesoderm and endoderm). Potential to become these three embryonic layers is in general are characteristics possessed by embryonal stem cells (ESCs) (Szablowska-Gadomska, 2011). According to Halim *et al* (2010), compared to progenitor cells that have unipotent properties, stem cells, in particular ESCs, has potential far greater differentiation. ESCs, derived from the inner cell mass (ICM) in blastocyst are pluripotent, so as to differentiate into various types of cells making up the body of the three embryonic layers, including nerve cells, blood cells, the cells making up the heart and immune system cells. In this study, MSCs, which are adult stem cells that are generally well characterized as multipotent, can express OCT4 and SOX2 genes, both genetically and phenotypically (Safitri *et al*, 2016; Prasetyo and Safitri, 2016) after being treated as hypoxic during *in vitro* culture. Therefore, in the future it is suspected that MSCs with hypoxic preconditioning have pluripotent potential. This suspicion is confirmed by the opinion of some studies (Takahashi *et al*, 2007; Yu *et al*, 2007; Takahashi and Yamanaka, 2006; Moheyldin *et al*, 2010), that the efficiency of reprogramming in iPS cells in hypoxic conditions is very high.

SUMMARY

Hypoxic precondition *in vitro* culture can be induced of pluripotency from MSCs like hypoxia niche 1-3% in rabbit's bone marrow reside. The matching of OCT4 gene sequenz with the data based on similarity significance value cut off 73% in culture 1% O_2 and 65% in culture 3% O_2, eventhough in normoxia culture 21% O_2 similarity significance value cut off only 43%. The matching of SOX2 gene sequenz with the data based on similarity significance value cut-off 85% in cultur 1% O_2 and 3% O_2, eventhough in normoxia culture 21% O_2 similarity significance value cut off only 50%. Conclusions of this study : Hypoxic precondition 1% and 3% O_2 and cultivation time 2, 4, 8 days *in vitro* culture can be induced of pluripotency from rabbit's MSCs based on genotype expression of OCT4 and SOX2 genes with similarity significance value cut off that must ≥ 60%.

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