

Penelitian

Peripheral Blood Mesenchymal Stem Cells Isolated from Indonesia Long Tailed Monkey (*Macaca fascicularis*)

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ABSTRAK

Sebuah penelitian untuk membandingkan pengaruh umur dari monyet ekor panjang *Macaca fascicularis* (Mf) sebagai sumber isolat darah tepi (DT) sel punca mesenkimal (SPM) dan mempelajari dampak konsentrasinya terhadap perkembangan sel darah tepi berinti tunggal (SDTBT) telah dilakukan. Dua belas ekor Mf jantan digunakan pada penelitian ini. Tiga kelompok umur monyet (bayi (A1), anak (A2), dan dewasa (A3)) dibandingkan sebagai perlakuan. Isolat SPMDT dibuat dengan mengambil 1, 5 atau 10 ml darah tepi Mf, mengolahnya menjadi SDTBT, mencacah, mengisolasi dan membiakkan, kemudian dilakukan subkultur agar diperoleh pelet sel, diekstraksi messenger ribonucleic acid (mRNA)nya, kemudian dilakukan reverse transcriptase-polymerase chain reaction (RT-PCR). Amplifikasi PCR dilakukan untuk melihat ekspresi gen SPM dan divisualisasi dengan Gel Doc. Rancangan kelompok tidak lengkap digunakan pada penelitian ini. Data yang diperoleh dianalisis secara deskriptif dan uji-T. Hasil penelitian menunjukkan bahwa jumlah pencacahan SDTBT dari isolat darah bayi, anak dan dewasa berturut-turut 6.78 – 7.28, 6.18 – 7.30, dan 6.01 – 7.34 log sel. Subkultur dan pelet hanya dapat dikerjakan pada isolate darah tepi Mf dewasa saja dengan marker positif yaitu CD 73, 90, 105, dan negatif yaitu CD 34, 45. Dari penelitian ini dapat disimpulkan bahwa darah tepi Mf jantan dewasa dapat digunakan sebagai sumber SPM.

Kata kunci: darah tepi, *Macaca fascicularis*, marker CD, sel punca mesenkimal

ABSTRACT

An experiment to compare age of *Macaca fascicularis* (Mf) as peripheral blood (PB) mesenchymal stem cell (MSC) isolate sources and the impact of its concentration on the peripheral blood mononucleous cells (PBMC) development has been conducted. Twelve male Mf were used in this experiment. Three different age groups (infant (A1), juvenil (A2) and adult (A3)) of the Mfs were compared as treatments. Isolate of peripheral blood MSC were created by taking 1 ml, 5 ml or 10 ml the Mfs peripheral blood, processed them into PBMC, counted, isolated, cultured, subcultured, pelleted, extracted for their messenger Ribonucleic Acid (mRNA). Reverse transcriptase - polymerase chain reaction (RT-PCR) were conducted to obtain complementary Deoxyribonucleic Acid (cDNA). PCR amplification were performed to look cluster differentiation (CD) of the MSC gene expression. Incomplete block design was used and the data were analysed using descriptive statistic and T-Test. The results showed that PBMC counted from infant, juvenil and adult were 6.78 – 7.28, 6.18 – 7.30, and 6.01 – 7.34 log cell, respectively. The subculture and pelleting cells were only obtained from A3 with positive 73, 90, 105 and negative 34, 45 CD markers. It is concluded that peripheral blood of adult Mf can be utilized as MSC source.

Keywords: CD marker, *Macaca fascicularis*, mesenchymal stem cell, peripheral blood

INTRODUCTION

The cell therapy studies using mesenchymal stem cells (MSC) transplantation had been started in 1940 (Stoltz *et al.*, 2015) and since then many research on cardiac muscle cells had been advanced (Marketou *et al.*, 2016). The MSC cell therapies had been studied *in vitro* and *in vivo* (Cantoni *et al.*, 2015). Strauer *et al.* (2003) explained the advantages of MSCs over other stem cells for variety of reasons namely for its non-controversial ethical issue, easy of growth in the culture, differentiate into specific multiple lineages such osteogenic, adipogenic and chondrogenic (Agata *et al.* (2009); Baksh *et al.* (2004); Krampera *et al.* (2006); Porada *et al.* (2006)) and so true for its well-characterized and well-identified using markers (Mackie *et al.* (2011); Rujkijyanont *et al.* (2012); Xie *et al.* (2015)).

Effort on searching MSC sources had been done by many scholars. The Bogor Primates Research Center, Research Institutions and Community Service, Bogor Agricultural University, as a laboratory with Bio Safety Level- 1 (BSL-1) facilities and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) had isolated adult MSC from umbilical cord, amniotic fluid, fat, dental pulp, skin of *Macaca fascicularis* (Mf) and *Macaca nemestrina* (Mn) successfully. Chong *et al.* (2014) had isolated and developed MSC from Mn embryo using *in vitro* and *in vivo* studies. Whereas, Ren *et al.* (2011) had isolated MSC from Mf bone marrow in an *in vitro* study. Peripheral blood is also possible as MSC isolate sources, however, their information is still limited.

Prior to human MSC therapy trials, animals were used as experiment laboratory model for several advantage reasons such as easy to control, homogen, repeatable, higher accessibility and its have been used in many studies of cardiac arrhythmia (Chui *et al.*, 2012). The common animal used as model were rodent species. They had been used about 90% of the total laboratory animal, even though they have only 31.9% approximately genetic similarity with humans (Disotell *et al.*, 2007).

In opposite, large animals such as the B group genus *Mf* originated from Mauritius (Liedigk *et al.*, 2015) had 93.5% similarity of mitochondrial deoxyribonucleic acid (mtDNA) to humans (Gibbs (2007); Shively *et al.* (2009)) although they had living 23- 25 million years earlier than human, the closest to human evolution, physiology, cognitive ability, anatomy nerves system, reproductive system, complex social life (Capitanio *et al.*, 2008), however, they are just only used about 0.28% (Carlsson *et al.*, 2004) in medicine

research. This genus were used as complimentary, not to replace other species (Hau *et al.* (2011); Phillips *et al.* (2014)). Study on the possibility of MSC as cardiac cell therapy need large animal as a model to fit sophisticated equipment.

Age of *Mf* influence the capacity of the animal as ppherical blood source for MSC isolate. It is not only because of the volume of blood that can be sampled, but also because the different of concentration of MSC and the development its ppherical blood mononucleous cells (PBMC). The objectives of the study were to compare the effect of age of male *Mf* on the PBMC isolate profiles and to seek the influence of the initial PBMC concentration on their development during culture.

MATERIAL AND METHODS

Experimental and Animal Conditions

The experiment was conducted from January to August 2016 in the Animal Laboratory of Bogor Primate Research Center, Bogor Agricultural University. The station applied bio safety level-1 (BSL-1) and have been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Twelve male *Mfs* from the centre were used in this experiment. The *Mfs* used in this experiment have had etic clearance from the Animal Care and Use Committee of the centre. The *Mfs* were divided into three groups. The groups were based on the age as used by Santosa *et al.* (2012) i.e. infant (0-18 months/ still breast feeding), juvenile (18 months-4 years old) and adult (> 4years).

Prior to treatment allocations, the animals were quarantined for 2 weeks in individual 80 x 80 x 90 cm³ cages, freed from tuberculosis disease, viral and bacterial infections. The animals were given water and fruits *ad libitum*. The cage were equipped with environmental control to fulfil the experimental requirement.

Peripheral Blood Sampling

The peripheral Blood were taken at different volume depend on the age group of the *Mfs*: 1 ml for infant, 5 ml for juvenil and 10 ml for adult. The blood was taken using ethylenediaminetetraacetic acid (EDTA) tube. The blood was harvested to find peripheral blood mononucleated cells (PBMNC), isolated and cultured into peripheral blood mesenchymal stem cells (PBMSC) using the same method as used by Trivanovic *et al.* (2013) and Ullah *et al.* (2015). The peripheral blood was collected from

the femoral vein of the *Mf* using a needle connected to a tube contained ethylenediaminetetraacetic acid (EDTA) anticoagulant. This stage was carried out by a veterinarian using the same procedures as used by Trivanovic et al. (2013) and approved by the Commission for Supervision of Animal Welfare and Veterinary Research, Primate Research Center, Bogor Agricultural University.

Media for Cell Growth

Cell growth medium was made according to Trivanovic et al. (2013) formula. The media used Dubelcco's Modified Eagle's medium (DME GIBCO™) which was supplemented with 20% fetal bovine serum (FBS). The GIBCO™ medium was added with penicillin-streptomycin antibiotic with concentration of 100 U/ml penicillin and streptomycin 100 ug/ml.

Isolation and Culture Conditions

Isolation of the PBMNC were conducted in a biosafety culture cells cabinet while cultures were done in 37°C temperature and 5% CO₂ concentration incubator. Blood samples were centrifuged at 700 *g* speed for 15 minutes. The Buffy coat layer which contained white blood cells were drawn and resuspended in 2 ml of phosphate buffered saline (PBS), then added with 3 ml of ficoll in underlayer. The tubes were centrifuged at 1100 *g* for 20 minutes. The white ring layer that contains mono nucleated cells were harvested then centrifuged at 600 *g* for 15 minutes in a medium without serum. The supernatant were removed, cell pellets were added to 5 ml of PBS and centrifuged at 700 *g* for 10 minutes. Again, the supernatant were removed and the pellet cells were added with 5 ml growth cells medium. The cells were calculated by hemacytometer on inverted lens microscope.

The cells were then grown in 6 wells tissue culture plates with concentration 10⁷ cells per well. The wells were incubated at a 37°C temperature and 5% CO₂ concentration incubator. The colonies cells were expected to grow after the 14th day of incubation.

The subculture cells were performed after the cells reached 80% confluent. The media was disposed and washed with 2 ml sterile PBS and then was added with 1 ml of 0,125% trypsin at each well. Then the plates were incubated at 37°C temperature and 5% CO₂ concentration incubator. After the cells are regardless, they were added with 1 ml growth cells medium for inactivated and resuspended of trypsin and they were accommodated in the 15 ml tube

centrifugation. After that the suspension cells were centrifuged at 700 *g* for 5 minutes, the supernatant were removed and the pellets cell were resuspended with 3 ml growth cells medium.

The cells were calculated by hemacytometer. Approximately 1 x 10⁵ cells are accommodated in a micro tube to make the pellet cells, while the remaining cells were let grown. The suspension cells in the micro tube were centrifuged at 4000 *g* for 1 minute and then the medium was removed. One ml PBS was added to wash the remaining media of the pelleting cells and then centrifuged again at 10,000 *g* for 1 minute. The cells washing was done twice. The pelleting cells were stored at -20°C for the extraction process of messenger ribonucleic acid (mRNA).

Mesenchymal Stem Cell mRNA Extraction

The mRNA extracting was conducted according to Lin et al. (2003) procedure. The extraction was performed using the RNeasy kit (Qiagen, USA). The RNA cells lysing and Tissues (RLT) buffers were added first with mercaptoethanol (100 : 1). The 350 µl RLT buffer was mixed with the result pelleting cells and then inserted into the column. Ethanol with a ratio of 1: 1 was added, then centrifuged at 10000 *g* for 1 minute. The column solution were removed and then added with 700 µl buffer remove (RW1), centrifuged at 10000 *g* for 1 minute. The column solution were removed again, added with 500 µl buffer washing membrane bounding RNA (RPE) and the centrifuged at 10000 *g* for 1 minute and added with buffer RPE. The cells collection tube were replaced with the new and centrifuged at 10000 *g* for 1 minutes without adding solvent. The cells collection tube were replaced with the vial and added with 20 µl RNase free water, centrifuged at 10000 *g* for 1 minute. The RNase free water was to obtain 40 µl mRNA.

Polymerase Chain Reaction

The Polymerase chain reaction (PCR) used in this experiment followed Viljoen et al. (2005) procedure. It consisted of reverse transcriptase (RT) and amplification. Detail of RT and amplification of the PCR are detailed below:

The RT-PCR was performed according to O'Connell (2002) procedure to obtain complementary deoxyribonucleic acid/ cDNA (Ying 2004) from a purified RNA sample, using a Superscript kit (Qiagen, USA). In the PCR reaction mixture first tube were added 1 µL Oligo (DT), 1 µL dNTPs, 1 µL dH₂O and 10 µl mRNA samples. The PCR reaction mixture first tube was

run on a PCR machine at 65°C for 5 minutes and 4°C for 1 minute. After that the results of the running of the first tube was inserted into a second mixed reaction tubes that contains 5 × 4 µL buffers, 1 µL dTT, 1 µL RNA sin and 1 µl superscript enzyme. The PCR reaction mixture second tube was run on a PCR machine at 50° C for 60 minutes, 70° C for 15 minutes and 4° C for 3 minutes, so the cDNA RT-PCR was stored at -20° C.

PCR Amplification was conducted according to van Pelt-Verkuil, *et al.* (2008) procedure. The PCR amplification was performed to see the positive mesenchymal stem cells gene expression of CD105, CD73, CD90 and the negative gene expression of CD34, CD45. Other gene expression used was glyceraldehyde-3-phosphate dehydrogenase/ GAPDH as a cDNA control of each sample. The PCR reagents were prepared. It consisted of 10 x PCR buffer, 50 mM MgCl₂, dNTP, Taq gold, RNase free water, primers (forward and reverse) and cDNA samples. The first is a pre-PCR process (Maddocks *et al.*, 2017) which aims to activate the polymerase enzyme at 94°C for 10 minutes and followed by a PCR stage that consist of denaturation at 94°C for 30 seconds; annealing at 54°C for 30 seconds; and elongation at 72°C for 30 seconds. The PCR amplification staging was repeated 40 cycles. After the last cycle completed, a 10-minute elongation at 74° C was extended. After the amplification process completed, a 10-minute elongation at 74° C was extended. After the amplification process completed, the PCR result was visualized by agarose gel electrophoresis with a 2% concentration that contain 0.1 µg / ml ethidium bromide. The DNA marker was 100 bp and the PCR result was added with loading buffer (1: 5), then incorporated into the gel well. The agarose gel electrophoresis processing was run on a 100 volt for 40 minutes and its results were visualized with Gel Doc (Lin *et al.*, 2003).

Statistical Analysis

This research uses two numerical variables with 12 samples. For normal distributed data, hypothesis test was done using independent T test of 2 group unpaired and followed by data distribution in Shapiro - Wilk table for the significant result. For skewed data distribution, transformation was

conducted prior to analysis. To analyze the numerical variables (age and blood count) as a categorized variable to other numerical variables (the cells growing), chi-square test was performed with the expected count < 5 must < 20%. For unfulfilled chi-square test, Kolmogorow Smirnov test was performed. Correlation analyses were performed to seek the relationship between PBMC initial concentration and their development during culture.

RESULTS

Peripheral blood mononuclear cells (PBMNC)

Concentration of PBMNC isolate from different groups of Mfs age groups are shown in Table 1. There was no significant effect of Mfs groups ages peripheral blood sources on concentration of PBMNC isolated (P = 0.348) due to high variation of PBMNC concentration within the treatment. The PBMNC (cells) isolats were greatly 1 - 22 x 10⁶ for adults, 1.5 - 20 x 10⁶ for Juvenile and 6-7.6 x 10⁶ for infant. Transformation into log cell showed a reduction in the variation. The distribution of adult PBMNCs was more stable at about 1-3 million cells in compare to juvenile and infant (figure 1).

Development of Peripheral Blood Mesenchymal Stem Cells (PBMSC)

The development of PBMSC from the PBMNC culture are shown in Table 2. The unpair t-tests have been done due to all data were numerical and significant difference between the growing versus the not-growing cells were found (p = 0.030). However, there was no significant difference between the age group of Mfs effect on cell growing (p = 0.491) were found. According to the production of primary cells culture and isolation procedures, the cells media should be replaced every 3-4 days and the cells growth should be seen at every 7th days in the reverse lens microscope. The observation results showed that 7 out of the 12 Mfs PBMNC samples became PBMSC and survived until passage 1 and 2. The 2 sample of juvenile PBMNC and the 3 sample of infant PBMNC were grown into PBMSC until passage 0 and 1 only, meanwhile the 3 sample of adult

Table 1 Peripheral blood mononuclear cells (PBMNC) isolated from different Mfs age groups

Parameters	Peripheral blood sources		
	Infant	Juvenile	Adult
PBMNC (Cell/ml)	6-7.6 x 10 ⁶	1.5 - 20 x 10 ⁶	1 - 22 x 10 ⁶
PBMNC (log cells/ml)	6.981 ± 0.267	6.742 ± 0.790	6.654 ± 0.483

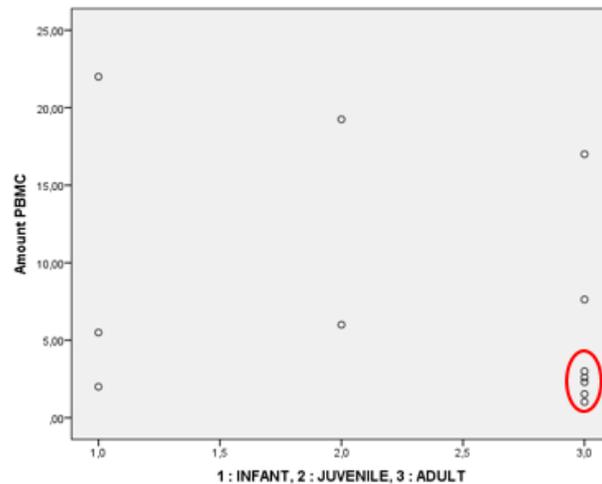


Figure 1 Relationship the peripheral blood mononuclear cells on different ages male *Macaca fascicularis*. The red circle shows the adult group.



Figure 2 Microscopic images of adult *Macaca fascicularis* 3 peripheral blood mesenchymal stem cells (P2D13Mf3 MSPRO)

PBMNC can be made into pellets PBMSC.

The morphology spindle like fibroblasts cell at 13th day by reversed lens microscope (Figure 2). It has reached 80% confluency of PBMSC at 23th to 27th day (Figure 3 and 4). This study identified positive markers CD73, CD90, CD105 (Domonici et al. 2006, Kundrotas 2012), negative markers CD34, CD45 (Mackie et al., 2011) and the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Figure 5 and 6). The GAPDH as a control of the presence of genes were examined complementary deoxyribonucleic acid (cDNA). The GAPDH gene was analyzed as control using the primers: 5- GAPDH forward and 5- GAPDH reverse. The GAPDH primers were used as a DNA amplification control of the Peripheral blood mesenchymal stem stem cells Mf (Tisato et al. 2007). The cDNA reflects the expression of specific cell genes (Ying 2004), whereas the DNA genomically represents all the genetic information in somatic cells.

Correlation between PBMNC concentration and PBMSC Production

Analysis correlation between PBMNC concentration to PBMSC pellet production showed a low negativ coefficient ($R = -0.304$, $P = 0.358$) which shows that the succesfull of PBMSC pellet formations did not depend on the concentration of PBMNC. Statistical analysis data were showed that the cells growing didnt depend on the highest male Mf PBMNC i.e.: the high PBMNC concentration sample ($9,2456 \times 10^6$) did not show any cell growing, in opposite to the lower ($2,2067 \times 10^6$) one.

DISCUSSION

Different result of PBMNC concentration found between and within group age of the Mfs were caused by several factors. The volume of blood sampled was one of this example. The number of blood samples

Table 2 The characteristic peripheral blood mononuclear cells of male *Macaca fascicularis* cultures

No	Sampling date	Treatments	Volume of blood (cc)	PBMNC (cell)	Passage 0	Passage 1	Passage 2	Noted
1	02-02-2016	Mf Adult 220409	10	2.2×10^7	02 - 21/02/2016	22 - 29/02/2016	Not done	not develop
2	02-02-2016	Mf Juvenil 140123	5	2×10^7	02 - 21/02/2016	22 - 29/02/2016	Not done	not develop
3	02-02-2016	Mf Infant 150721	1	6×10^6	02 - 21/02/2016	22 - 29/02/2016	Not done	not develop
4	02-02-2016	Mf Infant 151111	1	$1,925 \times 10^7$	02 - 21/02/2016	22 - 29/02/2016	Not done	not develop
5	03-03-2016	Mf adult	5	$1,7 \times 10^7$	03 - 23/03/2016 (2×10^5)	Not done	Not done	not develop
6	04-03-2016	Mf adult C2774	6	$1,025 \times 10^6$	04 - 23/03/2016 (6×10^5)	24/02 - 03/03/2016 (5×10^5)	03/04 - 13/06/2016	pelet
7	04-03-2016	Mf Juvenil 140625	5	$1,525 \times 10^6$	03 - 23/03/2016	Not done	Not done	not develop
8	04-03-2016	Mf Infant 150913A	1	$7,625 \times 10^6$	04 - 23/03/2016	Not done	Not done	not develop
9	22-03-2016	Mf adult C4939	2	$2,3 \times 10^6$	22/03 - 08/04/2016 (1×10^5)	Not done	Not done	not develop
10	20-04-2016	Mf adult	5	3×10^6	20/04 - 25/05/2016 (8×10^5)	25/05 - 13/06/2016 (6×10^5)	pelet	pelet
11	28-04-2016	Mf adult	10	$2,6 \times 10^6$	28/04 - 25/05/2016 (6×10^5)	25/05 - 13/06/2016 (6×10^5)	pelet	pelet
12	28-04-2016	Mf adult	10	$5,5 \times 10^6$	28/04 - 25/05/2016 (1×10^5)	Not done	Not done	not develop

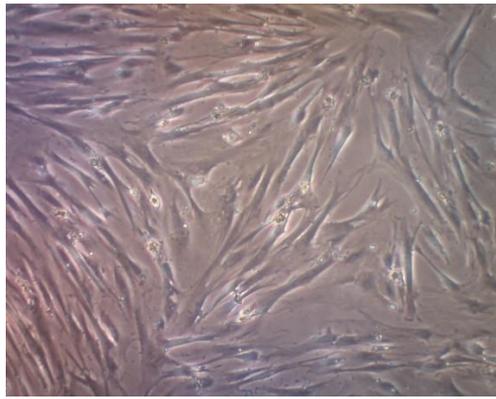


Figure 3 Microscopic images of adult *Macaca fascicularis* 5 peripheral blood mesenchymal stem cells (PoD24Mf5 MSPRO)

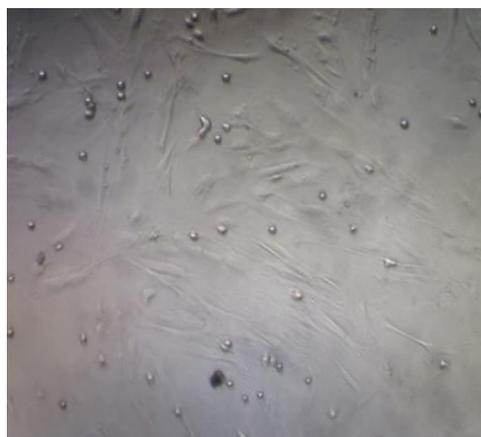


Figure 4 Microscopic images of adult *Macaca fascicularis* 6 peripheral blood mesenchymal stem cells (PoD27Mf6 MSPRO)

collected must appropriate for the primate animal laboratory safety and health. According to Diehl et al, (2001), the blood volume of circulating blood was 6% - 8% of the *Mf* body weight. The blood taken via an intravenous catheter in Vena Femoralis in this experiment were 1 ml for infant, 5 ml for juvenile and 10 ml for adult *Mfs*. The different volume of blood taken from each age group of the *Mfs* were due to different their body weight. However, the volume of blood sampled in this experiment were lower in comparison to Hai-jing et al. (2007) which used 15 ml of bone marrow for stem cells source isolate although it had known that bone marrow contains more stem cells than peripheral blood circulation due to its function for red blood cell production (Champlin et al., 2000). It explained why the 2 sample of peripheral blood male from Juvenil *Macaca fascicularis* and 3 samples of peripheral blood male Infant *Macaca fascicularis* can not be made PBMSC pellets.

Insufficient number of blood sampling and stress due to blood sampling process in infant and juvenile

are higher than adult *Mfs*. Some researcher advised to use general anesthesia that its to prevent the occurrence of hemoconcentration, lymphocytosis and neutrophilia (Wang et al. 2012). However, this condition could damage stem cells in the peripheral blood circulation (Ives et al., 1956).

Lack of information on mother condition during pregnancy was also another reason for the high variation of PBMNC concentration obtained. During pregnancy, *Mfs* need a comfortable environment (Clarke et al., 1994) and less stress. Attention to enrichment so as to avoid the psychic and physical stress that will affect the fetal *Macaca fascicularis* during the infant to juvenile periods (Cohen et al., 1992) should be in placed. The stressful state of the female parent *Macaca fascicularis* during pregnancy (Stavisky et al., 2003) increased the glucocorticoid hormone causing instability of *Macaca fascicularis* fetus, Infant and Juvenil (Kay et al., 2000); (Weinstock 1996). In mid-pregnancy, the hormone cortisol from the mother passed onto fetus through the placenta (Pepe et al. (1987); Walsh et al. (1979)), led to

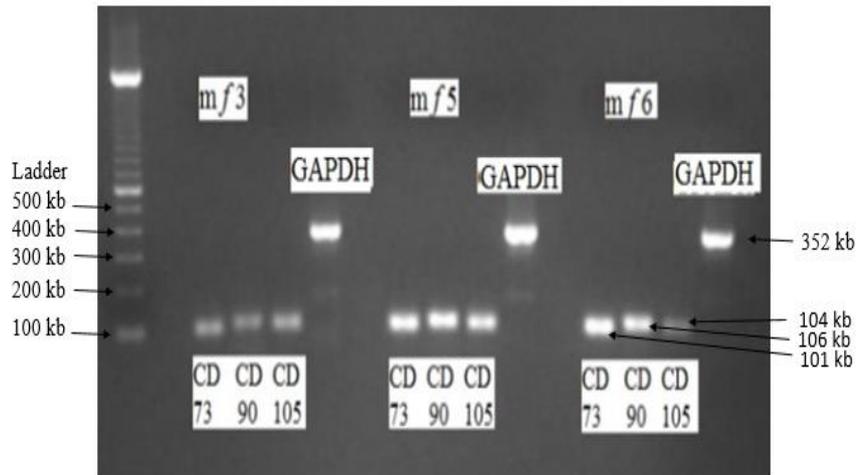


Figure 5 The positive markers of adult male *Macaca fascicularis* 3.5.6 peripheral blood mesenchymal stem cells

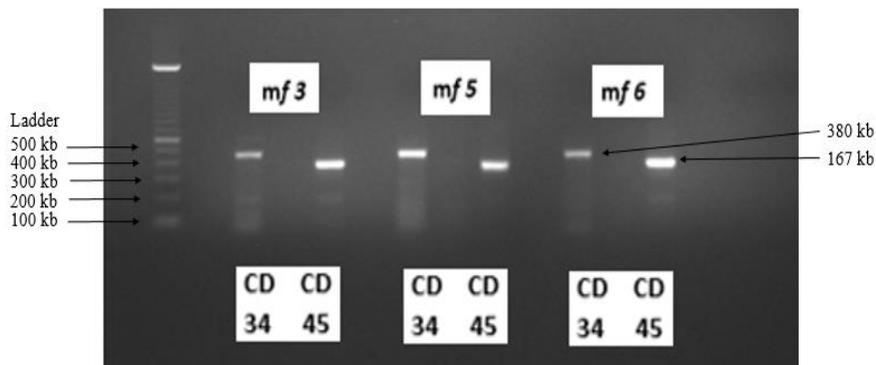


Figure 6 The negative markers of adult male *Macaca fascicularis* 3.5.6 peripheral blood mesenchymal stem cells

the suppression of receptors of cortisone in hippocampus and the axis of the adrenal pituitary hypothalamic fetus. Increasing cortisone hormone in fetuses reduced lymphocytes proliferation and cytolytic ability (Coe *et al.* (1996); Eishi *et al.* (1983); Kay *et al.* (1998); Klein *et al.* (1995); Murphy *et al.* (1994)), decreased receptor proinflammatory cytokines (Reyes *et al.* (1997); Scheinman *et al.* (1995)) such as interleukin IL-6, tumor necrosis factor (TNF) α and IL-1 β (Franchimont *et al.* (1999); Miller *et al.*, (1999a); Miller *et al.* (1999b)). Stress in the pregnant *Macaca fascicularis* can occur immediately (acute) during pregnancy and chronic (post partum), juvenile until age 2 years (Althaus *et al.*, 1986); (Reyes *et al.*, 1997).

Although quarantine prior to sampling have been conducted, however long-term effect of previous condition such as temperature, cage condition, age, genetic, feeding habit and nutrient status of the Mfs before, during and after pregnancy were also influence the amount of PBMNC harvested from

their offspring (Wang *et al.*, 2012). According Drevon-Gaillot *et al.* (2006), geography factor as origin of *Macaca fascicularis* have specific characteristic research that influence the results. The Mfs from Mauritania, Philippines and Vietnam produced different results.

The mesenchymal stem cells processing were started with peripheral blood harvesting to select PBMNC, followed by MSC isolation and identification using positive and negative markers, mRNA extraction and GAPDH as a control of cDNA (Baltimore (1970); Gillespie *et al.* (1965); Kessler (1992); Temin *et al.* (1970)). The time of 80% confluency PBMSC reached difference from the Haijing *et al.*, 2007 finding on Bone marrow stem cells/BMSC, but the morphology spindle cells were seen almost the same on day 18th. The quantification of mRNA extract obtained in this experiment were accurate due to the reverse transcription quantification polymerase chain reaction (RTqPCR) technique used.

According to Bustin (2008) and Murphy et al. (2009), the method was accurate due to its real-time RNA detection, rapid identification, screening, classification and RNA monitors.

From this study, it can be concluded that adult *Macaca fascicularis* peripheral blood can be harvested, isolated and cultured for peripheral blood mesenchymal stem cell source in this *in vitro* study. For future research, it is suggested to increase the number of infant and juvenile used as well as their mother pregnancy information record.

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“All authors declare that there are no conflicts of interest”.

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