ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

# Wharton's Jelly Derived Mesenchymal Stem Cells: A Comparison Study in Preterm vs. Term Deliveries and in FBS vs. PRP vs Mesencult Culture Media

**Gita Pratama[1, 2*] Ari Khusuma[3], Suhartiningsih[4], Silvia Werdhy Lestari[5], Indra Gusti Mansur[5], Angliana Chouw[6], Yanni Dirgantara[6], Cynthia Retna Sartika[6]**

1. *Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*
2. *Stem Cell Medical Technology, Integrated Service Unit, Dr. Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*
3. *Mataram Health Polytechnic, Ministry of Health, West Nusa Tenggara, Indonesia.*
4. *Regional Health Laboratory, Province of DKI Jakarta.*
5. *Department of Biology of Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*
6. *Prodia Stem Cell Indonesia, Jakarta, Indonesia.*

## Abstract

Background: Use of human mesenchymal stem cells (MSCs) for therapy in the field of degenerative medicine has been extensively studied. One of the potential sources of MSC which is easily available is from the human umbilical cords Wharton's jelly of the newborn baby. Wharton's jelly derived MSCs (WJ-MSCs) from full-term labor has been isolated and differentiated, but very limited data came from preterm labor. This study aimed to compare the proliferation and differentiation characteristics between human WJ-MSCs from preterm and term labor using xeno-free culture media, particularly platelet rich plasma (PRP). Methods: WJ-MSCs from preterm and term deliveries was cultured using culture medium supplemented with 10% PRP or MSC or FBS culture media. Cells were cultured until the fifth passages. Cumulative cell numbers and differentiation capacity of the MSCs was tested according to International Society for Cellular Therapy (ISCT) criteria. Results: The preterm WJ-MSCs grown in culture media is not significant differences with WJ-MSCs derived from full-term labor, but have higher number of cell populations. WJ-MSCs are able to differentiate into osteocyte, chondrocyte and adipocyte., and xeno-free media can be used to replace FBS. WJ J-MSCs from preterm labor can be used as a source for mesenchymal stem cells. Conclusion: WJ-MSCs collected from preterm samples to have a better proliferation rate compared to term samples, while possessing equal differentiation capacity. In addition, PRP can replace FBS as it a xeno-free material for WJ-MSCs production that required for regenerative medicine.

**Keywords:** *Mesenchymal stem cells, Wharton's jelly, Preterm labor, Proliferation, Differentiation.*

## Introduction

Stem cells have shown a great potential for treatment of numerous degenerative diseases [1].However due to the limited use of embryonic stem cells, researchers are looking for other alternatives source of stem cells, eg. From adipose and perinatal tissue. Mesenchymal stem cells (MSCs) are adult multipotent stem cells that have been isolated from many source of tissues [2, 3].Recently many studies have focused on MSCs from human umbilical cord (UC-MSCs), an essential embryo structure that provides the nutrients for the fetus during intrauterine development. The cord consists of two arteries and one vein, surrounded by a unique connective tissue stroma between umbilical cord blood vessels derived from an epiblast called Wharton's jelly [2, 4].Umbilical cord is considered as a waste and disposed during labor makes it interesting for regenerative therapy studies and development. The umbilical cord can be taken through non-invasive procedure without risking the donors during labor [4].

Wharton's jelly derived stem cells (WJ-MSCs) have lower immunogenecity compare to other cell in the body, therefore it does not require 100% HLA matched for allogeneic use [4, 5]. Stem cells populations which are collected from Wharton's jelly can be propagated and harvested easily and show high telomerase activity and self life [6]. Many studies have isolated MSC from full term labor Wharton's jelly used for clinical application of degenerative diseases. On the other hand, using Wharton's jelly umbilical cord from preterm labor is still rare. In addition, culture medium with serum supplementation such as fetal bovine serum (FBS) is performed, but it encloses animal serum therefore the application is diminished [7].

Platelet rich plasma (PRP), is a lysate consisting growth factors which play role in proliferation and differentiation of stem cells in vitro, could be an alternative culture medium for MSCs [8]. Application of PRP in WJ-MSCs is very less performed, therefore this research is essential to determine the effectiveness of PRP on WJ-MSCs culture compared to FBS and Mesencult as a commercial medium. This study was aimed to compare the proliferation and differentiation of WJ-MSCs from preterm and term labor in FBS, PRP and Mesencult media.

## Methods

This study was an in vitro cell culture experimental analytic study, conducted at Prodia Stemcell Indonesia Laboratory (ProStem), Jakarta, Indonesia from February 2016 until September 2016. Umbilical cords were collected from pregnant women who volunteered to donate the umbilical cord following cesarean section at Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. The samples were obtained from 5 preterm and 5 term samples. This research has been approved by Health Research Ethics Committee of Faculty of Medicine of Universitas Indonesia-Ciptomangunkusumo Hospital.

### Samples

The umbilical cord samples were cut with the length approximately 10 cm and stored inside sterile container containing transport medium. Samples were processed within 12-24 hours from labor in laboratory. WJ-MSCs were isolated aseptically under Biological Safety Cabinet Type II.

Samples were washed with PBS to remove the red blood cells amniotic fluid. The umbilical cord was cut into 3-5 cm length pieces and divided into half. The arteries and veins were removed and Wharton's jelly was collected into conical tube. Enzyme collagenases 3 mg/ml were added to the samples. Samples were incubated for one hour at 37°C at 5% $CO_2$ until the enzymatic digestion is completed to obtain single cells from Wharton's jelly. Complete medium was added at the end of the incubation period and samples were centrifuged at 2500 rpm for 5 min. Cell's pellet were seeded in culture plate to isolate the WJ-MSCs. (Figure 1 of supplement data)

### Culture of WJ-MSCs

The WJ-MSCs were cultured using DMEM-HG supplemented with 10% fetal bovine serum (FBS) or 10% platelet rich plasma (PRP), and commercial MSC differentiation medium Mesencult (Stem cell Technology, Canada). The PRP was obtained from Indonesian Red Cross. Antibiotic-antimycotic with concentration of 100U penicilin and 100 µg Streptomycin were added to the complete medium to prevent the growth of bacteria and fungus. Primary cells were cultured using 6 well plates containing culture medium according to each treatment.

Observations were conducted every day to see the cell growth and signs of contamination. Every 3-4 days the culture medium was replaced. Cultures were maintained for 21 days, until WJ-MSCs began to colonize and reach confluence. When the confluency reaches approximately 70-80%, cells were harvested and subcultured. Cell numbers were counted using hemocytometer. Subcultures was carried out until passage 5 with the calculation of population doubling time (PDT) in each passage.

### Characterization of WJ-MSCs

The characterization was performed by flowcytometry towards the surface marker of WJ-MSCs. Cells were harvested, filtered, pelleted and suspended ($10^6$) in 1 mL of PBS and put in a 5-mm tube for unstained. The sample was poured conjugated antibodies against CD73, CD90, CD105 as positive marker and CD11b, CD19, CD34 and CD45 as negative marker, and was incubated for 20 minutes in lightless condition.

The suspension, then, was washed off using 1 ml of PBS. After the washing, it was put in flow tube placed on BD FACSCanto II machine using BD FACS Diva software for further analysis (BD Biosciences, Europe).

### Differentiation of WJ-MSCs

Cells were harvested in appropriate differentiation medium, either for osteogenic (DMEM F12, FCS (10)%, $\beta$-glycerophosphate (10mM), dexamethasone (10 nM), L-ascorbic-acid (50 $\mu$M) ); chondrogenic (DMEM+Gluta MaxTM−1, FCS (2%), gentamicin (10 $\mu$g/mL−1), ITS (1%), ascorbic-acid (0.1mM), dexamethasone (10 nM), and transforming growth factor $\beta$1(TGF-$\beta$1) (10 ng/mL-1) ) and adipogenic (DMEM F12, FCS (10)%, insulin-transferrin-selenium-X (ITS) (1 %) (GibcoUK), isobutylmethylxanthine (0.5 $\mu$M) (Sigma, UK), dexamethasone (1 $\mu$M), and indomethacin (100 $\mu$M). (Mennan C et al., 2013) Then the cells were fixed and stained with Oil Red O (Abcam, United States) for adipogenesis, Alizarin Red S (Merck, Germany) for osteogenesis and for Alcian blue staining (Abcam, United States) for chondrogenesis.

### Statistical Analysis

The distribution of the data was analysed using Kolmogorof-Smirnov or Shapiro Wilk and homogenecity test. If the data was normally distributed then T-Independent Test was used, otherwise Mann-Whitney was used.

## Results

Microscopic observations were performed to see the growth of WJ-MSCs in culture plate with different supplemented media. DMEM + 10% PRP and Mesencult for xeno-free media and DMEM +10% FBS as control. On the first day following isolation, the cells look round and some cells began to attach on the bottom of the plate. Morphological changes on the 7th day after passage in the two samples did not give a different morphological picture of either passage 3 or passage 5.

Cells were able to grow on all uses of medium with fibroblastic cell morphology with sufficient cells to passage. Microscopic observations showed that WJ-MSCs preterm and term samples were able to grow after passage. The planted cells successfully grow to produce fibroblastic morphological cells both in passages 3 and 5. Fibroblastic cells are seen with a picture of cells that are long and flat and the others are slightly rounded with protruding cytoplasm.

Cell populations are small in size and some others show larger cell sizes. Cells also appear to colonize and accumulate in several parts (Figure 1).

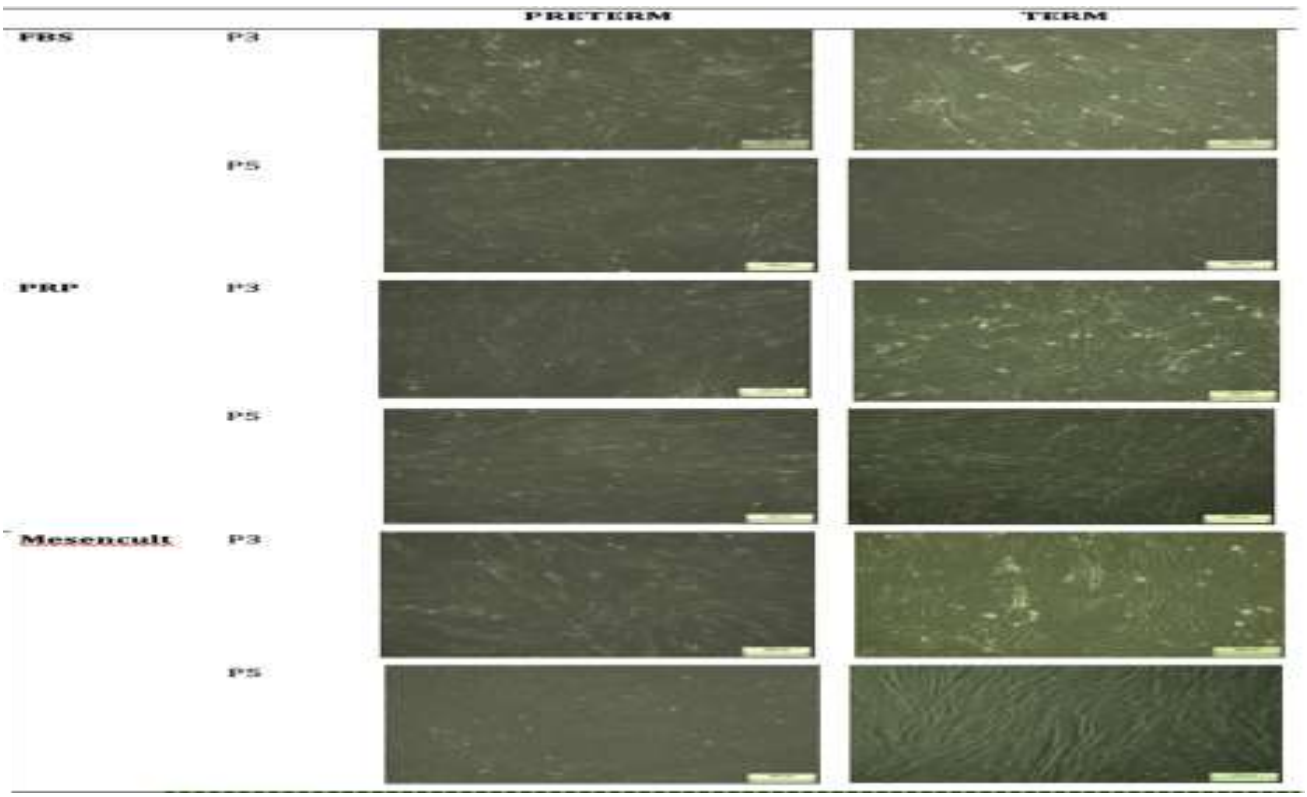



**Figure 1: WJ-MSCs morphology of passages 3 and 5**

WJ-MSCs from preterm sample were able to grow until passage 5 using DMEM supplemented with 10% FBS, supplemented with 10% PRP, and Mesencult with a total cell count of 61.20 x $10^6$, 91.09 x $10^6$, and 58.61 x $10^6$, respectively (Fig. 2A). High PDT values are shown in passage 2 and decreasing until passage 5. The use of PRP as supplementation yields the lowest PDT value (0.946) compared to Mesencult (1.006) and FBS (1.000) (Fig. 2B).

WJ-MSCs from term sample were able to grow until passage 5 with a total cell count of 42.95 x $10^6$ (FBS), 36.63 x $10^6$ (PRP), and 82.88 x $10^6$ (Mesencult) (Fig. 2A). Similar with the preterm samples, the PDT value is highest in passage 2 and continued to decrease until passage 5. The use of Mesencult medium suplementation resulted in the lowest PDT value (0.825) compared with FBS (0.914) and PRP (0.939)(Fig. 2B).

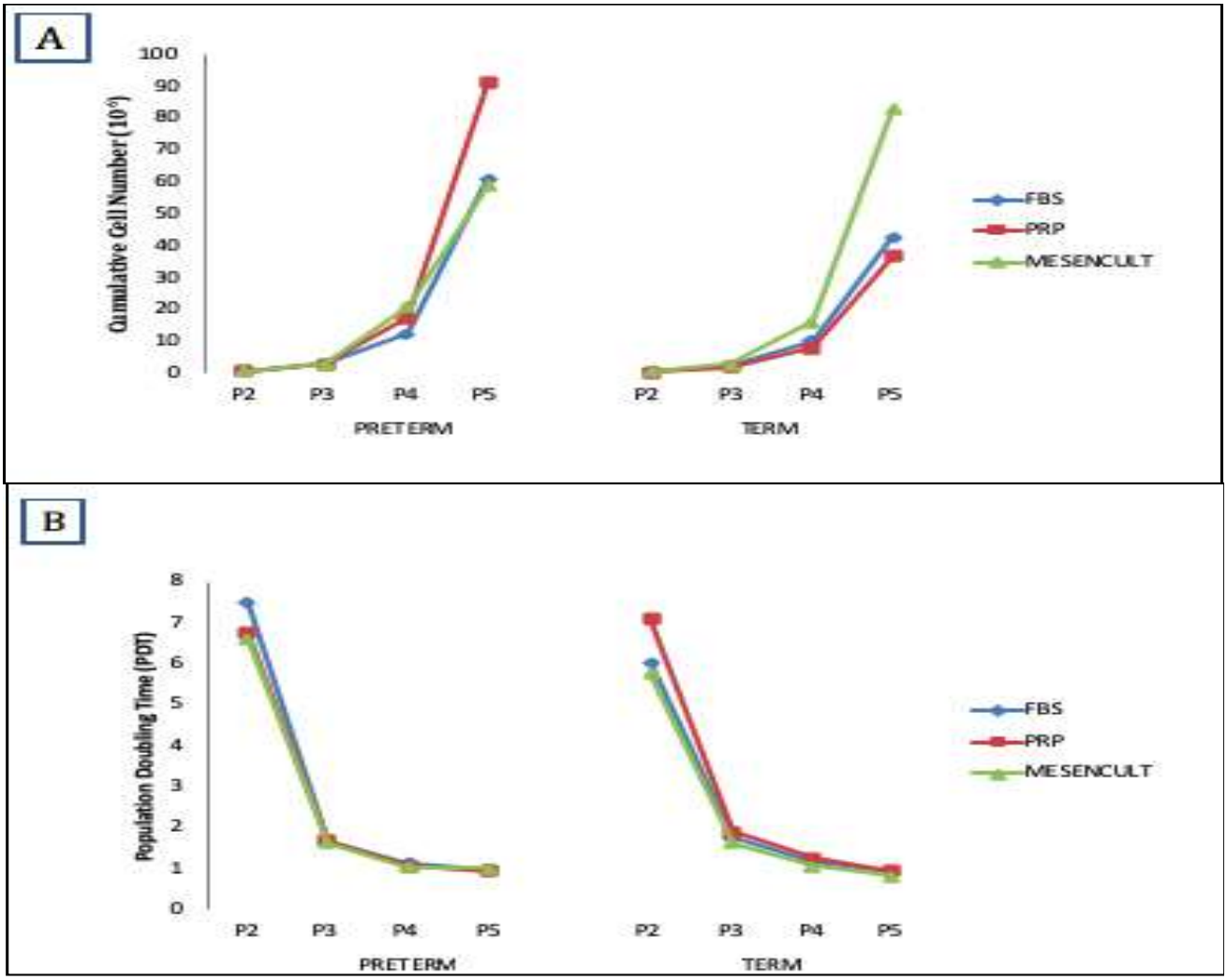



**Figure 2: Comparison of WJ-MSCs s in different supplemented media (A). Cumulative cell numbers (B). Population doubling time (PDT)**

In term samples, the used of Mesencult has the highest number of cells produce in the end of the passage 5, whereas in preterm samples, PRP produced the highest number of cells. Statistical analysis showed that there were no significant differences ($p>0.05$) between all supplemented media in each sample (Figure 2 of supplement data) A similar pattern is shown in the PDT value of each treatment. Mesencult has the lowest PDT value in the aterm WJ-MSCs, whereas the PRP has the lowest PDT value in the preterm WJ-MSCs.

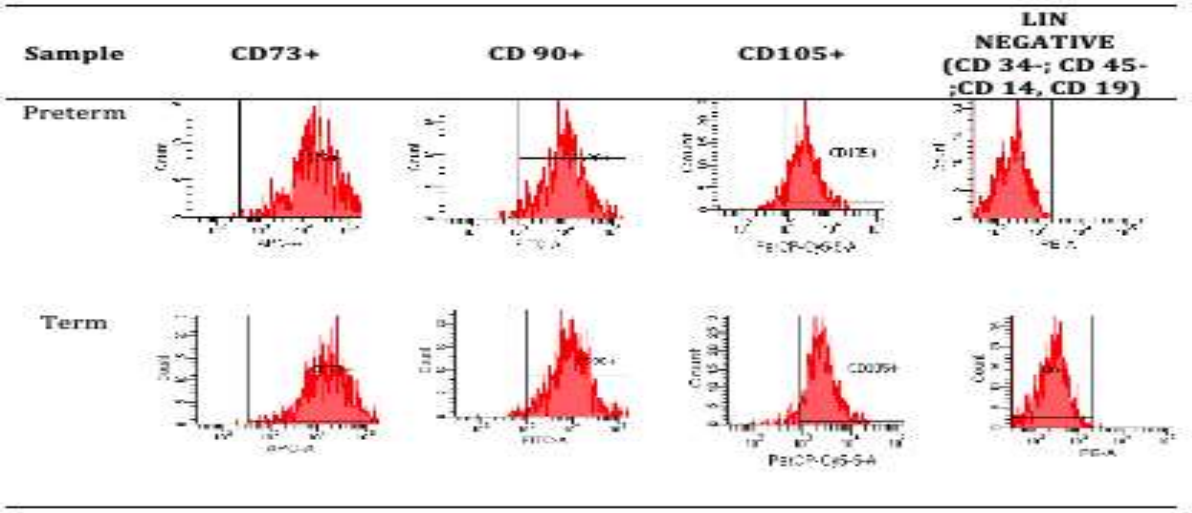
In addition, other analysis result show that there is no significant difference ($p>0.05$) between preterm and term samples. Although from the cumulative cell number, preterm WJ-MSCs appear to have a tendency to produce more cells than aterm WJ-MSCs.

## Characterization of WJ-MSCs

The characterization of WJ-MSCs was performed by flowcytometry. More than 90% of WJ-MSCs were positive for the MSC

markers, CD73+/CD90+/CD105+, and were negative for hematopoietic and endothelial cell markers, CD45-/CD34-/CD11b-/CD19 (Figure 3).

A




B

| Sample | CD73+ (%) | CD 90+ (%) | CD105+ (%) | LIN NEGATIVE (CD 34-; CD 45-;CD 14, CD 19) (%) |
|---|---|---|---|---|
| Preterm | 98.9 | 98.3 | 91.4 | 0 |
| Term | 99.2 | 97.6 | 93.7 | 0.1 |

**Figure 3: Characterization of WJ-MSCs**

## Differentiation of WJ-MSCs

The observation of cell differentiation on preterm and term WJ-MSCs in different supplemented media showed that the WJ-MSCs were able to undergo osteogenic, chondrogenic and adipogenic differentiation (Fig. 4).

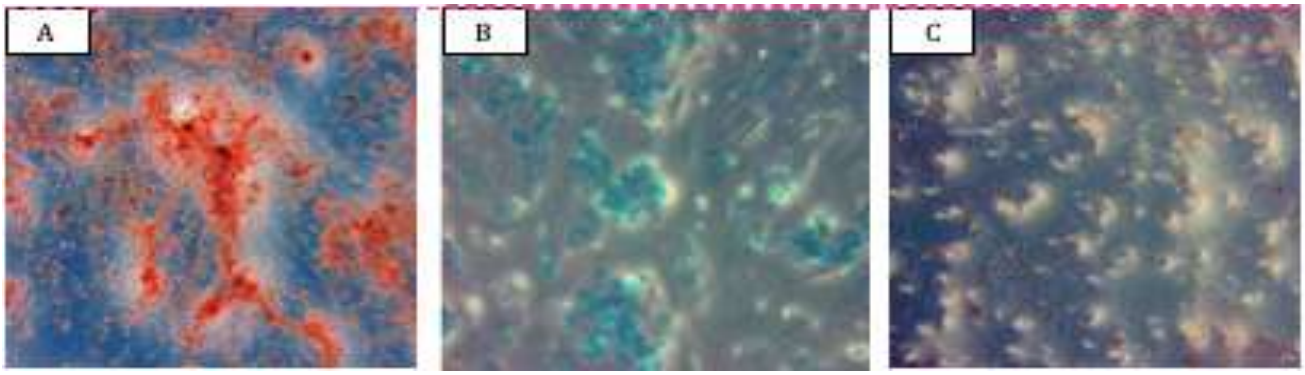



**Figure 4: WJ-MSCs differentiation (A). Osteocytic differentiation, stained with Alizarin Red S (B). Chondrocytic differentiation with Alcian blue staining.(C). Adipocytic differentiation, stained with Oil Red O**

Preterm and term samples of WJ-MSCs are able to differentiate into osteocyte, chondrocyte, and adipocyte cells. WJ-MSCs in passage 3 and 5 shows ability to differentiate into all specific lineages as International Society for Cellular Therapy (ISCT) recommendation.

## Discussion

In this study, isolation of WJ-MSCs was conducted with 5 samples each for both preterm and term samples. However, only 3 samples from preterm and 3 samples from term labor were successfully isolated. Cultures from other 4 samples were discarded due to contamination. Bacterial or fungal contamination often occurred although exclusion of infection risks such as vaginal delivery, premature rupture of the amnion membrane and chorioamnionitis was performed [6, 9].

## Proliferation in Preterm and Term

A low PDT value indicates that the time spent for cell to proliferate is faster. PDT values in term samples are lower than

preterm samples. These results are consistent with other studies examining angiopoietic factors found to be lower in UC-MSCs in preterm samples compared to term samples [10]. This circumstances triggers a pathological development of the tissues/ organs formed. However, Agarwal S et al demonstrated that MSCs derived from the term and preterm UC have similar characteristics [11].

The PDT value in both preterm and term WJ-MSCs of each passage is decreasing. In passage 5, WJ-MSCs still continue to proliferate but there has been no sign of increased PDT value compared to the passage before. This finding was similar with some studies which showed that umbilical cord derived MSCs is capable of proliferating until passages 10 [4, 12].The amount of time that needed for cells to proliferate is related to the metabolism rate of the cells. The more rapid the metabolism, the fasterthe proliferation rate of the cell.

This condition may be influenced by the growth medium used and the quality of the stem cell source itself. Cell proliferation occurs when cells establish intercellular communication and cell migration. The cell bonds that are formed will produce new cells and increase in number so as to increase the number of cells through cell attachment [13]. However, excessive cell growth is feared might trigger stem cell transformation toward the malignancy cells [14, 16].

**Proliferation in Different Medium**

We found that with the same seeding density, WJ-MSCs cultured in Mesencult growth medium grew more rapid compared to WJ-MSCs in DMEM with both PRP or FBS supplementation on culture day 7. This may be due to the content of growth factors and others within Mesencult which has been standardized and is stable so that it is appropriate to support the WJ-MSC proliferation.

However, in other studies, it has been proven that PRP shows more proliferation performance in cultures in the UC-MSCs passage 1-3, compared to Mesencult media [17]. In this study, WJ-MSC proliferation in DMEM with PRP supplementation was similar to FBS. These results are in accordance with other studies which state that PRP is similar to FBS in MSC proliferation in vitro [18]. This might to be due the standardization of growth factors in PRP has not been standardized so that it becomes less than optimal for cells to grow.

In addition, other causes can also be caused by cells in PRP competing with WJ-MSC to grow on culture plates. Proper monitoring of quality of PRP must be determined so that it can be used as an alternative growth media supplement for MSC that requires xeno-free conditions when it is used for clinical applications [19, 20].Our study also proves that WJ-MSCs have a high proliferation capacity to produce large numbers of cells that might be required for cell therapy.

**Characterization of WJ-MSCs**

In the flowcytometry characteristics test, it can be seen that both the term and preterm samples express positive for mesodermal antigens including CD73 + / CD90 + / CD105 +, and negative for hematopoietic antigens CD45- / CD34- / CD11b- / CD19, which means they meet the ISCT criteria. In this study, it turns out that both the term and preterm sample groups express specific surface antigens for mesenchymal stem cells and form a homogeneous cell population graph especially after passage 5.

This proves that WJ-MSCs cells from the preterm umbilical cord can be used as cell sources mesenchymal stem. The results of this characteristic test also prove that the expression of mesenchymal stem cell surface antigen in WJ is not affected by gestational age at the time the sample is taken.

This is in accordance with research conducted by Anzalone et al [21] and Messerli et al [22] which stated the same thing [21, 22].Positive mesodermal antigens in table 3.1 can be seen that the levels are increasing at passage 5, even exceeding 95%. Only on CD105 + values vary somewhat. This has also been reported by other researchers some even stated that CD105 expression depends on sample conditions [23].

**Differentiation of WJ-MSCs**

In WJ-MSCs differentiation study, both cell from preterm and term proliferate and become confluent after 12 days of incubation. The result of osteogenic differentiation is a visualization of red cells morphology which shows calcium deposits in the matrix with red.alizarin staining.

The concentration of calcium inside the cells can affect the cell becoming osteoblasts through the interaction of cell matrix with cells or between cells. The cell morphology is cuboid-shaped, mononuclear and have high-posterior alkaline activity [24].

The result of condrogenic differentiation is a condensed cells morphology in preterm and term WJ-MSC. This appearance is formed by the accumulation of glucosaminoglycans which are stained by alcian blue staining. Condensed cells have a core at the edges with a slightly rounded cytoplasm. This study show same result with Li G et al research that the condroblast cells will undergo certain matrix activities and develop cartilage [25].

Furthermore, in the differentiation of WJ-MSC into adipocytes, positive cell shown through small fatty grains in Oil Red O staining. Small fatty grains in the the accumulation of small lipid then developed into pre-adipocytes and turn into mature adipocytes. Higuchi M, et al successfully performed with the staining technique is an early marker for cell differentiation testing [26].

There is no known difference between the preterm and term WJ-MSCs tested for differentiation capacity of osteocytes, chondrocytes and adipocytes, which were confirmed through specific cell staining. All isolated WJ-MSCs are able to differentiate into mesodermal lineage. This is consistent with other studies and ISCT criteria for MSCs.[3, 6, 9].Other study stated that the differentiation capability of WJ-MSCs do not depend on the sex of the baby or methods of labor, but mostly affected by the regulation of transcription factors, cytokines, growth factors, and extra cellular components in the growth medium [27].

In addition, the time needed by the WJ-MSCs to differentiate is influenced by the gestational age of the samples [28]. The use of various supplements such as FBS, PRP, and Mesencult might affect the number of cells growth although in this study show no significant between all treatments. Cell differentation is not affected by those replacement of supplement [7, 29, 30].

## Conclusion

In conclusion, WJ-MSCs collected from preterm labor samples to have a better proliferation rate compared to WJ-MSCs collected from term samples, while possessing equal differentiation capacity into osteocyte, chondrocyte and adipocyte. Therefore, WJ-MSCs from preterm labor may be a potential source for cell therapy. In addition, PRP can replace FBS as it a xeno-free material for WJ-MSCs production required for regenerative medicine [31].

## References


1. Damayanti S, Triana R, Chouw A, Dewi NM (2017) Is Stem Cell a Curer or an Obstruction?. Mol Cell Biomed Sci., 1: 1(1):17.
2. Kim D-W, Staples M, Shinozuka K, Pantcheva P, Kang S-D, Borlongan CV (2013) Wharton's Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic Potential for Clinical Applications. Int J Mol Sci., 31: 14(6):11692-712.
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F., Krause DS, et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8(4):315-7.
4. Nekanti U, Mohanty L, Venugopal P, Balasubramanian S, Totey S, Ta M (2010) Optimization and scale-up of Wharton's jelly-derived mesenchymal stem cells for clinical applications. Stem Cell Res., 5(3):244-54.
5. Lina Y, Wijaya A (2012) Novel Sources of Fetal Stem Cells for Future Regenerative Medicine. Indones Biomed J., 1: 4(1):3.
6. Freshney RI, Stacey G, Auerbach JM, editors (2007) Culture of human stem cells. Hoboken, N.J: Wiley-Interscience; 343. (Culture of specialized cells).
7. Suryani D, Pawitan JA, Lilianty J, Purwoko RY, Liem IK, Damayanti L (2013) Comparison of fetal bovine serum and platelet-rich plasma on human lipoaspirate-derived mesenchymal stem

cell proliferation. Med. J. Indones., 22: 146.

8. Kocaemer A, Kern S, Kluter H, Biebeck K (2007) Human ab serum and thrombin-active PRP are suitable alternative to fetal calf serum for expantion of MSC from adipose tissue. Stem Cells, 25: 1270-8.
9. Kassis I, Zangi L, Rivkin R, Levdansky L, Samuel S, Marx G, et al (2006) Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplant, 37(10):967-76.
10. Szwajkiewicz DG, Ulanczyk Z, Zagrodnik E, Luczkowska K, Roginska D, Kawa MP, et al (2020) Differential secretion of angiopoietic factors and expression of microRNA in umbilical cord blood from health appropriate-for-gestational-age preterm and term newborns - in search of biomarkers of angiogenesis-related processes in preterm birth. International journal of molecular sciences, 21(1305):1-24.
11. Agarwal S, Winter C, Corral A, Mustafa SB, Hornsby and Moreira A (2018) Comparison of preterm and term Wharton's jelly-derived mesenchymal stem cell properties in differing oxygen tensions. Cell Tissues Organs, 205(3):137-150.
12. Mediana D, Liem I.K, Pawitan J.A, Goie N (2015) Passage effect on aging of human umbilical cord derived mesenchymal stem cell. Online Journal of Biological Sciences, 15(3):170.177
13. Bilic G, Zeisberger SM, Mallik AS, Zimmermann R, Zisch AH (2008) Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. Cell Transplant, 17(8):955-68.
14. Ren H, Sang Y, Zhang F, Liu Z, Qi N, Chen Y (2016) Comparative Analysis of Human Mesenchymal Stem Cells from Umbilical Cord, Dental Pulp, and Menstrual Blood as Sources for Cell Therapy [Internet]. Stem Cells International. [cited 2018 Dec 1]. Available from: https://www.hindawi.com/journals/sci/2016/3516574/
15. Jung S, Sen A, Rosenberg L, Behie LA (2012) Human mesenchymal stem cell culture: rapid and efficient isolation and expansion in a defined serum-free medium. J Tissue Eng Regen Med., 6(5):391-403.
16. Lou Z, Chen J (2006) Cellular senescence and DNA repair. Exp. Cell Res., 15: 312(14):2641-6.
17. Budiyanti E, Liem IK, Pawitan JA, Wulandari D, Jamaan T, Sumapradja K (2015) Umbilical cord derived mesenchymal stem cell proliferation in various platelet rich plasma and Xeno-material containing medium. International journal of research in pharmaceutical sciences, 6(1):7-13.
18. Hashemi SS, Raft AR (2016) Comparison between human cord blood serum and platelet rich plasma supplementation for human Wharton's jelly stem cells and dermal fibroblast culture. International Journal of medical research & health sciences, 5(8):191-196.
19. Neagu M, Suciu E, Ordodi V, Pâunescu V (2005) Human mesenchymal stem cells as basic tools for tissue engineering: isolation and culture. Romanian J. Biophys., 15(1-4):29-34.
20. Sang Hwa Lee, Young Sun Sohn, Yong Woon Choi, Nam Ho Park, Hyun Joong Yoon (2010) Culture of Mesenchymal Stromal Cells from Dental Pulp: Culture Medium Study for Effective Expansion and Characterization < 논문상세 < 페이퍼서치. Tissue Eng. Regen. Med., 7(2):248-54.
21. Anzalone R, Timoneri F, Rocca ML, Iacono ML, Amico G, Corsello T et al (2014) Isolation And Phenotypical Characterization of Mesenchymal Stem Cell From The Wharton's Jelly Of Preterm Human Umbilical Cord. I J A E., 119: 1.
22. Messerli M, Wagner A, Sager R, Mueller M, Baumann M, Surbek DV, et al (2013) Stem Cells From Umbilical Cord Wharton's Jelly From Preterm Birth Have Neuroglial Differentiation Potential. Reproductive Sciences, 20(12):1455-64.
23. Mafi P, Hindocha S, Mafi R, Griffin M, Khan WS (2011) Adult mesenchymal stem cell Surface Characterization-a systematic review of the literature. Open Orthop J., 5(2):253-60

24. Shetty P, Cooper K, Viswanathan C (2010) Comparison of proliferative and multilineage differentiation potentials of cord matrix, cord blood, and bone marrow mesenchymal stem cells. Asian J. Transfus. Sci., 4(1):14-24.

25. Li G, Zheng B, Meszaros LB, Vella JB, Usas A, Matsumoto T, et al (2011) Identification and characterization of chondrogenic progenitor cells in the fascia of postnatal skeletal muscle. J. Mol. Cell Biol., 1: 3(6):369-77.

26. Higuchi M, Dusting GJ, Peshavariya H, Jiang F, Hsiao ST-F, Chan EC, et al (2013) Differentiation of Human Adipose-Derived Stem Cells into Fat Involves Reactive Oxygen Species and Forkhead Box O1 Mediated Upregulation of Antioxidant Enzymes. Stem Cells Dev., 15;22(6):878-88.

27. Gupta A, El-Amin SF, Levy HJ, Sze-Tu R, Ibim SE and Maffulli N (2020) Umbilical cord-derived Wharton's jelly for regenerative medicine applications. Journal of orthopaedic surgery and research, 15(49):1-9.

28. Penolazzi L, Vecchiatini R, Bignardi S, Lambertini E, Torreggiani E, Canella A, et al(2009) Influence of obstetric factors on osteogenic potential of umbilical cord-derived mesenchymal stem cells. Reprod Biol Endocrinol RBE., 5 (7):106.

29. Rauch C, Feifel E, Amann E-M, Spötl HP, Schennach H, Pfaller W, et al (2011) Alternatives to the use of fetal bovine serum: human platelet lysates as a serum substitute in cell culture media. ALTEX, 28(4):305-16.

30. Pratama G, Vaghjiani V, Tee JY, Liu YH, Chan J, Tan C, et al (2011) Changes in culture expanded human amniotic epithelial cells: implications for potential therapeutic applications. PloS One, 6(11):e26136.

31. Mennan C, Wright K, Bhattacharjee A, Balain B, Richardson J, Roberts S (2013) Isolation and characterization of mesenchymal stem cells from different regions of the human umbilical cord. Biomed Research International, 1-8.