

A Comparative Assessment of CD271 Immunohistochemical Expression in Mesenchymal Stem Cells from Early-Term and Postdate Human Umbilical Cord

<https://doi.org/10.62940/als.v13i3.2994>

Issue: Volume 13, Issue 3

Received: 17-12-2023

Revised: 07-08-2026

Accepted: 26-08-2026

Published online: 30-09-2026

Keywords: Embryonic stem cell, Human umbilical cord, Wharton jelly, Biomarker p75NTR, Oxidative stress

Ali Shakir Abed^{1,*}, Haider Abdulrasool Jaafar²

1. College of Pharmacy, Al-Zahraa University for Women, Karbala, Iraq

2. Department of Anatomy, college of medicine, Al- Nahrain University, Baghdad, Iraq

* alish9921@gmail.com

ABSTRACT

Background: The CD271 biomarker has been identified with more specificity for the categorization and detection of Mesenchymal Stem Cells (MSCs) in the Human Umbilical cord (HUC). This marker appears to be insufficient for the separation of MSCs from various tissues, like the blood of the umbilical cord (UC), Wharton's jelly (WJ), and other regions. Objective of the study was to estimate quantitatively the immunohistochemical (IHC) expression of CD271 of (MSCs) in the early term and postdate of HUC.

Methods: The samples were taken from 50 normal vaginal deliveries. 25 of the UC samples were classified as normal early term labour and 25 as normal postdate labor. The newborn was born between 37 and 38 weeks (6 days) in both early-term HUCs. In a postdate HUC, delivery is granted an extension beyond 40 weeks plus one to 13 days (any time after the estimated due date of delivery). The age range of pregnant women was 18 to 30. The mother's pregnancy and vaginal delivery were normal, and they were singleton newborns. The sections of UC were stained with CD 271 IHC expression.

Results: IHC expression of CD271 in MSCs was significantly higher in the postdate UC (25295.08 ± 6062.32) compared to the early UC (20653.24 ± 5728.09). Because oxidative stress may coexist with the early term that shows decreased MSCs in UC, this is likely because oxidative stress destroyed the cells. The MSCs are distributed throughout the WJ in a jelly-like substance made of several proteoglycan isoforms and collagen fibers.

Conclusion: The IHC expression of CD271 has been the most expressed for MSCs detection. CD271 reveals an adequate method for insulation of the MSCs in UC. In response to oxidative stress, there was vascular endothelium damage to the UC, which led to less abundant MSCs in early term labor.

INTRODUCTION

The human Umbilical Cord (UC) is two arteries and a vein coated in an embryonic jelly connective tissue known as Wharton jelly (WJ), which is covered by amniotic epithelium. That contains a very interesting population of MSCs inside a rich extracellular matrix (ECM). UC contorted formation the joins fetus with placenta. The UC has inherent features perfect for obtaining MSCs because of their use without the need for ethical approval; it is easy to obtain, and is a rich source for MSCs, as shown by WJ and the blood of the UC [1,2]. Certain studies demonstrated that CD271 did not aid in the isolation of MSCs from the blood of UC [3]. The ineffectiveness of the CD271 label for the isolation of MSCs from WJ's prior culture [4]. Others studied the emphasis of CD271 in fragments of WJ, observing it to be expressed weakly [2].

METHODS

Human umbilical cord samples were procured from the labor rooms of Al- Imamain Al-Kadhimiyain Medical City and the Gynecology and Obstetrics Teaching Hospital in Karbala. The collection of samples from 50 umbilical cords (UC) associated with normal vaginal deliveries was partitioned into 25 UC identified as normal early term and 25 UC designated as normal postdate labour. In the early term, labour was recorded between 37 weeks and 38 weeks and 6 days, whereas deliveries of human umbilical cords that were postdate occurred beyond 40 weeks, extending up to 13 days. Expectant mothers were aged 18 to 30 years. A lone newborn resulting from a mother with an ordinary pregnancy and a routine vaginal delivery. Transverse sections of umbilical cord samples were stained immunohistochemically with CD271 marker for the expression of MSCs. The procedure was done according to what was received from the manufacturing company Biorbyt in China for the primary antibody for CD271 and the staining kit from Abcam in the United Kingdom. The results were evaluated using the Aperio of Image Scope V12 positive pixel total algorithm program, which measures the quantity of a certain color for detecting CD271 protein.

This study was specifically designed to compare umbilical cord characteristics between early term deliveries (37 weeks to 38 weeks and 6 days) and post term pregnancies (40 weeks, + 1 to 13 days). The aim was to investigate the potential effects of relatively shorter versus prolonged intrauterine exposure. Therefore, standard full-term pregnancies were not included, as they fall outside the primary comparison groups defined in this study.

Immunohistochemical Protocol for Anti-CD271

For antigen retrieval, tissue pieces were heated in a microwave for 20 minutes with a standardized citrate buffer (pH 6.0) prior to immunohistochemical analysis. After blocking endogenous peroxidase activity, the sections were incubated overnight at 4°C with a primary anti-CD271 antibody (Abcam, UK) at a dilution of 1:200. The sections were then treated with the secondary antibody, visualized with DAB staining chromogen and contrast stained with hematoxylin [5].

Statistical Analysis of Data

For all statistical analyses, the data was analysed using the SPSS software application (version 21). The data is presented as the mean $\pm$ a standard deviation (SD). A t-test with independent parameters was employed to evaluate the mean values [data presented as (M $\pm$ SD)]. P values > 0.05 proved to be statistically not significant and P values of 0.05 or under 0.05 were regarded as significant findings.

RESULTS

The immunohistochemical expression CD271 of mesenchymal stem cells reveals high significant values in postdate umbilical cord which recorded (25295.08 $\pm$ 6062.32) than that of early term umbilical cord which recorded (20653.24 $\pm$ 5728.09) (table1, figure 1).

Detection of the Mesenchymal Stem Cells in early term of UC

Immunohistochemical expression of CD271 of mesenchymal stem cells located in the early term of the umbilical cord appears less abundant cell among their matrix filled with Wharton's

jelly within intercellular spaces and evaluated by the Aperio software program (figure 2).

Detection of the Mesenchymal Stem Cells in Postdate of UC

Mesenchymal cells have been expressed CD271 that is specific for the stem cells as they present in postdate of umbilical cord and appear more abundant cells among their matrix that are filled with Wharton's jelly in intercellular spaces which had been evaluated by Aperio software program and these stem cells are originated from mesenchymal mesoderm, endothelium cells of blood vessels and cord blood cells (figure 3).

Figures

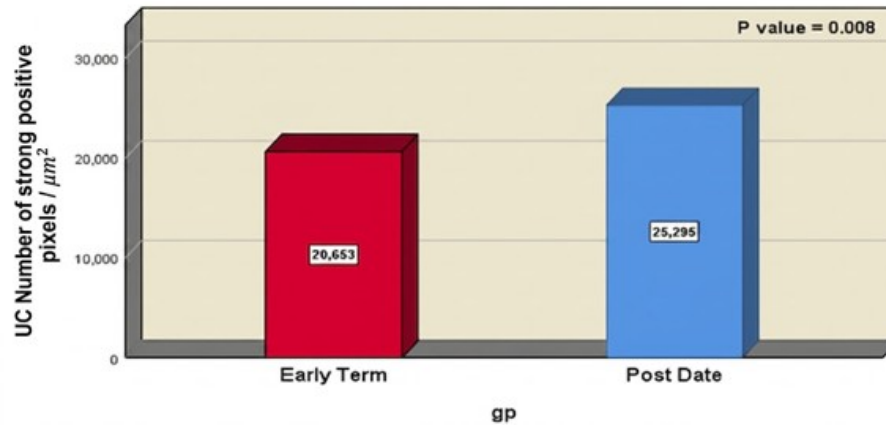


Figure 1: Immunohistochemical expression of CD271 of Mesenchymal Stem Cells in early term Umbilical Cord in comparison to the postdate umbilical cord.

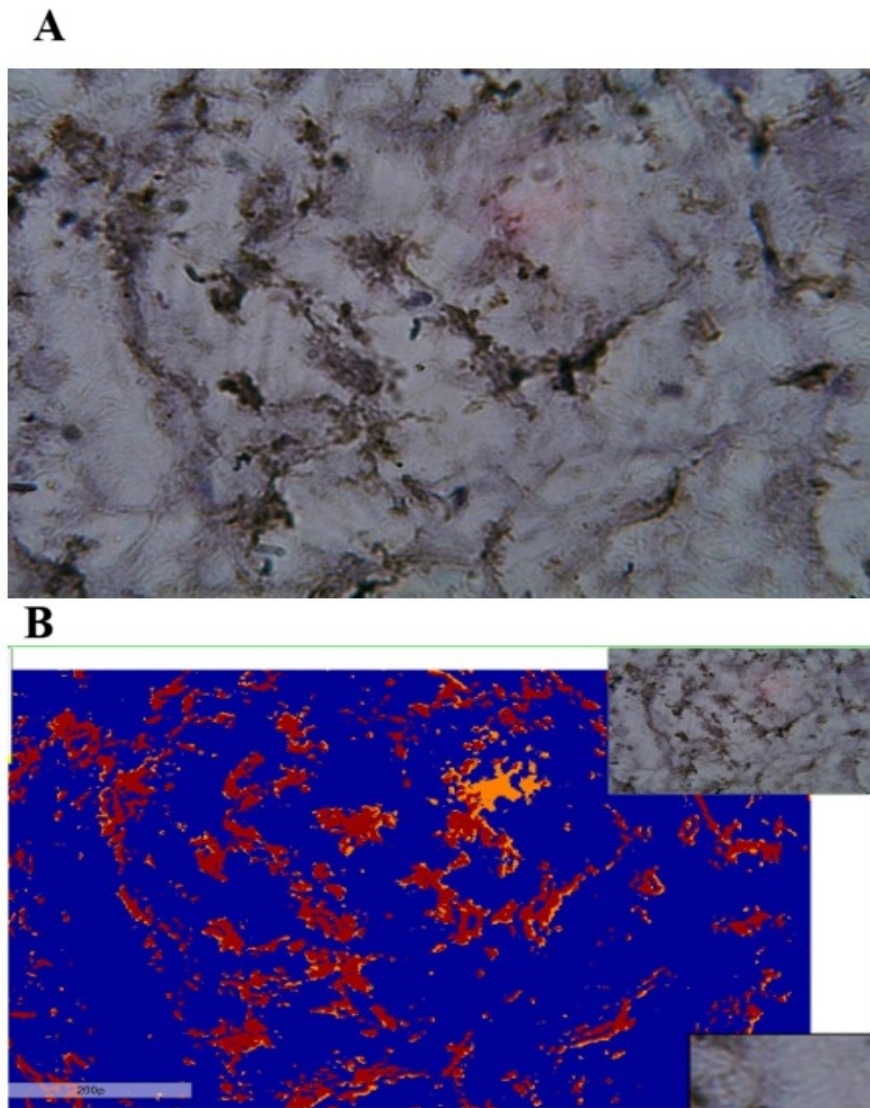


Figure 2: This figure reveals the brown coloration immunohistochemical expression of CD271 of mesenchymal stem cells in early term of umbilical cord disperse with WJ (A) [400X]. Evaluated by Aperio software Program (B).

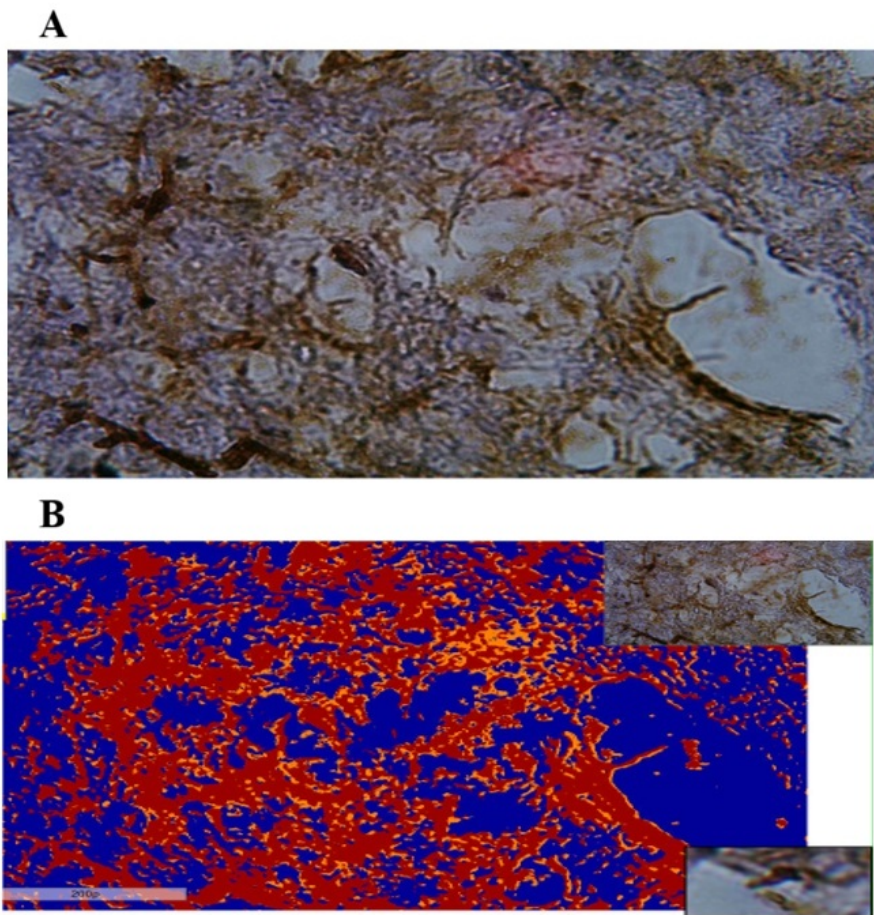


Figure 3: Cross-sectional view in the Umbilical cord showing the distribution of CD271 protein in the brown coloration the immunohistochemical expression of (MSCs) in postdate of (UC) disperse with WJ [400X]. Evaluated by Aperio software (B) program.

Tables

Group	Number	Mean ± SD	P Value
Early Term	25	20653.24 ± 5728.09	0.008
Post Date	25	25295.08 ± 6062.32	

Table 1: Immunohistochemical expression of CD271 of Mesenchymal Stem Cells within postdate Umbilical Cord in comparison to the early term umbilical cord. Independent t-test, P value of ≤ 0.05 is considered significant.

DISCUSSION

Specificity of Mesenchymal Tissue Stem Cells in Umbilical Cord Applying Immunohistochemical Expression CD271

In the current study, MSCs located in the UC cord were identified by immunohistochemical (IHC) expression of CD271. The results showed that there was significant expression of MSCs in both postdate and early-term umbilical cords, with higher expression in the postdate group. These results agree with previous reports that have described the presence of MSCs in many tissues such as BM, adipose cells, liver tissue, skeletal muscle tissue, amniotic fluid, blood from umbilical cords and oral pulp [2,6]. The wide distribution of MSCs in different tissues highlights their vital biological importance and supports the notion of UC as an important reservoir of these cells.

Wharton's Jelly Mesenchymal Stem Cells

The present investigation established the existence of MSCs in the Wharton's jelly of the HUC by the immunohistochemical expression of the CD271 marker. The results are comparable with earlier research which have shown the isolation of MSCs from various types of tissues including human umbilical cord [7]. The MSCs from Wharton's jelly are recognized as the major source for regeneration therapy because of their great self-regenerative ability, multi-lineage differentiation potential and immunomodulatory capabilities [8]. The jelly of the Wharton's is mostly proteoglycans and collagen fibers suspended in a gelatinous matrix. The major function of Wharton's jelly is to prevent the constriction and twisting of the umbilical veins throughout intrauterine life, while permitting appropriate blood flow, oxygen and nutrition delivery to the developing fetus and removal of carbon dioxide and metabolic waste products [9].

Mesenchymal stem cells extracted from the Wharton's jelly of the umbilical cord discarded have attracted considerable attention in tissue engineering applications and medical regeneration because of its easy accessibility and cryopreserved for future therapeutic applications. In the present study, CD271 expression was successfully detected in Wharton's jelly tissues. However, some previous studies reported weak expression of CD271 in freshly isolated Wharton's jelly samples and suggested that this marker may not be optimal for the isolation of MSCs before culture [2,4].

Previous investigations have shown that mesenchymal stem cells obtained from various tissue origins, such as bone marrow and umbilical cord tissues, typically express markers such as CD44 and CD105, yet lacking the hematopoietic markers CD34 and CD45. These cells also exhibit plastic adherence under standard culture conditions. In addition, CD271 has been identified as an important marker associated with MSCs characterization and isolation [10]

Mesenchymal Stem Cells in the Umbilical Cord

In the present study, we found that MSCs were found scattered throughout the umbilical cord tissue and that they had spindle-shaped morphology and were not arranged in a specific manner in either the cytoplasmic or nuclear regions. These finding are different from those of previous studies that showed that there were distinct stem cell populations in the umbilical cord, a feature that distinguishes them from other cell types that are derived from blood [11]. Several authors proposed that MSCs derived from umbilical cords have high molecular and morphological similarity with those derived from bone marrow, including the absence of hematopoietic cell markers and similar biological properties [11]. However, the existence of MSCs in umbilical cord blood (UCB) remains controversial. Some investigators have questioned the existence of MSCs in UCB, stating that MSCs are more prevalent in early fetal blood and are markedly reduced at later gestational ages, which limits the detection and isolation of MSCs from samples of umbilical cord blood obtained from term births [12]. The difficulties of extracting MSCs form umbilical cord blood has been attributed by alternative research as the reason for their comparatively diminished prevalence in relation to bone marrow-derived MSCs. This reduced abundance has raised concerns regarding the suitability of umbilical cord blood as a reliable source of MSCs. Nevertheless, a number of scientists have successfully extracted and defined mesenchymal stem cells from umbilical cord blood in vitro, proving their potential to develop into many cell lineages including osteogenic, chondrogenic, neurogenic and hepatogenic cells [13, 14]. In the present study, the CD271 marker was utilized to detect MSCs in the UC. In the present study, the CD271 marker was used to detect MSCs in the UC which is consistent with previous studies that used CD271 for the identification of these cells in umbilical cord blood (UCB). However, other studies have shown that CD271 is not a significant marker for the isolation of MSCs from UC blood suggesting that it is not sufficient for direct application to UC tissues [15]. The findings of the present study indicate a reduced yield of mesenchymal stem cells (MSCs) in umbilical cords (UC) from early-term labors, a phenomenon likely continuous with oxidative stress-induced cellular damage. This is in agreement with previous studies that physiological levels of reactivity oxygen species (ROS) are important modulators of multiple cellular functions, while a homeostatic equilibrium with antioxidants is maintained in normal conditions. Conversely, the excess generation of free radicals overcomes the capacity of the antioxidant defense system, leading to oxidative stress that impairs healthy cells. This oxidative environment leads to the degradation of RNA and DNA, subsequently deteriorating biological functions, accelerating cellular aging, and ultimately triggering cell death [16,17]. The present study shows reduced yield of UC MSCs in early-term labor, which may be mediated by oxidative stress. This finding is in agreement with previous reports on the effect of

fetal oxidative stress, where increased free radical production has been strongly associated with initiating early labor [18,19].

MSCs Of UC In Postdate and Early Term with Oxidative Stress Factors

We found that the number of MSCs was lower in early-term than in postdate deliveries, probably because of oxidative stress in early-term labor. This may give us a clue to the disease pre-eclampsia, a severe disorder of gestation that leads to hypertension, proteinuria and early labor. Defective placental development and impaired perfusion generate oxidative stress, releasing unbalanced pro- and anti-angiogenic factors into the maternal bloodstream. This circulation of factors damages the vascular endothelium, leading to clinical manifestations of pre-eclampsia. The decidua of the placenta acts as a principal origin of reactivity of oxygen species (ROS) that contribute to this endothelial injury. Crucially, the intrinsic cytoprotective properties of MSCs are activated in such vascular distress, indicating that placental and UC-MSCs are active players in the modulation of oxidative stress in normotensive and pre-eclamptic pregnancies [20]. Further studies have shown that MSCs can downregulate oxidative stress responses induced by inflammatory processes in several pathological situations, including acute kidney injury. Moreover, MSCs have been shown to downregulate senescence-associated protein expression, thereby promoting tissue repair and improving recovery in acute kidney injury cases [21].

The immunohistochemical expression of the CD271 marker described has been labeled as the most accurate indicator for mesenchymal stem cell detection. This marker is specifically shown by these cells; therefore, this marker reveals an adequate method for insulation of the mesenchymal stem cells in UC. The mesenchymal stem cells are among the WJ, which is a gelatinous material made up of multiple isoforms of collagen fiber and proteoglycans. It is a protector of the arteries and veins and prevents twisting and compression. It has a bidirectional flow, providing oxygen and nutrients while eliminating waste and carbon dioxide from the fetus. The umbilical cord blood of the UC reveals CD271 marker expression as stem cells, which are shared in hemopoiesis and angiogenesis in the UC, and the blood of the UC is a special and affluent resource for endothelial precursor cells. In response to oxidative stress, there was widespread vascular endothelium damage as MSCs in the UC were less abundant in the early term labor. Premature labor (early term) is likely due to oxidative stress; this therefore expands the width of the blood vessels as umbilical vein increases the amount blood returning to the fetus.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in publicizing this study.

AUTHOR CONTRIBUTIONS

Ali S.A. conceived and designed the study, performed the cell isolation and immunohistochemistry experiments, analyzed the data, and drafted the initial manuscript.

Haider A.J. participated in tissue sample collection, contributed to data interpretation and statistical analysis, supervised the research, and critically revised the manuscript. Both authors have read and approved the final version of the manuscript.

ETHICS STATEMENT

This research study is approved by the Research Ethical Committee of the College of Medicine, Al-Nahrain University, Iraq vide letter

Ref no. [UNCOMIRB20241240](#).

REFERENCES

1. Gervaso F, Boschetti F, Pennati G. Evaluation of the Wharton's jelly poroelastic parameters through compressive tests on placental and foetal ends of human umbilical cords. *Journal of the Mechanical Behavior of Biomedical Materials*, (2014); 35: 51–58.
2. Stoltz JF, Magdalou J, Netter P, Pinzano A, Margossian T, et al. Mesenchymal stem cells derived from Wharton's jelly: comparative phenotype analysis between tissue and in vitro expansion. *Bio-Medical Materials and Engineering*, (2012); 22(4): 243–254.
3. Attar A, Ghalyanchi Langeroudi A, Vassaghi A, Ahrari I, et al. Role of CD271 enrichment in the isolation of mesenchymal stromal cells from umbilical cord blood. *Cell Biology International*, (2013); 37(9): 1010–1015.
4. Alvarez-Viejo M, Menendez-Menendez Y, Blanco-Gelaz MA, Ferrero-Gutierrez A, Fernandez-Rodriguez MA, et al. *LNGFR (CD271) as marker to identify mesenchymal stem cells from different human*

- sources: Umbilical cord blood, Wharton's jelly and bone marrow. *Journal of Bone Marrow Research*, (2013); 1(4):132.
5. Magaki S, Hojat SA, Wei B, So A, Yong WH. An Introduction to the Performance of Immunohistochemistry. *Methods in Molecular Biology*, (2019); 1897:289-298.
 6. Hong SJ, Jia SX, Xie P, Xu W, Leung KP, et al. Topically delivered adipose derived stem cells show an activated-fibroblast phenotype and enhance granulation tissue formation in skin wounds. *PLoS ONE*, (2013); 8(1): e55640.
 7. Hassan G, Kasem I, Antaki R, Mohammad MB, AlKadry R, Aljamali M. Isolation of umbilical cord mesenchymal stem cells using human blood derivatives accompanied with explant method. *Stem Cell Investigation*, (2019); 6: 28.
 8. Drobiova H, Sindhu S, Ahmad R, Haddad D, Al-Mulla F, et al. Wharton's jelly mesenchymal stem cells: a concise review of their secretome and prospective clinical applications. *Frontiers in Cell and Developmental Biology*, (2023); 11: 1211217.
 9. Taghizadeh R, Cetrulo K, Cetrulo C. Wharton's jelly stem cells: future clinical applications. *Placenta*, (2011); 32(Suppl 4): S311–S315.
 10. Liu S, Hou KD, Yuan M, Peng J, Zhang L, et al. Characteristics of mesenchymal stem cells derived from Wharton's jelly of human umbilical cord and for fabrication of non-scaffold tissue-engineered cartilage. *Journal of Bioscience and Bioengineering*, (2014); 117(2): 229–235.
 11. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *British Journal of Haematology*, (2000); 109(1): 235–242.
 12. Yu M, Xiao Z, Shen L, Li L. Mid-trimester fetal blood-derived adherent cells share characteristics similar to mesenchymal stem cells but full-term umbilical cord blood does not. *British Journal of Haematology*, (2004); 124(5): 666–675.
 13. Zhang Y-N, Lie PC, Wei X. Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. *Cytherapy*, (2009); 11(5): 548–558.
 14. Liu G, Ye X, Zhu Y, Li Y, Sun J, et al. Osteogenic differentiation of GFP-labeled human umbilical cord blood derived mesenchymal stem cells after cryopreservation. *Cryobiology*, (2011); 63(2): 125–128.
 15. Watson JT, Foo T, Wu J, Moed BR, Thorpe M, et al. CD271 as a marker for mesenchymal stem cells in bone marrow versus umbilical cord blood. *Cells Tissues Organs*, (2013); 197(6): 496–504.
 16. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, (2008); 4(2): 89.
 17. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, et al. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, (2017); (1): 8416763.
 18. Prasad S, Tiwari M, Pandey AN, Shrivastav TG, Chaube SK. Impact of stress on oocyte quality and reproductive outcome. *Journal of Biomedical Science*, (2016); 23: 1–5.
 19. Schoots MH, Gordijn SJ, Scherjon SA, van Goor H, Hillebrands J-L. Oxidative stress in placental pathology. *Placenta*, (2018); 69: 153–161.
 20. Kusuma GD, Georgiou HM, Perkins AV, Abumaree MH, Brennecke SP, et al. Mesenchymal stem/stromal cells and their role in oxidative stress associated with preeclampsia. *Yale Journal of Biology and Medicine*, (2022); 95(1): 115.
 21. Rodrigues CE, Capcha JMC, de Bragança AC, Sanches TR, Gouveia PQ, et al. Human umbilical cord-derived mesenchymal stromal cells protect against premature renal senescence resulting from oxidative stress in rats with acute kidney injury. *Stem Cell Research and Therapy*, (2017); 8: 1–17.



This work is licensed under a Creative Commons Attribution- NonCommercial 4.0 International License. To read the copy of this license please visit: <https://creativecommons.org/licenses/by-nc/4.0/>