Evaluation of the Impact of Pregnancy-Associated Factors on the Quality of Wharton’s Jelly-Derived Stem Cells Using SOX2 Gene Expression as a Marker

Paulina Gil-Kulik, Małgorzata Świstowska, Arkadiusz Krzyżanowski, Alicja Petniak, Anna Kwaśniewska, Bartosz J. Plachno, Dariusz Galkowski, Anna Bogucka-Kocka and Janusz Kocki

1. Introduction

Mesenchymal stem cells (MSCs), especially obtained from perinatal tissues, including Wharton’s jelly of umbilical cord have been an excellent source of stem cells used in regenerative medicine during the last decade. MSCs derived from Wharton’s jelly (WJSC) possess a number of valuable properties including the high ability of self-renewal and differentiation, fast proliferation rate, immunosuppressive and paracrine properties and high expression of factors specific to embryonic stem cells e.g., SOX2 or POU5F1. Additionally, the umbilical cord is considered to be a safe and abundant resource for MSCs, and sampling does not raise any ethical concerns. In recent years WJSC have been the subject of numerous studies in both pre-clinical and clinical phases [1,2]. However, there is still a lack...
of in-depth information regarding the mechanisms affecting the regulatory properties of these cells, as well as the factors determining their therapeutic potential. The study focused on the evaluation of one expression of the pluripotency factors—SOX2 expression, at the mRNA level. The SOX2 gene (SRY-Related HMG-Box Gene 2) belonging to the SOX gene family was discovered and described by Stevanovic et al. in 1994. It has been mapped to 3q26.3-27 on the long arm of chromosome 3 [3]. It encodes the SOX2 protein consisting of 317 amino acids which contain the HMG domain, characteristic of all SOX proteins. The domain binds to the ATTGTT motif in the DNA [4–7]. In embryonic stem cells, the level of expression of transcription factors SOX2, OCT4 and NANOG impacts self-renewal, maintenance of pluripotency and reprogramming of somatic cells [8–10]. It also directs the differentiation of pluripotent stem cells to neural progenitors, as well as maintaining the properties of neural progenitor cells [11]. SOX2 is a vital gene participating in maintaining cell pluripotency [9,11]. Its mechanism of action involves activation and maintenance of the OCT4 gene expression [12]. The SOX2 protein, when interacting with the OCT4 forms a heterodimeric complex responsible for activation and silencing transcription processes of genes in control of cell differentiation [13]. The cell remains undifferentiated if there is an adequate level of SOX2 and even small changes in the SOX2 and OCT4 gene expression may cause the loss of pluripotency [11]. A drop in SOX2 expression leads to the differentiation of cells into trophoderm [14], while its increase causes differentiation into mesoderm and trophoderm [15]. There are numerous reports recording the presence of SOX2 gene expression in human stem cells derived from different tissues, including perinatal [16–18], adipose [19,20], bone marrow [19,21], dental pulp [22], mammary gland [23,24] skin and heart muscle [20]. Many factors may influence gene expression and SOX2 protein level. The effect depends, among other factors, on the type of cell and the degree of differentiation. SOX2 protein concentration is regulated at many levels, including transcription, post-transcription and post-translation [25]. Horizontal gene transfer occurs through a process of self-regulation involving feedback mechanism, as well as mutual cross-regulation between SOX2 and OCT4, OCT4 and NANOG [26]. Current research indicates that the high proliferation potential is caused (at least partially) by the inhibitory effect of SOX2 on genes involved in cell cycle regulation such as CCND1 and CDK4 genes [27].

SOX2 is considered irreplaceable and is required for the normal functioning of stem cells and their maintenance in an undifferentiated state as well as self-renewal [28]. In addition, it has been shown that SOX2 also affects migration and cell adhesion [29], SOX2 pituitary stem cells can hold additional roles in tissue expansion and homeostasis, acting as paracrine signaling centers to coordinate the proliferation of neighboring cells [30]. Research suggests that the presence of high levels of SOX2 is related to the therapeutic potential of stem cells, and the prospect of modulating SOX2 expression to achieve therapeutic benefit seems to be promising [31]. Understanding the molecular mechanisms governing SOX2 functions will facilitate the use of pluripotent stem cells for clinical and biomedical applications, with particular emphasis on the modeling and treatment of various neurological disorders [11].

Factors influencing the SOX2 gene expression in stem cells are still being intensively studied and there is a high demand for more data. The discovery of new factors influencing the expression of SOX2 may contribute to better utilization of the cells taken from the patient, as well as better preparation and maintenance of cells in vitro culture.

The effect of maternal and neonatal factors on the quality of umbilical cord blood stems cells have been a subject of numerous studies [31–40]. One of the deciding factors in the usefulness of cord blood is the number of CD34+ stem cells. Studies involving umbilical cord blood stem cells have shown, inter alia, that the number of CD34+ cells depends on the number of previous live births and the body weight of the newborn, and is better with good weight and first babies, and decreases with subsequent births [41]. It was also observed in several studies that the gestational age negatively correlates with the number of CD34+ cells [42,43], and the number of CD34+ stem cells is significantly higher in premature babies [44]. Bielec–Berek et al. assessed the correlations between
maternal age and selected properties of umbilical cord blood stem cells. Moreover, in this study, the correlation between the mode of delivery, the age of the mother and the quality of the obtained material for transplantation was assessed. The older the women from whom umbilical cord blood was collected, the lower the mean concentration of HSC cells in the material [37]. Aufderhaar et al. showed that perinatal factors such as low blood pH and prolonged first stage of labor correlate with increases in CD34+ cells and cord blood progenitor cells [45]. There is insufficient research related to the influence of these factors on the quality of mesenchymal stem cells of Wharton’s jelly of the umbilical cord. Available literature does not provide sufficient information on the influence of maternal age, birth weight and duration of pregnancy or the physicochemical properties of cord blood on the expression of the SOX2 gene in stem cells derived from umbilical cord Wharton’s jelly. Therefore, in our work, we have decided to evaluate these factors. The study aims to bring closer the knowledge of MSC biology, the combination of in vivo factors necessary to maintain the state of undifferentiation, self-renewal potential and stem cell proliferation. Finding the optimal maternal age and other perinatal parameters for collecting and banking the highest quality material.

In view of the potential therapeutic benefits that may result from mesenchymal stem cell transplantation widening viewers, understanding how to maintain cell viability and in vitro pluripotency trait is of primary importance, bridging the gap in the knowledge of the specific origin of MSCs, from Wharton’s jelly as opposed to into umbilical cord blood.

The aim of the study was to assess the expression of the SOX2 gene in mesenchymal stem cells of Wharton’s jelly at the transcript level. The study also examined the effect of a patient’s age, pregnancy length, birth weight and cord blood parameters on SOX2 gene expression in the examined material.

2. Results

2.1. Cell Culture and Cytometric Analysis

Using flow cytometry (Figures 1 and 2) and cell culture under adhering conditions (Figure 3A,B), mesenchymal character of the analyzed cells was demonstrated. The cytometric test confirmed the presence of surface antigens characteristic for MSC on the tested cells, such as: CD73, CD90, CD105 and CD145. During cell culture, the adherence capacity to the plastic walls and the fibroblast-like shape of the analyzed cells were confirmed.

2.2. SOX2 Gene Expression Analysis

The presence of the SOX2 gene transcript was shown in all examined mesenchymal Wharton’s jelly stem cells. The results showed that the expression of the SOX2 gene in WJSC varies significantly depending on maternal age. In women aged 34 years and younger, significantly higher expression of the SOX2 gene was recorded in comparison to women over 34 years of age (p = 0.005) (Figure 4A). The analysis of the correlation between gestational age and the SOX2 gene expression revealed a negative correlation (r = −0.55, p < 0.05) (Figure 5a). Significant differences in the expression of the SOX2 gene in WJSC have been noted in relation to the time of delivery. In the group of women who gave birth before the due date, the expression of the SOX2 gene in Wharton’s jelly mesenchymal stem cells was statistically significantly higher compared to women who gave birth in due course (p = 0.002) (Figure 4B). An analysis of a correlation between the SOX2 gene expression and the week of pregnancy in which the birth took place discovered a significant negative correlation (r = −0.43, p < 0.05) (Figure 5b). A statistically significant negative correlation between the SOX2 gene expression level and the birth weight (r = −0.47, p < 0.05) was noted (Figure 5c). An analysis of the SOX2 gene expression in relation to the umbilical cord blood pH showed a significantly negative correlation between the SOX2 gene expression level and the cord blood pH (r = −0.46, p < 0.05) (Figure 5d). Furthermore, it was observed that the SOX2 expression in WJSC is statistically significantly higher at pH 7.35 (p = 0.02) or lower (Figure 4c). An analysis of the SOX2 gene expression in relation to the oxygen and carbon dioxide pressure showed a statistically significant negative correlation of the SOX2 gene
expression with \( pO_2 \) \( (r = -0.44, p < 0.05) \) (Figure 5f) and a statistically significant positive correlation of the SOX2 expression level with \( pCO_2 \) \( (r = 0.57, p < 0.05) \) (Figure 5e). The investigation did not show the impact of the delivery route, drugs used during pregnancy and delivery on the SOX2 gene expression in WJSC.

![Figure 1](image1.png)

**Figure 1.** Expression of CD73, CD90, CD105, and CD146 surface antigens in Wharton’s jelly stem cells. Cytometric analysis carried out on a MoFlo XDP cell sorter (Beckman Coulter). Analysis of minimum 10,000 events was recorded for each probe.

![Figure 2](image2.png)

**Figure 2.** Single stem cells, antigens (CD90, CD73, CD146, CD105 positive and CD34, CD45-negative). Photographs of single samples of cells, presenting microscope image (BF) and fluorescence in channels, showing the expression of studied antigens (FlowSight f. Amnis flow cytometer).

![Figure 3](image3.png)

**Figure 3.** (A). Stem cells from a 4-day culture (B). Stem cells from a 10-day culture; bright field microscopy (BF), 100× magnification (Xcellence RT system with an IX81 inverted microscope Olympus).
Figure 4. Average expression of SOX2 gene (logRQ ± SE) in WJSC (A). depending on the parturient’s age; (B). depending on delivery time; (C). depending on umbilical cord blood pH. *, $p < 0.05$ U Mann Whitney test.
Figure 5. SOX2 gene expression (logRQ) on (a) the parturient’s age (b) the week of pregnancy in which the birth took place, (c) newborn’s birth weight, (d) pH of the umbilical cord blood, (e) carbon dioxide pressure, (f) oxygen pressure. * The Spearman Rank Order.

3. Discussion

The authors present the study showing the expression of the SOX2 gene, regarded as one of the main factors of Wharton’s jelly stem cell pluripotency factors to be contingent on the parturient’s age, the maternal age, birth weight, the pH of the umbilical cord blood, carbon dioxide pressure and oxygen pressure.

The SOX2 expression in WJSC was statistically significantly higher at the pH of umbilical cord blood equal to or lower than 7.35. They also have shown a correlation between the SOX2 gene expression and the physicochemical parameters of umbilical cord blood. The SOX2 expression was increased at lower O2 and higher CO2 levels of umbilical cord blood. The SOX2 level was increased with a decrease in cord blood pH. Obradovic et al. showed that Wharton’s jelly stem cells cultured in vitro at 3% oxygen concentration showed higher
expression of SOX2 compared to cells cultured at 21% oxygen concentration. It was also noted that lower oxygen concentration increases in vitro migration ability of culture and enhances the activity of proteolytic enzymes, as well as protecting the cell from harmful factors. The authors theorize that low oxygen concentration enhances WJ-MSC multipotency by stimulating their self-renewal and increasing the expression of the pluripotency factor which can boost the therapeutic potential of WJSC [46]. As previously explained in the literature, it has been shown that higher SOX2 expression in stem cells is associated with stemness, greater self-reinforcement potential, better proliferative properties, and probably also increases cell migration and adhesion, and influences their paracrine properties [28–31].

Mesenchymal stem cells require adequate oxygen concentration for their physiological function. The balance between differentiation, apoptosis and self-mood which is characteristic of stem cells must be achieved through regulation by the microenvironmental niche in which stem cells reside. Oxygen concentration is an important factor to consider when growing stem cells in tissue engineering and regenerative medicine [47]. In their research, Halim et al. focused on finding a combination of factors in vitro necessary to control stem cell proliferation, which would allow them to remain viable and undifferentiated, by analyzing, among other things, oxygen concentration [48]. The research conducted by Widowati et al. showed that Wharton’s jelly MSCs, cultured under hypoxic conditions, have a higher rate of proliferation but show no difference in surface markers from cells grown under normoxic conditions [49]. Yamamoto et al. demonstrated that low oxygen pressure enhances proliferation and increases the number of growth factors secreted by stem cells derived from adipose tissue. The authors would like to emphasize the effectiveness of hypoxic cultures for ASC expansion and maintenance of an undifferentiated state for further therapeutic use [50]. Zhao et al. show that HSPCs of the umbilical cord blood maintain stemness better under hypoxic conditions [51]. It is difficult to compare our research with the research presented in the literature, due to the use of various units by the authors, moreover, in our study, we assess the possible effect of the pressure of carbon dioxide and oxygen in the umbilical cord blood on the level of SOX2 expression in MSC, while the level of CO₂ and O₂ in culture cellularity was constant at 5% and 15%. Safitri et al. studied the effect of oxygen concentration on the level of SOX2 expression in the MSC of rabbit bone marrow. They observed that low in vitro oxygen pressure conditions increased OCT4 and SOX2 expression compared to conventional or hyperoxic conditions [52]. Bae et al. suggest that SOX2 is a gene that is exceptionally amplified under hypoxic conditions [53]. The presented research agrees with findings from other studies. It demonstrates that lower oxygen pressure is linked to higher SOX2 gene expression. Our results indicate that mesenchymal stem cells show higher expression of the SOX2 gene in a more acidic environment with lower oxygen pressure and higher carbon dioxide pressure. We imply that in vivo the pH of umbilical cord blood, oxygen and carbon dioxide concentration are important factors regulating stem cells by influencing SOX2 expression. This fact may prove to be valuable information used in the stem cell collection process as well as during the handling of the cells.

The authors have demonstrated that the expression of the SOX2 gene in WJSC is statistically significantly higher in women aged 34 years and younger compared to women over 34 years of age. It is also shown that there is a statistically significant moderate negative correlation between maternal age and the SOX2 gene expression. It was established in several studies that the parturient’s age affects the quantity and quality of stem cells, however, the studies focused mainly on cord blood stem cells [36,39,40,54]. Alrefaei et al. evaluated the effect of maternal age on the expression of the mesenchymal stem cell markers CD105 and CD29 in various regions of the human umbilical cord and showed that there were significant negative correlations between maternal age and CD29 and CD105 expression [55]. In studies with rats, Asmuda et al. observed that the expression profile of Sox-2 in bone marrow derived MSCs of old rats was significantly lower compared to that of young rats [56]. Huang et al. suggested that the younger donor umbilical
cord is a relatively effective source of MSC. The authors speculated that the older donor’s umbilical cord cells showed reduced differentiation capacity, and this could be attributed to the decreased functional status of the older maternal organs, which play a supporting role and the microenvironment enabling the development of umbilical cord MSC [57].

No studies have been found with regard to the influence of the parturient’s age on the SOX2 expression level in WJSC. In the authors’ previous research, it was shown that the expression of the POU5F1 gene [58] and the expression of the BIRC2, BIRC3 and BIRC5 genes [59] decrease with maternal age. It was noted that the expression of the SOX2 gene is statistically significantly higher in WJSC of babies born prematurely, and the level of the SOX2 expression correlated positively with the length of pregnancy. The earlier the birth took place the higher the SOX2 expression in WJSC. The study also showed a negative correlation between the SOX2 expression and the birth weight. It was observed that the lower the birth weight, the higher the SOX2 gene expression. Researching the influence of the birth weight and the time of delivery on the quality of stem cells is focused mainly on the umbilical cord blood. A number of studies have demonstrated that higher birth weight and, consequently, a larger volume of the placenta, acts as a stimulus on the number of stem cells in the umbilical cord blood [36,38–40,60]. On the other hand, other researchers note that the size of the placenta is related to the number of pregnancies, and so the first pregnancy is usually associated with the weakening of the vascularization of the placenta, while the more births, the larger the size of the placenta in multi-family mothers, thus providing a greater volume of umbilical cord blood and more cells. CD34 [61,62]. However, these studies looked only at the number of stem cell

Looking at the delivery time, the reports vary. Some studies report a high number of stem cells collected during term deliveries [34]. Others suggest that during preterm births the number of CD34+ cord blood cells is higher compared to the predicted due date [33,63]. In our study, we accepted preterm deliveries before our 37th week of pregnancy. The earliest born child was at 35 weeks of pregnancy. However, no studies on the influence of birth weight and delivery time on WJSC quality or expression of the SOX2 gene in WJSC have been found in the available literature.

Low birth weight may result from preterm birth and/or intrauterine growth restriction (IUGR). Premature birth (PT) and low birth weight (LBW) are associated with numerous health and social consequences, both short-term and long-term. These infants have an increased risk of death in the perinatal period and are at increased risk of developing chronic diseases [64]. According to some authors these two adverse pregnancy outcomes, PT and LBW should be investigated together [65]. Kotowski et al. found that the count of cord blood non-HSCs/VSELs is inversely associated with the birth weight of preterm infants. They also noticed that a high number of cord blood HSCs is strongly associated with a lower risk of prematurity complications [66]. The conducted research provides valuable information in the context of possible compensation mechanisms for babies born prematurely and with low birth weight. Further research is needed to evaluate the umbilical cord in babies with low birth weight and premature births, due to the possible better therapeutic potential of the collected cells with regard to increased expression of the SOX2 gene.

In the author’s previous work, it was demonstrated that the expression of the second key factor responsible for maintaining the state of pluripotency, POU5F1 in stem cells of Wharton’s jelly cord, is dependent on the age of the gravida, the manner of delivery, the method of delivery and the use of oxytocin. MSCs derived from Wharton’s jelly (WJSC) taken from younger women and during their first childbirth as well as from patients who received oxytocin showed higher expression of POU5F1 [58]. In the authors’ previous studies, it was also observed that MSCs derived from Wharton’s jelly (WJSC) collected from younger women who were giving birth naturally and in an acidic umbilical cord blood environment are characterized by higher expression of inhibitor of apoptosis protein (IAP): BIRC2, BIRC3 and BIRC5 genes, making them more resistant to apoptosis. IAPs have multidirectional effects and a wide range of cellular functions; in addition to promoting
cell survival, they are also involved in signal transduction, cell differentiation, cell response to damage, and cell division [59].

4. Conclusions

The authors concluded that the younger the woman and the earlier the birth takes place, the lower the birth weight and the higher the SOX2 gene expression in WJSC. In addition, it has been noticed that the physicochemical parameters of umbilical cord blood, such as O2, pressure, CO2 pressure and pH are the factors that regulate the expression of the SOX2 gene in WJSC. However, the correlation coefficients obtained in our study, although significant, are quite low (around 0.5), thus, the study should be continued with a larger number of patients. Due to functions performed by the SOX2 in stem cell biology, it is possible to draw a conclusion that increased expression is likely to translate to higher stem cell effectiveness. Stem cells with high SOX2 expression have a lower degree of differentiation. They have higher proliferative potential, which makes them more clinically useful. Our findings look promising and warrant the need for further research.

5. Materials and Methods

The study was conducted on a group of 30 women hospitalized in the Department of Obstetrics and Pathology of Pregnancy of the Independent Public Clinical Hospital No. 1 in Lublin. The age range of the patients was 24–46 years. The women had a section of the umbilical cord sampled soon after delivery. The statistics with regard to the examined group are presented in Table 1. The research was carried out with the consent of the Bioethics Committee at the Medical University of Lublin no. KE-0254/128/2014. All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects and/or their legal guardian(s).

Table 1. Statistics of pregnancy related factors.

| Parameter                  | N   | Mean    | Median | Minimum | Maximum | SD    |
|----------------------------|-----|---------|--------|---------|---------|-------|
| maternal age [years]       | 32.3| 32.000  | 24.000 | 46.000  | 5.098   |       |
| number of pregnancies      | 2.000| 2.000   | 1.000  | 8.000   | 1.414   |       |
| week of pregnancy          | 38.786| 39.000 | 35.000 | 41.000  | 1.548   |       |
| number of deliveries       | 1.862| 2.000   | 1.000  | 7.000   | 1.187   |       |
| newborn’s weight [g]       | 3383.793| 3300.000| 2140.000| 4740.000| 584.089|       |
| pH                        | 7.326| 7.341   | 7.182  | 7.434   | 0.066   |       |
| pCO2 [mmHg]               | 45.268| 43.800  | 32.600 | 66.500  | 8.098   |       |
| PO2 [mmHg]                | 24.643| 23.200  | 14.600 | 42.700  | 7.887   |       |
| cHCO3 [mmol/L]            | 22.729| 23.100  | 19.200 | 26.700  | 2.045   |       |

* Blood acid-base balance indicators were determined on an ABL90 FLEX gas analyzer (Radiometer, Denmark).

The study was carried out on mesenchymal stem cells of umbilical cord Wharton’s jelly. The stem cells were obtained using the explant method. A section of an umbilical cord removed soon after delivery was placed in a sterile container with an antibiotic and culture medium. Next, the umbilical cord was sectioned into smaller fragments in the laboratory and cultured for 10 days. Cell culture conditions: culture medium: DMEM (1×) + GlutaMAX[+] 1 g/L D-Glucos [+], Pyruvate; Gibco, Paisley, UK; Serum: Heat Inactivated FBS; Gibco, Carlsbad, CA, USA; Antibiotics: Amphotericin B 250 µg/mL + Penicillin/Streptomycin (100×); PAA, Austria; Temperature: 37 °C; O2 concentration: 15%; CO2 concentration: 5%. The isolation procedure is described in the authors’ previous work [67]. The obtained cells were subjected to a cytometric analysis in order to confirm mesenchymal character using antibodies against CD73 (PE-Cy7-labeled), CD90 (FTC-labeled) and CD105 (C7-labeled), CD146 (C5-labeled), CD34 (ECD), and CD45 (APC-A750-labeled) surface antigens (DURACLONE SC Mesenchymal Tube, Beckman Coulter, France). The procedure of cytometric analysis is presented in paper [68]. Total cellular RNA was isolated from the cells using the modified method of Chomczynski and Sacchi [69]. The following reagents
were used for the isolation: TRI Reagent (Sigma-Aldrich, St Louis, MO, USA), Chloroform (Sigma-Aldrich, USA), Isopropanol (Sigma-Aldrich, USA) and Ethanol (Poch, Poland). Subsequently, reverse transcription was performed to obtain complementary DNA. The cDNA synthesis was carried out in accordance with the manufacturer’s recommendation using the High-Capacity cDNA Transcription Kits (Applied Biosystems, Foster City, CA, USA). The synthesized cDNA was amplified in the qPCR reaction, using commercially available TaqMan Hs0153049_s1 probes for the SOX2 gene and Hs99999905_m1 for GAPDH endogenous control (Applied Biosystems, Foster City, CA, USA). The analysis of the obtained results was performed using Expression Suite Software. The \( \Delta \Delta C_t \) method [70] was used to determine the relative level of expression of the examined gene. The statistical analysis was performed using Statistica 13 software. Mann Whitney’s U test was used to assess the differences in the studied groups. The correlation was assessed with Spearman’s rank test. The \( p \)-value was set at \( p < 0.05 \).

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**References**

1. Watson, N.; Divers, R.; Kedar, R.; Mehindru, A.; Mehindru, A.; Borlongan, M.; Borlongan, C.V. Discarded Wharton jelly of the human umbilical cord: A viable source for mesenchymal stromal cells. *Cytotherapy* **2015**, *17*, 18–24. [CrossRef] [PubMed]
2. Davies, J.E.; Walker, J.T.; Keating, A. Concise Review: Wharton’s Jelly: The Rich, but Enigmatic, Source of Mesenchymal Stromal Cells. *Stem Cells Transl. Med.* **2017**, *6*, 1620–1630. [CrossRef] [PubMed]
3. Stevanovic, M.; Zuffardi, O.; Collignon, J.; Lovell-Badge, R.; Goodfellow, P.N. The cDNA sequence and chromosomal location of the human SOX2 gene. *Mamm. Genome* **1994**, *5*, 640–642. [CrossRef] [PubMed]
4. Liu, A.; Yu, X.; Liu, S. Pluripotency transcription factors and cancer stem cells: Small genes make a big difference. *Chin. J. Cancer* **2013**, *32*, 483–487. [CrossRef] [PubMed]
5. Weiss, M.A. Floppy SOX: Mutual induced fit in hmg (high-mobility group) box-DNA recognition. *Mol. Endocrinol.* **2001**, *15*, 353–362. [CrossRef] [PubMed]
6. Badis, G.; Berger, M.F.; Philippakis, A.A.; Talukder, S.; Gehrke, A.R.; Jaeger, S.A.; Chan, E.T.; Metzler, G.; Vedenko, A.; Chen, X.; et al. Diversity and Complexity in DNA Recognition by Transcription Factors. *Science* **2009**, *324*, 1720–1723. [CrossRef]
7. Kondoh, H.; Kamachi, Y. SOX-partner code for cell specification: Regulatory target selection and underlying molecular mechanisms. *Int. J. Biochem. Cell Biol.* **2010**, *42*, 391–399. [CrossRef]
8. Chambers, I.; Silva, J.C.R.; Colby, D.; Nichols, J.; Nijmeijer-Winter, B.; Robertson, M.; Vrana, J.; Jones, K.; Grotewold, L.; Smith, A. Nanog safeguards pluripotency and mediates germline development. *Nature* **2007**, *450*, 1230–1234. [CrossRef]
14. Chew, J.-L.; Loh, Y.-H.; Zhang, W.; Chen, X.; Tam, W.-L.; Yeap, L.-S.; Li, P.; Ang, Y.-S.; Lim, B.; Robson, P.; et al. Reciprocal
15. Yoon, D.S.; Kim, Y.H.; Jung, H.S.; Paik, S.; Lee, J.W. Importance of Sox2 in maintenance of cell proliferation and multipotency of
16. Navarro, P.; Festuccia, N.; Colby, D.; Gagliardi, A.; Mullin, N.P.; Zhang, W.; Karwacki-Neisius, V.; Osorno, R.; Kelly, D.; Robertson,
17. Kamachi, Y.; Kondoh, H. Sox proteins: Regulators of cell fate specification and differentiation.
18. Twigger, A.-J.; Hepworth, A.R.; Lai, C.T.; Chetwynd, E.; Stuebe, A.M.; Blancafort, P.; Hartmann, P.E.; Geddes, D.T.; Kakulas, F.
19. Izadpanah, R.; Trygg, C.; Patel, B.; Kriedt, C.; Dufour, J.; Gimble, J.M.; Bunnell, B.A. Biologic properties of mesenchymal stem
20. Fong, Y.W.; Inouye, C.; Yamaguchi, T.; Cattoglio, C.; Grubisic, I.; Tjian, R. A DNA Repair Complex Functions as an Oct4/Sox2
21. Rizzino, A.; Wuebben, E.L. Sox2/Oct4: A delicately balanced partnership in pluripotent stem cells and embryogenesis.
22. Liu, L.; Wei, X.; Ling, J.; Wu, L.; Xiao, Y. Expression Pattern of Oct-4, Sox2, and c-Myc in the Primary Culture of Human Dental
23. Hassiotou, F.; Beltran, A.; Chetwynd, E.; Stuebe, A.M.; Twigger, A.-J.; Metzer, P.; Trengove, N.; Filgueira, L.; Blancafort,
24. Gonzalez, R.; Maki, C.; Pacchiarotti, J.; Csontos, S.; Pham, J.; Slepkov, N.; Patel, A.; Silva, F. Pluripotent marker expression
25. Kopp, J.L.; Ormsbee, B.D.; Desler, M.; Rizzino, A. Small Increases in the Level of Sox2 Trigger the Differentiation of Mouse
Embryonic Stem Cells. Stem Cells 2008, 26, 903–911. [CrossRef] [PubMed]
26. Masui, S.; Nakatake, Y.; Toyooka, Y.; Shimosato, D.; Yagi, R.; Takahashi, K.; Okochi, H.; Okuda, A.; Matoba, R.; Sharov, A.A.; et al. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat. Cell Biol. 2007, 9, 625–635. [CrossRef]
27. Niwa, H.; Miyazaki, J.-I.; Smith, A.G. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet. 2000, 24, 372–376. [CrossRef]
28. Adachi, K.; Suemori, H.; Nakatsuji, N.; Kawase, N. The Role of SOX2 in Maintaining Pluripotency and Differentiation of
29. Trivanovic, D.; Kocic, J.; Mojsilovic, S.; Krstic, A.; Ilic, V.; Okic-Djordjevic, I.; Santibanez, J.; Jovcic, G.; Terzic, M.; Bugarski,
30. Russell, J.P.; Lim, X.; Santambrogio, A.; Yianni, V.; Kemkem, Y.; Wang, B.; Fish, M.; Haston, S.; Grabek, A.; Hallang, S.; et al. Reciprocal Transcriptional Regulation of Pou5f1 and Sox2 via the Oct4/Oct2 Complex in Embryonic Stem Cells. Mol. Cell. Biol. 2005, 25, 6031–6046. [CrossRef]
31. Abdelrazik, A.M.; El Said, M.N.; Abdelaziz, H.E.M.; Badran, H.M.; Elal, E.Y.A.A. The impact of fetal and maternal physiologic
factors on umbilical cord blood quality as a source of stem cells in Egyptian population. Transfusion 2015, 55, 2882–2889. [CrossRef] [PubMed]
32. Nunes, R.D.; Zandavalli, F.M. Association between maternal and fetal factors and quality of cord blood as a source of stem cells. Rev. Bras. Hematol. Hemoter. 2015, 37, 38–42. [CrossRef]
33. Wu, J.-Y.; Liao, C.; Chen, J.-S.; Xu, Z.-P.; Gu, S.-L.; Wu, S.-Q.; Lu, Y.; Xie, G.-E. Analysis of maternal and neonatal factors associated with hematopoietic reconstruction potential in umbilical cord blood units. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2010, 18, 1535–1541. [PubMed]
34. Chandra, T.; Afreen, S.; Kumar, A.; Singh, U.; Gupta, A. Does umbilical cord blood-derived CD34+ cell concentration depend on the weight and sex of a full-term infant? J. Pediatr. Hematol. 2012, 34, 184–187. [CrossRef] [PubMed]
35. Caughey, A.B.; Sundaram, V.; Kaimal, A.J.; Cheng, Y.W.; Gienger, A.; Little, S.E.; Lee, J.F.; Wong, L.; Shaffer, B.L.; Tran, S.H.; et al. Maternal and neonatal outcomes of elective induction of labor. Evid. Rep. Technol. Assess. 2009, 176, 1–257.
36. Mousavi, S.M.; Abroun, S.; Zarrabi, M.; Ahmadipanah, M. The effect of maternal and infant factors on cord blood yield. Pediatr. Blood Cancer 2016, 64, e26381. [CrossRef]
37. Bielec-Berek, B.; Jastrzębska-Stojko, Z.; Drosdzol-Cop, A.; Jendyk, C.; Boruczkowski, D.; Oldak, T.; Nowak-Brzezińska, A.; Stojko, R. Maternal predictors and quality of umbilical cord blood units. Cell Tissue Bank. 2018, 19, 69–75. [CrossRef]
38. Mancinelli, F.; Tamburini, A.; Spagnoli, A.; Malerba, C.; Suppo, G.; Lasorella, R.; de Fabritiis, P.; Calugi, A. Optimizing Umbilical Cord Blood Collection: Impact of Obstetric Factors Versus Quality of Cord Blood Units. Transplant. Proc. 2006, 38, 1174–1176. [CrossRef]
39. Santos, S.V.; Marti, L.; Ribeiro, A.A.F.; Conti, F.; Barros, S.M. A cross-sectional study of umbilical cord blood donor profiles and their influence on umbilical cord blood collection in a Brazilian hospital. Cytotherapy 2011, 13, 1120–1127. [CrossRef]
40. Al-Deghaiiter, S.Y. Impact of maternal and neonatal factors on parameters of hematopoietic potential in umbilical cord blood. Saudi Med. J. 2015, 36, 704–712. [CrossRef]
41. Al-Deghaiiter, S.Y. Impact of maternal and neonatal factors on parameters of hematopoietic potential in umbilical cord blood. Saudi Med. J. 2015, 36, 704–712. [CrossRef]
42. Nakagawa, R.; Watanabe, T.; Kawano, Y.; Kanai, S.; Suzuya, H.; Kaneo, M.; Watanabe, H.; Okamoto, Y.; Kuroda, Y.; Nakayama, T.; et al. Analysis of maternal and neonatal factors that influence the nucleated and CD34+ cell yield for cord blood banking. Transfusion 2004, 44, 262–267. [CrossRef] [PubMed]
43. Ballen, K.; Wilson, M.; Wuu, J.; Ceredona, A.; Hsieh, C.; Stewart, F.; Popovsky, M.; Quesenberry, P. Bigger is better: Maternal and neonatal predictors of hematopoietic potential of umbilical cord blood units. Bone Marrow Transplant. 2001, 27, 7–14. [CrossRef] [PubMed]
44. Lin, Y.; Weisdorf, D.J.; Solovey, A.; Hebbel, R.P. Origins of circulating endothelial cells and endothelial outgrowth from blood. J. Clin. Invest. 2000, 105, 71–77. [CrossRef] [PubMed]
45. Aulderhaar, U.; Holzgrewe, W.; Danzer, E.; Tichelli, A.; Troeger, C.; Shaffer, B.L.; Tran, S.H.; et al. Maternal and neonatal factors on umbilical cord blood stem cell banking. J. Perinat. Med. 2003, 31, 317–322. [CrossRef]
46. Obradovic, H.; Krstic, J.; Trivanovic, D.; Mojsilovic, S.; Okic, I.; Kukolj, T.; Ilic, V.; Jaukovic, A.; Terzic, M.; Bugarski, D. Improving stemness and functional features of mesenchymal stem cells from Wharton’s jelly of a human umbilical cord by mimicking the native, low oxygen stem cell niche. Placenta 2019, 82, 25–24. [CrossRef]
47. Santos, S.V.; Marti, L.; Ribeiro, A.A.F.; Conti, F.; Barros, S.M. A cross-sectional study of umbilical cord blood donor profiles and their influence on umbilical cord blood collection in a Brazilian hospital. Cytotherapy 2011, 13, 1120–1127. [CrossRef]
48. Nakagawa, R.; Watanabe, T.; Kawano, Y.; Kanai, S.; Suzuya, H.; Kaneo, M.; Watanabe, H.; Okamoto, Y.; Kuroda, Y.; Nakayama, T.; et al. Analysis of maternal and neonatal factors that influence the nucleated and CD34+ cell yield for cord blood banking. Transfusion 2004, 44, 262–267. [CrossRef] [PubMed]
49. Widodo, M.A. Quantity of umbilical cord blood units. Transplant. Proc. 2006, 38, 1174–1176. [CrossRef]
50. Yamamoto, Y.; Fujita, M.; Tanaka, Y.; Kojima, I.; Kanatani, Y.; Ishihara, M.; Tachibana, S. Low oxygen tension enhances proliferation and maintains stemness of adipose tissue-derived stromal cells. Stem Cell Investig. 2020, 7, 5. [CrossRef]
51. Zhao, D.; Liu, L.; Chen, Q.; Wang, F.; Li, Q.; Zeng, Q.; Huang, J.; Luo, M.; Li, W.; Zheng, Y.; et al. Hypoxia with Wharton’s jelly mesenchymal stem cell culture maintains stemness of umbilical cord blood-derived CD34+ cells. Stem Cell Res. Ther. 2018, 9, 158. [CrossRef]
52. Safitri, E. Effect of low oxygen tension on transcriptional factor OCT4 and SOX2 expression in New Zealand rabbit bone marrow-derived mesenchymal stem cells. Vetér. World 2020, 13, 2469–2476. [CrossRef]
53. Bae, K.-M.; Dai, Y.; Vieweg, J.; Siemann, D.W. Hypoxia regulates SOX2 expression to promote prostate cancer cell invasion and sphere formation. Am. J. Cancer Res. 2016, 6, 1078–1088. [PubMed]
54. Alrefaei, G.I.; Al-Karim, S.; Ayuob, N.N.; Ali, S.S. Does the maternal age affect the mesenchymal stem cell markers and gene expression in the human placenta? What is the evidence? Tissue Cell 2015, 47, 406–419. [CrossRef] [PubMed]
55. Alrefaei, G.I.; Al-Karim, S.; Ayuob, N.N.; Ali, S.S.; Alkarim, S. Effects of maternal age on the expression of mesenchymal stem cell markers in the components of human umbilical cord. Folia Histochem. Cytobiol. 2015, 53, 259–271. [CrossRef] [PubMed]
56. Asmund, F.; Chase, P.B. Age-related changes in rat bone-marrow mesenchymal stem cell plasticity. BMC Cell Biol. 2011, 12, 44. [CrossRef]
57. Huang, S.; Feng, C.; Wu, Y.; Yang, S.; Ma, K.; Wu, X.; Fu, X. Dissimilar characteristics of umbilical cord mesenchymal stem cells from donors of different ages. Cell Tissue Bank. 2013, 14, 707–713. [CrossRef]
58. Gil-Kulik, P.; Chomik, P.; Krzyżanowski, A.; Radzikowska-Büchner, E.; Maciejewski, R.; Kwaśniewska, A.; Rahnama, M.; Kocki, J. Influence of the Type of Delivery, Use of Oxytocin, and Maternal Age on POU5F1 Gene Expression in Stem Cells Derived from Wharton’s Jelly within the Umbilical Cord. *Oxidative Med. Cell. Longev.* 2019, 2019, 1027106. [CrossRef]

59. Gil-Kulik, P.; Świstowska, M.; Kondracka, A.; Chomik, P.; Krzyżanowski, A.; Kwaśniewska, A.; Rahnama, M.; Kocki, J. Increased Expression of *BIRC2, BIRC3, and BIRC5* from the IAP Family in Mesenchymal Stem Cells of the Umbilical Cord Wharton’s Jelly (WJSC) in Younger Women Giving Birth Naturally. *Oxidative Med. Cell. Longev.* 2020, 2020, 9084790. [CrossRef]

60. Mazzoccoli, G.; Miscio, G.; Fontana, A.; Copetti, M.; Francavilla, M.; Bosi, A.; Perfetto, E.; Valoriani, A.; De Cata, A.; Santodirocco, M.; et al. Time related variations in stem cell harvesting of umbilical cord blood. *Sci. Rep.* 2016, 6, 21404. [CrossRef]

61. Thame, M.; Osmond, C.; Bennett, F. Wzrost płodu jest bezpośrednio związany z antropometrią matki i objętością lożycka. *Eur. J. Clin. Nutr.* 2004, 58, 894. [CrossRef]

62. Ballen, K.K.; Gluckman, E.; Broxmeyer, H.E. Umbilical cord blood transplantation: The first 25 years and beyond. *Blood* 2013, 122, 491–498. [CrossRef]

63. Page, K.M.; Mendizabal, A.; Betz-Tablein, B.; Wease, S.; Shoulars, K.; Gentry, T.; Prasad, V.K.; Sun, J.; Carter, S.; Balber, A.E.; et al. Optimizing donor selection for public cord blood banking: Influence of maternal, infant, and collection characteristics on cord blood unit quality. *Transfusion* 2014, 54, 340–352. [CrossRef] [PubMed]

64. Vrijens, K.; Tsamou, M.; Madhloom, N.; Gysselaers, W.; Nawrot, T.S. Placental hypoxia-regulating network in relation to birth weight and ponderal index: The ENVIRONAGE Birth Cohort Study. *J. Transl. Med.* 2018, 16, 2. [CrossRef] [PubMed]

65. Taha, Z.; Hassan, A.A.; Wikkeling-Scott, L.; Papandreou, D. Factors Associated with Preterm Birth and Low Birth Weight in Abu Dhabi, the United Arab Emirates. *Int. J. Environ. Res. Public Health* 2020, 17, 1382. [CrossRef] [PubMed]

66. Kotowski, M.; Safranow, K.; Kawa, M.P.; Lewandowska, J.; Klös, P.; Dziedziejko, V.; Paczkowska, E.; Czajka, R.; Celcewicz, Z.; Rudnicki, J.; et al. Circulating hematopoietic stem cell count is a valuable predictor of prematurity complications in preterm newborns. *BMC Pediatr.* 2012, 12, 148. [CrossRef]

67. Gil-Kulik, P.; Krzyżanowski, A.; Dudzińska, E.; Karwat, J.; Chomik, P.; Świstowska, M.; Kondracka, A.; Kwaśniewska, A.; Cioch, M.; Jojczuk, M.; et al. Potential Involvement of BIRC5 in Maintaining Pluripotency and Cell Differentiation of Human Stem Cells. *Oxid. Med. Cell. Longev.* 2019, 2019, 8727925. [CrossRef]

68. Walecka, I.; Gil-Kulik, P.; Krzyżanowski, A.; Czop, M.; Galkowski, D.; Karwat, J.; Chomik, P.; Świstowska, M.; Kwaśniewska, A.; Bogucka-Kocka, A.; et al. Phenotypic Characterization of Adherent Cells Population CD34+ CD90+ CD105+ Derived from Wharton’s Jelly. *Med. Sci. Monit.* 2017, 23, 1886–1895. [CrossRef]

69. Chomczynski, P.; Sacchi, N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 1987, 162, 156–159. [CrossRef]

70. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT Method. *Methods* 2011, 25, 402–408. [CrossRef]