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ARTICLE

Effect of milk and whey on proliferation and differentiation of placental stromal cells

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Abstract Fetal bovine serum (FBS), which is widely used in cell culture media, has the potential to cause medical and ethical problems. Here, an experimental study using milk or whey proteins containing essential nutrients and growth factors is presented to limit the use of FBS in cell culture media produced for cell and tissue regeneration. Study groups were formed by culturing human placenta mesenchymal stem cells, known to have high proliferation and differentiation capacity, with milk or whey solution at increasing concentrations, alone or in combination with FBS. Osteogenic and adipogenic differentiation capacities of proliferating cells were observed in FBS, milk or whey groups. Milk, whey or FBS groups obtained in P3 and after differentiation were separately analyzed for protein mRNA expression by reverse transcriptase-polymerase chain reaction (RT-qPCR). Fibroblast Growth Factor 2 (FGF2), Octamer-binding Transcription Factor 4 (OCT4), Bone Morphogenetic Protein 6 (BMP6), and adipogenic differentiation marker Peroxisome Proliferator-Activated Receptor Gamma (PPARG) were analysed by RT-qPCR. Proliferation was more pronounced in FBS alone and in its combinations with milk-whey compared to the groups in which only milk and whey were used. OCT4 mRNA and FGF2 mRNA expression decreased in differentiated cells. BMP6 mRNA expression increased with osteogenic and adipogenic stimuli. As expected, PPRG expression also increased with adipogenic stimulation. With this experimental study, evidence has been obtained that milk or whey can provide nutritional support to the culture media of repair cells and preserve the functional capacity of the cells, with a slightly more limited capacity than FBS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10616-023-00585-z.

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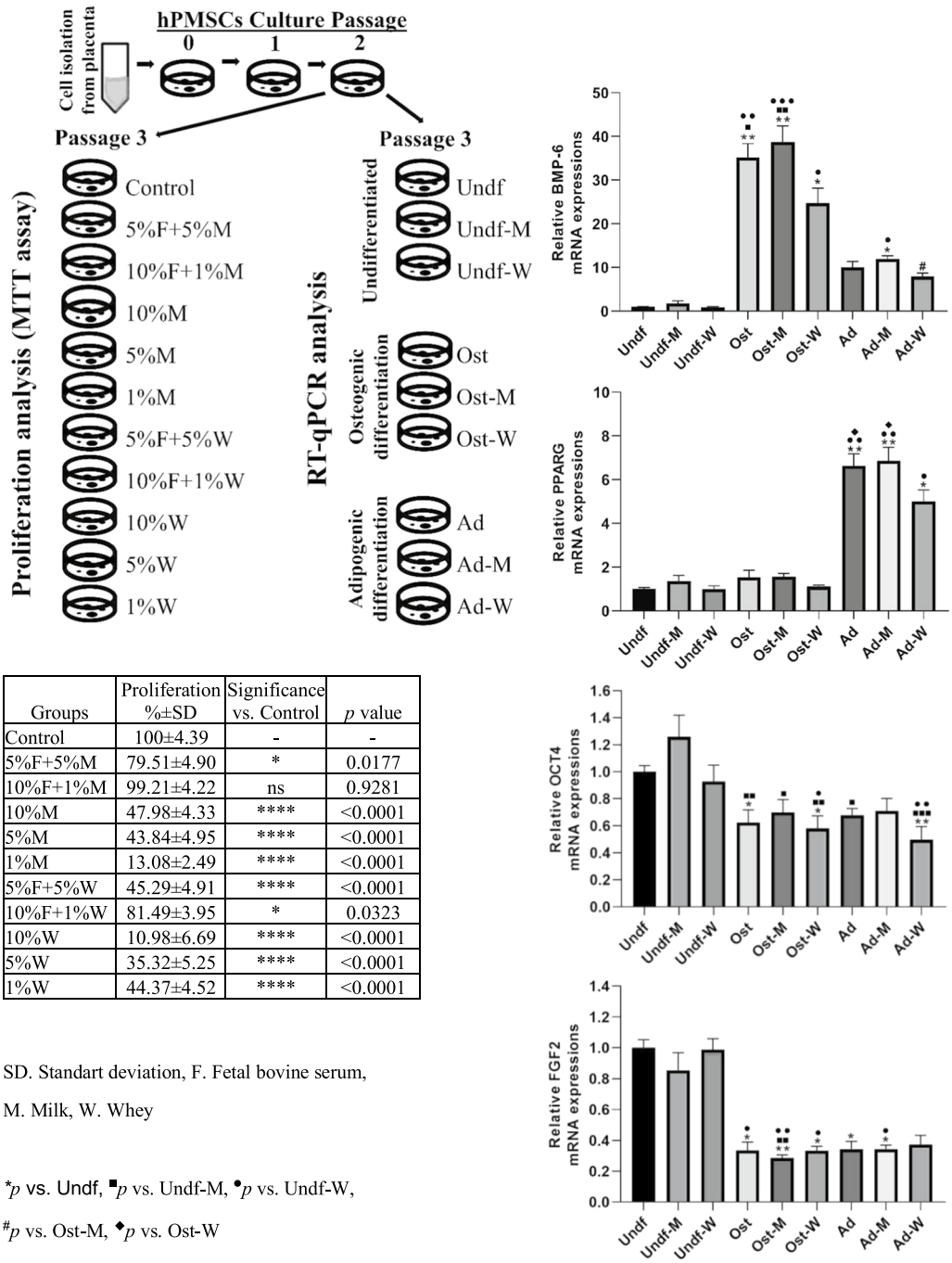
Graphical abstract



Groups	Proliferation %±SD	Significance vs. Control	*p* value
Control	100±4.39	-	-
5%F+5%M	79.51±4.90	*	0.0177
10%F+1%M	99.21±4.22	ns	0.9281
10%M	47.98±4.33	****	<0.0001
5%M	43.84±4.95	****	<0.0001
1%M	13.08±2.49	****	<0.0001
5%F+5%W	45.29±4.91	****	<0.0001
10%F+1%W	81.49±3.95	*	0.0323
10%W	10.98±6.69	****	<0.0001
5%W	35.32±5.25	****	<0.0001
1%W	44.37±4.52	****	<0.0001

SD. Standart deviation, F. Fetal bovine serum,

M. Milk, W. Whey

*p vs. Undf, ■p vs. Undf-M, •p vs. Undf-W,

#p vs. Ost-M, ◆p vs. Ost-W

Keywords Mesenchymal stem cell · Milk · Whey · Proliferation · Differentiation

Introduction

Adult stem cells, such as human mesenchymal stem cells (MSCs), grown in culture can provide a better understanding of fatal diseases, produce vaccines, and are useful in gene therapy (Berebichez-Fridman and Montero-Olvera 2018; Squillaro et al. 2016). One of the most interesting uses for stem cell culture is the repair of damaged tissues and organs (Rushkevich et al. 2015; Redondo et al. 2018). However, the biological activity of mesenchymal stem cells may vary depending on their source and production conditions (Ma et al. 2014). Human placental mesenchymal stem cells (hPMSCs) are considered among the most accessible source of multipotent cells (Kmiecik et al. 2013; Karlsson et al. 2012; Marongiu et al. 2010).

Growing cells in culture requires incubation in growth-sustaining nutrients. The most basic nutritional supplement for cell culture is fetal bovine serum (FBS), which contains nutrients and growth factors after blood coagulation (Tekkatte et al. 2011; Electricwala 1992). The use of FBS in culture media has raised some ethical concerns because one million calf fetuses are euthanized annually for this purpose. FBS may also transmit viruses, bacteria and endotoxins or cause an allergic reaction (Tonarova et al. 2021). On the other hand, whey or milk nutrients can provide the development and survival of multipotent cells in the breast tissue (Patki et al. 2010; Hassiotou et al. 2013; Xu 2009). Milk contains multiple nutrients and promotes growth, and pasteurized milk could be used as a sterile source of nutrients (Hassiotou et al. 2013). Likewise, whey is rich in amino acids and minerals, improves muscle tissue, and can contribute to wound healing (Almeida et al. 2016). We hypothesize that adding these substances to a cell culture environment may facilitate cell growth, the need to use FBS can be restricted, and proliferative ability and self-reneval capacity of the MSCs may be maintained without causing ethical problems.

In this study, we investigated how the multipotency or differentiation-related gene expression levels and functional properties of cells were affected by the use of whey/ milk nutrients in hPMSCs culture medium.

Materials and methods

Study plan

Human placental stromal cells were isolated following a standard procedure (Beeravolu et al. 2017). Following the first passage (P1), hPMSCs cells obtained from samples of P1 were expanded in a standard culture medium until passage 2 (P2). The morphology, proliferative capacity, and immunophenotype of the cells from P2 were characterized by inverted microscopy, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium (MTT) test, and flow cytometry, respectively (Präbst et al. 2017; Kozanoglu and Maytalman 2012). Milk, whey or FBS groups obtained in P3 and were separately analyzed for protein mRNA expression after differentiation by reverse transcriptase-polymerase chain reaction (RT-qPCR). Fibroblast Growth Factor 2 (FGF2) and Octamer-binding Transcription Factor 4 (OCT4) were used in the analysis for stemness gene expression (Dev et al. 2012; Dvorak et al. 2005). Osteogenic differentiation marker Bone Morphogenetic Protein 6 (BMP6) and adipogenic differentiation marker Peroxisome Proliferator-Activated Receptor Gamma (PPARG) were also analysed by RT-qPCR. Histochemical staining assessed the osteogenic and adipogenic differentiation potential (Carreira et al. 2015; Yuan et al. 2016).

This study was approved by The Scientific and Technological Research Council of Turkey on Noninterventional Clinical Research (Project No: 1,689,301,304,147). In our study, cell culture processes and analyses were conducted at Quality Control Laboratory and Molecular Biology-Genetic Laboratory of Center of Adana Adult Bone Marrow Transplantation Center of Baskent University.

Isolating and culture of placenta membran cells

Mothers who with no known hereditary and chronic diseases and healthy babies in the follow-up were included in the study. Placenta samples were not taken from mothers with health problems and pregnancy complications. The placenta samples (n = 3: 1 baby boy and 2 girls) were obtained after consent of the mother and prepared by researchers as described previously (Marongiu et al. 2010; Kozanoglu and Maytalman 2012). The cells obtained from placental

membranes were cultured at the initial concentration of 1×10^6 cells/mL in T75 flasks using MesenCultTM Proliferation Kit (Human) (Stemcell Technology, Vancouver, Canada). The cell passage was performed when cells proliferated to cover 85–90% of the flask's surface. Experiments were done with passage 3 (P3) cells.

Cell proliferation assays

The methyl thiazolyl tetrazolium (MTT) analysis was performed on the cells obtained after P2 (P3 transition) to assess the effects of whey and milk effect on cell proliferation and for dose determination. For each sample, eight control wells were prepared; in addition, eight test wells were prepared for each dose with 1000 cells/0.1 mL per well. The cells were fed using a medium prepared by whey or milk with or without FBS at concentrations of 5%FBS–5%milk, 10%FBS–1%milk, 10% milk, 5% milk, 1% milk, 5%FBS–5% whey, 10%FBS–1%whey, 10 whey, 5% whey, 1% whey. The cells were incubated at 5% CO_2 and 95% humidity for 7 days by feeding at every 48 h using a fresh medium. Briefly, the cells were cultured in 96-well plates; 10 µL of MTT (5 mg/mL) in 90 mL fresh medium were added to each well after removing the medium, including milk and whey. The cells were incubated 4 h again then formazan crystals formed were dissolved with dimethylsulfoxide. The plates were read at 490 nm in a Bio-Rad 680 ELISA reader (Bio-Rad Laboratories, CA). Results were presented as mean optic density readings derived from triplicate plates.

Flow cytometry

The expression levels of antigens CD73, CD105, CD34 and CD45 in hPMSC populations were assessed by immunofluorescence using flow cytometry. Cells were analyzed using FACS DIVA software (BD Biosciences) after acquisition. The cells were marked with CD73 (PE), CD105 (FTIC), CD45 (APC-H7), and CD34 (PE-Cy7) for isotype control (Kozanoglu and Maytalman 2012).

Experiments groups

The groups to be studied for RT-qPCR were determined as follows.

Undif. represents undifferentiated and cultured in standard medium (10% FBS); Undif-M. Undifferentiated and cultured in medium containing 5% FBS plus 5% milk; Undif-W. Undifferentiated and cultured in medium containing 5% FBS plus 5% whey; Ost./Ad. Differentiated as standard with osteogenic/adipogenic differentiation medium; Ost-M./Ad-M. Differentiated with osteogenic/adipogenic differentiation medium supplemented with milk (5%);

Ost-W./Ad-W. Differentiated with osteogenic/adipogenic differentiation medium supplemented with whey (5%).

Differentiation of hPMSCs

A test for osteogenic and adipogenic differentiation of hPMSCs was planned to show how differentiation is affected by experimental media. For this purpose, the cells obtained at the transition to P3 were inoculated to T25 flasks at a concentration of 2×10^4 cells/mL. The cell cultures were maintained by standard (FBS) and experimental media until cell proliferation to cover 85–90% of the flask surface. After adding prepared mediums, the cell cultures were maintained for 21 days by feeding every 3 days. MesenCultTM Osteogenic Stimulatory Kit (Human) (Stemcell Technologies, Vancouver, Canada) was used for osteogenic differentiation while MesenCultTM Adipogenic Differentiation Medium (Human) (Stemcell Technologies, Vancouver, Canada) was used for adipogenic differentiation. Cell proliferation was achieved in two distinct flasks, one for microscopic evaluation and one for PCR analysis. Under the microscope (Nikon Eclipse TS100, Tokyo, Japan), osteogenic differentiation was observed by intracellular calcium deposits stained using Alizarin Red S (Sigma, St. Louis, MO), while adipogenic differentiation was observed by vesicles stained using Oil Red O (Sigma, St. Louis, MO).

Quantitative real-time polymerase chain reaction

The RT-qPCR analysis was performed using primers (Table S1-Supplementary File) representing specific mRNA gene regions to demonstrate the stemness effects and the differentiation potential of the cells. For this purpose, Total RNA was isolated from undifferentiated and differentiated groups at passage 3

using High Pure RNA Isolation Kit (Roche, Germany). RNA concentration was measured by Nanodrop 2000 (Thermo Scientific, Wilmington, DE). The cDNA samples were produced by a thermal cycle device (Palm Cycler, New Delhi, India) using Ipsogen RT Kit (Qiagen, Hilden, Germany). The cDNA samples were used as template in SYBR Green-based RTqPCR analysis performed using Rotor-Gene (Qiagen, Hilden, Germany) and obtained threshold cycle (CT) values. The CT values were used to calculate the fold change of mRNA expressions.

For stemness markers FGF2 and OCT4, osteogenic differentiation marker BMP6, adipogenic differentiation marker PPARG as well as (beta-actin) as a hausekeeping gene, RT-qPCR analysis of cDNAs were performed. The analysis of the up- or down-regulated genes in the different groups was summarized in images created with Experiment Viewer software (Rotor-Gene, Q software version 2.0, Corbett Research, Australia) (Gilliland et al. 1990). Each sample was measured in triplicates. Fold change in gene expression for each sample and experimental condition was calculated as $2^{-\Delta\Delta CT} \pm$ SD.

Statistical analysis and fold change calculations

To analyze whey or milk in cell culture media, obtained data from the MTT test were compared using one-way ANOVA with post hoc Bonferroni. Data regarding expression behaviors for each donor were separately obtained for each cell group by RTqPCR analysis. The CT values of the undifferentiated group (Undif.) were normalized according to housekeeping gene B-actin, and fold change values of groups ($2^{(-\Delta\Delta CT)}$) were calculated. To assess expression behavior at all stages, all groups were compared with each other by statistical means. The normality of distribution was assessed by the Shapiro–Wilk test. Since the groups were obtained from each other from the initial passage, they were evaluated as independent groups for statistical analysis. For expression analysis, non-parametric data were compared by the Kruskal Wallis test.

A p value < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 9.0 (La Jolla, CA).

Results

The cells provided the morphologically expected mesenchymal cell appearance. Flow cytometric analyzes revealed that the generated cell lines were quite weak for the expressions of the hematopoietic cell markers CD34 ($0.19 \pm 0.06\%$) and CD45 ($0.98 \pm 0.83\%$), whereas they were positive for the mesenchymal cell markers CD73 ($97.95 \pm 1.12\%$) and CD105 ($96.71 \pm 2.28\%$).

MTT results showed that cell proliferation obtained at different concentrations of milk or whey alone was less than that of the control (FBS) group. Preliminary data indicated that there was a concentration dependent increase in proliferation in culture medium containing milk, with a maximum of $47.98 \pm 4.33\%$ with 10% milk, as compared to control ($100 \pm 4.39\%$). In contrast, increasing whey concentration decreased cell proliferation by a maximum of 44.37% for 1% whey, as compared to control ($100 \pm 4.39\%$) (Table 1). The viability of cell preparation was greater than 90% in all analyzed samples.

However, in microscopic analysis, only 10% of the whey groups showed cell loss in the W 10% group. Possibly, this concentration showed a toxic effect in terms of content. There was no increase in

Table 1 MTT Results

Groups	Proliferation % ± SD	Significance vs. Control	*p* value
Control	100 ± 4.39		NA
5%F + 5%M	79.51 ± 4.90	*	0.0177
10%F + 1%M	99.21 ± 4.22	ns	0.9281
10%M	47.98 ± 4.33	****	< 0.0001
5%M	43.84 ± 4.95	****	< 0.0001
1%M	13.08 ± 2.49	****	< 0.0001
5%F + 5%W	45.29 ± 4.91	****	< 0.0001
10%F + 1%W	81.49 ± 3.95	*	0.0323
10%W	10.98 ± 6.69	****	< 0.0001
5%W	35.32 ± 5.25	****	< 0.0001
1%W	44.37 ± 4.52	****	< 0.0001

Statistical significance for MTT analysis results are given in the table versus control only

SD Standart deviation, *F* Fetal bovine serum, *M* Milk, *W* Whey

The difference was statistically significant in all groups except 10% F + 1%M group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

cells in other whey groups, but vitality continued. In the milk groups, although the cells were alive in the 1%M group, there was almost no proliferation. There was no toxic evidence for milk at high concentrations, but it was seen that 1 M% was not sufficient to increase cells. Although proliferation can occur for milk and other concentrations of whey used alone, the best results were seen at concentrations with FBS added. Addition of 1%M with FBS resulted in proliferation almost equivalent to the control. However, the addition of 1%W somewhat limited the proliferation. While some proliferation was achieved using 5 and 1%M alone, a limiting effect was exerted when used together with FBS. Cell proliferation capacity reached up to 79.51 ± 4.9% with 5%F + 5%M group in which the FBS ratio was reduced. Only 45.29 ± 4.9% proliferation was achieved in the 5%F + 5%W group, and the difference between them was statistically significant ($p = 0.0011$). The difference was statistically significant in the other groups compared with each other ($p < 0.05$ for all).

Milk-, whey-, and FBS-hPMSC treated with osteogenic medium successfully differentiated osteogenic lineages: calcium phosphate deposits were stained with Alizarin Red S. The cells were photographed under an inverted microscope. These cells treated

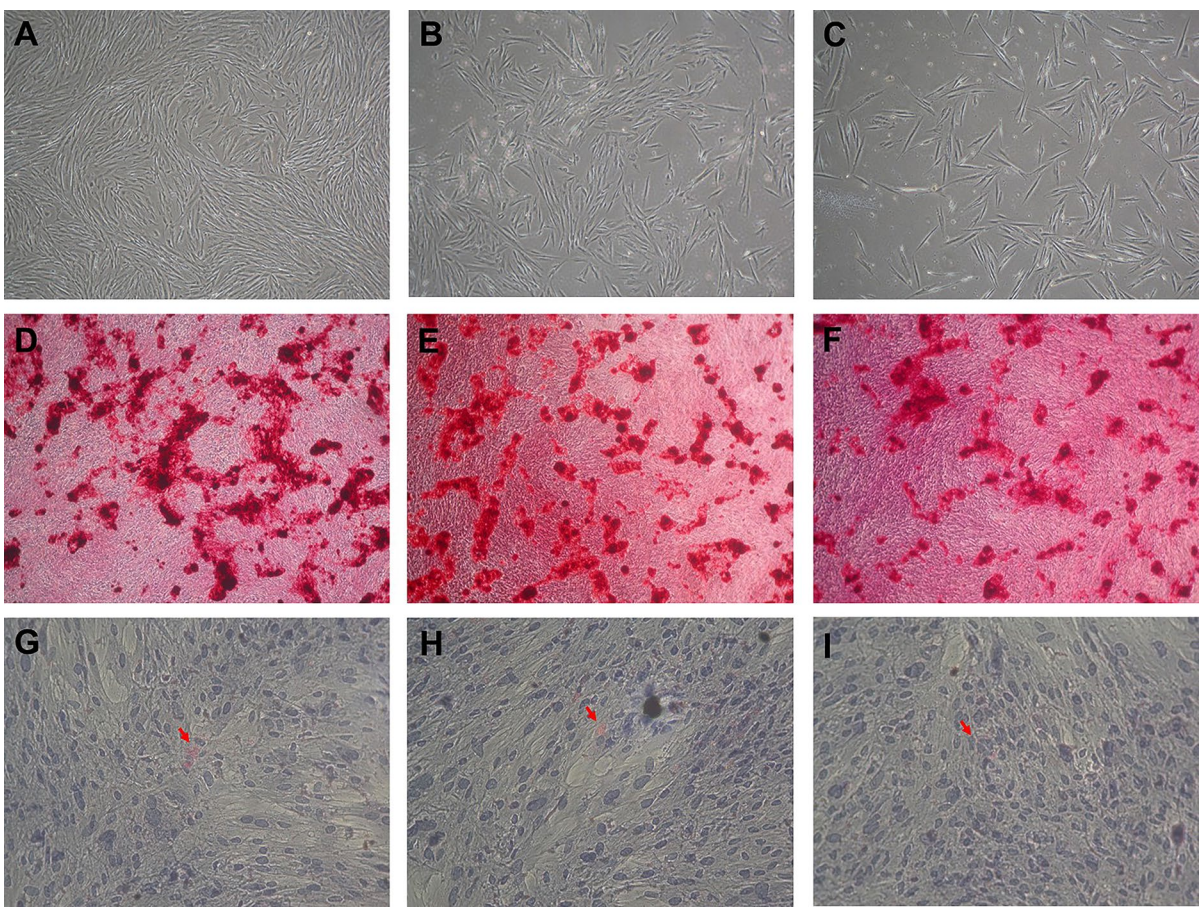


Fig. 1 The proliferation capacities of FBS, milk and whey treated undifferentiated hPMSCs at day 7 were different from each other. **A** 10% FBS, **B** 5% FBS + 5% milk, **C** 5% FBS + 5% whey. FBS-, milk- and whey-hPMSCs treated with osteogenic medium successfully differentiated osteogenic lineages (calcium phosphate deposits were stained with Alizarin Red S). **D** Standard osteogenic differantiation medium (SODM), **E** SODM + 5% milk, **F** SODM + 5% whey. Adipogenic differentiation was limited to very few cells (oil vesicles were stained Oil Red O). **G** Standard adipogenic differantiation medium (SADM), **H** SADM + 5% milk, **I** SADM + 5% whey. The cells were photographed under an inverted microscope (**A**–**F**: ×4, **G**–**I**: ×10)

with adipogenic media did not differentiate into adipogenic lineages hsitologically enough. Adipogenic differentiation was microscopically limited to very few cells (Fig. 1).

The osteogenic transformation was more observable microscopically than the adipogenic transformation in groups that underwent RT-qPCR analysis. We demonstrated that the differentiation of these cells is slower and limited compared to bone marrow cells. In particular, the microscopic appearance of the adipogenic transformation was observed to be faint. However, the expression results of BMP-6 and PPRG show that differentiation of these cells is induced and achieved at the molecular level.

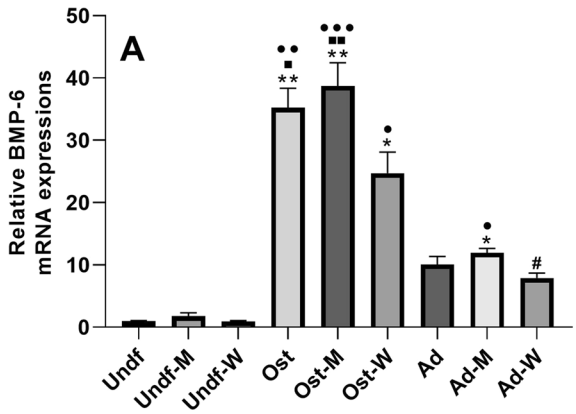
Our results showed that BMP-6 mRNA expression increased at very high coefficients in all osteogenic differentiated groups in which standard differentiation medium was applied and milk/whey was added versus all undifferentiated groups (Fig. 2A). We found that milk further increased the expression of BMP-6 mRNA, whereas whey somewhat limited the increase. The increase in BMP-6 mRNA expression was also observed in adipogenically differentiated groups, mostly in the milk group.

PPRG mRNA expression was also significantly increased in all adipogenically differentiated groups. No increase was observed in the osteogenic differentiated groups. For PPRG, the expression level obtained with milk was the highest (Fig. 2B). These results show that milk can better stimulate differentiation.

Gene analysis demonstrated that the expression of the stemness genes OCT4 and FGF-2 was maintained during the growth of undifferentiated hPMSCs in the media tested (Fig. 3A and B).

Discussion

In addition to its nutritional components, milk contains factors responsible for the growth and differentiation of cells, such as granulocyte-macrophage stimulating factor, epidermal growth factor and platelet-derived growth factor (Electricwala 1992). However, milk and whey are also rich in amino acids, vitamins, and growth factors like FGF-2 and insulin-like growth factor. One liter of milk contains 5.5 g of whey protein, and the major proteins are α-lactalbumin (19%), β-lactoglobulin (48%), immunoglobulin (10%), and serum albumin (7%) (Hassiotou et al. 2012). These natural substances are sterile, ready to use, and inexpensive. Therefore, it is logical to use them in cell culture medium. Studies have shown that if whey protein is added to the culture medium at concentrations of 0.02 and 0.1 mg/mL, whey protein can stimulate the proliferation and differentiation of osteoblasts cultured in different concentrations of whey protein (xu 2009; Almeida et al.



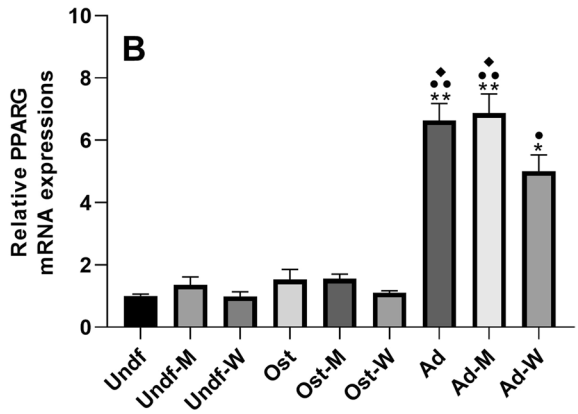


Fig. 2 **A** BMP-6 mRNA Expressions. BMP-6 mRNA showed higher expression in all osteogenic differentiated groups than undifferentiated groups. Milk increased BMP-6 mRNA expression more than whey. The increase in BMP-6 mRNA expression was also observed in adipogenically differentiated groups, mostly in the milk group. **B** PPARG mRNA Expressions. PPRG mRNA expression was significantly increased in all adipogenically differentiated groups compared to undifferentiated groups. This effect was most observed in the milk groups. Data are shown as means ± SD. **p* vs. Undf, ^{n}p vs. Undf-M, ^{l}p vs. Undf-W, $^{\#}p$ vs. Ost-M, ^{u}p vs. Ost-W

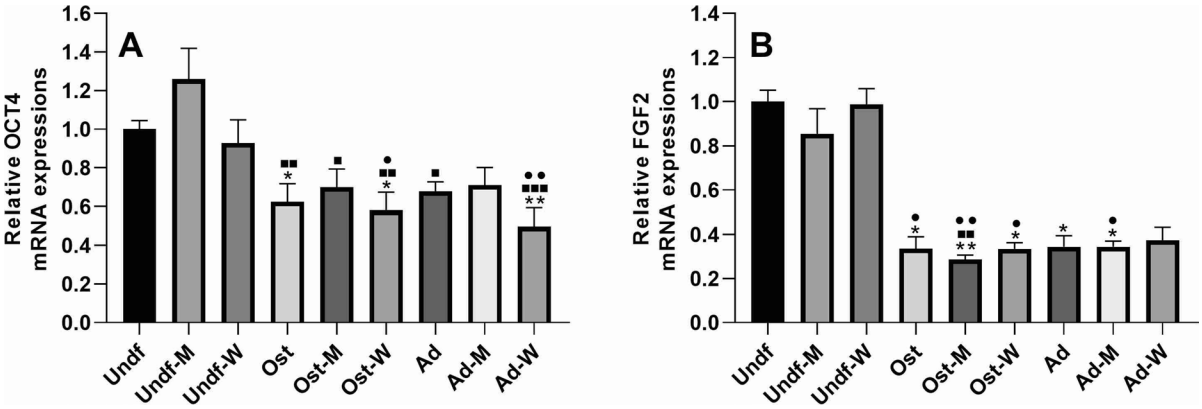


Fig. 3 **A** OCT4 mRNA Expressions. OCT-4 mRNA expressions were decreased in cells in osteogenic and adipogenic differentiated groups. **B** FGF2 mRNA Expressions. FGF2 mRNA expressions were decreased in cells in osteogenic and adipogenic differentiated groups. Data are shown as means ± SD. **p* vs. Undf, ^{n}p vs. Undf-M, ^{l}p vs. Undf-W.

2016; Carreira et al. 2015). Whey protein may also have a lipolytic effect on muscle cells (D'Souza et al. 2020). These observations supported the thesis that whey protein may play an important role in bone formation and have a potential therapeutic role in osteoporosis by activating osteoblasts.

Human placental mesenchymal stem cells are counted among the most active adult stem cells. It can be easily obtained without affecting donor safety (Soncini et al. 2007). Growth factors are essential elements in commonly used cell culture medium, such as FBS. There are reports that milk and whey contain such growth factors (xu 2009; Almeida et al. 2016; Grafe et al. 2018). Because of these features, we chose these cells as the study material. In this experimental study, we tried to isolate whey- and milk-hPMSCs and analyze their multipotency gene expression and function.

In this study, it was determined that if whey or milk is used alone instead of FBS or with FBS in cell culture medium, it can ensure the proliferation of hPMSCs and maintain cell viability in other passages. This study encouraged the development of a new culture medium using milk or whey without FBS supplementation, which effectively preserves stemness in vitro hPMSCs. However, without FBS supplementation, milk and whey alone were not sufficient for the growth of cells. In a study using the GPK cell line, it was reported that the use of milk with FBS supplementation provided sufficient (75–85%) cell production to reach (Electricwala 1992). The results are similar to our study. Undoubtedly, the source, preparation and storage conditions of milk and whey should be considered more in future studies.

In the microscopic analysis, cell loss of the 10%W group was observed. It could possibly be argued that whey proteins may be due to the toxic effects associated with their content at this concentration. Although it is claimed that cysteine in the whey protein molecules may cause S–S redistribution and be toxic to cell viability, especially in relation to storage conditions, it is reported that cysteine-containing whey proteins are not toxic to human umbilical vein endothelial cells (Yang et al. 2021). Another claim is that trace elements such as zinc may interact with whey protein and lead to a relaxation of the structural volatility of whey protein (Yuan et al. 2022). A cytotoxic α-lactalbumin-oleic acid complex named "HAMLET," (Human Alpha-Lactalbumin Made Lethal to Tumor cells) is a variant of alpha-lactalbumin that is bound to oleic acid. This complex may have toxicity to undifferentiated cells via triggering apoptotic mechanisms (Chetta et al. 2021).

Stem cells might respond to exogenous growth factors or chemicals. These growth can send intracellular signals to regulate or maintain stemness or to induce differentiation (Grafe et al. 2018). They might positively regulate the expression factors OCT4 and FGF2, which are key molecules in hPMSC stemness and might contribute to the undifferentiated growth of stem cells (Dev et al. 2012; Dvorak et al. 2005). As expected, the milk- and whey-hPMSCs did not

change stemness gene expression during undifferentiated growth. The expression of FGF-2 and OCT4 remained unchanged under conditions that induce hPMSC proliferation, but decreased after stimulation of hPMSC differentiation, implicating the role of FGF-2 and OCT4 in the self-renewal of hPMSCs.

Cell differentiation depends on several signalling pathways. Bone morphogenetic proteins (BMPs) are signal molecules that stimulate ectopic bone formation. The activation of BMPs is important for the osteogenic differentiation of MSCs harboring all BMP receptors (Carreira et al. 2015). Peroxisome proliferator-activated receptor c (PPARc) belongs to nuclear hormone receptor family and acts as a trigger for differentiation, which is abundantly expressed in adipogenic modulation of MSCs (Yuan et al. 2016).

The adipogenic marker PPARG regulated adipocyte differentiation. Milk-, whey- and FBS-hPMSCs expressed more PPARG after adipogenic induction than after osteogenic induction. It has been reported that the differentiation of human placental mesenchymal cells with adipogenic stimulation may be difficult or delayed in demonstrating by histological staining (Maytalman et al. 2022). Although histological staining failed to demonstrate adipogenic differentiation, *PPARG* gene expression might reflect the adipogenic differentiation potential. Low BMP6 expression under conditions that induce adipogenic differentiation might reflect osteogenic gene expression is FGF-2 dependent (Yuan et al. 2016; Muruganandan et al. 2009).

It is known that the osteogenic marker BMP6 induces osteogenic differentiation together with FGF-2. A notable finding of this study was that osteogenic induction with milk-, or FBS-hPMSCs resulted in greater BMP6 expression than with whey-hPMSCs. Our findings are supported by a report that bovine milk whey active protein facilitated osteoblastic differentiation in normal human osteoblasts. This report may suggest that the mechanism involves activating (JNK)-activating transcription factor 4 (ATF4) and extracellular signal-regulated kinase (ERK) (20, 21).

One limitation of this study was a need for more data related to further passages and other cell types. It is also necessary to optimize the densities of milk and whey in the medium.

In conclusion, in parallel with the reduction of FBS in the culture medium, the proliferation ability of multipotent hPMSCs also decreases. However, even in the absence of FBS, whey- and milk nutrients were effective in maintaining the stemness properties of hPMSCs in vitro. This observation can provide support for experimental and clinical studies in the fields of cell biology and regenerative medicine. It could also potentially reduce the use of serum in culture media.

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Author contributions The project idea was put forward by BB and MA. BB, MA, and EM jointly designed and conducted the laboratory phases of the study. IK provided laboratory support. The writing phase of the study was done by all the authors.

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Declarations

Competing interests The authors declare no competing interests.

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