FULL ARTICLE

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Stem cells derived from different tissues have similar morphology, proliferation, viability, plasticity, and mitotic potential

Células-tronco derivadas de diferentes tecidos possuem morfologia, proliferação, viabilidade, plasticidade e potencial mitótico semelhantes

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ABSTRACT

Studies suggest that stem cells derived from different tissues can give rise to heterogeneous populations of cells, and the adverse effects of successive passages on the mitotic activity of these cells are discussed. Therefore, this study evaluated the differences in morphology, proliferation, plasticity, and viability of porcine MSCs derived from bone marrow (BM-MSCs) and adipose (ADMSCs) tissue and the impact of culture until the 10th passage in their cell cycle. MSCs derived from pig adipose tissue and bone marrow were cultured in a D-MEM medium supplemented with fetal bovine serum, non-essential amino acids, L-glutamine, and penicillin-streptomycin. These cells were expanded over 10 passages, morphologically and immunophenotypically characterized, and evaluated for viability, proliferation, plasticity, and cell cycle. Our results identified two cell types, ADSCs and BMMSCs, which exhibited spindle-shaped morphology, expression of CD90+/CD105+/CD14- and similar characteristics of proliferation (ADMSCs: 2.34 ± 1.0x105; BM-MSCs: 2.20 ± 0.62 x105), viability (ADMSCs: 84.6%; BM-MSCs: 80.9%) and plasticity. Furthermore, both cell groups showed an increasing concentration of cells in G0/G1 from the sixth passage onwards. In conclusion, the morphology, proliferation, viability, plasticity, and immunophenotype of porcine ADMSCs and BMMSCs are similar. Furthermore, it is possible to suggest that both groups of cells have a gradual reduction in mitotic potential from the sixth passage onwards.

Keywords: Adipose-derived mesenchymal stem cells. Stromal cells. Pigs. Senescence.

RESUMO

As células-tronco mesenquimais (CTM) são células somáticas multipotentes, amplamente estudadas devido à sua plasticidade e imunomodulação. Estudos sugerem que células-tronco derivadas de diferentes tecidos podem originar populações heterogêneas de células e, ainda, discutem sobre os efeitos negativos de sucessivas passagens sobre a atividade mitótica dessas células. Diante disso, este estudo avaliou as diferenças na morfologia, proliferação, plasticidade e viabilidade de MSCs suínas derivadas de medula óssea (BM-MSCs) e tecido adiposo (ADMSCs) e o impacto da cultura até a 10ª passagem em seu ciclo celular. MSCs derivadas de tecido adiposo suíno e medula óssea foram cultivadas em meio D-MEM suplementado com soro fetal bovino, aminoácidos não essenciais, L-glutamina e penicilina-estreptomicina. Essas células foram expandidas em dez passagens, caracterizadas morfologicamente e imunofenotipicamente e avaliadas quanto à viabilidade, proliferação e plasticidade e ciclo celular. Nossos resultados identificaram dois tipos de células, ADSCs e BMMSCs, que exibiram morfologia fusiforme, expressão de CD90+/CD105+/CD14- e características semelhantes de proliferação (ADMSCs: 2,34 ± 1,0x105; BM-MSCs: 2,20 ± 0,62 x105), viabilidade (ADMSCs: 84,6%; BM-MSCs: 80,9%) e plasticidade. Além disso, ambos os grupos de células apresentaram concentração crescente de células em G0/G1 a partir da sexta passagem. Em conclusão, a morfologia, proliferação, viabilidade, plasticidade e imunofenótipo de ADMSCs e BMMSCs suínos são semelhantes. Além disso, é possível sugerir que ambos os grupos de células apresentam redução gradual do potencial mitótico a partir da 6ª passagem.

Palavras-chave: Células-tronco mesenquimais derivadas de tecido adiposo. Células estromais. Suínos. Senescência.

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Introduction

Mesenchymal stem cells (MSC) are multipotent somatic cells (Crisan et al., 2008) that exhibit key characteristics in vitro, including self-renewal capacity and differentiation into specialized cell types, making them a relevant therapeutic alternative in animals (Kalra & Tomar, 2014). MSCs have applications in regenerative medicine to repair, replace, or regenerate diseased tissues and organs (Williams et al., 2008) and can be easily obtained from humans and animals. Bone marrow is the point of the greatest prevalence of MSC, but a variety of tissues can supply these cells (Vacanti et al., 2005), including adipose tissue, which is considered an essential source of MSC (Luck et al., 2020).

MSCs derived from bone marrow and adipose tissue are frequently used in preclinical and clinical tissue regeneration (Liu & Holmes, 2021). BMMSCs were the first type of MSC to be characterized and are the most widely used (Caplan, 1991). However, Adipose tissue-derived mesenchymal stem cells (ADMSCs) are more frequent, are easier to obtain and with less damage to the donor site (Reumann et al., 2018), and can be used in various clinical applications. Their collection is not restricted by ethical issues (Shingyochi et al., 2015), as in mitigating cardiac functional deterioration after myocardial ischemia (Dariolli et al., 2013). Some authors suggest that MSCs obtained from different tissues constitute a heterogeneous population of cells, with significant differences between cell types (Liu & Holmes, 2021), making it necessary to create standards for characterizing MSCs (Kozlowska et al., 2019).

The extensive number of passages in stem cell culture may affect the quality of these cells. Changes in telomeres and reduced telomerase activity, which compromises the oxidative balance of these cells, are some of the damages caused by extensive culture (Romano et al., 2010). However, adequate quantities of ADMSCs for therapeutic applications, for example, require extensive in vitro expansion of these cells (Yin et al., 2020). Human ADMSCs cultured in vitro significantly reduced proliferation in the 15th passage. However, mitochondrial activity was reduced from the 5th to the 10th passage (Truong et al., 2019). Human mesenchymal stem cells derived from bone marrow rapidly declined cell proliferation at passages 7 to 9, being considered extensively expanded (Bartosh & Ylostalo, 2019).

Porcine MSCs exhibited characteristics reminiscent of cellular aging, such as reduced plating efficiency, which suggests gradual changes in cell membrane properties and cytoskeletal structures, starting from the 15th passage. However, this study only evaluates the 5th and 15th passages (called early and late), leaving a gap regarding cell damage accumulated in the time interval between the 5th and 15th passages (Vacanti et al., 2005). In light of this, low-passage cultures are recommended for clinical-scale expansion of cultures (Lechanteur et al., 2016). However, more evidence is needed to determine the ideal number of passages to maintain the ideal characteristics of MSC.

This study evaluated the differences in morphology, proliferation, plasticity, and viability of porcine MSCs derived from bone marrow and adipose tissue and the impact of culture until the 10th passage in their cell cycle.

Material and Methods

The present study follows the guidelines set by the Brazilian College of Animal Experimentation (COBEA) and received ethical approval from the Ethics Commission on the Use of Animals (CEUA/UFPI) under authorization number 269/16.

Animals

To collect subcutaneous adipose tissue and bone marrow samples, healthy pigs (n=5) with an average age and weight of 70 ± 8.4 days and 29.6 ± 10.2 kg, respectively, were used. The animals came from the Animal Science sector of the Federal University of Piauí (UFPI) and were fed daily with a diet of three kilograms of for pigs in the initial phase, along with water ad libitum (Rodríguez Del Águila & González-Ramírez, 2014).

Reagents

Dulbecco's Modified Eagle's (D-MEM Glutamax®), Penicillin-Streptomycin, trypsin, type I collagenase, Ficoll-Histopaque, and HEPES buffer solution, medium StemPro Differentiation Kit, paraformaldehyde (Vetec®), dyes alizarin red, oil red, alcian Blue and trypan blue were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Specific markers for the characterization of stem cells CD105 (Anti-CD105 PE,10:100, Ab53318) and CD90 (Anti-CD90 APC, 1:100, code 555596) were obtained from Abcam Cambridge, USA and CD14 (Anti-CD14 FITC, 1:100, code. C7673) from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The company of other chemicals and equipment was mentioned in the text.

Sample collection and cell isolation

The animals were initially anesthetized to collect the adipose tissue and bone marrow samples, the collection sites were shaved, and antisepsis was performed. The collection and isolation of stem cell samples derived from bone marrow were carried out according to the methodology of Costa et al. (2017) with some modifications. In that case, subcutaneous adipose tissue was collected by a 4 cm skin incision near the manubrium region's sternum. After the skin was incised, 1g fragments of subcutaneous adipose tissue were collected and placed in 50 ml conical tubes (TPP®, Trasadingen, Switzerland) for subsequent isolation. The pieces of adipose tissue were washed twice in phosphate-buffered saline (PBS) solution with 10% antibiotic (Penicillin-Streptomycin) and mechanically dissociated for 5 min using two sterile scalpel blades (No. 24) in a sterile Petri dish. For enzymatic digestion, 1 mg/ml of type I collagenase previously diluted in a 1:3 ratio in a D-MEM culture medium was added. The material was incubated in a humidified incubator (Thermo Scientific series II Water Jacket, USA) at 37 °C with 5% CO_2 and 95% humidity for 30 min. The enzymatic reaction was then neutralized by adding a complete basal culture medium.

The collection and isolation of stem cell samples derived from bone marrow were carried out according to the methodology of Argôlo Neto et al. (2016) with some modifications. Briefly, bone marrow samples were collected through the puncture of the iliac crest, after trichotomy and antisepsis, the region was palpated and 20 ml of bone marrow were aspirated using a 24G needle attached to a heparinized luer lock syringe. The bone marrow aspirate was separated by density gradient, and the mononuclear cell layer was collected and aliquoted into a Falcon tube containing complete media. The content was washed in a centrifuge for 10 min at 1500 rpm; the pellet was resuspended in 2 ml of medium and cultured in a 25 cm^2 flask.

Culture and expansion of MSCs

Both cell types were cultured in Dulbecco's Modified Eagle's (D-MEM) supplemented with 20% fetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, and 1% penicillin-streptomycin at 37 °C and 5% CO_2. The cell isolation phase was called passage 0 (P0), characterized by heterogeneous morphology cells, tacky or not. The expansion phase (passage) began after obtaining 80% confluence and was performed by enzymatic dissociation by trypsin (trypsinization), with a raised rate of 1:2, according to previous descriptions (Neves et al., 2021). The primary culture of MSC was obtained from the first passage (P1), characterized by a uniform monolayer of adherent cells of fibroblastoid or fusiform morphology. The cells were continuously expanded and submitted to in vitro assays until the 10th pass (P10).

Immunophenotypic characterization of ADMSCs and BMMSC

The recommendation of the International Society for Cellular Therapy with Stem Cells (ISSCT) was adopted to characterize cells using specific markers. We used CD105 markers (10:100), CD90 (1:100), and CD14 (1:100). Briefly, aliquots of 106 cells/ml of ADMSCs and BMMSC in P4 were washed with FACS buffer (Dulbeco Phosphate Buffer Solution) containing 0.1% BSA, centrifuged and incubated at room temperature with antibodies. They were then washed with FACS buffer and analyzed in a flow cytometer (FACScanto® II) and BD FACSDiva Software (Version 6.1.3), obtaining 30,000 events per sample tested.

In our study we did not characterize MSC lineages, the nomenclatures ADMSCs and BMMSC were used only to define their anatomical isolation niche.

Colony Forming Unit Assay (CFU)

Heterogeneous cell samples were divided, plated, and incubated for 20 days. The colonies were fixed with 4% paraformaldehyde for 30 min and stained with Giemsa for 10 min at room temperature. The colonies were counted on the surface of the 25 cm^2 culture flask. The cells were measured using a micrometer grid in ImageJ software.

Cellular plasticity assay

Aliquots of 104 cells/ml of MSCs derived from adipose tissue (ADMSCs) and bone marrow (BMMSC) in P2 were induced adipogenic, chondrogenic, and osteogenic

differentiation, using a differentiation-inducing medium StemPro Differentiation Kit. The cultures were incubated in induction media at 37 °C with 5% CO_2 and 95% moisture over 21 days, renewing the culture medium every 72 h.

After 14 (for adipogenic and chondrogenic) and 21 (for osteogenic) days of induction, the cells were fixed in 4% buffered paraformaldehyde for 30 min at room temperature. Following fixation, cells were rinsed with distilled water. Adipose inducted cells were stained with Oil Red O solution for 15 min to identify the presence of intracytoplasmic lipid droplets indicative of adipogenic differentiation. The excess stain was washed away with 60% isopropanol. Chondrogenic inducted cells were stained with Alcian Blue solution for 30 min to visualize chondroitin sulfate in the extracellular matrix, a marker of chondrogenic differentiation. Osteogenic inducted cells were stained with Alizarin Red S solution for 20 min at room temperature. Excess stain was washed away with distilled water, and red-stained mineral deposits indicated successful osteogenic differentiation.

Cellular proliferation assay

A growth curve by saturation was performed in triplicate for ADMSCs and BMMSCs in P4. For this, aliquots of 104 cells/ml were sowed in culture plates of 24 wells and kept incubated in a culture medium supplemented at 37 °C with 5% CO_2 and 95% humidity. Daily, a sample of ADMSCs and BMMSC was trypsinized and centrifuged, and the pellet was resuspended in 1 ml of PBS. An aliquot of the solution was diluted in 0.2% trypan blue dye in the 1:1 ratio to evaluate cell viability and counted in a hemocytometer under optical microscopy. The the procedure was repeated daily for each sample over 20 days.

Cell viability test

Aliquots of 104 cells/ml of ADMSCs and BMMSC in P4 were sowed, in triplicate, in culture plates of 24 wells and kept incubated in a culture medium supplemented at 37 °C with 5% CO_2 and 95% humidity. Every three days, the culture medium of three wells of each cell lineage was replaced by a 3-bromide solution (4,5-dimethyl-2-thiazolil)-2,5-diphenyl-2H-tetrazolium (MTT) and culture medium in the proportion of 1:9 and kept incubated for 24 h. Then, the solution was discarded, dimethyl sulfoxide (DMSO) was added, the cells were transferred to an ELISA plate, and absorbance was measured in a spectrophotometer using a wavelength of 550nm. The procedure was repeated over 20 days. As a control, the viability evaluation was performed using trypan blue dye, as described above (Argôlo Neto et al., 2016).

Cell cycle evaluation

The ADMSCs and BMMSCs in P2, P3, P5, P6, and P9 were trypsinized, washed twice with cooled PBS, and then resuspended in 1X binding buffer at a concentration of 1x106 cells/ml. Then transferred 100µl of the solution and added 5µl of 7-AAD detection kit I Annexin V: PE Apoptosis (BD PharmigenTM) with light agitation and incubated for 15 min at room temperature in the dark. With the addition of 400µl of binding buffer in each tube, they were analyzed in FACScanto® II flow cytometer and BD FACSDiva software (Version 6.1.3), obtaining around 20,000 events per tested sample, enabling the classification and measurement of cells in phases G0/G1, S and G2/M.

Statistical analysis

Statistical analysis was performed using the data spreadsheet (Excel®) and the statistical analysis program version 8.0 (SAS®). The means and standard deviations of the percentage of viability, prolificacy, and cell cycle of the cultures were evaluated (Rodríguez Del Águila & González-Ramírez, 2014).

Results

The total period of cell isolation lasted 25 days, with the first adhesive cells of ADMSCs identified at 15 ± 4.6 days and BMMSC cells at 10 ± 2.3 days of culture. In both lines, the MSCs presented an average length of 46.34 ± 13.50µm and an average width of 7.61 ± 1.23µm. In the CFU assay (Figure 1A), we identified the formation of 187 ± 4.6 colonies of ADMSCs and 90.86 ± 3.27 BMMSC colonies. The cells maintained their fusiform morphological characteristics (Figure 1B), showed radial growth, and tended to merge between the colonies, forming a single layer of cells.

The ADMSCs and BMMSC strains showed positive plasticity for adipogenic, osteogenic, and chondrogenic induction. Osteogenic morphological alterations were observed after 18 ± 1.3 days, represented by irregular cytoplasmic limits, formation of cellular prolongations, and flattened aspect. After staining with Alizarin Red, the nucleus presented dark red coloring, and the cytoplasm was more basophilic and well-defined, with calcium deposits inside the cells. Adipogenic alterations were identified after 20 ± 2.4 days, characterized by large, hexagonal, irregular cells that exhibited birefringent cytoplasmic granules. When stained with Oil Red, it was observed that the cytoplasm granules were small fat vacuoles spread and colored brown. The chondrogenic alterations were identified after 15 ± 5.2 days, represented by rounded cells with polygonal to oval shapes, varying in size, surrounded by an extracellular matrix intensely colored with Alcian Blue.

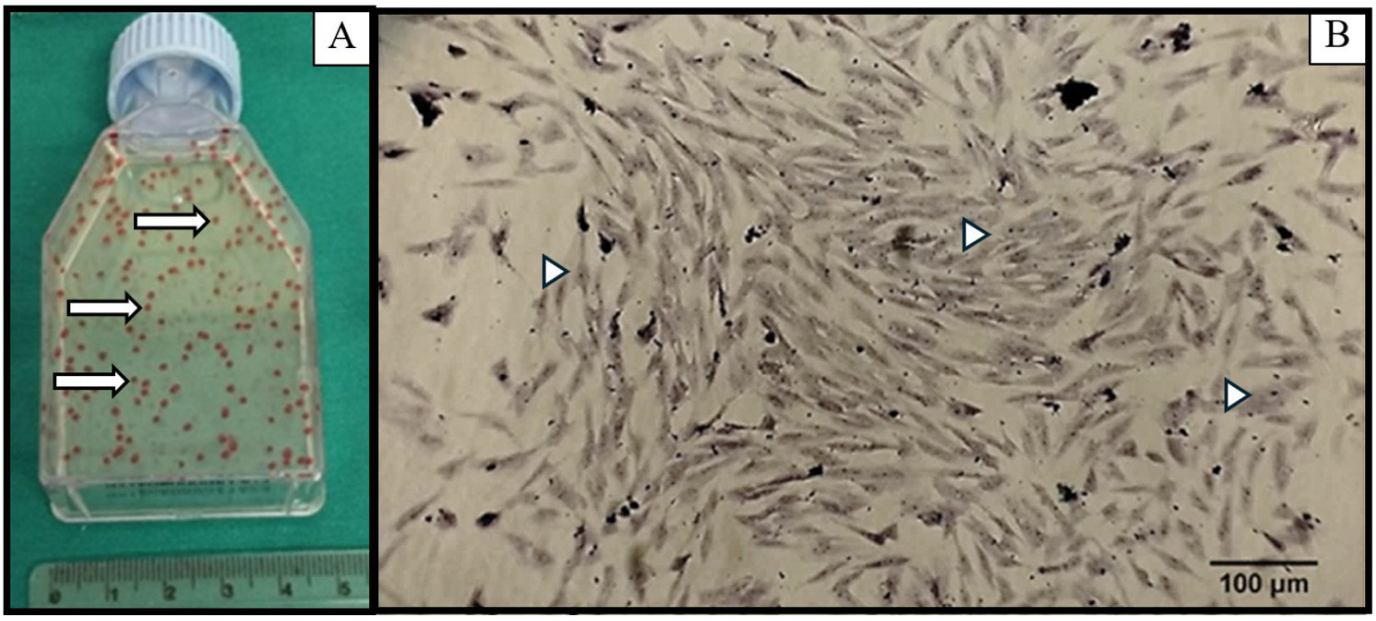

Figure 1 – Cellular Characteristics of ADMSCs and BMMSC. (A) Colony Forming Unit (CFU) Assay; (B) Photomicroscopy of cells ADMSCs and BMMSC with fusiform morphology. cells observed during the CFU assay. White arrows indicate colonies of ADMSCs and BMMSC; Arrowheads indicate ADMSCs and BMMSC with fusiform morphology and few peripheral fibroblastoid figures.

During the proliferation assay with growth curve by saturation, the ADMSCs exhibited an average concentration of 2.34 ± 1.0x105 cells per well cultured, with average viability of 84.6%, and BMMSC, 2.20 ± 0.62 x105 cells per well cultured and average initial viability of 80.9%. Similarly, the cells exhibited the phases of latency (lag), log growth (log), and exhaustion (decline). The initial lag phase varied from the first to the fourth day between the strains, presenting low cell proliferation. The log phase varied on average 11 ± 2.1 days, reaching its climax of prolificacy on the 12th and 14th day in ADMSCs and BMMSC, respectively. None of the lineages exhibited a stationary phase (plateau). From then on, the cultures showed a decline phase, with a progressive reduction of cell concentration.

The viability assay indicated total average absorbance of 2.39 ± 0.72 and 1.45 ± 0.6202 for ADMSCs and BMMSC, respectively. During the log phase, the mean absorbance observed was 2.89 ± 2.16 and 1.88 ± 1.52 for ADMSCs and BMMSC. The decline phase presented the lowest averages, 1.78 ± 0.36 and 0.85 ± 0.65, respectively.

Both lineages showed positive markings for CD90 and CD105 antibodies: 8.12% and 99.43% for ADMSCs and 10.2% and 95.13% for BMMSCs, respectively. Both ADMSCs and BMMSCs did not display CD14 markings.

Both lines, in P2 and P5, were predominantly in the S phase of the cell cycle. In P9, most cells were in phase G0/G1. However, the percentage of f cells in mitosis was similar in all the evaluated passages (Table 1).

Table 1 – Cell cycle analysis of mesenchymal stem cells (MSC) derived from adipose tissue (ADMSCs) and bone marrow (BMMSC) of pigs, over six distinct passages, by quantifying DNA content using detection kit I Annexin V.

Passage	Phase G_0/G_1 (%)	Phase S (%)	Phase G_2/M (%)
P2	19.63 ± 8.3	56.70 ± 0.4	12.02 ± 5.2
P3	23.32 ± 6.1	58.72 ± 0.6	14.14 ± 2.1
P5	11.41 ± 1.1	67.10 ± 1.4	19.21 ± 0.4
P6	32.24 ± 4.7	24.36 ± 2.0	19.70 ± 1.0
P9	60.54 ± 1.4	13.51 ± 1.3	18.91 ± 1.6

Discussion

Our study results in important information that reinforces the need to use culture time (number of passages) as a selection parameter for using stem cells derived from bone marrow or porcine adipose tissue and provide information on the morphological and developmental characteristics of stem cells of different origins. Even though we did not observe differences regarding the morphology, plasticity, proliferation, and viability of these different cell types, both present a higher concentration of cells in the G0/G1 phase from the sixth passage onwards.

Despite the cell isolation time, cells derived from adipose or bone marrow tissue exhibited indistinguishable morphology, fusiform morphology, and few peripheral fibroblastoid figures. Previous studies have described morphological variation between MSC obtained from

different anatomical niches (Mushahary et al., 2018; Soundararajan & Kannan, 2018). However, the predominant description is uniform morphology, regardless of the tissue in the collection (Sousa et al., 2021; Souza et al., 2016). Our findings corroborate the hypothesis that the morphology of MSCs is indistinguishable regardless of the tissue of origin, as they are from the same cell lineage (Caplan & Correa, 2011). However, studies are still needed to compare the functional differences observed in MSCs from different tissues, which remain unexplained (Caplan, 2017).

In our results, ADMSCs had longer isolation times and a greater number of colonies compared to BMMSCs. In this case, we suggest that adipose tissue provided a higher concentration of MSC than bone marrow-derived blood, as previously described (Carvalho et al., 2015; Costa et al., 2017; Esteves et al., 2017). It is estimated that MSCs represent only 0.001% of the total mononuclear blood cells in the bone marrow of animals (Argôlo Neto et al., 2016; Silva Filho et al., 2014). Diametrically, it is commonly reported that one gram of adipose tissue can contain at least 106 cells (Costa et al., 2017). In this study, the culture of adipose tissue explants yielded approximately 109 cells. The perivascular location of ADMSCs in adipose tissue would justify such findings.

The multipotent characteristics of ADMSCs and BMMSCs did not differ in our study, in line with previous studies (human: Aliabouzar et al., 2018; Arjmand et al., 2018; canine: Alves et al., 2017). These cells' osteogenic, adipogenic, and chondrogenic plasticity is currently well-defined. However, subtle differences in differentiation induction protocols may reflect functional peculiarities of MSCs from different tissues, generating different results. In this context, human BMMSCs can be induced to chondrogenic differentiation by the addition of transforming growth factor beta (TGF-β). In contrast, human ADMSCs require, in addition to TGF-β, the addition of bone morphogenetic protein (BMP). Differentiation (Silva Filho et al., 2014). It is not yet known whether such previous descriptions represent a fact or a methodological artifact, but they denote the relevance of studies with plasticity tests. In this study, ADMSCs and BMMSCs exhibited induction plasticity with the same differentiation medium, raising the inference that the possible functional differences between them did not interfere with the manifestation of plasticity in vitro.

The immunophenotypic characterization identified in this study revealed high expression of CD105+, low expression of CD90+, and absence of expression of the hematopoietic marker CD14-, excluding the possibility of isolation of another cell type. The low expression of CD90 may be associated with the use of non-specific antibodies for the swine species, in addition to the possibility of variation of the antigenic composition according to the collection site as described in other investigations (human: Gronthos et al., 2001; rat: Kaewkhaw et al., 2011). In the immunophenotyping of stem cells derived from Dasyprocta prymnolopha adipose tissue, for example, the expression of CD 105 was low despite the high expression of CD 90+ (Rocha et al., 2019), which reflects the variation in the expression of these markers according to the species or fabric used. Still, the expression of CD105+ and CD90+ is typical for all animal and human MSCs, although slight variations in expression magnitude can be observed among different tissues (Carvalho et al., 2015; Freitas Siqueira Silva et al., 2020; Sousa et al., 2021) the predominance of this expression reiterates the probable intimate molecular relationship between ADMSCs and porcine BMMSCs.

The proliferation of ADMSCs and BMMSCs in P4 was similar to our study and corroborated what Carvalho et al. (2015) described in cellular progenitors obtained from the dental pulp of agouti. Associated with these results, both cell groups showed high viability. Related to these results, analysis of the cell cycle of ADMSCs and BMMSCs demonstrated that cells in P5 were predominantly in the S phase. In contrast, the number of cells in G0/G1 gradually increased from P6, being higher in P9 compared to all other passages evaluated. This result suggests that from the sixth passage onwards, there is a reduction in mitotic potential, which may be related to cell death (Sousa et al., 2021; Wagner et al., 2008). According to these authors, cell senescence can induce decreased gene expression that controls the cell cycle, reducing its activation and increasing the number of cells at rest (G0). However, further gene expression studies with ADMSCs and porcine BMMSCs must confirm this hypothesis.

The description of the differences and similarities of stem cells derived from adipose tissue or porcine bone marrow, as performed in our study, is essential for developing appropriate cellular therapeutic methods based on MSCs. The potential for using stem cells derived from adipose tissue and bone marrow in cell therapy can be seen by observing research focused on regenerative and reparative medicine (Wang et al., 2008), such as the regeneration of bone tissue (porcine ADMSCs: Monaco et al., 2011) and of kidney tissue (human ADMSCs and BMMSCs: Pool et al., 2020)

Conclusion

In conclusion, the morphology, proliferation, viability, plasticity, and immunophenotype of porcine ADMSCs and BMMSCs are similar. Furthermore, it is possible to suggest

that both groups of cells have a gradual reduction in mitotic potential from the sixth passage onwards. Figure 1: Cellular Characteristics of ADMSCs and BMMSC. A. Colony Forming Unit (CFU) Assay -B. Photomicroscopy of cells ADMSCs and BMMSC with fusiform morphology, cells observed during the CFU assay. White arrows indicate colonies of ADMSCs and BMMSC; Arrowheads indicate ADMSCs and BMMSC with fusiform morphology and few peripheral fibroblastoid figures.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethics Statement

The present study follows the guidelines set by the Brazilian College of Animal Experimentation (COBEA) and received ethical approval from the Ethics Commission on the Use of Animals (CEUA/UFPI) under authorization number 269/16.

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