German Journal of Veterinary Research
eISSN:2703-1322

Research article

Comparative *in vitro* characterization of adipose tissue and bone marrow mesenchymal stem cells (MSCs) from sheep and goats for potential applications in stem cell therapy and improved reproduction

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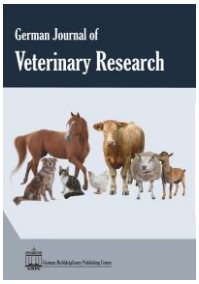

Article History:
Received: 7-Oct-2015
Accepted: 23-Nov-2025
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Abstract

Small ruminants, i.e., sheep and goats, are not only an important component of livestock production, but also a promising model for translational research in reproductive and regenerative medicine using mesenchymal stem cells (MSCs). MSCs, known for their pleiotropic effects and ability to secrete regulatory factors, are increasingly used in various fields of biomedicine and veterinary research. Here, a comparative study of the characteristics of MSCs from different tissues of sheep and goats, i.e., adipose tissue (AT) and bone marrow (BM), was performed, including the analysis of their *in vitro* isolation features, culturing characteristics, and interspecies differences. We found that MSC cultures obtained from AT and BM exhibit species-specific differences in proliferation rate and culturing time. We describe long-term culture of ovine (up to 40 passages) and caprine (up to 20 passages) MSCs for the first time. Growth curves were constructed, and morphological differences between the cultures and changes in them with increasing passage number were documented. Induction of adipogenic and osteogenic differentiation demonstrated that MSCs isolated from different sources (AT vs. BM) require different times for differentiation; however, interspecies differences in differentiation time were not apparent. The isolation, culture, and differentiation protocols reported here provide a stable method for generating MSC populations from AT and BM in sheep and goats. Sheep MSCs demonstrated a greater proliferative activity compared to goats, but goat MSCs had an earlier proliferation peak. Unlike ovine MSCs, goat AT- and BM-MSCs did not alter their morphology during long cultivation. Neither sheep nor goat MSCs had spontaneous mesenchymal differentiation, but both AT-MSCs displayed faster adipogenesis, with BM-MSCs predisposed to osteogenesis. The results of the study contribute to understanding the interspecies features of MSCs *in vitro* and lay the groundwork for the practical, cutting-edge applications of small ruminant MSCs across various biomedical and veterinary fields.

Keywords: Cell culturing, Differentiation, Long *in vitro* cultivation, Morphology, Proliferation, Small ruminants

Citation: Korochkina, E. A., Trifonova, A. V., Stafeev, I. S., Lavrik, A. A., Pushkina, V. S., Griffin, D. K., and Romanov, M. N. 2025. Comparative *in vitro* characterization of adipose tissue and bone marrow mesenchymal stem cells (MSCs) from sheep and goats for potential applications in stem cell therapy and improved reproduction. Ger. J. Vet. Res. 5 (4): 26–41. https://doi.org/10.51585/gjvr.2025.4.0161

Introduction

The production and breeding of small ruminants, i.e., sheep (*Ovis aries*) and goats (*Capra hircus*), are among the most attractive and promising areas for the development of the commercial and

private livestock sectors (Pulina et al., 2018; Morgan-Davies et al., 2024). To date, they are considered valuable farm animals because of their rapid rate of herd formation (Liptac et al., 2025), specifically due to the reproductive systems of sheep and goats (Al-Jaryan et al., 2023). Moreover, their products include high-value milk, wool, dairy, and meat products, which leverage the advantages of the small ruminant industry and contribute to its high profitability (Gadekar et al., 2023; Chadda et al., 2025).

In translational research, sheep and goats also offer several advantages, including large bones with structures, biochemistry, and mineral composition similar to those of humans (Kandziora et al., 2001). In addition, their skeletal size and basic anatomy, weight, joint structure, and bone/cartilage healing mechanisms are generally comparable to humans (Wilke et al., 1997; Pearce et al., 2007; Viateau et al., 2007; Martini et al., 2001). This makes them pivotal models primarily in orthopedics (Martini et al., 2001). There are also, however, new and emerging areas of translational research (Dias et al., 2022; Harness et al., 2022; Alvites et al., 2021). In particular, small ruminants are attractive models for translational research in reproductive and regenerative medicine using mesenchymal stem cells (MSCs), also known as multipotent stromal cells (Mohamad-Fauzi et al., 2015; Anatolitou et al., 2024). That is, MSCs have been used to identify factors affecting the efficacy of artificial insemination (Almeida et al., 2021; Souza-Fabjan et al., 2023), to improve sperm quality (Jia et al., 2024; Stewart and Shipley, 2021; Balaro et al., 2022; Paredes et al., 2024; Sakhatsky et al., 1987; Linnik et al., 2010), and to enhance male fertility (Segunda et al., 2024; Tanrıkulu et al., 2025). Moreover, goats have generated considerable interest as models for dairy animal genetic engineering and stem cell therapy for bone and cartilage restoration and implantation (Mohamad-Fauzi et al., 2015).

In recent years, MSCs have been identified, isolated, and characterized from various species (Mohamad-Fauzi et al., 2015; Anatolitou et al., 2024) and have been found to have further applications, serving as a useful source of regulatory substances (Korochkina et al., 2023, 2024a). Among other practical examples, the efficacy and safety of MSCs and their derivatives seem encouraging in treating mare endometritis (Del Prete et al., 2024, 2025; Wong et al., 2024; Tongu et al., 2024; Mambelli et al., 2013; Rink et al., 2018; Cabezas et al., 2018; Navarrete et al., 2020), chronic vesicular adenitis in bulls (Severo et al., 2025) and mastitis in dairy cows (Ghai et al., 2022), facilitating allogenic transplantation in bull recipient testes (Segunda et al., 2024), modulating the inflammatory response to spermatozoa in mares (Ferris et al., 2014) and implicating for other reproductive biotechnologies (Mattei et al., 2024; Cortez et al., 2024; Motta et al., 2023; Baouche et al., 2023; Segunda et al., 2019; Bezerra et al., 2019; Maia et al., 2017; Opiela et al., 2017; Lange-Consiglio et al., 2017; Mançanares et al., 2015).

Of particular interest is the use of MSCs to improve male reproductive capacity and confer cryoprotective effects on sperm (Scassiotti et al., 2023; Segunda et al., 2024; Tanrıkulu et al., 2025). According to data from the last 10 years, MSCs derived from umbilical cord, adipose tissue (AT), and bone marrow (BM) showed the best results in restoring male fertility. For instance, Hajihoseini and Vahdati (2017) and Mehrabani and Hassanshahi (2015) conducted studies on the effects of bone marrow-derived MSCs (BM-MSCs) and adipose tissue-derived MSCs (AT-MSCs) on induced azoospermia in guinea pigs and rats, respectively. Panahi and Keshavarz (2015) evaluated rat testes as an azoospermic animal model for stem cell transplantation when using busulfan to induce azoospermia. Following this model approach, Tamadon et al. (2015) injected BM-MSCs into the azoospermic hamster testes. In 35 days, the testes into which MSCs were injected became morphologically normal. Hereby, spermatogenesis was detected in a proportion of testicular tubules that received stem cells. Other studies reported trans-differentiation of MSCs into spermatogenic cells under appropriate conditions (Cakici and Buyrukcu, 2013). In an investigation by Kim et al. (2018), MSCs reduced chemotherapy-induced lesions, premature ovarian failure, and polycystic ovary syndrome in mice. These positive results can be explained, at least in part, by MSCs' effects on granulosa cells, as well as their anti-inflammatory, anti-apoptotic, regenerative, and potential paracrine actions (Yang et al., 2020). In studies by Sun et al. (2013) and Omar et al. (2016), MSCs reduced chemotherapy-induced damage and premature ovarian failure. These results can be explained by the fact that multiple mechanisms are involved when using MSCs. After MSC migration to the damaged ovary, MSCs

modulate proliferation, apoptosis, immunization, autophagy, oxidative stress, and fibrosis in ovarian cells (Li et al., 2021). AT- and BM-derived goat MSCs can differentiate into osteogenic, adipogenic, and chondrogenic types (Mohamad-Fauzi et al., 2015). Mohamad-Fauzi et al. (2015) described goat MSCs and demonstrated that MSCs obtained from different tissues and from the same tissue might differ significantly.

In our previous studies, we preliminarily assessed the positive effects of BM-MSCs and AT-MSCs on spermatozoa quality parameters in rams (Korochkina et al., 2024a,f), buck-goats (Korochkina et al., 2023), and bulls (Korochkina et al., 2024d). We further demonstrated that the reliability of results on stem cell applications in animals using species-specific cells is of great importance. It follows then that a more thorough characterization of MSCs obtained from sheep and goats is of further interest and significance, particularly as it seems to be relatively lacking in the literature (Mohamad-Fauzi et al., 2015). With this in mind, we carried out a detailed study of the features of MSCs isolated from different sources, i.e., AT and BM, and determined the characteristics and behavior of cells in culture for sheep and goats. An inter-specific comparison of these parameters in MSC cultures completed our investigations.

Materials and methods

Ethical approval

Approval for this study was obtained from the Local Ethics Committee established at the Federal State Budgetary Educational Institution of Higher Education "St. Petersburg State University of Veterinary Medicine" (Fsbeihe Spsuvm), St. Petersburg, Russia (Protocol No. 1 dated 20 January 2025). The study was approved for conducting within the framework of the research project entitled "The Influence of MSCs from Adipose Tissue and Bone Marrow of Small Ruminants on the Quality Indicators of Their Spermatozoa before and after Cryopreservation" at the Fsbeihe Spsuvm.

Animals and samples

Samples of AT and BM tissues were collected after slaughter of the males, including four rams and four goat bucks kept in a private farm in Leningrad Oblast. All animals were about two years old and clinically healthy. AT samples were isolated from subcutaneous fat immediately after slaughter. To isolate BM samples, rib fragments were taken from the animals. The samples were placed in sterile tubes filled with 25 mL of the following transferring medium: phosphate-buffered saline (PBS; BioloT, St. Petersburg, Russia) containing 200 µg/mL ceftriaxone-AKOS (Sintez, Kurgan, Russia), 3 µg/mL amphotericin B (Sintez), 100 U/mL penicillin (Gibco, Billings, MT, USA), and 100 µg/mL streptomycin (Gibco).

MSC isolation from adipose tissue and bone marrow

MSC isolation protocols were adopted from Dar et al. (2021). Under laminar flow conditions, all biomaterial was thoroughly washed with sterile PBS buffer supplemented with 200 µg/mL ceftriaxone-AKOS and 3 µg/mL amphotericin B. AT samples were minced and incubated with 500 U/ml collagenase (type 2) (PanEco, Moscow, Russia) for 45 min at 37 ^{0}C with constant shaking. After that, cells were pelleted by centrifugation at 400 g for 10 min. Pellets were resuspended in α-MEM medium (BioloT). For BM cell isolation, rib fragments were thoroughly washed with sterile PBS buffer supplemented with 200 µg/mL ceftriaxone-AKOS and 3 µg/mL amphotericin B. Then, BM cells were washed out of the rib fragments under pressure using a 25-gauge syringe. The resultant cell suspension was transferred to a sterile tube and collected by centrifugation (400 g for 10 min).

Resuspended cells from AT and BM were filtered through a cell filter with a pore size of 100 µm, and the filtrate was centrifuged again. The pellet was diluted with α-MEM medium supplemented with 10% HyClone™ fetal bovine serum (FBS; Cytiva, Marlborough, MA, USA), 100 U/ml penicillin, and 100 µg/mL streptomycin and seeded into cell culture flasks (SPL Life Sciences, Naechon-myeon, Pocheon, Korea). Cell cultures were placed in a CO_2 incubator at 37 °C and 5% CO_2. After 24 hours, the medium was changed, and non-adherent cells were removed by washing. The medium was refreshed every 3 days until an 80–90% confluent monolayer was achieved, which was formed on the 7th–10th day of cultivation. Cells were then treated using 0.05% trypsin (PanEco) in Versen solution (PanEco). After two isolations, all sheep and goat AT- and BM-MSC cultures obtained were cryopreserved. This involved equilibration in a cryoprotective medium containing FBS and 10% DMSO (PanEco) for 20–30 min at room temperature, followed by slow cooling at 10

°C/min in an isopropanol-based freezing chamber to −80 °C, and subsequent storage in liquid nitrogen (−196 °C). The produced MSC cultures were deposited in the cell culture biobank maintained at Stem Renovatio LLC (St. Petersburg, Russia).

Finally, randomly selected cultures of AT- and BM-MSCs obtained from one sheep and one goat were thawed to evaluate their properties. To study the growth properties, MSC cultures were seeded in 25 cm^2 flasks and cultured in α-MEM medium supplemented with 10% FBS and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin).

Proliferation assay for sheep and goat MSCs

To plot the growth curves and compute the population doubling time (td), we regularly seeded sheep MSCs at 2 × 10^3 cells/cm^2 and goat MSCs at 4 × 10^3 cells/cm^2, and counted the cells removed after 72–96 h of growth. Based on these data, we calculated the proliferation index (PI), i.e., the ratio of the number of grown cells (N_t) to the number of seeded (N_0) as described by Frausto et al. (2019). We also determined t_d using the following formula: $t_d = t/\log_2 (N_t/N_0)$, where t is the culture growth time (Uzbekov, 2004). Sheep AT- and BM-MSC cultures were passaged until passage 40, and goat cultures until passage 20. Cell morphology in each culture was also assessed during cultivation using an inverted microscope (Zeiss Axio Observer A1, Zeiss, Oberkochen, Germany) and image capture.

Adipogenic and osteogenic differentiation potential of MSCs

The ability of MSCs to differentiate into adipogenic lineage *in vitro* was studied using the StemPro™ Adipogenesis Differentiation Kit (Gibco) according to the manufacturer's instructions. Osteogenesis potential evaluation was performed according to Mahajan et al. (2022). For osteogenesis induction, we used a medium based on DMEM HG (4.5 g/L, Gibco) supplemented with 10% HyClone™ FBS (Cytiva), 1% L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 50 U/mL penicillin, and 50 µg/mL streptomycin. To this medium, the following osteogenic inducers were also added: 50 µg/mL ascorbic acid (Sigma-Aldrich), 10 µM dexamethasone (Sigma-Aldrich), and 10 mM β-glycerophosphate (Sigma-Aldrich).

At passage 4, the cells were transferred to 24-well plates at a density of 4 × 10^5 cells/cm^2. After 24 h, the medium in the wells was replaced with induction (n = 3) or control (n = 3) media for each culture and lineage. Then, the media were replaced every 3–4 days. The differentiation efficiency was assessed at 14, 21, and 28 days of cultivation. For this purpose, cells were fixed with ice-cold methanol and stained with specific dyes. Lipid droplets in the cytoplasm were labeled using Oil Red O staining (Sigma-Aldrich), and nuclei were stained with Carazzi's hematoxylin (PanEco). Osteogenic differentiation efficiency was analyzed using Alizarin Red S (Abcam, Waltham, MA, USA). To assess possible spontaneous differentiation in MSC cultures, monolayer staining was performed as described above and without differentiation at passage 30 for sheep MSCs and at passage 20 for goat MSCs.

Statistical analysis

The analyzed data were represented as mean ± standard error of the mean (SEM) for each group. The Mann-Whitney rank-sum U-test was used to estimate the difference between two independent groups at the $p < 0.05$ significance level. Graphs were plotted using Microsoft Excel and GraphPad Prism 8.0 (Dotmatics, Boston, MA, USA; GraphPad Software, 2024).

Results

Proliferation of sheep and goat MSCs

At the first study step, we analyzed the proliferative activity of goat and sheep MSCs isolated from AT and BM tissues. Proliferative activity is a crucial feature of MSCs that can be used directly in cell-based therapeutic approaches and in a cell-free format for secretome production (Patel et al., 2017; Vizoso et al., 2017; Mushahary et al., 2018; Eleuteri and Fierabracci, 2019; Han et al., 2019). In both cases, active proliferation is a very important characteristic. As can be seen in Figure 1, sheep MSCs from AT and BM had significantly higher PI than goat MSCs, while goat AT-MSC proliferation was higher than that of goat BM-MSCs.

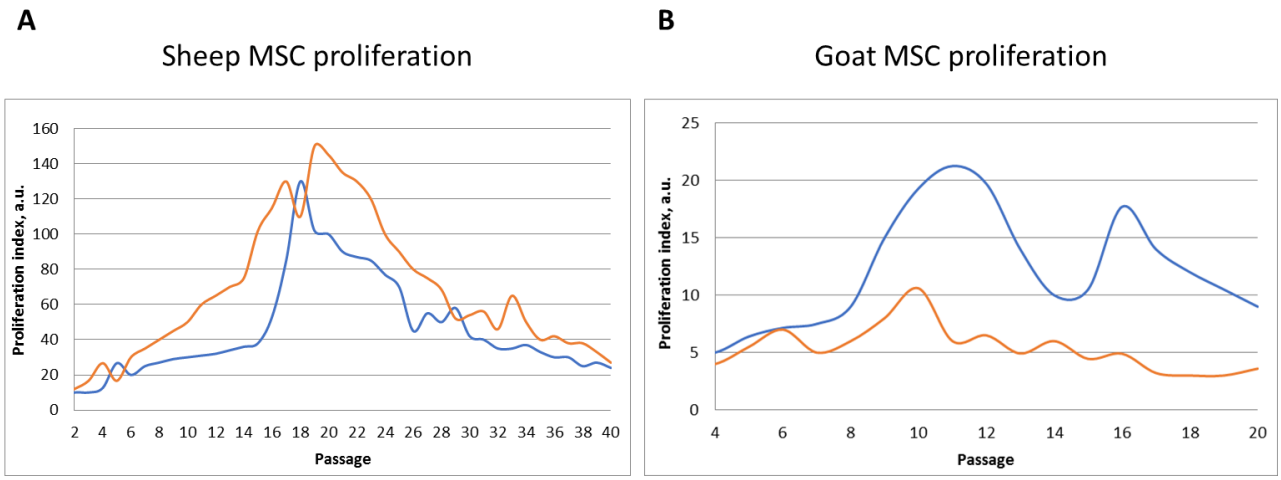

Figure 1: Small ruminant culture growth curves of mesenchymal stem cells (MSCs) from adipose tissue (AT) and bone marrow (BM) showing changes in proliferation index (PI) depending on the duration of cultivation. (A) The representative PI curves for sheep MSCs; (B) the same for goat MSCs. Blue curve, AT-MSCs; red curve, BM-MSCs; a.u., arbitrary units. Data are presented as mean values.

We demonstrated that AT- and BM-derived sheep MSC cultures had the peak proliferation rate at passages 15–22. The population doubling time at the initial cultivation stage (i.e., passages 3–6) for both AT- and BM-MSC cultures was 20–26 h, whereas it was 10–14 h at the peak of cell growth and 14–19 h by passage 40. Overall, it should be noted that the proliferation kinetics for AT- and BM-derived sheep MSCs were similar, although PI of the AT-MSCs culture was lower than that of BM-MSCs (Figure 1A). In goat MSCs, we found that AT-MSCs demonstrated significantly higher proliferative potential than BM-MSCs. The population doubling time for goat AT-MSCs was 30 h at the initial cultivation stage (i.e., at passages 3 to 6) and 18–20 h at the peak of proliferative activity (passages 9–13), returning to 30 h up to passage 20. The PI for goat BM-MSCs remained very low throughout passaging. The population doubling time was 31 h during the first 15 passages and grew up to 60 h by passage 20 (Figure 1B). Direct comparisons of the absolute values of proliferation dynamics between goat and sheep MSCs revealed that both AT- and BM-MSCs of sheep had significantly higher proliferative potential than goat MSCs (Figure 1). In summary, sheep MSCs demonstrated greater proliferative activity in comparison to goat MSCs, although goat MSCs had an earlier proliferation peak. These findings suggest that sheep MSCs have greater potential for use in cell-based and cell-free biotechnological applications, for instance, in veterinary medicine. Among goat MSCs, AT-MSCs had greater potential for future biotechnological applications.

Morphology of freshly isolated sheep and goat MSCs

Cell morphology in MSCs is a key parameter for assessing cell homeostasis and subsequent cell fate (Colter et al., 2001; Sekiya et al., 2002; Haasters et al., 2009; Al-Azab et al., 2022). Morphology analysis after isolation allows for checking for possible contamination by mesothelial cells or other cell types. The cell morphology in sheep AT-MSC and BM-MSC cultures at passages 0–3 was heterogeneous. In the AT-MSC culture, spindle-shaped cells with fibroblast-like morphology were observed. Also, small round cells, single colonies of polygonal cells with epithelioid morphology, and single large squamous cells were encountered (Figure 2). In the BM-MSC culture, in addition to fibroblast-like cells, round cells of varying sizes were observed (Figure 2). We hypothesize that this might be the adhesive fraction of hematopoietic stem cells.

In the case of goat cells, AT-MSC and BM-MSC cell morphology was homogenous, since there were only single cells with fibroblast-like morphology. At the beginning, we detected single polygonal cells among AT-MSCs and single round-shaped cells among BM-MSCs. However, these cells were eliminated at passage 2, and the cell cultures became homogenous (Figure 2). Goat BM-MSCs and AT-MSCs exhibited distinct growth landscapes. In particular, goat AT-MSCs formed small spiral-shaped colonies, whereas BM-MSCs were more round-shaped and did not form a distinct growth pattern (Figure 2).

Collectively, freshly isolated sheep MSCs were heterogeneous, whereas goat MSCs after isolation had a homogenous morphology.

Sheep and goat MSC morphology during long cultivation

One of the crucial questions in biotechnology and cell subculturing is how cells change over long cultivation and passaging. It is especially important to determine the fate of the cell secretome and its components (Al-Azab et al., 2022; Chouaib et al., 2023; Siraj et al., 2023; Hughes et al., 2024). Accordingly, in this study, we examined changes in cell morphology in sheep AT- and BM-MSCs over a long cultivation period, as shown in Figure 3. At passage 4, sheep AT- and BM-MSC cultures appeared more homogeneous, with fewer foreign cells. The cell shape became fibroblast-like with characteristic 2–4 pseudopodia, depending on the cell density. It should be noted that AT-MSCs were visually larger than BM-MSCs. All differences between the two culture types were evened out at passage 15, when the cultures actively proliferated, and the monolayer acquired the maximal density. At passage 30 (Figure 3), when the culture growth rate decreased, the cell morphology significantly changed compared to the culture at passage 3. Spiral-shaped colonies became indistinct, while the cell pseudopodia in both cultures became less elongated, with several short appendices. At the same time, cells with two long, thin pseudopodia appeared. In the AT-MSC culture, we observed many small, round cells, whereas in the BM-MSC culture, we observed clusters of polygonal and round cells (Figure 3).

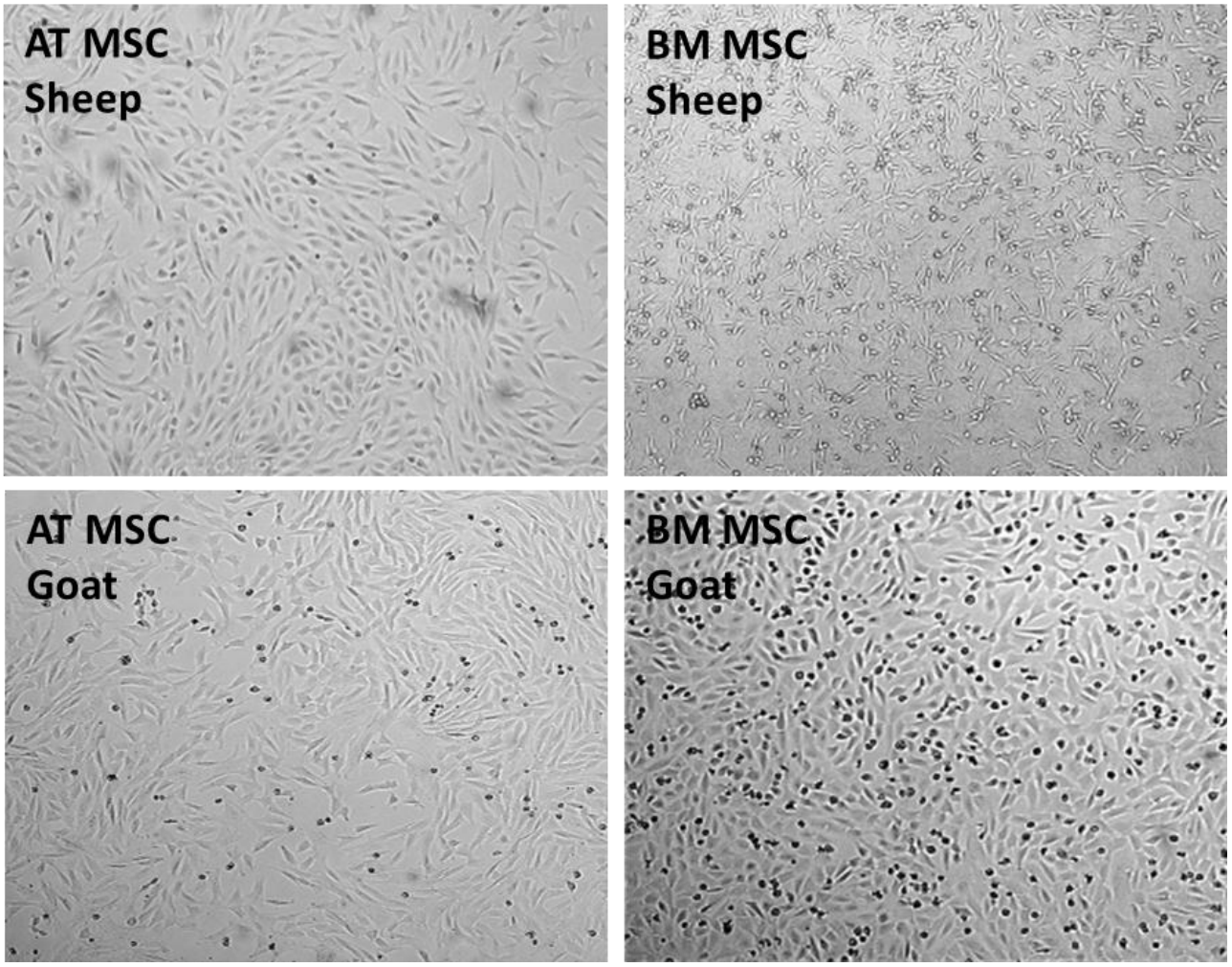

Figure 2: Morphology of freshly isolated sheep and goat MSCs at 7 days after isolation. Phase-contrast microscopy, 40×.

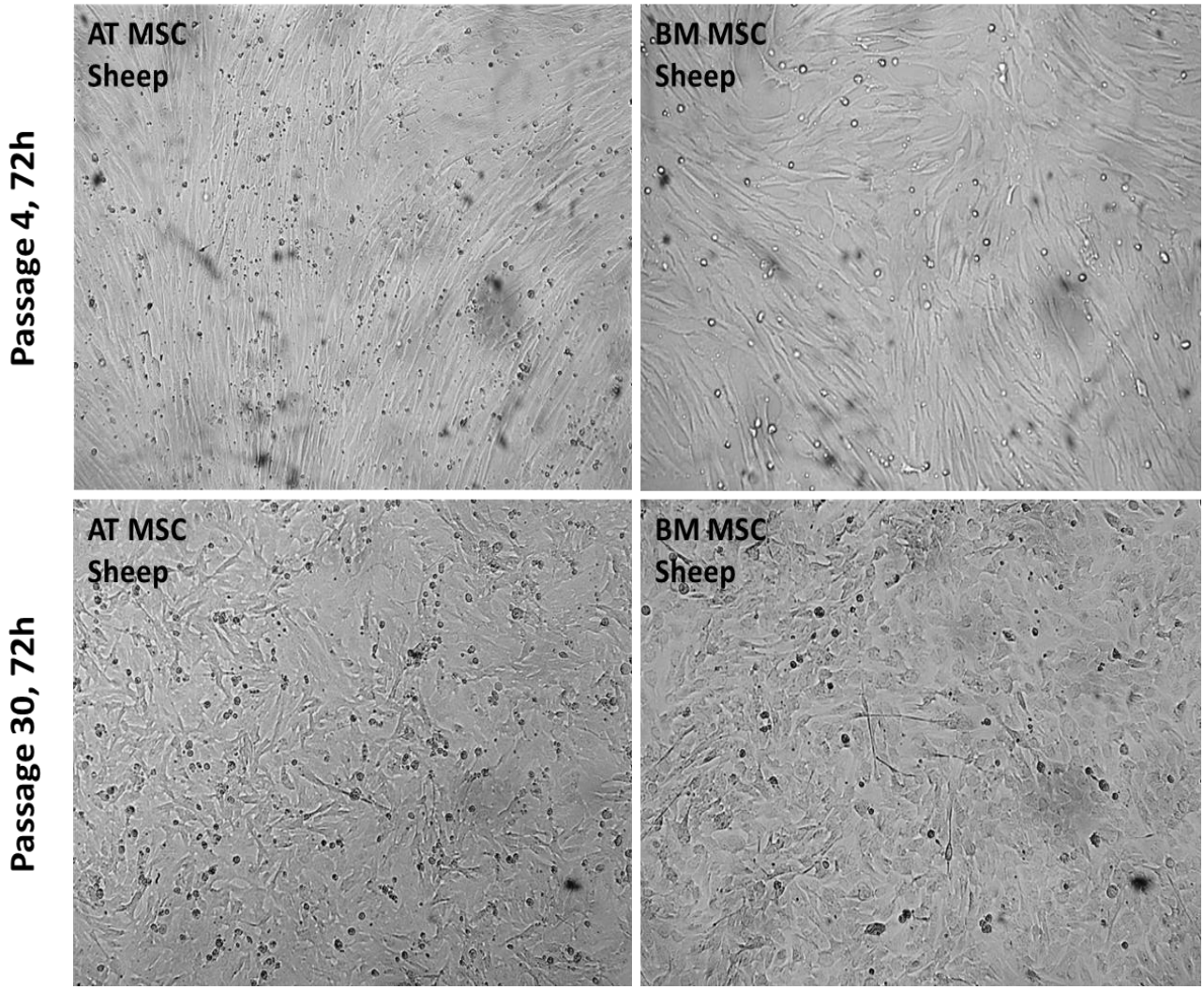

Figure 3: Morphology of cultures from sheep adipose tissue (AT) and bone marrow (BM)-derived mesenchymal stem cells (MSCs) at later passages. Ovine MSCs altered their morphology during prolonged cultivation, losing multiple pseudopodia and forming two sharp pseudopodia (phase contrast microscopy, 100×).

In summary, sheep AT- and BM-MSCs, upon prolonged cultivation, significantly altered their morphology, with a more sharply defined cell shape. The question of secretome equivalence across different subculturing stages remains open for sheep MSCs, as significant morphological changes are often associated with reorganized transcription, translation, and secretion (Sanjurjo-Rodriguez et al., 2017; Infante and Rodríguez, 2018; Harman et al., 2021; Campagna et al., 2025).

In addition to sheep MSC morphology, we also explored the long cultivation-related morphology changes in goat MSCs. It was found that goat AT- and BM-MSCs did not alter their morphology during long cultivation (Figure 4). Goat MSCs at passage 4 had a fibroblast-like, homogeneous morphology. Long subculturing did not significantly alter cell morphology, even though the cell growth rate decreased up to passage 20. Hereby, the cells and the monolayer did not change their morphology and did not form any specific landscape (Figure 4). This observation may suggest that MSCs exhibit a more stable secretome profile, which may be more beneficial for biotechnology applications. In contrast, sheep MSCs underwent pronounced morphological remodeling.

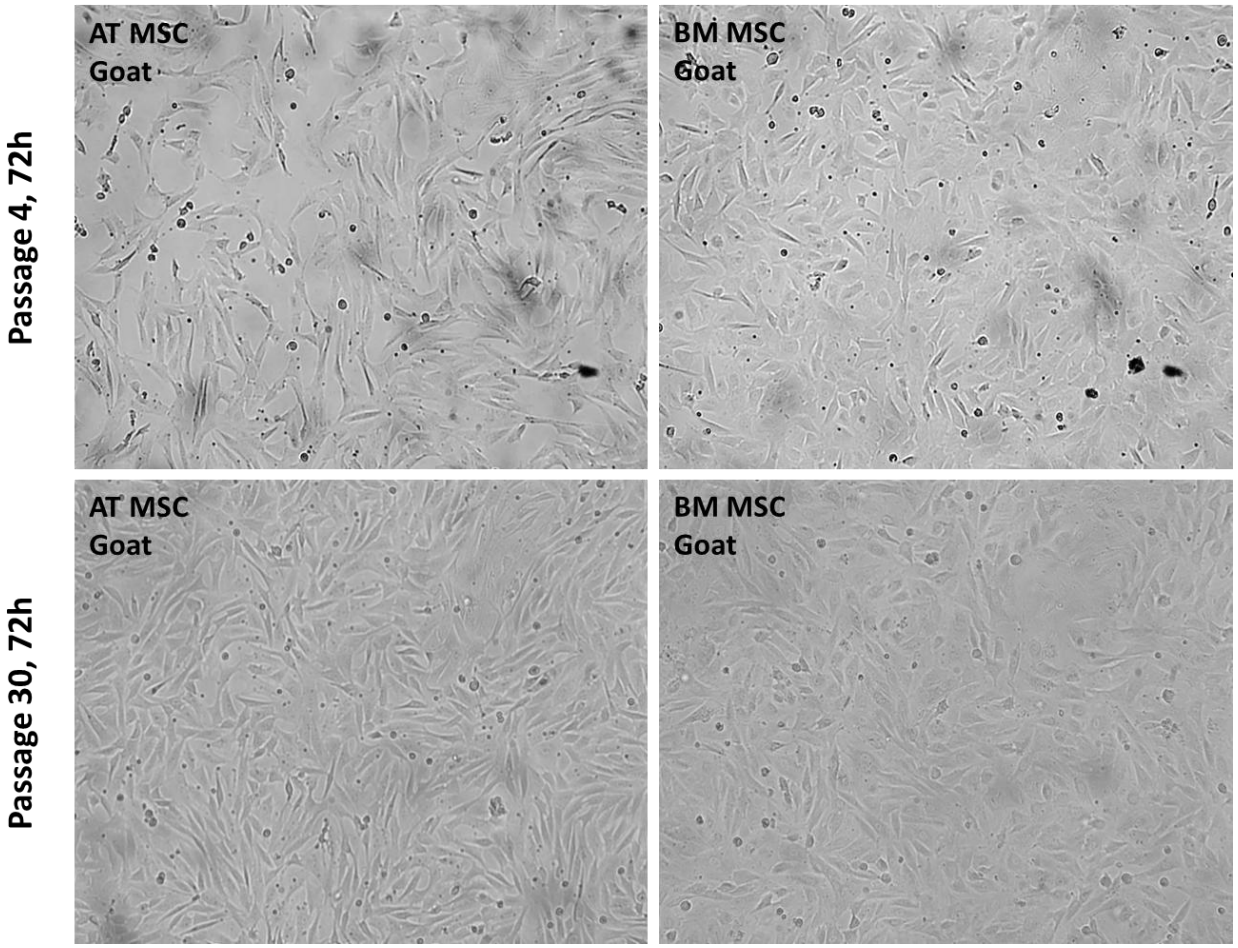

Figure 4: Morphology of cultures from goat adipose tissue (AT) and bone marrow (BM) derived mesenchymal stem cells (MSCs) at later passages. Goat MSCs did not change their morphology during long-term cultivation: phase-contrast microscopy, 100×.

Sheep and goat MSC differentiation

Our results demonstrated that both sheep and goat MSCs did not have spontaneous mesenchymal differentiation. Herewith, AT-MSCs from sheep and goats had faster adipogenesis, while their BM-MSCs were predisposed to osteogenesis.

Use of specific dyes to lipid droplets (Oil Red O for adipogenesis) and calcium inclusions (Alizarin Red S for osteogenesis) in sheep and goat MSC monolayers at passage 30 and 20, respectively, did not reveal any features of spontaneous differentiation in the adipogenic and osteogenic lineages (data not shown). Despite prolonged subculturing, sheep and goat MSCs from AT and BM retained their multipotency.

We evaluated the mesenchymal phenotype of isolated cells based on their overall ability to differentiate into adipogenic and osteogenic lineages. Accordingly, the MSC differentiation efficacy was assessed using Oil Red O and Alizarin Red S staining, as shown in Figure 5.

During adipogenesis induction, all MSCs formed cells with lipid droplets, which could be characterized as adipocytes. Nevertheless, the adipogenesis induction period was significantly less for both sheep and goat AT-MSCs. Lipid droplets in the cells from AT- and BM-MSCs were detected respectively at 14 and 21 days after induction (Figure 5, upper panel). This was observed in both sheep and goat MSCs.

The osteogenesis induction also stimulated the formation of calcium inclusions, so that the cells with calcium inclusions were determined as osteoblasts. Calcium inclusions in both sheep and goat AT- and BM-MSCs were detected respectively at 28 and 21 days after induction (Figure 5, lower panel).

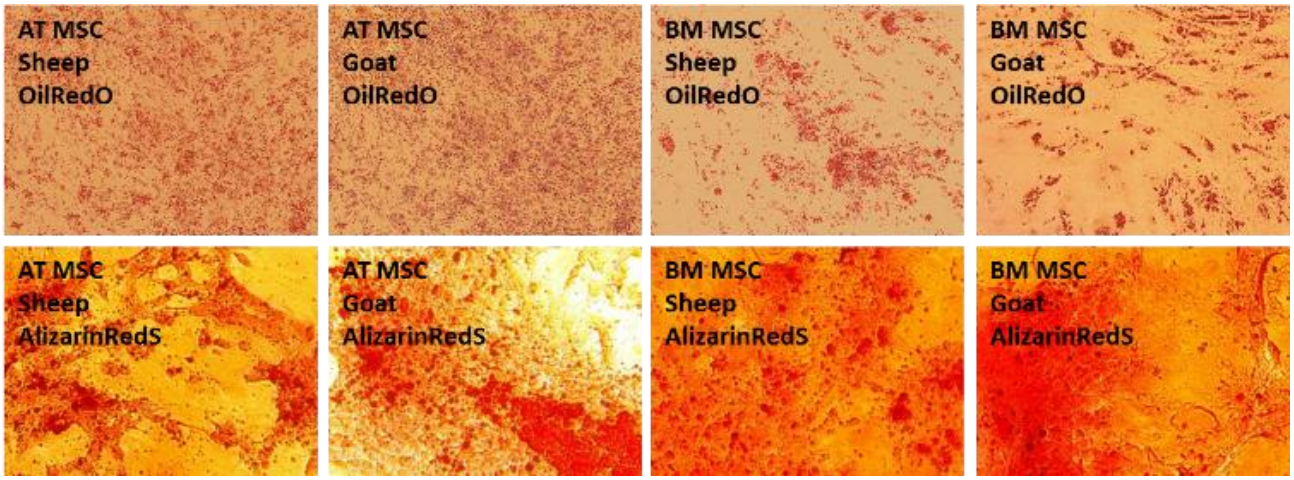

Figure 5: Micrographs of stained cultures of sheep and goat MSCs after differentiation in the adipogenic and osteogenic lineages. Adipogenic samples were stained with Oil Red O, and osteogenic samples with Alizarin Red S. Phase-contrast microscopy, 40×.

This study, therefore, confirmed the MSC mesenchymal phenotype and demonstrated that tissue-specific MSCs are predisposed to lineage-specific differentiation. That is, sheep and goat AT-MSCs were predisposed to adipogenesis, whereas BM-MSCs were predisposed to osteogenesis.

Discussion

The successful breeding and improvements of farm animals are based on a number of factors, including preserving their fertility, including issues such as implantation success (Mohamad-Fauzi et al., 2015), as well as maintaining and preserving their genetic diversity (Scherf, 1995; Deniskova et al., 2024; Bondarenko and Khvostik, 2020). Given the current trend in global agriculture, the primary task for the further development of the small ruminant industry is the preservation of existing sheep and goat breeds (Deniskova et al., 2025; Romanov et al., 2021; Tarasova et al., 2021). Several approaches use sperm banks that employ cutting-edge techniques to cryopreserve sperm from high-value rams and goats (Berean et al., 2024; Korochkina and Pushkina, 2024; Korochkina et al., 2024e; Korochkina, 2025). Taking into account the reproductive characteristics of small ruminants of both sexes (Michael et al., 2025), but, in particular, in the search for factors affecting the success of artificial insemination (Almeida et al., 2021; Souza-Fabjan et al., 2023; Artemenko et al., 2010), it is relevant to study methods for improving the andrological quality of sperm before and after cryopreservation (Jia et al., 2024). MSCs can be used for this (Stewart and Shipley, 2021; Balaro et al., 2022; Paredes et al., 2024) and thereby enhance male reproductive capacity (Segunda et al., 2024; Tanrıkulu et al., 2025). In previous studies, we preliminarily established a short-term positive effect of goat MSCs components (lysate) on the quality of their spermatozoa (Korochkina et al., 2024b), as well as an effective protocol for using conditioned medium of mesenchymal stromal cells from ram AT and BM and spermatozoa in terms of improving the quality of sperm cells (Korochkina et al., 2024c). Moreover, the role of sheep and goats as models for genetic engineering in dairy species, including stem cell therapy (e.g., for bone and cartilage restoration), suggests that more research and investment are likely in the fields of reproduction and MSCs for these small ruminant species (Mohamad-Fauzi et al., 2015).

Although MSCs from different species are widely used in research, unlike in humans, there are no universally accepted minimal criteria for their identification in other animals. Heidari et al. (2013) demonstrated that, in contrast to human and mouse MSCs, ovine MSCs derived from both BM and AT exhibit low expression of CD90 and CD105, but like human and murine cells, they express CD44 and CD166. CD34 and CD45, however, remain negative across all four species examined. In contrast, caprine MSCs were reported to express CD90, CD105, and CD73, while lacking the hematopoietic marker CD45 (Mohamad-Fauzi et al., 2015). Ovine MSCs characterized by Vivas et al. (2018) expressed CD44, CD90, CD140a, CD105, and CD166. More recently, Al-Mutheffer et al. (2023) demonstrated

variable expression of CD29 (22.11–99.93%) and CD44 (48.96–99.73%) in BM-derived MSCs from 13 sheep, while all donors consistently showed low expression of CD45 and CD31. In this study, due to the absence of species-specific markers, MSC identity was verified by directed bilineage differentiation into adipogenic and osteogenic lineages.

Osteogenic differentiation in our cultures required 21–28 days, longer than the 14–21 days usually observed for human MSCs, depending on their tissue source (BM or AT). Prolonged osteogenic differentiation of ovine MSCs compared with human MSCs was first reported by Kulneva et al. (2010b). Kalaszczynska et al. (2013) also found significant interspecies differences, suggesting that standard *in vitro* osteogenic protocols may not be suitable for all species. These authors further reported that phosphate source influences ovine osteogenesis, with monosodium phosphate being required for successful differentiation. Both their data and ours point to strong interindividual and interbreed heterogeneity among ovine MSCs. Later studies employing β-glycerophosphate (β-GlyP) and monosodium phosphate (NaH_2PO_4) supplemented with BMP-2 also found that ovine BM-MSCs had weaker osteogenic potential than their human counterparts (Bottagisio et al., 2015). Similarly, Al-Mutheffer et al. (2023) reported donor-dependent variation in ovine BM-MSC osteogenesis, though extracellular matrix mineralization was achieved by day 21, consistent with our results. Haddouti et al. (2020) provided a semi-quantitative comparison of human and ovine MSC mineralization, finding comparable results by Alizarin Red S staining in both AT-MSCs and BM-MSCs after 21 days. Optical density measurements, however, revealed that mineralization rates were consistently higher in human MSCs. Inorganic phosphate (Pi) release was comparable between human and ovine BM-MSCs, whereas ovine AT-MSCs released approximately half as much as human AT-MSCs. In contrast, Westerkowsky et al. (2023) reported enhanced osteogenic differentiation in ovine BM-MSCs compared with human BM-MSCs.

Reports on adipogenic differentiation of ovine MSCs are inconsistent. Kulneva et al. (2010a, 2010b) found that standard adipogenic media failed to induce adipogenesis in BM-MSCs and even induced cell death. Reducing dexamethasone and IBMX concentrations tenfold enabled Oil Red O-positive adipocytes to appear by day 21, increasing by day 28. Modified protocols have also been required for AT-MSCs, as shown by Heimann et al. (2023), who proposed a rosiglitazone-based induction medium that improved adipogenesis across several mammalian species, including sheep. Haddouti et al. (2020) compared human and ovine MSC adipogenesis under standard induction conditions, showing lipid-rich vacuoles stained with Oil Red O by day 21 in both BM- and AT-derived cultures. Human MSCs, however, exhibited more than double the adipogenic capacity of ovine MSCs. Other studies also reported low or absent adipogenesis in ovine BM-MSCs, despite variations in protocol (Rentsch et al., 2010; Adamzyk et al., 2013). Westerkowsky et al. (2023) confirmed these findings, observing more lipid vacuoles in human BM-MSCs than ovine BM-MSCs by day 27, with ovine BM-MSCs rarely showing typical lipid droplet morphology. McCarty et al. (2009) demonstrated adipogenesis in ovine BM-MSCs after four weeks in human-targeted induction medium, though the number of Oil Red O-positive vacuoles remained low. Similarly, Al-Mutheffer et al. (2023) found weak adipogenesis in BM-MSCs after 17 days in DMEM-HG–based adipogenic medium, with only one donor culture showing strong lipid accumulation, while two showed none. Our results, obtained with StemPro™ medium, are consistent with Korovina et al. (2019), who also reported successful but limited adipogenesis in ovine MSCs.

Here, by comparing MSC differentiation capacity in sheep and goats, we established that longer induction times were required to achieve adipogenic and osteogenic differentiation compared to human MSCs. A small number of studies have addressed goat MSCs. Mahajan et al. (2022) reported that AT-MSCs from the infrapatellar fat pad differentiated into adipogenic, chondrogenic, and osteogenic lineages, with osteogenesis observed by day 28 and adipogenesis by days 14–21. Abraham et al. (2024) also showed osteogenesis in goat AT-MSCs after 21 days in StemPro™ medium. Mohamad-Fauzi et al. (2015) reported weak osteogenesis in AT-MSCs but significant mineralization in BM-MSCs. They also found much higher adipogenesis in AT-MSCs, with 9-fold more Oil Red O-positive cells than BM-MSCs by day 21. Elkhenany et al. (2016) demonstrated both osteogenic and adipogenic induction in goat

MSCs at passage 3, while also documenting reduced differentiation capacity at later passages, up to passage 11 — the latest passage reported for goat MSCs. In contrast, ovine MSCs are commonly studied at passages 3–5. Thus, our characterization of ovine MSCs at passage 40 and goat MSCs at passage 20 provides the first published description of their proliferative and morphological traits at such late stages.

Cellular heterogeneity following MSC isolation from different tissues, as observed in our study, has also been reported by many groups (Phinney et al., 1999; Sekiya et al., 2002; Meirelles and Nardi, 2003; Tropel et al., 2004; Silva Filho et al., 2014), regardless of whether density gradient separation was used. This reflects the complex bone marrow microenvironment, where HSCs coexist with stromal, endothelial, adipose, and mesenchymal cells. HSC–MSC interactions, mediated by adhesion molecules, regulate quiescence and differentiation. During MSC isolation, many HSCs are removed by washing, but a small fraction may remain transiently associated with MSCs (Grenier et al., 2021; Omatsu, 2023). Reported times for achieving pure MSC cultures range from 8 days (Silva Filho et al., 2014) to 30 days (Tropel et al., 2004).

In this study, MSCs from both sheep and goats maintained visual morphological homogeneity for at least 20 passages; however, late-passage ovine MSCs showed reduced growth (over twofold lower compared with peak proliferation at passage 20 ± 3) and marked morphological alterations. Previous studies demonstrated spontaneous oncogenic transformation of ovine MSCs at ≥40 passages, characterized by loss of fibroblast-like morphology, failure of adipogenic differentiation, karyotype abnormalities (trisomies, polyploidy), elevated telomerase activity, upregulation of c-myc and VEGF, and tumorigenic potential *in vivo* (Popov et al., 2009; Bersene et al., 2005). Thus, the high proliferative rates and altered morphology of late-passage ovine MSCs in our study may indicate immortalization, though this requires further investigation.

Overall, our data show that ovine and caprine MSCs differ substantially in their proliferation and differentiation characteristics. Ovine MSCs displayed high proliferative capacity up to passage 40, whereas goat MSCs, particularly BM-MSCs, declined in growth by passage 20. These findings concur with prior reports of higher proliferation in ovine MSCs compared with human MSCs (Haddouti et al., 2020; Westerkowsky et al., 2023) and with Abraham et al. (2024), who reported a population doubling time of 44 hours in early-passage goat AT-MSCs, slightly longer than in our study.

The features of AT- and BM-MSCs we identified in both sheep and goats will be instrumental for further research to elucidate the influence of these small ruminant MSCs on the quality of their spermatozoa before and after cryopreservation to develop advanced reproductive biotechnologies, as well as their role as models for stem cell therapies.

Conclusions

Overall, our findings are consistent with those of other, albeit limited, studies on ovine and caprine MSCs (e.g., Mohamad-Fauzi et al., 2015). The described protocols for isolation and culture reliably yielded MSC populations from both AT and BM, as confirmed by successful bilineage differentiation. For the first time, we documented the long-term cultivation of ovine and caprine MSCs, generated growth curves, and documented morphological changes at late passages. We also provided the first direct comparison of the proliferative potential of MSCs from small ruminants, demonstrating that MSCs derived from different sources (AT vs. BM) require different induction times for differentiation; however, no interspecies differences in differentiation time were detected.

With the ongoing development of sheep and goat farming in Russia, artificial insemination is particularly important. Its success relies heavily on cryopreservation techniques, specifically the maintenance of viable and fertile spermatozoa during freezing and transport without loss of fertilizing capacity. Many studies have sought to mitigate the impact of environmental stress and temperature fluctuations on male gametes. In this context, MSCs hold the potential for applications in reproduction through their dynamic antioxidant activity, mediated by endogenous production of heme oxygenase-1 (HO-1) and glutathione, and their capacity to limit cellular generation of reactive oxygen species (ROS). Since ram and buck spermatozoa are especially vulnerable to oxidative stress due to their membranes being rich in polyunsaturated fatty acids (PUFAs), which are highly sensitive to lipid peroxidation, the protective properties of MSCs provide tolerance

against oxidative damage. Consequently, the detrimental effects of ROS on sperm motility and viability during cryopreservation and thawing may be mitigated by incorporating MSCs into extenders or by developing optimized storage and transport media containing MSCs. Such strategies could significantly advance reproductive biotechnology and expand sheep and goat breeding worldwide. Considering the role of small ruminant MSCs in the field of stem cell therapy, the results described herein provide valuable information for furthering the cause of treatment for bone and cartilage disorders.

Article Information

Conflict of interest. The author declared no conflict of interest.
Acknowledgement. The authors acknowledge the use of ChatGPT (GPT-5, OpenAI, San Francisco, CA, USA) as an English language-support tool for translation and editorial refinement of the Discussion and Conclusions sections. The authors confirm that ChatGPT was not used to generate scientific content, analyze data, or interpret results. All scientific ideas, data, and conclusions are the sole responsibility of the authors.
Authors contribution. E. A. K.: Conceptualization, methodology, formal analysis, resources, writing – review & editing, funding acquisition. A. V. T.: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, writing – original draft, writing – review & editing, visualization. A. A. L.: Methodology, data curation, project administration. V. S. P.: Writing – review & editing. D. K. G.: Writing – review & editing, supervision. M. N. R.: Writing – review & editing, visualization.
Funding. The research was funded by the Russian Science Foundation, Grant No. 23-26-00157 (https://rscf.ru/project/23-26-00157/).
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References

Abraham, M., Kori, I., Vishwakarma, U., Goel, S., 2024. Comprehensive assessment of goat adipose tissue-derived mesenchymal stem cells cultured in different media. Scientific Reports 14, 8380. https://doi.org/10.1038/s41598-024-58465-1

Adamzyk, C., Emonds, T., Falkenstein, J., Tolba, R., Jahnen-Dechent, W., Lethaus, B., et al., 2013. Different culture media affect proliferation, surface epitope expression, and differentiation of ovine MSC. Stem Cells International 2013, 387324. https://doi.org/10.1155/2013/387324

Al-Azab, M., Safi, M., Idiiatullina, E., Al-Shaebi, F., Zaky, M.Y., 2022. Aging of mesenchymal stem cell: machinery, markers, and strategies of fighting. Cellular & Molecular Biology Letters 27, 69. https://doi.org/10.1186/s11658-022-00366-0

AL-Jaryan, I.L., AL-Thuwaini, T.M., Merzah, L.H., Alkhammas, A.H., 2023. Reproductive physiology and advanced technologies in sheep reproduction. Reviews in Agricultural Science 11, 171–180. https://doi.org/10.7831/ras.11.0_171

Al-Mutheffer, E.A.A., Reinwald, Y., El Haj, A.J., 2023. Donor variability of ovine bone marrow-derived mesenchymal stem cells: implications for cell therapy. International Journal of Veterinary Science and Medicine 11, 23–37. https://doi.org/10.1080/23144599.2023.2197393

Almeida, V.M. de, Batista, L.F., Santos, J.F.N., 2021. Artificial insemination at a fixed time in small ruminants: challenge of a commercial program. Ciência Animal 31(1 Suppl 1), 14–24. https://revistas.uece.br/index.php/cienciaanimal/article/view/9548/7724 accessed 6 October 2025.

Alvites, R.D., Branquinho, M.V., Sousa, A.C., Lopes, B., Sousa, P., Mendonça, C., et al., 2021. Small ruminants and their use in regenerative medicine: Recent works and future perspectives. Biology 10, 249. https://doi.org/10.3390/biology10030249

Anatolitou, A., Sideri, K., Mavrogenis, A., Psalla, D., Markou, M., Krystalli, A., et al., 2024. Cartilage extracellular matrix collagen type II and aggrecan expressions in rabbit cartilage following mesenchymal stem cell implantation. German Journal of Veterinary Research 4, 197–207. https://doi.org/10.51585/gjvr.2024.3.0110

Artemenko, A.B., Tagirov, M.T., Tereshchenko, A.V., 2010. The effectiveness of the method of artificial insemination of waterfowl. Poultry Farming 66, 129–134.

Balaro, M.F.A., Cosentino, I.O., Ribeiro, A.C.S., Brandão, F.Z., 2022. Ultrasound diagnosis in small ruminants: occurrence and description of genital pathologies. Veterinary Sciences 9, 599. https://doi.org/10.3390/vetsci9110599

Baouche, M., Krawczenko, A., Paprocka, M., Klimczak, A., Mermillod, P., Locatelli, Y., et al., 2023. Feline umbilical cord mesenchymal stem cells: Isolation and *in vitro* characterization from distinct parts of the umbilical cord. Theriogenology 201, 116–125. https://doi.org/10.1016/j.theriogenology.2022.11.049

Berean, D.I., Bogdan, L.M., Cimpean, R., 2024. Advancements in understanding and enhancing antioxidant-mediated sperm cryopreservation in small ruminants: Challenges and perspectives. Antioxidants 13, 624. https://doi.org/10.3390/antiox13060624

Bersenev, A.V., 2005. Study of spontaneous oncogenetic transformation of human mesenchymal stem cells in culture. Cell Transplantology and Tissue Engineering 1, 14–16. https://elibrary.ru/item.asp?id=9236128 accessed 6 October 2025

Bezerra, M.É.S., Monte, A.P.O., Barberino, R.S., Lins, T.L.B.G., Junior, J.O., Santos, J.M.S., et al., 2019. Conditioned medium of ovine Wharton's jelly-derived mesenchymal stem cells improves growth and reduces ROS generation of isolated secondary follicles after short-term *in vitro* culture. Theriogenology 125, 56–63. https://doi.org/10.1016/j.theriogenology.2018.10.012

Bondarenko, Yu.V., Khvostik, V.P., 2020. Improving the productivity of meat and egg chickens through domestic selection. Bulletin of Sumy National Agrarian University, Series Livestock 2, 29–32. https://doi.org/10.32845/bsnau.lvst.2020.2.5

Bottagisio, M., Lovati, A.B., Lopa, S., Moretti, M., 2015. Osteogenic differentiation of human and ovine bone marrow stromal cells in response to β-glycerophosphate and monosodium phosphate. Cellular Reprogramming 17, 235–242. https://doi.org/10.1089/cell.2014.0105

Cabezas, J., Rojas, D., Navarrete, F., Ortiz, R., Rivera, G., Saravia, F., et al., 2018. Equine mesenchymal stem cells derived from endometrial or adipose tissue share significant biological properties, but have a distinctive pattern of surface markers and migration. Theriogenology 106, 93–102. https://doi.org/10.1016/j.theriogenology.2017.09.035

Cakici, C., Buyrukcu, B., Duruksu, G., Haliloglu, A.H., Aksoy, A., Isık, A., et al., 2013. Recovery of fertility in azoospermia rats after injection of adipose-tissue-derived mesenchymal stem cells: the sperm generation. BioMed

Research International 2013, 529589. https://doi.org/10.1155/2013/529589

Campagna, C.E., Larey, A.M., Daga, K.R., Roos, M., Ghosh, S., Grimsey, N., et al., 2025. Development of a high-throughput morphological assay for evaluating mesenchymal stromal cell-derived extracellular vesicle modulation of brain pericyte secretory phenotype. Stem Cell Reviews and Reports 1–15. https://doi.org/10.1007/s12015-025-10940-6

Chadda, A., Meena, D.C., Singh, J., 2025. Exploring the value chain of small ruminants: A review. Indian Journal of Small Ruminants 31, 1–5. https://doi.org/10.5958/0973-9718.2025.00019.7

Chouaib, B., Haack-Sørensen, M., Chaubron, F., Cuisinier, F., Collart-Dutilleul, P.Y., 2023. Towards the standardization of mesenchymal stem cell secretome-derived product manufacturing for tissue regeneration. International Journal of Molecular Sciences 24, 12594. https://doi.org/10.3390/ijms241612594

Colter, D.C., Sekiya, I., Prockop, D.J., 2001. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proceedings of the National Academy of Sciences 98, 7841–7845. https://doi.org/10.1073/pnas.. 141221698

Cortez, J., Torres, C.G., Parraguez, V.H., De los Reyes, M., Peralta, O.A., 2024. Bovine adipose tissue-derived mesenchymal stem cells self-assemble with testicular cells and integrates and modifies the structure of a testicular organoids. Theriogenology 215, 259–271. https://doi.org/10.1016/j.theriogenology.2023.12.013

Dar, E.R., Gugjoo, M.B., Javaid, M., Hussain, S., Fazili, M.R., Dhama, K., et al., 2021. Adipose tissue- and bone marrow-derived mesenchymal stem cells from sheep: culture characteristics. Animals 11, 2153. https://doi.org/10.3390/ani11082153

Del Prete, C., Montano, C., Cocchia, N., de Chiara, M., Gasparrini, B., Pasolini, M.P., 2024. Use of regenerative medicine in the treatment of endometritis in mares: A systematic review and meta-analysis. Theriogenology 227, 9–20. https://doi.org/10.1016/j.theriogenology.2024.07.006

Del Prete, C., Gaspari, G., Kosior, M.A., Merlo, B., Iacono, E., Longobardi, C., et al., 2025. Effects of Wharton's jelly mesenchymal stromal/stem cells-derived conditioned medium and platelet-rich plasma on *in vitro* induced equine endometrial inflammation. Theriogenology 241, 117423. https://doi.org/10.1016/j.theriogenology.2025.117423

Deniskova, T.E., Dotsev, A.V., Abdelmanova, A.S., Petrov, S.N., Frolov, A.N., Platonov, S.A., et al., 2024. Genetic diversity in the Orenburg goat breed revealed by single-nucleotide polymorphism (SNP) analysis: Initial steps in saving a threatened population. Genes 15, 1375. https://doi.org/10.3390/genes15111375

Deniskova, T.E., Dotsev, A.V., Koshkina, O.A., Solovieva, A.D., Churbakova, N.A., Petrov, S.N., et al., 2025. Examination of runs of homozygosity distribution patterns and relevant candidate genes of potential economic interest in Russian goat breeds using whole-genome sequencing. Genes 16, 631. https://doi.org/10.3390/genes16060631

Dias, I.E., Viegas, C.A., Requicha, J.F., Saavedra, M.J., Azevedo, J.M., Carvalho, P.P., et al., 2022. Mesenchymal stem cell studies in the goat model for biomedical research—A review of the scientific literature. Biology 11, 1276. https://doi.org/10.3390/biology11091276

Eleuteri, S., Fierabracci, A., 2019. Insights into the secretome of mesenchymal stem cells and its potential applications. International Journal of Molecular Sciences 20, 4597. https://doi.org/10.3390/ijms20184597

Elkhenany, H., Amelse, L., Caldwell, M., Abdelwahed, R., Dhar, M., 2016. Impact of the source and serial passaging of goat mesenchymal stem cells on osteogenic differentiation potential: implications for bone tissue engineering. Journal of Animal Science and Biotechnology 7, 16. https://doi.org/10.1186/s40104-016-0074-z

Ferris, R.A., Frisbie, D.D., McCue, P.M., 2014. Use of mesenchymal stem cells or autologous conditioned serum to modulate the inflammatory response to spermatozoa in mares. Theriogenology 82, 36–42. https://doi.org/10.1016/j.theriogenology.2014.02.015

Gadekar, Y.P., Shinde, A.K., Banerjee, R., Barbuddhe, S.B., 2023. Present status and future prospects of meat production from small ruminants: An overview. Indian Journal of Small Ruminants 29, 185–197. https://doi.org/10.5958/0973-9718.2023.00052.1

Ghai, S., Saini, S., Ansari, S., Verma, V., Chopra, S., Sharma, V., Devi, P., Malakar, D., et al., 2022. Allogenic umbilical cord blood-mesenchymal stem cells are more effective than antibiotics in alleviating subclinical mastitis in dairy cows. Theriogenology 187, 141–151. https://doi.org/10.1016/j.theriogenology.2022.05.001

GraphPad Software, 2024. GraphPad Prism 8 User Guide: What's new in Prism 8? GraphPad Software, LLC, Dotmatics, Boston, MA, USA. https://www.graphpad.com/guides/prism/8/user-guide/new-organization.htm accessed 6 October 2025.

Grenier, J.M., Testut, C., Fauriat, C., Mancini, S.J., Aurrand-Lions, M., 2021. Adhesion molecules involved in stem cell niche retention during normal haematopoiesis and in acute myeloid leukaemia. Frontiers in Immunology 12, 756231. https://doi.org/10.3389/fimmu.2021.756231

Frausto, R.F., Chung, D.D., Boere, P.M., Swamy, V.S., Duong, H.N., Kao, L., et al., 2019. ZEB1 insufficiency causes corneal endothelial cell state transition and altered cellular processing. PLoS One 14, e0218279. https://doi.org/10.1371/journal.pone.0218279

Haasters, F., Prall, W.C., Anz, D., Bourquin, C., Pautke, C., Endres, S., et al., 2009. Morphological and immunocytochemical characteristics indicate the yield of early progenitors and represent a quality control for human mesenchymal stem cell culturing. Journal of Anatomy 214, 759–767. https://doi.org/10.1111/j.1469-7580.2009.01065.x

Hajihoseini, M., Vahdati, A., Ebrahim Hosseini, S., Mehrabani, D., Tamadon, A., 2017. Induction of spermatogenesis after stem cell therapy of azoospermic guinea pigs. Veterinarski Arhiv 87, 333–350. https://doi.org/10.24099/vet.arhiv.151209

Han, Y., Li, X., Zhang, Y., Han, Y., Chang, F., Ding, J., 2019. Mesenchymal stem cells for regenerative medicine. Cells 8, 886. https://doi.org/10.3390/cells8080886

Harman, R.M., Marx, C., Van de Walle, G.R., 2021. Translational animal models provide insight into mesenchymal stromal cell (MSC) secretome therapy. Frontiers in Cell and Developmental Biology 9, 654885. https://doi.org/10.3389/fcell.2021.654885

Harness, E.M., Mohamad-Fauzi, N.B., Murray, J.D., 2022. MSC therapy in livestock models. Translational Animal Science 6, txac012. https://doi.org/10.1093/tas/txac012

Haddouti, E.M., Randau, T.M., Hilgers, C., Masson, W., Walgenbach, K.J., Pflugmacher, R., et al., 2020. Characterization and comparison of human and ovine mesenchymal stromal cells from three corresponding sources. International Journal of Molecular Sciences 21, 2310. https://doi.org/10.3390/ijms21072310

Heidari, B., Shirazi, A., Akhondi, M.M., Hassanpour, H., Behzadi, B., Naderi, M.M., et al., 2013. Comparison of proliferative and multilineage differentiation potential of sheep mesenchymal stem cells derived from bone marrow, liver, and adipose tissue. Avicenna Journal of Medical Biotechnology 5, 104–117. https://pmc.ncbi.nlm.nih.gov/articles/PMC3689554/pdf/AJMB-5-104.pdf accessed 6 October 2025

Heimann, M., Elashry, M.I., Klymiuk, M.C., Eldaey, A., Wenisch, S., Arnhold, S., 2023. Optimizing the adipogenic induction protocol using rosiglitazone improves the physiological parameters and differentiation capacity of

adipose tissue-derived mesenchymal stem cells for horses, sheep, dogs, murines, and humans. Animals 13, 3224. https://doi.org/10.3390/ani13203224

Hughes, A.M., Kuek, V., Oommen, J., Kotecha, R.S., Cheung, L.C., 2024. Murine bone-derived mesenchymal stem cells undergo molecular changes after a single passage in culture. Scientific Reports 14, 12396. https://doi.org/10.1038/s41598-024-63009-8

Infante, A., Rodríguez, C.I., 2018. Secretome analysis of *in vitro-aged human mesenchymal stem cells reveals IGFBP7 as a putative factor* promoting osteogenesis. Scientific Reports 8, 4632. https://doi.org/10.1038/s41598-018-22855-z

Jia, B., Allai, L., Li, C., Liang, J., Lv, C., Wu, G., et al., 2024. A review on the functional roles of trehalose during cryopreservation of small ruminant semen. Frontiers in Veterinary Science 11, 1467242. https://doi.org/10.3389/fvets.2024.1467242

Kalaszczynska, I., Ruminski, S., Platek, A.E., Bissenik, I., Zakrzewski, P., Noszczyk, M., et al., 2013. Substantial differences between human and ovine mesenchymal stem cells in response to osteogenic media: how to explain and how to manage? BioResearch Open Access 2, 356–363. https://doi.org/10.1089/biores.2013.0029

Kandziora, F., Pflugmacher, R., Scholz, M., Schnake, K., Lucke, M., Schröder, R., et al., 2001. Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. Spine 26, 1028–1037. https://doi.org/10.1097/00007632-200105010-00008

Kim, G.A., Lee, Y., Kim, H.J., Oh, H.J., Kang, S.K., Ra, J.C., et al., 2018. Intravenous human endothelial progenitor cell administration into aged mice enhances embryo development and oocyte quality by reducing inflammation, endoplasmic reticulum stress, and apoptosis. Journal of Veterinary Medical Science 80, 1905–1913. https://doi.org/10.1292/jvms.18-0242

Korochkina, E.A., 2025. Mesenchymal stem cells from adipose tissue and bone marrow as a component of a diluent for cryopreservation of goats. Veterinary Medicine Journal 2, 29–33. https://doi.org/10.30896/0042-4846.2025.28.2.29-33

Korochkina, E.A., Pushkina, V.S., 2024. The influence of mesenchymal stem cells on the quality of ram sperm after cryopreservation. Veterinary Medicine and Feeding 4, 55–59. https://doi.org/10.30917/ATT-VK-1814-9588-2024-4-10

Korochkina, E.A., Trifonova, A.V., Finageev, E.Y., Glavatskaya, D.E., Pushkina, V.S., 2023. The effect of mesenchymal stem cells from the adipose tissue and bone marrow of goats on the quality of their sperm cells. International Journal of Veterinary Medicine 4, 421–430. https://doi.org/10.52419/issn2072-2419.2023.4.421

Korochkina, E.A., Finageev, E.Yu., Glavazkaya, D.E., Pushkina, V.S., Trifonova, A.V., Ivanovskaya, M.M., 2024a. The influence of MSCs from adipose tissue and bone marrow of sheep on the quality parameters of their spermatozoa. Veterinary Medicine Journal 1, 37–41. https://doi.org/10.30896/0042-4846.2024.27.1.37-41

Korochkina, E.A., Trifonova, A.V., Finageev, E.Y., Pushkina, V.S., Glavatskaya, D.E., 2024b. The influence of goat mesenchymal stem cells' components on the quality parameters of spermatozoa. Veterinary Medicine and Feeding 2, 40–44. https://doi.org/10.30917/ATT-VK-1814-9588-2024-2-9

Korochkina, E.A., Trifonova, A.V., Nechaev, A.Yu., Pushkina, V.S., Glavatskaya, D.E., 2024c. The influence of the conditioned environment of sheep mesenchymal stromal cells on the qualitative parameters of sperm cells. International Journal of Veterinary Medicine 2, 276–284. https://doi.org/10.52419/issn2072-2419.2024.2.276

Korochkina, E.A., Trifonova, A.V., Nikitin, V.V., Glavatskaya, D.E., Pushkina, V.S., 2024d. Prospects for using component mesenchymal stem cells as a way to improve the quality of bulls' sperm. Veterinary Medicine Journal 4, 486–492. https://doi.org/10.52419/issn2072-2419.2024. 4.486

Korochkina, E.A., Finageev, E.Yu., Pushkina, V.S., Glavatskaya, D.E., Trifonova, A.V., 2024e. Possibility of mesenchymal stromal cells using in the cryopreservation ram's sperm. Veterinary Medicine Journal 11, 43–47. https://doi.org/10.30896/0042-4846.2024.27.11.43-47

Korochkina, E.A., Trifonova, A.V., Finageev, E.Y., Pushkina, V.S., Glavatskaya, D.E., 2024f. Effect of ram mesenchymal stem cell (MSC) lysate on sperm quality indicators. Bulletin of KSAU 12, 94–100. https://doi.org/10.36718/1819-4036-2024-12-94-100

Korovina, D.G., Volkova, I.M., Vasilieva, S.A., Gulukin, M.I., Savchenkova, I.P., 2019. Multipotent mesenchymal stem cells derived from sheep bone marrow: isolation and cryopreservation. Cell and Tissue Biology 13, 161–169. https://doi.org/10.1134/S1990519X19030052

Kulneva, E.I., Devrishov, D.A., Teplyashin, A.S., Korzhikova, S.V., 2010a. Characteristics of multipotent mesenchymal stromal cell-like cells isolated from the ovine bone marrow. Veterinary Medicine 3-4, 102–104. https://elibrary.ru/item.asp?id=15564812 accessed 6 October 2025.

Kulneva, E.I., Devrishov, D.A., Teplyashin, A.S., Korzhikova, S.V., 2010b. The effective adipogenic differentiation of MMSCs isolated from ovine bone marrow after treatment with media containing inductors in different concentrations. Veterinary Medicine 3-4, 104–105. https://elibrary.ru/item.asp?id=15564813 accessed 6 October 2025

Lange-Consiglio, A., Perrini, C., Bertero, A., Esposti, P., Cremonesi, F., Vincenti, L., 2017. Isolation, molecular characterization, and *in vitro* differentiation of bovine Wharton jelly–derived multipotent mesenchymal cells. Theriogenology 89, 338–347. https://doi.org/10.1016/j.theriogenology.2016.09.042

Li, Z., Zhang, M., Tian, Y., Li, Q., Huang, X., 2021. Mesenchymal stem cells in premature ovarian insufficiency: mechanisms and prospects. Frontiers in Cell and Developmental Biology 9, 718192. https://doi.org/10.3389/fcell.2021.718192

Linnik, T.P., Dyubko, T.S., Martynyuk, I.N., Tereschenko, A.V., 2010. Interactions of cryoprotectants with liposomes from total lipids of the fowl spermatozoa. Problems of Cryobiology and Cryomedicine 20, 34–46. https://cryo.org.ua/journal/index.php/probl-cryobiol-cryomed/article/view/215 accessed 6 October 2025

Liptac, A.P., Pila, M., Stanciu, S., 2025. The small ruminant sector in Romania: Developments, challenges, and market concentration. Economics and Applied Informatics 1, 91–97. https://doi.org/10.35219/eai15840409489

Mahajan, A., Hazra, S., Arora, A., Katti, D.S., 2022. Isolation, expansion, and differentiation of mesenchymal stem cells from the infrapatellar fat pad of the goat stifle joint. Journal of Visualized Experiments 186, e63617. https://doi.org/10.3791/63617

Maia, L., de Moraes, C.N., Dias, M.C., Martinez, J.B., Caballol, A.O., Testoni, G., et al., 2017. A proteomic study of mesenchymal stem cells from equine umbilical cord. Theriogenology 100, 8–15. https://doi.org/10.1016/j.theriogenology.2017.05.015

Mambelli, L.I., Winter, G.H.Z., Kerkis, A., Malschitzky, E., Mattos, R.C., Kerkis, I., 2013. A novel strategy of mesenchymal stem cells delivery in the uterus of mares with endometrosis. Theriogenology 79, 744–750. https://doi.org/10.1016/j.theriogenology.2012.11.030

Mançanares, C.A.F., Oliveira, V.C., Oliveira, L.J., Carvalho, A.F., Sampaio, R.V., Mançanares, A.C.F., et al., 2015. Isolation and characterization of mesenchymal stem cells from the yolk sacs of bovine embryos. Theriogenology 84, 887–898. https://doi.org/10.1016/j.theriogenology.2015.05.031

Martini, L., Fini, M., Giavaresi, G., Giardino, R., 2001. Sheep model in orthopedic research: a literature review.

Comparative Medicine 51, 292–299. https://pubmed.ncbi.nlm.nih.gov/11924786/ accessed 6 October 2025

Mattei, D.N., Harman, R.M., Van de Walle, G.R., Smith, R., Grivel, J.C., Abdelalim, E.M., et al., 2024. Effect of pregnancy on isolation efficiency and *in vitro* proliferation of equine peripheral-blood derived mesenchymal stromal cells. Theriogenology 224, 107–118. https://doi.org/10.1016/j.theriogenology.2024.05.015

McCarty, R.C., Gronthos, S., Zannettino, A.C., Foster, B.K., Xian, C.J., 2009. Characterisation and developmental potential of ovine bone marrow derived mesenchymal stem cells. Journal of Cellular Physiology 219, 324–333. https://doi.org/10.1002/jcp.21670

Mehrabani, D., Hassanshahi, M.A., Tamadon, A., Zare, S., Keshavarz, S., Rahmanifar, F., et al., 2015. Adipose tissue-derived mesenchymal stem cells repair germinal cells of seminiferous tubules of busulfan-induced azoospermic rats. Journal of Human Reproductive Sciences 8, 103–110. https://doi.org/10.4103/0974-1208.158618

Meirelles, L.D.S., Nardi, N.B., 2003. Murine marrow-derived mesenchymal stem cell: isolation, *in vitro* expansion, and characterization. British Journal of Haematology 123, 702–711. https://doi.org/10.1046/j.1365-2141.2003.04669.x

Michael, A., Bayne, J., Siepker, C., Almeida, M., 2025. Small ruminant reproductive loss investigation: a retrospective analysis and recommendations for optimizing diagnostic outcomes. Journal of the American Veterinary Medical Association 263, 54–64. https://doi.org/10.2460/javma.24.12.0806

Mohamad-Fauzi, N., Ross, P.J., Maga, E.A., Murray, J.D., 2015. Impact of source tissue and ex vivo expansion on the characterization of goat mesenchymal stem cells. Journal of Animal Science and Biotechnology 6, 1. https://doi.org/10.1186/2049-1891-6-1

Morgan-Davies, C., Tesnière, G., Gautier, J.M., Jørgensen, G.H.M., González-García, E., Patsios, S.I., et al., 2024. Review: Exploring the use of precision livestock farming for small ruminant welfare management. Animal 18, 101233. https://doi.org/10.1016/j.animal.2024.101233

Motta, L.C.B., Pereira, V.M., Pinto, P.A.F., Mançanares, C.A.F., Pieri, N.C.G., de Oliveira, V.C., et al., 2023. 3D culture of mesenchymal stem cells from the yolk sac to generate intestinal organoid. Theriogenology 209, 98–106. https://doi.org/10.1016/j.theriogenology.2023.06.003

Mushahary, D., Spittler, A., Kasper, C., Weber, V., Charwat, V., 2018. Isolation, cultivation, and characterization of human mesenchymal stem cells. Cytometry Part A 93, 19–31. https://doi.org/10.1002/cyto.a.23242

Navarrete, F., Saravia, F., Cisterna, G., Rojas, F., Silva, P.P., Rodríguez-Alvarez, L., et al., 2020. Assessment of the anti-inflammatory and engraftment potential of horse endometrial and adipose mesenchymal stem cells in an *in vivo* model of post-breeding induced endometritis. Theriogenology 155, 33–42. https://doi.org/10.1016/j.theriogenology.2020.06.010

Omar, F., Amin, N., Elsherif, H., Mohamed, D., 2016. Role of adipose-derived stem cells in restoring ovarian structure of adult albino rats with chemotherapy-induced ovarian failure: a histological and immunohistochemical study. Journal of Carcinogenesis & Mutagenesis 7, 254. https://doi.org/10.4172/2157-2518.1000254

Omatsu, Y., 2023. Cellular niches for hematopoietic stem cells in bone marrow under normal and malignant conditions. Inflammation and Regeneration 43, 15. https://doi.org/10.1186/s41232-023-00267-5

Opiela, J., Samiec, M., Romanek, J., 2017. *In vitro* development and cytological quality of inter-species (porcine→bovine) cloned embryos are affected by trichostatin A-dependent epigenomic modulation of adult mesenchymal stem cells. Theriogenology 97, 27–33. https://doi.org/10.1016/j.theriogenology.2017.04.022

Panahi, M., Keshavarz, S., Rahmanifar, F., Tamadon, A., Mehrabani, D., Karimaghai, N., et al., 2015. Busulfan induced azoospermia: Stereological evaluation of testes in rat. Veterinary Research Forum 6, 273–278. https://pmc.ncbi.nlm.nih.gov/articles/PMC4769331/ accessed 6 October 2025

Paredes, J., Czochara, L., Villagomez, D., King, A., 2024. Disorders of sexual development in small ruminants. Clinical Theriogenology 16, 10273. https://doi.org/10.58292/ct.v16.10273

Patel, D.B., Gray, K.M., Santharam, Y., Lamichhane, T.N., Stroka, K.M., Jay, S.M., 2017. Impact of cell culture parameters on production and vascularization bioactivity of mesenchymal stem cell-derived extracellular vesicles. Bioengineering & Translational Medicine 2, 170–179. https://doi.org/10.1002/btm2.10065

Pearce, A.I., Richards, R.G., Milz, S., Schneider, E., Pearce, S.G., 2007. Animal models for implant biomaterial research in bone: a review. European Cells and Materials 13, 1–10. https://doi.org/10.22203/ecm.v013a01

Phinney, D.G., Kopen, G., Isaacson, R.L., Prockop, D.J., 1999. Plastic adherent stromal cells from the bone marrow of commonly used strains of inbred mice: variations in yield, growth, and differentiation. Journal of Cellular Biochemistry 72, 570–585. https://pubmed.ncbi.nlm.nih.gov/10022616/ accessed 6 October 2025

Popov, B.V., Petrov, N.S., Mikhailov, V.M., Tomilin, A.N., Alekseenko, L.L., Grinchuk, T.M., et al., 2009. Spontaneous transformation and immortalization of mesenchymal stem cells *in vitro*. Cell and Tissue Biology 3, 110–120. https://doi.org/10.1134/S1990519X09020023

Pulina, G., Milán, M.J., Lavín, M.P., Theodoridis, A., Morin, E., Capote, J., et al., 2018. Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. Journal of Dairy Science 101, 6715–6729. https://doi.org/10.3168/jds.2017-14015

Rentsch, C., Hess, R., Rentsch, B., Hofmann, A., Manthey, S., Scharnweber, D., et al., 2010. Ovine bone marrow mesenchymal stem cells: isolation and characterization of the cells and their osteogenic differentiation potential on embroidered and surface-modified polycaprolactone-co-lactide scaffolds. *In Vitro* Cellular & Developmental Biology - Animal 46, 624–634. https://doi.org/10.1007/s11626-010-9316-0

Rink, B.E., Kuhl, J., Esteves, C.L., French, H.M., Watson, E., Aurich, C., et al., 2018. Reproductive stage and sex steroid hormone levels influence the expression of mesenchymal stromal cell (MSC) markers in the equine endometrium. Theriogenology 116, 34–40. https://doi.org/10.1016/j.theriogenology.2018.04.034

Romanov, M.N., Zinovieva, N.A., Griffin, D.K., 2021. British sheep breeds as a part of world sheep gene pool landscape: looking into genomic applications. Animals 11, 994. https://doi.org/10.3390/ani11040994

Sakhatsky, N.I., Tereshchenko, A.V., Artemenko, A.B., 1987. Rapid method for evaluating the fertilizing ability of frozen-thawed semen of poultry. Agricultural Biology 12, 77–80. https://www.cabidigitallibrary.org/doi/full/10.5555/19880165689 accessed 6 October 2025

Sanjurjo-Rodriguez, C., Castro-Vinuelas, R., Hermida-Gomez, T., Fernandez-Vazquez, T., Fuentes-Boquete, I.M., de Toro-Santos, F.J., et al., 2017. Ovine mesenchymal stromal cells: morphologic, phenotypic, and functional characterization for osteochondral tissue engineering. PLoS One 12, e0171231. https://doi.org/10.1371/journal.pone.0171231

Scassiotti, R.F., de Paula Coutinho, M., Santos, S.I.P., Pinto, P.A.F., de Almeida, M.F., Karam, R.G., et al., 2023. Adipose and amniotic-derived mesenchymal stem cells: Extracellular vesicles characterization and implication for reproductive biotechnology. Theriogenology 198, 264–272. https://doi.org/10.1016/j.theriogenology.2022.12.012

Scherf, B.D., ed. World Watch List for Domestic Animal Diversity. 2nd ed. Rome, Italy: FAO, UNEP; 1995. https://web.archive.org/web/20151126034309/http://www.fao.org/ag/againfo/programmes/en/lead/toolbox/Ind

ust/wwl.pdf accessed 6 October 2025

Segunda, M.N., Bahamonde, J., Muñoz, I., Sepulveda, S., Cortez, J., De Los Reyes, M., et al., 2019. Sertoli cell-mediated differentiation of bovine fetal mesenchymal stem cells into germ cell lineage using an *in vitro* co-culture system. Theriogenology 130, 8–18. https://doi.org/10.1016/j.theriogenology.2019.02.034

Segunda, M.N., Cortez, J., Díaz, C., Arancibia, R., Torres, C.G., Parraguez, V.H., et al., 2024. Potential of mesenchymal stromal/stem cells and spermatogonial stem cells for survival and colonization in bull recipient testes after allogenic transplantation. Theriogenology 230, 192–202. https://doi.org/10.1016/j.theriogenology.2024.09.022

Sekiya, I., Larson, B.L., Smith, J.R., Pochampally, R., Cui, J.G., Prockop, D.J., 2002. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells 20, 530–541. https://doi.org/10.1634/stemcells.20-6-530

Severo, N.C., de Assumpção, T.I., Peixer, M.A.S., da Cunha Xavier, M., Malard, P.F., Brunel, H.D.S.S., et al., 2025. Effectiveness of intraglandular allogeneic mesenchymal stem cell administration for treating chronic vesicular adenitis in bulls. Theriogenology 241, 117419. https://doi.org/10.1016/j.theriogenology.2025.117419

Silva Filho, O.F.D., Argôlo, N.M., Carvalho, M.A.M.D., Carvalho, Y.K.D., Diniz, A.D.N., Moura, L.D.S., et al., 2014. Isolation and characterization of mesenchymal progenitors derived from the bone marrow of goats native from northeastern Brazil. Acta Cirurgica Brasileira 29, 478–484. https://doi.org/10.1590/s0102-86502014000800001

Siraj, Y., Galderisi, U., Alessio, N., 2023. Senescence induces fundamental changes in the secretome of mesenchymal stromal cells (MSCs): implications for the therapeutic use of MSCs and their derivates. Frontiers in Bioengineering and Biotechnology 11, 1148761. https://doi.org/10.3389/fbioe.2023.1148761

Souza-Fabjan, J.M.G., Oliveira, M.E.F., Guimarães, M.P.P., Brandão, F.Z., Bartlewski, P.M., Fonseca, J.F., 2023. Review: Non-surgical artificial insemination and embryo recovery as safe tools for genetic preservation in small ruminants. Animal 17, 100787. https://doi.org/10.1016/j.animal.2023.100787

Stewart, J.L., Shipley, C.F., 2021. Management of reproductive diseases in male small ruminants. Veterinary Clinics: Food Animal Practice 37, 105–123. https://doi.org/10.1016/j.cvfa.2020.10.005

Sun, M., Wang, S., Li, Y., Yu, L., Gu, F., Wang, C., et al., 2013. Adipose-derived stem cells improved mouse ovary function after chemotherapy-induced ovary failure. Stem Cell Research & Therapy 4, 80. https://doi.org/10.1186/scrt231

Tamadon, A., Mehrabani, D., Rahmanifar, F., Jahromi, A.R., Panahi, M., Zare, S., et al., 2015. Induction of spermatogenesis by bone marrow-derived mesenchymal stem cells in busulfan-induced azoospermia in hamster. International Journal of Stem Cells 8, 134–145. https://doi.org/10.15283/ijsc.2015.8.2.134

Tanrıkulu, M.D., Cevik, M., Yüce, M., Taşlı, P.N., Yıldırım, K., 2025. Cryoprotective effects of mesenchymal stem cell and seminal plasma-derived extracellular vesicles on canine sperm. Theriogenology 244, 117480. https://doi.org/10.1016/j.theriogenology.2025.117480

Tarasova, E.I., Frolov, A.N., Romanov, M.N., Lebedev, S.V., 2021. History, breeding, selection, and genetics of the Orenburg goat breed. In: Molecular Genetic Technologies for Analysis of Gene Expression Related to Animal Productivity and Disease Resistance, Materials of the 3rd International Scientific and Practical Conference, Moscow, Russia: Sel'skokhozyaistvennye tekhnologii; 2021, 450-454. https://doi.org/10.18720/SPBPU/2/z21-43

Tongu, E.A.O., Segabinazzi, L.G., Alvarenga, M.L., Monteiro, A., Papa, F.O., Alvarenga, M.A., 2021. Allogenic mesenchymal stem cell-conditioned medium does not affect sperm parameters and mitigates early endometrial inflammatory responses in mares. Theriogenology 169, 1–8. https://doi.org/10.1016/j.theriogenology.2021.03.019

Tropel, P., Noël, D., Platet, N., Legrand, P., Benabid, A.L., Berger, F., 2004. Isolation and characterisation of mesenchymal stem cells from adult mouse bone marrow. Experimental Cell Research 295, 395–406. https://doi.org/10.1016/j.yexcr.2003.12.030

Uzbekov, R.E., 2004. Analysis of the cell cycle and a method employing synchronized cells for study of protein expression at various stages of the cell cycle. Biochemistry (Moscow) 69, 485–496. https://doi.org/10.1023/b:biry.0000029845.11184.30

Viateau, V., Guillemin, G., Bousson, V., Oudina, K., Hannouche, D., Sedel, L., et al., 2007. Long-bone critical-size defects treated with tissue-engineered grafts: a study on sheep. Journal of Orthopaedic Research 25, 741–749. https://doi.org/10.1002/jor.20352

Vizoso, F.J., Eiro, N., Cid, S., Schneider, J., Perez-Fernandez, R., 2017. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. International Journal of Molecular Sciences 18, 1852. https://doi.org/10.3390/ijms18091852

Vivas, D., Caminal, M., Oliver-Vila, I., Vives, J., 2018. Derivation of multipotent mesenchymal stromal cells from ovine bone marrow. Current Protocols in Stem Cell Biology 44, 2B.9.1–22. https://doi.org/10.1002/cpsc.43

Westerkowsky, E.K., Soares de Almeida, A.M., Selle, M., Harms, O., Bundkirchen, K., Neunaber, C., et al., 2023. Characterization of human, ovine, and porcine mesenchymal stem cells from bone marrow: critical *in vitro* comparison with regard to humans. Life 13, 718. https://doi.org/10.3390/life13030718

Wilke, H.J., Kettler, A., Wenger, K.H., Claes, L.E., 1997. Anatomy of the sheep spine and its comparison to the human spine. Anatomical Record 247, 542–555. https://doi.org/10.1002/(SICI)1097-0185(199704)247:4<542::AID-AR13>3.0.CO;2-P

Wong, Y.S., Mançanares, A.C., Navarrete, F., Poblete, P., Mendez-Pérez, L., Rodriguez-Alvarez, L., et al., 2024. Short preconditioning with TGFβ of equine adipose tissue-derived mesenchymal stem cells predisposes towards an anti-fibrotic secretory phenotype: A possible tool for treatment of endometrosis in mares. Theriogenology 225, 119–129. https://doi.org/10.1016/j.theriogenology.2024.05.018

Yang, J.H., Liu, F.X., Wang, J.H., Cheng, M., Wang, S.F., Xu, D.H., 2020. Mesenchymal stem cells and mesenchymal stem cell-derived extracellular vesicles: Potential roles in rheumatic diseases. World Journal of Stem Cells 12, 688–705. https://doi.org/10.4252/wjsc.v12.i7.688