Ann. Anim. Sci., Vol. 24, No. 2 (2024) 367–377 *DOI: 10.2478/aoas-2023-0055*

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MULTI-FACETED CHARACTERIZATION OF MESENCHYMAL STEM CELLS AND THEIR APPLICATION AS A POWERFUL TOOL FOR AGROBIOTECHNOLOGY, ASSISTED REPRODUCTIVE TECHNOLOGIES, AND VETERINARY AND REGENERATIVE BIOMEDICINE – A REVIEW*

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Abstract

A broad spectrum of cytologically specific immunophenotypic, biochemical, physiological and molecular attributes of neonatal and adult mesenchymal stem cells (MSCs) exists. Taking into account the biological traits of these types of multipotent stem cells, they play a pivotal role in the wide utilization of MSC-mediated engineering and MSC-based assisted reproductive technologies (ARTs) for animal production, veterinary research, regenerative and reconstructive medicine, and cell/tissue transplantology. Consequently, this paper sought to comprehensively provide research highlights, and insightful interpretation of current goals and future targets related to the agricultural, embryological and biomedical application of MSC-assisted biotechnological strategies and MSC-dependent interdisciplinary research techniques.

Key words: mammalian, livestock species, mesenchymal stem cell, agrobiotechnology, assisted reproductive technology, embryology, biomedicine, MSC-based therapeutics

The biological and biotechnological background predetermining the practical applicability of MSC-based therapies

Stem cells are the non-specialized cells that give rise to the formation of all of the differentiated types of fetal and adult somatic cells originating from various germ layers (ectoderm, endoderm and mesoderm) and inhabiting various organ systems (Nowicka, 2005). It is worth noting that, on the one hand, the stem cells constitute a bioreservoir of cells, replacing and supplementing individual niches inhabited by specialized (i.e., partially or terminally differentiated) cells. On the other hand, at certain stages of ontogenetic development, stem cells also fill the gaps created by differentiated somatic cells that are eliminated from the aging organism as a result of necrosis, apoptosis or carcinogenesis. Stem cells include totipotent, pluripotent, multipotent and unipotent stem cells (Kangari et al., 2020). The only totipotent cells are the cells of the embryo, which can form all types of cells, including the placenta (Xu and Liang, 2022). Pluripotent stem cells, such as embryonic stem cells, display the ability to form cells from all three germ layers, excluding the placenta (Yamanaka, 2020). Cells that can form all cell types within one germ layer are multipotent cells, typical representatives of which are adult stem cells such as bone marrow-derived mesenchymal stem cells (BM-MSCs) or adipose tissue-derived MSCs (AT-MSCs) (Sobhani et al., 2017). Unipotent stem cells, in turn, are a natural reservoir of one type of cell (Sobhani et al., 2017). The use of adult stem cells creates an opportunity to treat many diseases using the patient's own cells, which reduces the risk of an immune reaction (Kangari et al., 2020).

MSCs are characterized by the ability to differentiate into a wide variety of mesodermal cell types such as adipocytes, chondrocytes and osteoblasts. Additionally, MSCs are suitable for use in cell therapy due to their easy isolation, high expansion, low immunogenicity, susceptibility to genetic modification and multipotent nature (Almalki et al., 2017). Unlike most other allogeneic cell therapies, allogeneic MSC-based therapies can be used

*The present study was financially supported by the research grant No. 01-19-11-21 from the National Research Institute of Animal Production in Balice near Kraków, Poland to Monika Trzcińska.

without concomitant immunosuppression due to an MHC class II protein deficiency and a reduced propensity to induce an immune response (Staff et al., 2019). Moreover, MSCs exhibit anti-inflammatory and immunomodulatory effects. The immunosuppressive effect of MSCs arises from their ability to inhibit T cell proliferation and impair T cell functionality (Uccelli et al., 2008), and reprogram M1 macrophages (Kim and Hematti, 2009). In addition, the interaction of MSCs with macrophages has been shown to contribute to the formation of a new type of macrophage with a potentially significant role in tissue repair. This confirms that MSCs can influence immune cells and regulate their cytokine secretion (Kim and Hematti, 2009).

MSC-based cell/tissue transplantation is used for therapeutic procedures targeted at approximately 90 diseases, including the treatment modalities for lymphoblastic leukemia, cardiomyopathy, COVID-19, neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, cerebral palsy), type 1 and type 2 diabetes, stroke, myocardial infarction, autoimmune diseases (lupus erythematosus, Hashimoto's disease) and a wide variety of oncological diseases (Staff et al., 2019; Kouchakian et al., 2021). Due to their ability to differentiate into somatic cells of various provenance, MSCs can play a pivotal role in the regeneration/reconstruction of tissues and organs that have been damaged either by anatomo- and histopathological changes or by biological mechanisms underlying the processes of oncogenic transformation of the cells (i.e., benign/non-malignant and malignant tumorigenesis) and the capacity of neoplastic cells to metastasize (Al Najar et al., 2017). MSCs have also been found to display a capability to promote hepatocyte proliferation and exert a protective activity against liver damage (Liu et al., 2019).

Collectively, according to the newest information included in the database www.clinicaltrials.gov, the current status of clinical trials involves over 1,400 biomedical programs based on therapeutic procedures using MSC-dependent cell and tissue therapies.

Immunophenotypic, cytobiochemical, cytophysiological and molecular characterization of MSCs

MSCs can be isolated from a variety of tissues including bone marrow, adipose tissue, cord blood or placental tissue. At the present stage of investigations, BM and AT provide the main sources of MSCs for the purposes of veterinary and human medicine (Chung et al., 2012). Cell culture engineering with the use of BM-MSCs offers 1) a low share of these subpopulations of stem cells (ranging from 0.001% to 0.01%) in the total pool of cells inhabiting individual compartments of the medullary cavity of the long and flat bones, and 2) a high invasiveness of the surgical procedure of recovering red BM and the accompanying isolation of BM-derived biopsies constituting a bioreservoir of MSCs (Fraser et al., 2006). Therefore, taking the above into account, an alternative source of MSCs (in terms of both the abundance of MSCs and the low invasiveness of the tissue biopsy isolation procedure itself) appears to be adipose tissue (Zuk et al., 2001; Sachs et al., 2012). In turn, the umbilical cord (UC) provides a bioreservoir of two sources of MSCs deposited in umbilical cord-derived blood and umbilical cord-derived mesenchymal tissue, anatomohistologically designated as Wharton's jelly. Cord blood is collected non-invasively and UC-MSCs exhibit a lower immunogenicity than MSCs isolated from other histological compartments, making them an excellent source that is suitable for cell/tissue engineering-based therapies in regenerative and reconstructive medicine and transplantation surgery. Although MSCs are routinely retrieved from the umbilical cord in humans, mice and birds, little is known about these cells in livestock species.

Placentas are the organs that are usually discarded post-partum. Furthermore, their post-parturient recovery is a non-invasive and atraumatic procedure, which does not raise any bioethical concerns (Araújo et al., 2017). Studies focused on the comparative analysis of neonatal and adult sources of MSCs have provided empirical evidence that cord blood-, umbilical cord-, placenta-, amnion- or chorion-derived MSCs display an immunophenotypic characterization that is similar to their bone marrow- and adipose tissue-derived counterparts. Nonetheless, the proliferative, immunosuppressive and/or regenerative capacity of neonatal MSCs is superior compared with MSCs originating from adult tissues (Zhang et al., 2022). Another important advantage of neonatal tissues is that they provide immature cells, which have a lower risk of mutations, and they exhibit augmented proliferative activity and an enhanced capacity to self-renew, which is also reflected in their increased differentiability, lineage commitment, cytological/histological colonization and engraftment potency, and simultaneously in their diminished immunogenicity (Zhang et al., 2022).

Cytologically and cytobiochemically specific attributes of mammalian BM- and AT-MSCs encompass multipotency and high capabilities to self-renew and differentiate, inter alia, into fibroblastic cells, muscle cells (myoblasts), adipose tissue cells (adipocytes), cartilage cells (chondroblasts/chondrocytes), bone cells (osteoblasts/osteocytes), tendon cells and nerve tissue cells (neurons), and also into cells of the heart muscle (cardiomyocytes) (Yang et al., 2015; Lian et al., 2021; Almalki et al., 2017). Recognizing the species-specific mesenchymal stemness of the cells isolated either from red BM or from AT is confirmed by the positive expression of surface markers (clusters of differentiation) such as integrin β1 (CD29), hyaluronan receptor (CD44), transferrin receptor 1 (CD71), ecto-5'-nucleotidase (CD73), thymus cell antigen 1 (CD90), endoglin (CD105), tumor necrosis factor receptor 1 (CD120a) and interleukin-4 receptor (CD124) (Uder et al., 2018). At the same time, BM- and AT-MSCs display a negative expression of surface antigens characteristic of endothelial cells: αM integrin (CD11b), platelet endothelial cell adhesion molecule 1

(CD31) and prominin 1 (CD133), as well as the lack of expression of antigens typical for hematopoietic cells: lipopolysaccharide receptor (CD14), antigen of hematopoietic progenitor cell antigen (CD34) and leukocyte-specific antigen (CD45) (Uder et al., 2018). Cumulatively, the expression profile of surface markers of myeloid and adipogenic MSCs shares many features, but it is not identical in both subpopulations of these stem cells. Adipogenic MSCs express the membrane antigen CD49d and lack the expression of the CD106 antigen, while myeloid MSCs express the surface biomarker CD106 and lack the expression of the CD49d biomarker (Ranera et al., 2011). In addition, a difference in the differentiative capacity of myeloid and adipogenic MSCs was observed. Studies performed by Niemeyer et al. (2010) confirmed that, as a result of reimplantation of adipogenic MSCs into the medullary cavity of the tibia of domestic sheep, the AT-MSCs exhibited a diminished potential for their cytodifferentiation into bone-forming cells (osteoblasts) and their terminal derivatives (osteocytes). This may be due to the fact that undifferentiated MSCs obtained from adipose tissue may have a higher predisposition to differentiate into the terminal cells of lipid tissue (adipocytes). In addition, it is worth noting that the differentiation capacity of adipogenic MSCs into chondrocytes is lower than that of myeloid MSCs (Molnar et al., 2022).

In 2006, the International Society for Cell Therapy published minimal criteria to define human MSCs (Horwitz et al., 2005). They report that MSCs are designated as the type of adherent cells that have been isolated from BM or other sources and exhibit a high incidence of functional and molecular plasticity. Secondly, MSCs are characterized by their enhanced capabilities to differentiate towards osteoblasts/osteocytes, adipocytes and chondroblasts/chondrocytes under *in vitro* culture conditions. Thirdly, MSCs display the immunophenotypic expression of CD105, CD73 and CD90 antigens and are deprived of the expression of CD45, CD34, CD14, CD11b, CD79α, CD19 and HLA-DR surface markers (Dominici et al., 2006). However, MSCs originating from a variety of mammalian species exhibit species-specific differences in the immunophenotypic extent and profile. Analogously, MSCs that have been isolated from different tissues have been also found to differ remarkably in the immunophenotypic signatures and this variability is often characterized by a high degree of histological specificity (Paebst et al., 2014). A wide array of MSC-specific clusters of differentiation has been presented in Table 1.

Bovine MSCs isolated from different adult and fetal tissues have been shown to be immunophenotypically positive for CD29, CD44, CD73, CD90, CD105, CD166, CD49d, CD13 and MHC-I antigens and negative for CD45, CD34, CD14, CD31, CD117, CD74, CD11b, CD80, CD86, CD106 and MHC-II surface markers (Merlo et al., 2022; Wang et al., 2022, Cahuascanco et al., 2019).

In contrast, equine MSCs do not commonly exhibit the expression of CD45, CD14, CD11b, CD79, CD19 and MHC class II antigens, they variably express different surface markers such as CD29, CD90, CD44, CD166, CD13, CD146, CD117, CD40 and CD80. A wide range of analyses for the expression levels of CD13, CD73, CD105 and CD34 have been inconsistent, which can be explained by the differences in the cell/tissue sources, harvesting methods and the antibodies used (Gugjoo et al., 2019). The *ex vivo* expanded equine MSCs originating from BM, AT and the umbilical cord have been found to exhibit high positivity for CD90, CD44 and CD105. On the other hand, they have been shown to be characterized not only by the lack of CD34-specific expression, but also by the low frequency of occurrence pinpointed for MHC- class II antigens (Barberini et al., 2014). Moreover, Paebst et al. (2014) have demonstrated that CD29 and CD44 surface antigens were the only markers that could be consistently detected in the plasma membrane of equine MSCs. In contrast, CD73, CD14, CD34, CD45 and MHC-II antigens were undetectable in the plasmalemmas of a vast majority of equine MSCs. Additionally, immunoprofiling targeted at the CD90 and CD105 markers has been positively confirmed only on the surface of the plasma membrane in AT-MSCs (at the levels of 24% and 40%, respectively).

Taking porcine BM-MSCs into consideration, immunophenotyping has been found to be positive for CD90, CD44, CD29, CD49d and CD49f antigens, and simultaneously negative for CD45, CD34 and CD14 antigens (Noort et al., 2012). In turn, AT-MSCs have been proven to be highly positive for MSC-specific markers such CD73, CD90 and CD105, and they are simultaneously characterized by almost undetectable signatures noticed for typical hematopoietic markers such as CD34 and CD45 (Almalki et al., 2017). Moreover, porcine AT-MSCs have been shown to be negative for CD14 and CD45 surface markers (Almalki et al., 2017).

Table 1. Detailed characterization of cluster of differentiation (CD)-dependent immunophenotypic profile noticed for mesenchymal stem cells of bone marrow-specific and other provenance

CD-mediated immunophenotypic landscapes pinpointed for MSCs in selected species of farm animals		Livestock species	References
Positive expression	Negative expression		
CD29, CD44, CD73, CD90, CD105, CD166	CD34, CD45	Cattle	Merlo et al., 2022; Wang et al., 2022
CD29, CD44, CD90, CD105, MHC-I	CD14, CD34, CD45, CD73, MHC-II	Horse	Gugjoo et al., 2019
CD29, CD44, CD49d, CD49f, CD90, CD105	CD14, CD34, CD45	Pig	Almalki et al., 2017; Noort et al., 2012
CD9, CD29, CD44, CD54, CD 166	CD14, CD31, CD34, CD45	Sheep	Sanjurjo-Rodríguez et al., 2017; Zhao et al. 2019; Dar et al., 2021

In sheep, the selection of antibodies is a major obstacle, taking into account the research focused on determining the quantitative profiles of expression noticed for MSC-specific clusters of differentiation. Although the immunophenotypic landscapes of ovine MSCs are similar to human MSCs, most antibodies reacting with human MSCs did not exhibit the reactivity with the surface antigens of ovine MSCs. The investigations performed by Dar et al. (2021) have shown that ovine BM- and AT-derived MSCs are simultaneously characterized by the increased expression levels recognized for CD73 and CD90 markers, and the absence of CD34 and CD45 antigens. Other studies undertaken to identify the immunophenotypic landscapes specific for ovine BM-MSCs have confirmed the high expression of CD29, CD166 and CD44 antigens and the lack of expression of the CD45 marker (Sanjurjo-Rodríguez et al., 2017). However, taking into account ovine UC-MSCs, they have been found to be positive for CD13, CD29, CD44, CD90 and CD106 markers, and negative for cell CD45 antigen (Zhao et al., 2019).

A wide range of analyses of the expression levels of various genes and proteins have been performed for MSCs. An analysis for detecting the transcriptional activities of genes encoding matrix metalloproteinase proteins 2 (MMP-2), tissue inhibitors of metalloproteinases 2 (TIMP-2), transcriptional coactivator with a PDZ-binding motif (TAZ) and myocyte enhancer factor 2A (MEF2a) was performed to more comprehensively understand the mechanisms of MSC differentiation (Opiela et al., 2016). The results of the previously mentioned study confirmed that, at the transcript level, the differentiation of MSCs towards adipocytes may involve MMP-2, TIMP-2 and TAZ. In turn, the osteogenic differentiation of MSCs may involve TIMP-2, MEF2a and TAZ. Simultaneously, no significant differences have been detected in the semi-quantitative profile of proapoptotic Bcl-2-associated death promoter (BAD) protein between porcine BM-MSCs, and their osteogenic and adipogenic derivatives (Opiela et al., 2013). Romanek et al. (2017) analyzed the impact of high hydrostatic pressure (HHP) applied before cryopreservation on the survival rate, proliferative activity, multipotency extent and apoptosis incidence in porcine post-thaw MSCs. The abovementioned research showed that BM-MSCs undergoing 40 MPa of HHP displayed improvements in survivability and proliferative activity. However, applying 50 and 60 MPa of HHP remarkably affected only the survival rates of MSCs (Romanek et al., 2017). In the study by Romanek et al. (2018), exposure to HHP did not induce the symptoms of apoptotic cell death in porcine BM-MSCs. This has been confirmed by the lack of significant variability in the expression of each analyzed apoptosis-related protein between the cells subjected to HHP (at the levels of 40 MPa or 60 MPa) and their HHP-untreated counterparts (Romanek et al., 2018).

MSC-mediated cell/tissue engineering serves as a modern agrobiotechnological approach to veterinary research, regenerative and reconstructive medicine, and transplantology

MSCs derived from adult tissues and organs are used in regenerative and reconstructive medicine based on the procedures of transplantation of cells and tissues of allogenic, isogenic and autogenic origin. This opens up a number of therapeutic possibilities in treatment modalities intended for patients afflicted with hereditary disorders and acquired diseases. The therapies using allogeneic sources of MSCs frequently result in significantly decreasing the risk/probability of hyperacute, acute and delayed rejection reactions of cell-based allotransplants (Staff et al., 2019).

For the best response to cell engineering-based therapies, consideration should be given to the patient's overall health, application time, number of applications, type of cells applied and the route of delivery. Cryopreservation of MSCs can provide a ready source of abundant autologous stem cells. Cryopreservation of bovine MSCs may be achieved successfully with no change in the characteristics between fresh and thawed cells (de Moraes et al., 2016). The results of the study by Martinello et al. (2010) confirmed that cryopreservation of equine MSCs provides a promising tool for different *in vitro* applications, because cryopreserved cells possess the same stemness-related characteristics as freshly isolated cells. Similar research results were obtained in analyses of the influence of cryopreservation on the quality of porcine MSCs (Dariolli et al., 2013).

Although liver transplant surgery is the main treatment strategy for liver degenerative diseases caused by hepatotoxic agents, the deficit of liver cells and tissues for allotransplantation has stimulated the search for alternative sources of transplantation in therapies based on innovative tissue engineering strategies using MSCs (Iansante et al., 2018). MSCs have been shown to promote hepatocyte proliferation and protect against acute liver injury in mouse models (Liu et al., 2019). Additionally, they are characterized by hepatoprotective properties in the event of liver-dependent cytotoxicity induced by a combination of such toxicological factors as concanavalin A, α-galactosylceramide and *D*-galactosamine (Ramanathan et al., 2017; Gazdic et al., 2018). In turn, in phase I/II clinical trials encompassing the therapies aimed at the treatment of liver cirrhosis-related symptoms, by using the modalities focused on the transplantation of autologous MSCs, an improvement in the indicators of liver-specific physiological capacity has been demonstrated (Kharaziha et al., 2009). Furthermore, in a phase II study, MSC-mediated transplantation mitigated the incidence of hepatotoxic degenerative changes triggered by hepatic fibrosis, which was reflected in a diminishment of the liver insufficiency in patients afflicted with alcoholic cirrhosis (Suk et al., 2016). MSCs that have been subjected to procedures of targeted extra-

corporeal differentiation are used in various subfields of regenerative and reconstructive medicine, medical transplantology and transplantation immunology. They are also used in replacement therapies of either myocardial tissues in patients afflicted with myocardial infarction (Zhang et al., 2009) or peripheral nerve issues (Park et al., 2012), or endothelial cell-mediated engineering and angioplasty of blood vessels damaged by atherosclerotic changes (Wang et al., 2020).

The dairy industry is a multi-billion-dollar industry, producing approximately 887 million tons of milk in 2021 (www.fao.org). Every year the demand for milk and dairy products is growing. Mastitis significantly reduces milk production and adversely affects animal welfare (Sharma et al., 2017). The use of antibiotic therapy in the treatment of mastitis is not satisfactory due to the prevalence of antibiotic resistance in dairy herds. An alternative therapy may be the use of MSCs. Administration of umbilical cord-derived MSCs via the intravenous or topical routes has been found to be safe and effective in alleviating mastitis in dairy cows (Ghai et al., 2022). Additionally, intra-mammary administration of repeated doses of allogenic bovine MSCs did not induce immune rejection; moreover, it reduced the *Staphylococcus aureus* bacterial count in milk (Peralta et al., 2020). Ghai et al. (2022) have shown that allogeneic MSCs are more effective than antibiotics in relieving mastitis in dairy cows. Moreover, transplantation of MSCs for the repair of mammary tissue re-established milk production in goats with mastitis (Costa et al., 2019).

In various cutaneous wound models, MSCs have improved healing, although the results are currently inconclusive. However, *in vivo* studies validating such differences are currently very limited. Thus far, there is a paucity of concurrent clinical reports in the existing literature, especially concerning cattle teat injuries. Dar et al. (2022) concluded that MSCs display an augmented capability to improve the healing of full-thickness cutaneous wounds and/or fistulae.

In large animal clinics, MSCs are used mainly to treat musculoskeletal diseases in horses such as tendon injuries or bone and cartilage damage. The incidence of nonunion fractures ranges from 5 up to 10% of fractures. As the lack of adhesions remains a significant issue, there has been increased interest in using tissue engineering strategies to enhance bone fracture repair (Toosi et al., 2018). Regenerative medicine-based efforts have been undertaken to utilize MSCs to heal fractures due to their remarkable role in promoting bone healing. MSCs can secrete trophic factors that favor the recruitment of other healing cells. Moreover, secreted trophic factors enhance angiogenesis, thus playing a significant role in osteoinduction and subsequent bone regeneration (Peer et al., 2022). A study by Wilson et al. (2010) has shown that porcine MSCs administered intravenously migrate to the site of bone injury. Regardless of the method of injection (intravenous or local), MSCs were able to significantly heal and regenerate porcine bone defects within 4 weeks of surgery (Wilson et al., 2010). Fracture is one of the most life-threatening injuries in horses, where bone healing is slow, taking a minimum of 6–12 months. Additionally, impaired bone healing in horses is a common problem contributed to by implant failure, deficient stabilization of the fracture, infection, age and drug treatment (McDuffee et al., 2014). Transplanted equine MSCs are characterized by an enhanced *in vivo* capacity to osteogenically differentiate and, as a consequence, to induce a generation/propagation and multiplication of osteoblasts/osteocytes within bone tissue-formed cytological niches or histological compartments in a short time frame, specifically when fibrin glue is used as a scaffold. The benefits arising from MSC-based reconstructive medicine and allo-/iso-/auto-transplantology may be reflected in the increased reliability and feasibility of a wide spectrum of treatment modalities targeted at osteogenous regeneration/repair and tissue engineering bringing about the removal of bone injuries; this should be further evaluated in an orthotopic model (McDuffee et al., 2014; Seo et al., 2014). There are no data focused on the use of MSCs for bone repair in cattle (Hill et al., 2019). However, fractures can result in the loss of meat and milk production and interfere with the reproductive performance of these animals.

Taking into account the research aimed at other mammalian species, the use of MSC-mediated therapy to treat bone damage has the potential to reduce the recovery time of animals, which will reduce the associated economic losses associated with reduced milk and meat production. Goats are commonly used as large animal models for orthopedic therapies. Elkhenany et al. (2015) have shown that oxidized graphene supports the proliferation and osteogenic differentiation of adult MSCs isolated from caprine bone marrow aspirates. These investigations suggest that the combination of graphene and caprine MSCs provides a promising construct for bone tissue engineering (Elkhenany et al., 2015).

The therapeutic modalities most frequently used for bone reconstruction involve the procedures of transplanting autologous MSCs. Nevertheless, there are several limitations to the use of autologous cell sources such as insufficient cell numbers or the unavailability of cells. An alternative is related to the use of allogeneic or xenogeneic MSCs. Peer et al. (2022) have demonstrated that the MSC-based implantation improved bone healing in the treatment group compared to the control, irrespective of the cell source. It is worth noting that the regenerative procedures using the re-implantation of differentiated or undifferentiated MSCs led to comparable bone healing. However, the strategies aimed at transplanting undifferentiated and osteogenically differentiated MSCs of allogeneic provenance may produce better bone healing than its counterpart mediated by xenogeneic MSCs (Peer et al., 2022).

At present, there have been many studies focused on the treatment of cartilage injury with MSC transplantation in sheep and goats. All of the published studies

have shown positive effects and an absence of complications in the animals following the implantation of MSCs (Caminal et al., 2014). In order to improve their regeneration potential, MSCs that are pre-differentiated in the chondrogenic direction are recommended. In a model of surgically induced osteoarthritis (OA) in sheep, pre-differentiated BM-MSCs were introduced intra-articularly and compared with intact BM-MSC and a control group without cells. The data showed that the injection of intact or pre-differentiated *in vitro* BM-MSCs may retard the progression of osteoarthritis in the sheep model; however, pre-differentiated cells showed better results, especially in the process of meniscus regeneration (Al Faqeh et al., 2012). The study by Abdalmula et al. (2017) confirmed the regenerative potential of MSCs collected from ovine ovaries in a sheep monomorphic model. Injection of MSCs resulted in a significant reduction in lameness, pain in the affected joints and swelling in the test animals compared to the control group. Treatment also attenuated histopathological changes including the activation of synovial stromal tissue, accumulation of $CD4^+$ T cells and monocytes/macrophages in the synovium, and synovial angiogenesis. Within the period of 3 days following MSC injection into the anatomotopographic regions causing symptoms of arthropathy, a significant decrease in circulating blood neutrophils and plasma activin A levels were observed. On the basis of the obtained results, MSCs isolated from the ovaries can alleviate the anatomohistopathological changes related to OA progression and reduce the concentration of some inflammatory mediators in the blood responsible for the destruction of joint tissue (Abdalmula et al., 2017). In a model of a medial condyle defect of the femoral bone and trochlear notch in goats, the MSCs and/or stromal vascular fraction (SVF) seeded onto a collagen I/III scaffold resulted in better cartilage healing after 4 months, compared to the animals treated with the acellular collagen I/III scaffold. Jurgens et al. (2013) reported that non-significant differences in the healing between the specimens treated with MSCs and SVF were observed.

In cattle, chronic osteoarthritis has been reported to be a significant cause of infertility in bulls, leading to economic loss and a decrease in animal value. Chronic OA is a degenerative disease of the articular cartilage, which causes the release of pro-inflammatory cytokines (Heinola et al., 2013). In this species, there are no current clinical data; however, some studies have demonstrated the isolation of MSCs and their potential to differentiate into the chondrogenic lineage.

In horses, the effectiveness of stem cells in osteoarthritis treatment was confirmed for cells isolated from bone marrow and adipose tissue. Additionally, autologous, allogeneic and xenogeneic MSCs were injected into the joints of six healthy horses. The results have shown that both therapeutic modalities with the use of auto- and allogeneic MSCs in horses appear to be safe and feasible. The most prominent diseases treated represented tendon lesions, which showed improvement after the injection of autologous MSCs directly into the site of the lesion. There were no reports of side effects due to the MSC application in any of the studies. A total of 13 out of 18 horses returned to racing after the injection of autogeneic BM-MSCs into ligament and tendon injuries (Renzi et al., 2013) and 9 out of 11 horses after MSC treatment of superficial toe flexor tendon injuries (Pacini et al., 2007). Therefore, equine MSCs can be clinically utilized for the treatment of inflammatory conditions, and acute and chronic degenerative diseases of the tendons, cartilage and bone. Overall, the clinical use of both auto- and allogeneic MSCs in several livestock species, including horses, has been shown to be safe and without adverse events.

To sum up, MSCs may serve as a potent tool for use in regenerative medicine to control incurable diseases in human and animals. In cattle, there is no genetic basis for diabetes (Tajima et al., 1999). This means that other factors must be responsible for the induction of this disease. It has been shown that, in cattle infected with the foot-and-mouth disease virus and viral diarrhea, there were individuals suffering from diabetes (Tajima et al., 1999). Gao et al. (2019) demonstrated that bovine pancreatic MSCs are characterized by a high capability to self-renew and differentiate under *in vitro* conditions. Taking into account the abovementioned functional attributes, recovering MSCs from the pancreas will provide a source of a new type of stem cell that can be used for cell therapy in diabetes and tissue engineering (Gao et al., 2019). Additionally, studies undertaken to induce the 3-week differentiation of goat MSCs into insulin-secreting pancreatic islet-like cells demonstrated enhancements of not only the expression profiles pinpointed for pancreatic endoderm-specific mRNA transcripts such as pancreatic and duodenal homeobox 1 (*PDX1*), neurogenin-3 (*NGN3*), paired box 6 (*PAX6*), paired box 4 (*PAX4*), insulin gene enhancer protein Islet 1 (*ISL-1*; ISL LIM Homeobox 1 also termed as LIM-homeodomain transcription factor) and glucose transporter 2 (*GLUT2*), but also the relative abundances of proteins such as PDX1, INS and ISL-1. The latter is essential for promoting pancreatic islet proliferation, maintaining endocrine cell survival in embryonic and postnatal pancreatic islets, and perpetuating mature islet cell homeostasis (Dubey et al., 2022).

Applicability of MSCs to the strategies of *in vitro* embryo production and reproductive biotechnology

Despite the progress in terms of the development of new culture media, embryos produced *in vitro* still display a lower quality compared with their counterparts produced *in vivo*. One of the methods of improving the efficiency of *in vitro* embryo production is the development of systems based on co-culture with different types of homogenous (non-mixed) or heterogenous (mixed) populations of differentiated somatic cells or undifferentiated stem cells (the so-called feeder monolayers), or embryo culture in the media conditioned by cell secretory activity (so-called conditioned media). All of the

endocrinologically active protein/polypeptide factors secreted by MSCs, including not only hematopoietic and non-hematopoietic growth factors, but also interleukins and chemokines, may be suitable for increasing and perpetuating the totipotency extent of the embryos. In turn, this promotes improvements in their developmental capabilities and molecular quality parameters under *ex vivo* conditions.

Ascari et al. (2018) investigated the effect of the co-incubation of bovine oocytes or embryos with rat BM-MSC-formed feeder monolayers in the systems of *in vitro* oocyte maturation (IVM) and/or *in vitro* embryo culture (IVC). There were no significant differences in the rates of *ex vivo* matured (metaphase II-stage) oocytes and the apoptosis incidence between the BM-MSC-mediated co-culture and the mono-culture deprived of BM-MSC-formed feeder monolayers. Moreover, the BM-MSC-mediated co-culture system had no impact on not only the cleavage activity and blastocyst formation rates of bovine embryos, but also their cytological quality parameters (Ascari et al., 2018). In turn, Nejat-Dehkordi et al. (2021) elaborated on and optimized the strategies of the co-culture of *in vitro* fertilized cattle zygotes, originating from *ex vivo* matured oocytes, with bovine amniotic membrane-derived MSCs (bAM-MSCs) or bovine oviductal epithelial cells (BOECs). The blastocysts that had been generated following two systems of embryo co-culture were subjected to vitrification procedures, followed by assessment of post-thaw survival rates.

The results of the research by Nejat-Dehkordi et al. (2021) proved the considerable increase in the survival rates of bovine vitrified/thawed embryos undergoing co-culture on bAM-MSC-based feeder monolayers compared with the embryos subjected to the monoculture system. This contributed to the cryo-survivability of embryos undergoing co-culture with bAM-MSCs at levels similar to that noticed for the embryos co-cultured with BOECs. In the study by Jasmin et al. (2016), murine blastocysts co-cultured with rat BM-MSCs displayed numbers of cells within the inner cell mass per embryo that were significantly higher than those estimated for their mono-cultured embryo counterparts. Romanek et al. (2018) analyzed the influence of the heterologous system of porcine BM-MSC-mediated co-culture on the *in vitro* developmental competences of bovine *in vitro* fertilized embryos and their cytological quality parameters at the blastocyst stage. The abovementioned research demonstrated that the co-culture of bovine IVF-derived embryos with porcine BM-MSCs adversely affected the developmental capability of bovine embryos to reach the blastocyst stages without a simultaneous negative impact on the quality parameters of the blastocyst produced (Romanek et al., 2018).

In a study by Maldonado et al. (2018), murine cumulus–oocyte complexes (COCs) that had undergone IVM via either culture in a microenvironment composed of human UC-MSC-conditioned G2-PLUS medium or the direct co-culture on UC-MSC-based feeder monolayers were characterized by the highest frequencies of occurrence of nuclear (meiotic) maturity. Furthermore, it is worth highlighting that the outcome of IVM in female patients afflicted with polycystic ovary syndrome (PCOS) is relatively or extremely low. For this reason, Jafarzadeh et al. (2018) elaborated the murine model of females with induced symptoms of human PCOS followed by the recovery of meiotically immature oocytes at the dictyotene stage of prophase I (i.e., germinal vesicle/GV stage) from the ovaries of female mice afflicted with PCOS. The abovementioned investigators found that the enrichment of IVM media with 50% human BM-MSC-conditioned media resulted in remarkable improvements in the attributes related to the nuclear and cytoplasmic maturity of *in vitro* cultured oocytes collected from the ovaries of mice with a prompted phenotypic background of human PCOS. The cytoplasmically and meiotically matured oocytes derived from such IVM systems mediated by human BM-MSC-conditioned media have been shown to exhibit significantly increased fertilization rates identified for IVF-derived zygotes. This was followed by augmentation of the cleavage activities pinpointed for cultured embryos and the capacity to complete their *in vitro* development to the blastocyst stages (Jafarzadeh et al., 2018).

Having an undifferentiated status and multipotency, in addition to molecular and epigenomic plasticity, are all biological attributes of paramount importance. This is because they predetermine a priori MSCs as an especially valuable source of nuclear donors for somatic cell nuclear transfer (SCNT)-based cloning in a wide variety of mammalian species, including rodents and livestock. What is particularly interesting is the provenance of this type of nuclear donor cells (NDCs). They are closely related to mesenchymal stemness and augmented *ex vivo* capability to cyto- and transdifferentiate into the terminally specialized somatic cells of both mesodermal origin and ecto- or endodermal origin. This superiorly determines the improved reverse susceptibility of MSC-inherited nuclei to undergo efficient and faithful reprogramming of their epigenomic and transcriptomic signatures in SCNT-generated embryos (Olivera et al., 2018; Samiec et al., 2019). This reversible process of MSC-descended nuclei to be completely dedifferentiated in the blastomeres of developing cloned embryos, in order to restore and recapitulate the molecular phenotype, is strongly correlated with the totipotency-dependent biological clock of embryonic cells (Nazari et al., 2016; Zhai et al., 2018). Thus far, undifferentiated MSCs of different origin have been successfully utilized – as an alternative source of NDCs in relation to partially differentiated fetal or adult dermal fibroblast cells – for the propagation and multiplication of SCNT-derived embryos. This is either by intra-species somatic cell cloning in pigs (Faast et al., 2006; Jin et al., 2007; Lee et al., 2010; Li et al., 2013; Samiec et al., 2015), cattle (Nazari et al., 2016), goats (Kwong et al., 2014), buffalo (Sadeesh et al., 2016) and horses (Olivera et al., 2016, 2018) or by inter-species somatic cell

cloning in the heterologous system pigs→cattle (Opiela et al., 2017). Characterization of *in vitro* developmental competences and/or cytological and molecular quality landscapes were estimated by TUNEL-mediated analysis of overall cell counts and RT-qPCR-mediated evaluation of pluripotency-related mRNA transcripts (*Oct4*, *Nanog*), respectively. They frequently confirmed the values of these parameters noticed for SCNT-derived embryos reconstructed with MSCs were higher than those for SCNT-derived embryos reconstructed with fetal or adult cutaneous fibroblast cells (Kumar et al., 2007; Samiec et al., 2015; Sadeesh et al., 2016; Zhai et al., 2018). Additionally, the research efforts undertaken by Samiec et al. (2019) have resulted in the elaboration of a novel strategy of molecular rejuvenation of BM-MSCs. This has been prompted by epigenomic modulation of their transcriptional profiles with exposure to trichostatin A (TSA; a representative of non-specific histone deacetylases). This strategy has brought about *Sox2*-, *Rex1*-, *c-Myc*- and *Nanog*-triggered enhancements in terms of pluripotency extent and *Survivin*-induced resilience to epigenetic senescence (i.e., TERT-dependent aging) of the *ex vivo*-expanded porcine BM-MSCs. This has been reflected in the increased epigenomic reprogrammability and molecular dedifferentiability of nuclear genomes inherited from TSA-modulated MSCs in SCNT-generated pig embryos (Samiec et al., 2019). As a consequence, the latter displayed simultaneous augmentation of blastocyst formation rates and the improvements in parameters of their cytological and molecular quality. This has been proven by diminished numbers of late-apoptotic (TUNEL-positive) cells and increased total cell numbers and transcriptional profiles pinpointed for the selected pluripotency-associated genes (Samiec et al., 2015, 2019).

Cumulatively, at the present stage of investigations, the efficiency of somatic cell cloning in mammals remains disappointingly low; it ranges from approximately 0.5% to an average of 5% as a percentage of the progeny generated in relation to the total number of oocytes reconstructed with different types of NDCs, including MSCs. For this reason, multi-faceted exploration and comprehensive identification of molecular factors affecting the reprogrammability of the epigenomic and transcriptomic landscapes of donor cell nuclei in cloned embryos, conceptuses and offspring is indispensable to successfully improve the efficacy of SCNT-based cloning in a variety of mammalian species (Gorczyca et al., 2021; Zhang et al., 2021; Samiec, 2022). This is also a sine qua non condition that is inevitable for broadening the biological and biotechnological applicability of MSC culture engineering to SCNT-mediated ARTs focused on propagating and multiplying monogenetic and monosexual specimens; this is for the purposes of transgenic research, regenerative and reconstructive therapeutic treatments, transplantation immunology, and the medical and biopharmaceutical industries (Li et al., 2013; Lee et al., 2020; Perisse et al., 2021; Singh et al., 2021; Skrzyszowska and Samiec, 2021; Samiec et al., 2022).

Conclusions, future directions and goals

The reasons for the occurrence of variability in the differentiation potential of myeloid and adipogenic MSCs are not fully understood. Clarity should be sought in the cell-specific variability of the epigenomic profile at the level of global DNA methylations (methylome), reflected in the cytodifferentiation and transdifferentiation pathways (Samsonraj et al., 2017). Therefore, detailed recognition regarding the molecular mechanisms responsible for the abovementioned processes of cyto- and transdifferentiation of both subpopulations of MSCs may increase the possibility of practical application of innovative therapeutic procedures in the near future. These would be based on MSC-dependent cell and tissue engineering techniques in human regenerative and reconstructive medicine and auto-/iso-/allo-/xenotransplantology.

Finally, the attractiveness of current and future research directions is related to the wide spectrum of application possibilities of MSCs in 1) agrobiotechnology; 2) reproductive biotechnology focused on innovative approaches to embryonic genome engineering such as somatic cell cloning and transgenic research; 3) regenerative medicine, medical auto-, iso- allo- and xenotransplantology, transplantation immunology and cell/tissue replacement therapies intended for reconstruction of the nervous system, cardiovascular system, dermo-integumentary system and advanced hepatology-specific therapies; 4) cell/tissue engineering-based treatment modalities suitable for neuroplasty, angioplasty, dermoplasty, cardiomyoplasty, hepatoplasty and a wide spectrum of organ-specific therapies intended not only for patients undergoing post-traumatic, post-accident and post-operative repair/elimination of tissue/organ injuries, but also for patients afflicted with oncological diseases, inheritable disorders and acquired diseases.

Acknowledgments

Author contributions – conceptualization: Monika Trzcińska and Marcin Samiec; writing the article – original draft: Joanna Romanek, Monika Trzcińska and Marcin Samiec; writing the article – review and editing: Monika Trzcińska and Marcin Samiec; tabular documentation: Joanna Romanek, Monika Trzcińska and Marcin Samiec; supervision: Monika Trzcińska and Marcin Samiec; funding acquisition: Monika Trzcińska.

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Received: 13 III 2023
Accepted: 27 IV 2023