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A mini-review on bovine stromal mesenchymal cells sustainability and innovativeness

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Abstract Animal experiments are conducted to demonstrate proof-of-concept and mechanisms of action, provide safe cell culture methods, carry out tissue engineering, and study infectious disease pathogenesis. Regarding the sustainable principles of the 3Rs, namely, refinement, reduction, and replacement, animal and human biological systems have been used. By definition, the Wharton's jelly (WJ) layer is a connective tissue localized in the middle of the umbilical vessels and amniotic epithelium. In this review, we would like to state that these cells belong to the division of primitive stromal cells. Because the WJ layer is an economical source of cells, the use of this resource would improve areas such as regenerative medicine, biotechnology, and agronomics worldwide in accordance with sustainability.

Keywords: cattle, connective tissue, stem cells, umbilical cord

1. Introduction

In bovine species, mesenchymal stem cells (MSCs) have been isolated from several compartments of the umbilical cord (UC): UC blood, umbilical vein subendothelium, and the WJ layer (Calloni et al 2014). Within the WJ layer and of concern to human studies, WJ-MSCs have been collected from three nearly indistinct segments: the perivascular area, the intervascular area, and the subamnion (Figure 1). However, it is uncertain whether MSCs isolated from the different regions of the UC represent diverse populations (Anzalone et al 2010; Baksh et al 2004; Corrao et al 2013; Cremonesi et al 2011; Fan et al 2011; Frausin et al 2015; Iacono and Merlo 2015; Li et al 2015; Pham et al 2016; Taghizadeh et al 2011). Studies on the isolation and characterization of MSCs from fetal adnexa in humans are rapidly advancing in contrast with similar studies in bovine species, including small ruminants (Carlin et al 2006; Corradetti et al 2008; Corradetti et al 2013; Cortes et al 2013; Cremonesi et al 2008; Özmert and Arslan 2020; Somal et al 2016). According to reports from some scientists, MSCs secluded in the WJ layer of the human umbilical cord can be used in the treatment of certain degenerative illnesses, even in neoplasia cases (Barczewska et al 2019; Bojanic et al 2020; Carvalho et al 2011; Ding et al 2007; Fu et al 2006; Gärtner et al 2012; De Miguel et al 2012). The bovine model could offer an enormous advantage in researching fetal-associated MSCs sources, especially since humans and bovines have similar gestational times.

Previous studies reported the isolation of bovine WJ-MSCs (bWJ-MSCs) from the UC at birth that were then successfully grown in culture without fetal calf serum and showed a capacity for pluripotency (Cardoso et al 2012a; Gungjoo et al 2019; Lange-Consiglio et al 2016). MSCs are fixed within the WJ layer during the first two weeks of embryonic development and stay there for the entire gestation period. Thus, they can be collected during pregnancy or after birth (Karahuseyinoglu et al 2007; Raoufi et al 2011; Troyer and Weiss 2008). Accordingly, bovine Wharton's jelly-derived mesenchymal stem cells (bWJ-MSCs) that form during the initial ontogenetic term exhibit substantial expansion potential (Troyer and Weiss 2008; Weiss et al 2008).

Overall, the major biological characteristic of MSCs is their immunosuppressive behavior. In this context, some authors recently described the intricacy of investigating the immunological attributes of bWJ-MSCs *in vitro*, in addition to the hard work of determining the most advantageous gestational stages during which to harvest these cells (Cardoso et al 2017). Moreover, bWJ-MSCs harvested from bovine navel strings during gestation have revealed an immune-privileged status, which suggests that allogeneic cells have the potential to be used with no chance of immune rejection, an interesting perspective for the same studies in humans.

The scope of this paper is to provide (i) a brief perspective on the source and stem phenotype of bWJ-MSCs, (ii) a description of the attributes of bWJ-MSCs, (iii) a discussion of their biotechnology application with special reference to bovine

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species and neuron-like cell differentiation, (iv) a report on the potential advances in WJ-MSCs in human and bovine diseases and (v) a delineation of the limitations and applications of the 3Rs principle.

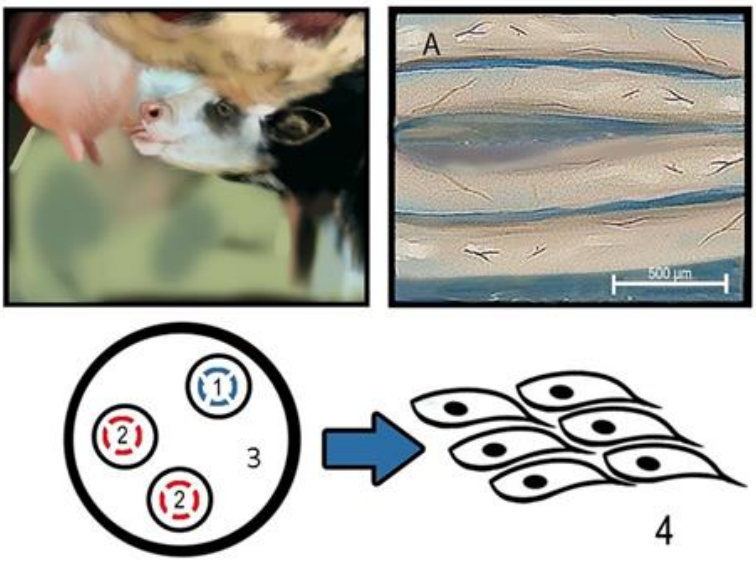

Figure 1 Overview of bovine umbilical cord obtained after parturition. The illustration on the top left shows a *Bos taurus* cow with a female calf. A) Representation of the bovine umbilical cord morphology. The lower left image shows a schematic of the components of the bovine umbilical cord: (1) umbilical vein; (2) umbilical cord blood; (3) Wharton's jelly region; and 4) bWJ-MSCs.

2. bWJ-MSCs Source and Phenotyping

In the literature, only two references describe enzymatic and nonenzymatic methods to obtain bWJ-MSCs *in vitro* (Cardoso et al 2012a; Lange-Consiglio et al 2016; Figure 2). The source of bWJ-MSCs does not appear to be from umbilical blood but rather from the matrix among the umbilical blood vessels. Earlier studies denominated these cells as "umbilical cord matrix stem cells" to separate them from cells isolated from other umbilical vein endothelial cells or UC blood. However, bWJ-MSCs fit the pattern for stem cells: they self-renew and can differentiate into various cell types, including adipocytes, chondrocytes/osteocytes, and neuron-like cells, and have adherence capacity. There have only been two descriptions of these stem cells in the literature after the first report using bovine UC. This source benefits the cells are isolated from the fetal formation in the perinatal stage and, perhaps similarly to UC blood, and they can have improved tolerance to transplantation with minor rates of graft-versus-host disease (Weiss et al 2008; Lu et al 2011).

Cultured bWJ-MSCs have a fibroblastic spindle shape and a high plastic adherence and expansion potential, showing a mean doubling time of 34 hours (Lange-Consiglio et al 2016). Molecular characterization and flow cytometry analysis have shown that bWJ-MSCs must express embryonic octamer-binding transcription factor 4 (POU5F1 or OCT-4), intersectin-1 (ITSN1), integrin beta-1 (CD29), ecto-5'-nucleotidase (CD73), 25–37-kDa heavily N-glycosylated glycophosphatidylinositol (CD90), and the type I membrane glycoprotein on cell surfaces that is part of the TGF (tissue growth factor) beta receptor complex (CD105). Moreover, bWJ-MSCs must be negative for CD34, which indicates the receptor attachment of hematopoietic stem cells to bone marrow extracellular matrix or directly to stromal cells, and for CD45, a marker expressed on the surface of all human leukocytes (lymphocytes, eosinophils, monocytes, basophils, and neutrophils). These parameters are well established in the International Society for Cellular Therapy (ISCT) manual (Dominici et al 2006). According to another study, these morphological characteristics and gene expression patterns are well preserved for 60 successive passages (Cardoso et al 2012a). When maintained in three-dimensional serum-free culture, bWJ-MSCs can produce several spheroids of approximately 300 µm in diameter each. bWJ-MSCs are pluripotent; they can be induced to undergo phenotypic differentiation through the osteogenic, chondrogenic, adipogenic and neurogenic pathways *in vitro.*

3. Response to Inflammation

A variety of stimuli might cause inflammatory situations. The most frequent pro-inflammatory factors are microbe infections, neoplasia development, and traumas (Mukonoweshuro et al 2014; Poncelet et al 2007; Prasanna et al 2010). The balance between pro-inflammatory and anti-inflammatory cytokine release represents an important factor in the battle between pathogens and the host system. Therefore, according to the previously described results, bWJ-MSCs show immunosuppressive attributes, mainly due to their lower MHC-I/MHC-II expression (Cardoso et al 2017). bWJ-MSCs possess a distinguished immune status that would indicate the capacity of these cells to be used with no risk of immune rejection. Considering all the properties of bWJ-MSCs described above (the ability to adhere to plastic culture surfaces, characterize as

mesoderm-derived cells, and undergo in vitro differentiation through osteogenic, chondrogenic, and adipogenic pathways) confirms the stem cell potential of these cells, as illustrated in Figure 3. This important phenomenon turns MSCs into an exciting model for comparative tissue research.

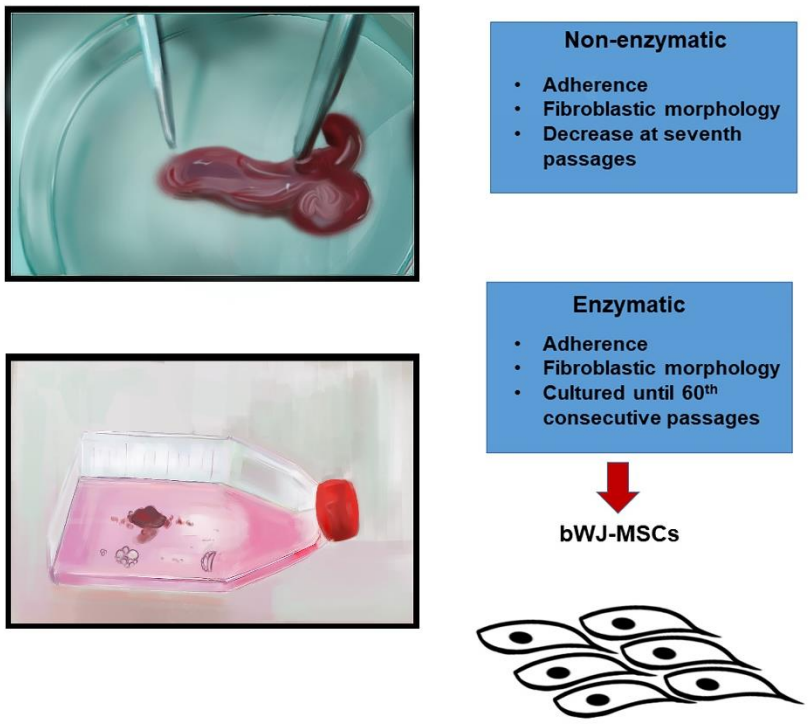

Figure 2 Illustration of two methods for obtaining bWJ-MSCs (enzymatic and nonenzymatic).

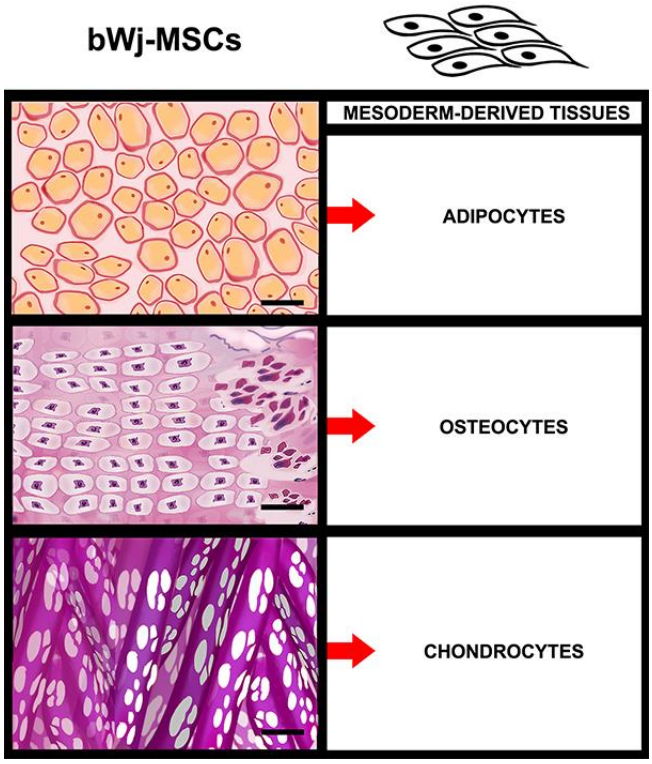

Figure 3 Schematic overview of bWJ-MSC differentiation into mesodermal tissues (scale bar: 40 µm).

4. The 3Rs principle

The 3Rs principle, defined as refinement, reduction, and replacement, is currently desired for any scientific procedure. A more comprehensive application concerning the original definition is necessary to avoid animal mistreatment during biological experiments (Hamilton et al 2018). The importance of regulatory guidance is to revise or reduce regulatory guidance, optimize the balance between scientifically relevant data and animal well-being, or reduce animal use. Limiting animal use as much as possible in all biological studies makes using *in vitro* models essential from an ethical point of view. In general, there are some viable methods: (i) cell line-based models, which raise no ethical concerns; (ii) primary cell-based models, with less ethical impact in animals; and (iii) organotypic ex vivo-based models, which raise moderate ethical concerns related to laboratory animal use. In this respect, bWJ-MSCs are considered a cell-based model collected from disposable biological material with the advantage of animal use (Cardoso et al 2012b; Geuna et al 2016). However, contamination during bovine UC harvest is an important challenge for researchers. Despite other sources of bovine stem cells, bWJ-MSCs are considered pluripotent, exhibit immune-privileged behavior, are easily cultured, and have no ethical concern related to animal use.

5. Innovative applications

5.1. bWJ-MSC neurodifferentiation

The ability to expand bWJ-MSCs in vitro denotes their applicability in extramesodermal tissues, such as ectoderm-like cells (Cardoso et al 2012b). Likewise, bWJ-MSCs tend to differentiate into neuron- and astrocyte-like cells after induction in a specific medium. However, these cells show a variable expression of neurogenic markers, as demonstrated in Figure 4. In each time interval, there are specific expression patterns exhibited by bWJ-MSCs. However, more accurate research is necessary to ensure their application in brain therapy, neuroinflammation, neurovirology, and neuroplasticity (Gungjoo et al 2019). Therefore, one unique study searched for ten neuronal/microglial cell markers in cultured neuron-like cells derived from bWJ-MSCs, which confirmed a valuable method displaying the principles of the 3Rs (Cardoso et al 2012b).

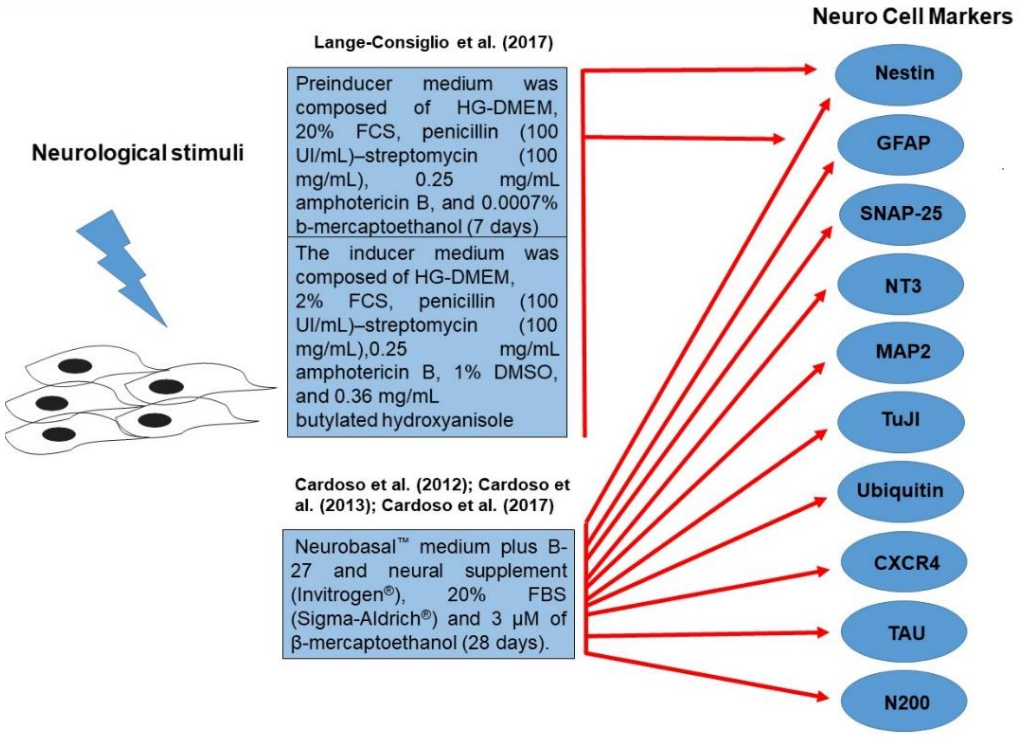

Figure 4 Neurogenic and microglial cell markers after neuronal induction of bWJ-MSCs. Nestin, glial fibrillary acidic protein (GFAP), synaptosomal-associated protein, 25 kDa (SNAP-25), NT-3 (a neurotrophic factor), MAP2 (a microtubule-associated protein), TuJI (or b-tubulin III/class III β-tubulin), ubiquitin, CXCR4 (chemokine receptor 4), tau proteins, and N200 (a neurofilament protein) are shown.

5.2. Neuronal cell markers

To confirm bWJ-MSC neurodifferentiation, cell markers, the cytoskeleton, and active functional proteins were investigated. The first cell marker, nestin, is considered an intermediate filament found in peripheral nerves responsible for radial nerve growth. Another cell marker is NT-3, a neurotrophic factor belonging to the neurotrophin family. Moreover, NT-3 is a growth factor protein with peripheral activity in the peripheral and central nervous systems, facilitating neuronal survival

and differentiation. In addition, microtubule-associated protein 2 (MAP2) is a neuron-specific cytoskeletal protein enriched in dendrites and is also expressed in neuron-like cell-derived bWJ-MSCs. Tau proteins are a class of six exceptionally soluble protein isoforms whose primary function is to preserve the cohesion of microtubules in axons. These proteins are plentiful in the neurons of the central nervous system. Another cytoskeletal protein, class III β-tubulin, alternatively known as βIII-tubulin (β3-tubulin), β-tubulin III or TuJI, is found in almost all differentiated neurons. Neurofilament 200 kDa (N200) is a structural component found in bipolar, multipolar and unipolar neurons (Cardoso et al 2012b).

In evaluating the neuronal function, synaptosomal-associated protein 25 kDa (SNAP-25), responsible for directly interfering with synaptic vesicle and plasma membranes fusion, was detected in neuron-like cells derived from bWJ-MSCs. In addition, chemokine receptor 4 (CXCR4) is typically detected in newly generated neurons throughout embryogenesis and adulthood. Moreover, ubiquitin is a small protein that is conserved among eukaryotic cells. Ubiquitin, considered an anti-inflammatory, immune modulator, and an endogenous opponent of pro-inflammatory events, has been detected among neuron-like cells derived from bWJ-MSCs (Cardoso et al 2012b). Considering all this information together, it is possible to infer that neuron-like cells derived from bWJ-MSCs represent a promising *in vitro* model for future studies.

5.3. Glial cell markers

The last cell marker, glial fibrillary acidic protein (GFAP), is considered a type III intermediate filament protein expressed by many cell types, such as astrocytes and ependymal cells of the central nervous system. GFAP is one of the most frequently utilized markers of mesenchymal cell neurodifferentiation in human and animal studies (Pereira et al 2014; Wu et al 2018).

6. Latest Advances

In addition, in large ruminants, this biotechnology has some barriers to face, such as the high cost of research and maintenance and the preference of using MSCs in reproduction instead of other activities in these animals. Given these hurdles, many studies use bWJ-MSCs to treat multiple conditions and diseases, such as mastitis, laminitis, nerve injuries, bovine spongiform encephalopathy (BSE), and oxidative stress, are still in progress (Mediano et al 2015; Pereira et al 2014;). An example of bWJ-MSCs in healthcare is the differentiation of neuron-like cells, as observed in dogs (Oda et al 2013; Uranio et al. 2011).

MSCs have been a practicable option for many applications because of their immunomodulatory properties, regeneration capacities, and tissue repair in human and animal diseases. Coronavirus disease 2019 (COVID-19) is a highly infectious disease caused by the coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Studies have shown that the use of MSCs can attenuate the symptoms related to SARS-CoV-2 infection, end fluid build-up, and support regeneration following alveolar injury, all in a secure and efficient method.

5. Final considerations

In conclusion, bWJ-MSCs can be acquired from several components of the umbilical cord of cattle, especially the WJ layer, and have a notable capacity for self-renewal and for undergoing cell differentiation. Their facility of acquisition soon after delivery and the accordance of their use with ethical principles make it possible to use them as an experimental model in *in vitro* studies according to the principles of the 3Rs. In addition, these qualities make their future applications in cell therapy, gene therapy, and animal biotechnology interesting. In large animals, the costs for bWJ-MSC use are still high, and studies remain focused mainly on their reproductive scope. The recent investigation of the immunomodulatory and repair effects of bWJ-MSCs in the recovery of alveolar damage caused by SARS-CoV-2 suggests applicability and importance in human medicine, which may arouse interest in more studies based on previous results to obtain further results.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Anzalone R, Lo Iacono M, Corrao S, Magno F, Loria T, Cappello F, Zummo G, Farina F, La Rocca G (2010) New emerging potentials for human Wharton's jelly mesenchymal stem cells: immunological features and hepatocyte-like differentiate capacity. Stem Cells and Development 19:423–38.

Baksh D, Song L, Tuan RS (2004) Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. Journal of Cellular and Molecular Medicine 8:301-16.

Barczewska M, Grudniak M, Maksymowicz S, Siwek T, Ołdak T, Jezierska-Woźniak K, Gładysz D, Maksymowicz W (2019) Safety of intrathecal injection of Wharton's jelly-derived mesenchymal stem cells in amyotrophic lateral sclerosis therapy. Neural Regeneration Research 14:313-18.

Bojanic C, To K, Zhang B, Mak C, Khan WS (2020) Human umbilical cord derived mesenchymal stem cells in peripheral nerve regeneration. World Journal of Stem Cells 12:288-302.

Calloni R, Viegas GS, Turck P, Bonatto D, Henriques JAP (2014) Mesenchymal stromal cells from unconventional model organisms. Cytotherapy 16:3-16.

Cardoso TC, Ferrari HF, Garcia AF, Novais JB, Silva-Frade C, Ferrarezi MC, Andrade AL, Gameiro R (2012a) Isolation and characterization of Wharton's jelly derived multipotent mesenchymal stromal cells obtained from bovine umbilical cord and maintained in a define serum-free three dimensional system. BMC Biotechnology 12:18.

Cardoso TC, Novais JB, Antello TF, Silva-Frade C, Ferrarezi MC, Ferrari HF, Gameiro R, Flores EF (2012b) Susceptibility of neuron-like cells derived from bovine Wharton's jelly to bovine herpesvirus type 5 infections. BMC Veterinary Research 8:242.

Cardoso TC, Okamura LH, Baptistella JC, Gameiro R, Ferreira HL, Marinho M, Flores EF (2017) Isolation, characterization and immunomodulatory-associated gene transcription of wharton's jelly-derived multipotent mesenchymal stromal cells at different trimesters of cow pregnancy. Cell and Tissue Research 367:243-56.

Carlin R, Davis D, Weiss M, Schuktz B, Troyer D (2006) Expression of early transcription factor Oct-4, Sox-2 and Nanog by porcine umbilical cord (PUC) matrix cells. Reproductive Biology and Endrocrinology 4:8.

Carvalho MM, Teixeira FG, Reis RL, Sousa N, Salgado AJ (2011) Mesenchymal stem cells in the umbilical cord: phenotypic characterization, secretome and applications in central nervous system regenerative medicine. Current Stem Cell Research & Therapy 6:221-28.

Corradetti B, Lange-Consiglio A, Cremonesi F, Bizzaro D (2008) Isolation, in vitro culture and characterization of foetal umbilical cord stem cells at birth. Veterinary Research Communications 32:139-142.

Corradetti B, Meuci A, Bizzaro D, Cremonesi F, Lange-Consiglio A (2013) Mesenchymal stem cells from amnion and amniotic fluid in the bovine. Reproduction 145:391-400.

Corrao S, La Rocca G, Lo Iacono M, Corsello T, Farina F, Anzalone R (2013) Umbilical cord revisited: from Wharton's jelly to mesenchymal stem cells. Histology and Histopathology 28:1235-44.

Cortes Y, Ojeda M, Araya D, Dueñas F, Fernández MS, Peralta AO (2013) Isolation and multilineage differentiation of bone barrow mesenchymal stem cells from abattoir-derived bovine fetuses. BMC Veterinary Research 9:133.

Cremonesi F, Corradetti B, Lange-Consiglio A (2011) Fetal adnexa derived stem cells from domestic animal: progress and perspectives. Theriogenology 75:1400-15.

Cremonesi F, Violini S, Lange-Consiglio A, Ramelli P, Ranzenigo G, Mariani P (2008) Isolation, in vitro culture and characterization of foal umbilical cord stem cells at birth. Veterinary Research Communications 32:139-42.

De Miguel MP, Fuentes-Julián S, Blázquez-Martinez A, Pascual CY, Aller MA, Arias J, Arnalich-Montiel F (2012) Immunosuppressive properties of mesenchymal stem cells: advances and applications. Current Molecular Medicine 12:574-91

Ding DC, Shyu WC, Chiang MF, Lin SZ, Chang YC, Wang HJ, Su CY, Li H (2007) Enhancement of neuroplasticity through upregulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. Neurobiology Disease 27:339-53.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for cellular therapy position statement. Cytotherapy 8:315-17.

Fan CG, Zhang QJ, Zhou JR (2011) Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. Stem Cells Reviews and Reports 7:195-207.

Frausin S, Viventi S, Verga Falzacappa L, Quattromani MJ, Leanza G, Tommasini A, Valencic E (2015) Wharton's jelly derived mesenchymal stromal cells: Biological properties, induction of neuronal phenotype and current applications in neurodegeneration research. Acta Histochemica 117:329-38.

Fu YS, Cheng YC, Lin MY, Cheng H, Chu PM, Chou SC, Shih YH, Ko MH, Sung MS (2006) Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism. Stem Cells 24:115-24.

Gärtner A, Pereira T, Simões MJ, Armada-da-Silva PA, França ML, Sousa R, Bompasso S, Raimondo S, Shirosaki Y, Nakamura Y, Hayakawa S, Osakah A, Porto B, Luís AL, Varejão AS, Maurício AC (2012) Use of hybrid chitosan membranes and human mesenchymal stem cells from the Wharton jelly of umbilical cord for promoting nerve regeneration in an axonotmesis rat model. Neural Regeneration Research 7:2247-58.

Geuna S, Raimondo S, Fregnan F, Haastert-Talini K, Grothe C (2016) In vitro models for peripheral nerve regeneration. European Journal of Neuroscience 43:287-96.

Gungjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2019) Mesenchymal stem cell: Basic research and potential applications in cattle and buffalo. Journal of Cellular Physiology 234:8618-35.

Hamilton N, Sabroe I, Stephen A. Renshaw SA (2018) A method for transplantation of human HSCs into zebrafish, to replace humanised murine transplantation models. F1000Research 7:594.

Iacono E, Merlo B (2015) Stem cells from foetal adnexa and fluid in domestic animals: an update on their features and clinical application. Reproduction in Domestic Animals 50:353-64.

Karahuseyinoglu S, Cinar O, Kilic E, Kara F, Akay GG, Demiralp DO, Tukun A, Uckan D, Can A (2007) Biology of the stem cells in human umbilical cord stroma: in situ and in vitro surveys. Stem Cells 25:319–31.

Lange-Consiglio A, Perrini C, Bertero A, Esposti P, Cremonesi F, Vincenti L (2016) Isolation, molecular characterization, and in vitro differentiation of bovine Wharton jelly–derived multipotent mesenchymal cells. Theriogenology 89:338-47.

Li T, Xia M, Gao Y, Chen Y, Xu Y (2015) Human umbilical cord mesenchymal stem cells: an overview of their potential in cell-based therapy. Expert Opinion on Biological Therapy 15:1293-1306.

Lu T, Huang Y, Wang H, Ma Y, Guan W (2011) Multi-lineage potential research of bone marrow-derived stromal cells (BMSCs) from cattle. Applied Biochemistry Biotechnology 172:21-35.

Mediano DR, Sanz-Rubio D, Ranera B, Bolea R, Martin-Burriel I (2015) The potential of mesenchymal stem cell in prion research. Zoonoses and Public Health 62:165-78.

Mukonoweshuro B, Brown CJF, Fisher J, Ingham E (2014) Immunogenicity of undifferentiated and differentiated allogenic mouse mesenchymal stem cells. Journal of Tissue Engineering 5:2041731414534255.

Oda Y, Tani K, Kanei T, Haraguchi T, Itamoto K, Nakazawa H, Taura Y (2013) Characterization of neuron-like cells derived from canine bone marrow stromal cells. Veterinary Research Communications 37:133-38.

Özmert E, Arslan U (2020) Management of retinitis pigmentosa by Wharton's jelly derived mesenchymal stem cells: preliminary clinical results. Stem Cell Research & Therapy 11:25.

Pereira T, Gärtner A, Amorim I, Almeida A, Caseiro AR, Armada-da-Silva PA, Amado S, Fregnan F, Varejão AS, Santos JD, Bartolo PJ, Geuna S, Luís AL, Mauricio AC (2014) Promoting nerve regeneration in a neurotmesis rat model using poly (DL-lactide-ε-caprolactone) membranes and mesenchymal stem cells from the Wharton's jelly: in vitro and in vivo analysis. BioMed Research International 2014:302659.

Pham PV, Truong NC, Le P T-B, Tran T D-X, Vu NB, Bui K H-T, Phan NK (2016) Isolation and proliferation of umbilical cord tissue derived mesenchymal stem cells for clinical applications. Cell Tissue Banking 17:289-302.

Poncelet AJ, Vercruysse J, Saliez A, Gianello P (2007) Although pig allogenic mesenchymal stem cells are not immunogenic in vivo, intracardiac injection elicits an immune response in vivo. Transplantation 83:783-90.

Prasanna SJ, Gopalakrishnan D, Shankar RS, Vasandan, AB (2010) Pro-inflammatory cytokines, IFNγ and TNFα, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. PLoS One 5:e9016.

Raoufi MF, Tajik P, Dehghan MM, Eini F, Barin A (2011) Isolation and differentiation of mesenchymal stem cells from bovine umbilical cord blood. Reproduction in Domestic Animals 46:95-9.

Somal A, Bhat IA, Pudey S, Pauda Bs, Thakur N, Sarkar M, Chaudra V, Saikumar G, Sharma GT (2016) A comparative study of growth kinetics, in vitro differentiation potential and molecular characterization of fetal adnexa derived caprine mesenchymal stem cells. PLoS One 11:e015681.

Taghizadeh RR, Cetrulo KJ, Cetrulo CL (2011) Wharton's jelly stem cells: Future clinical applications. Placenta 32:311-15.

Troyer DL, Weiss ML (2008) Concise review: Wharton's Jelly-derived cells are primitive stromal cell population. Stem Cells 26:591-99.

Uranio MF, Valentini L, Lange-Consiglio A, Caira M, Guaricci AC, Lábbate A, Catachio CR, Ventura M, Cremonesi F, Del Aquila ME (2011) Isolation, proliferation, cytogenetic, and molecular characterization and in vitro differentiation potency of canine stem cells from foetal adnexa: a comparative study of amniotic fluid, amnion, and umbilical cord matrix. Molecular Reproduction and Development 78:361-73.

Weiss ML, Anderson C, Medicetty S, Seshareddy KB, Weiss RJ, VanderWerff I, Troyer D, McIntosh KR (2008) Immune Properties of human umbilical cord Wharton's Jelly-derived cells. Stem Cells 26:2865-74.

Wu KJ, Yu SJ, Chiang CW, Lee YW, Yen BL, Hsu CS, Kuo LW, Wang Y (2018) Wharton' jelly mesenchymal stromal cell therapy for ischemic brain injury. Brain Circulation 4:124-127.