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Derivation and Potential Applications of Pluripotent Stem Cells for Regenerative Medicine in Horses

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ABSTRACT

***Background*:** The ability to create tissues using pluripotent stem cells to repair or replace tissue lost due to damage, i.e. regenerative medicine, is developing very rapidly in many fields of human medicine. For veterinarians, regenerative medicine has focused mainly in the use of stem cells for arthritis and tendon ligament repair, indicating a need for treating musculo-skeletal injuries. Our objective is to review the available approaches being used to derive pluripotent stem cells and discuss their potential use for regenerative medicine in the horse.

***Review*:** Adult adipose- and bone marrow-derived mesenchymal stem cells (MSC) are being used in practice to treat injuries in horses. However, there is scarce scientific evidence of their effectiveness and little is known of the mechanisms by which such cell preparations improve the healing process. For instance, although early healing response of articular cartilage injury was improved by treatement with injection of MSC, they did not enhance the long-term tissue response, indicating that cell proliferation was attenuated. Better protocols for the isolation and clinical testing of equine MSC are required to confirm healing properties. In contrast to MSC, embryonic stem cells (ESC) derived from the inner-cell- mass (ICM) of blastocyst stage embryos carry the ability to proliferate indefinitely *in vitro* and, given appropriate and favorable conditions, can differentiate into any tissue in the body. Parthenogenesis (PG) and somatic cell nuclear transfer (SCNT) are used to obtain a genetic match to the host animal and, thereby, eliminate the risk of inducing immune rejection of the grafted tissue. However, apart from the typical markers of pluripotency, equine ESC also express markers of trophoblastic tissues, indicating that they are different and possibly less able to differentiate than the ESC lines obtained in other species. Consequently, further studies are underway to identify conditions to obtain fully pluripotent ESC lines from equine SCNT embryos. To overcome the limitations of ESC lines derived from equine embryos, induced pluripotent stem cells (iPSC) were derived using a *piggyBac* transposon-based method to deliver transgenes containing the reprogramming factors Oct4, Sox2, Klf4 and c-Myc, expressed in a temporally controlled fashion. Our established fetal-derived iPSC lines express hallmark pluripotency markers, display a stable karyotype after prolonged culture, and are able to form teratomas in immunodeficient mice containing tissues from all three embryonic layers. By establishing a protocol for deriving stable iPSC lines in the horse, we expect that new opportunities will be shortly developed for regenerative therapies in this species.

***Conclusion*:** It is possible to derive autologous pluripotent stem cells in horses by using both ESC and iPSC-derived approaches. Although ESC lines are generally the gold standard of pluripotency, further research is required to improve the proliferative and pluripotency characteristics for clinical applications. On the other hand, equine iPSC show excellent stability during prolonged *in vitro* culture and have the capacity to differentiate into the three germ layers *in vivo*, suggesting that they could soon be used in pre-clinical trials. Therefore, further studies need to be performed to establish reliable protocols for assessing the regenerative properties of iPS and ESC for equine muscle-skeletal injuries.

Keywords: Pluripotent, Stem Cells, ESC, iPSC, MSC, Regeneration, Horse.

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I. INTRODUCTION

Regenerative medicine is an emerging field of medicine focused on repairing and replacing damaged cells and tissues either by tissue engineering (producing organs *in vitro* and implanting them in a living organism), or by cell therapy (injecting undifferentiated cells to help stimulate restoration of diseased organs). In the last 20 years the new science consisting of molecular imaging and biotechnology have led to an explosion in the knowledge of the biological processes of the human body. The field of regenerative medicine has grown tremendously. Often, this involves harnessing the properties of stem cells, which are capable of self-renewal and differentiation into many other cell types. Stem cell research provides the basis for the development of future medical procedures in a broad range of human diseases enabling the regeneration of many tissues and organs, including muscles, bone, heart and nerve.

When compared to human applications, the progress of regenerative medicine in veterinary medicine is in its infancy. For instance, one of the chief current therapies of human stem cell based regenerative medicine is to treat leukemia and other types of blood related cancer, requiring myeloablative chemotherapy followed by hematopoietic stem cell induced recovery of the immune system [26]. As mentioned above, these applications rarely apply to animals and they usually remain untreated for the sake of the animal's welfare, as well as monetary reasons. However, being a relatively young and emerging field, stem cell research for human and veterinary medicine remain fundamentally attached to one another. For instance, animal models are used to study the properties and potential of stem cells for future human medicine therapies. Moreover, various stem cell treatments for animal patients are currently being developed and some, like the treatment of equine tendinopathies with mesenchymal stem cells (MSC), have successfully entered the market for this purpose [32].

Stem cells are generically defined as undifferentiated cells that are capable of self-renewal through replication as well as differentiation into specific cell lineages from at least one of the three germ layers [36]. Depending on the developmental stage and tissue from which they are obtained, they can be classified as embryonic, extra-embryonic or adult. There are three measures of potency used to describe levels of plasticity associated with the various kinds of stem cells. Totipotency is used for cells that can form all cells or tissues that contribute to the formation of an organism (ex: the fertilized egg or zygote). Pluripotency is for cells that can differentiate into most but not all cells lines of an organism (ex: embryonic stem cells). Lastly, multipotency can form a small number of cells/tissues that are usually restricted to a particular germ layer (e.g. hematopoietic or mesenchymal stem cells) [29].

Many sources of stem cells exist and when choosing the appropriate one for the effective, stable and long-lasting repair of damaged tissue, a few common criteria should be considered. First a sufficient number of cells must be generated in order to fulfill the treatment. Such cells must also be capable of differentiation towards the right phenotype, and remain in that state. Also, they should be structurally and mechanically compliant with the native tissue and successfully avoid immunological rejection. Finally, they should adopt the appropriate cellular organization with extracellular matrix production, with or without the presence of structural support, and be able to integrate completely with the damaged tissue.

Adult-derived stem cells are somewhat easily accessible, are found in various tissues of the living organism, and when used autologously do not require treatment to lower the risks of graft rejection. However, MSC have a low proliferative potential, making the production of a large number of cells difficult, and their differentiation is often restricted to a specific cell lineage. Embryonic stem (ES) cells, on the other hand, have unlimited self-renewing abilities as well as multilineage differentiation potential but their derivation is more complicated, requiring the production and destruction of embryos. Their clinical use is also risky since high plasticity may lead to

uncontrolled teratoma formation. Currently, adult-derived stem cells are the most commonly used cells in the clinical field, and scientists are still conducting experimental transplantation therapies in animal models to assess the safety and long-term stable functioning of transplanted cells.

Horses are pioneering the application of regenerative medicine in several veterinary fields. Not only do horses hold enormous potential as a model for a various of medical conditions found in humans, such as injuries or diseases related to muscles, tendons, ligaments and joints, they also represent substantial commercial value in sport and recreational fields. One of the most common injuries in these large animals involves the musculoskeletal system causing serious consequences due to poor response to standard treatment used successfully in other species. In the case of bone fracture, casting and long-term immobilization is either impossible or accompanied by high risks of devastating secondary complications, such as damaged cartilage, tendons and ligaments that have a low capacity to heal. Similar complications are commonly observed in other species, including humans. Successful grafting therapies have recently been developed in the horse using autologous MSCs [10]. Although equine MSCs show improvement in the early healing response of articular cartilage lesions, they do not enhance long-term tissue repair and clinical treatments have yet to do so.

The objective of this review is to highlight our current understanding of stem cell biology, with particular emphasis on equine studies, by comparing the various sources of stem cells (Figure 1). Current and potential applications of equine regenerative medicine will also be reviewed.

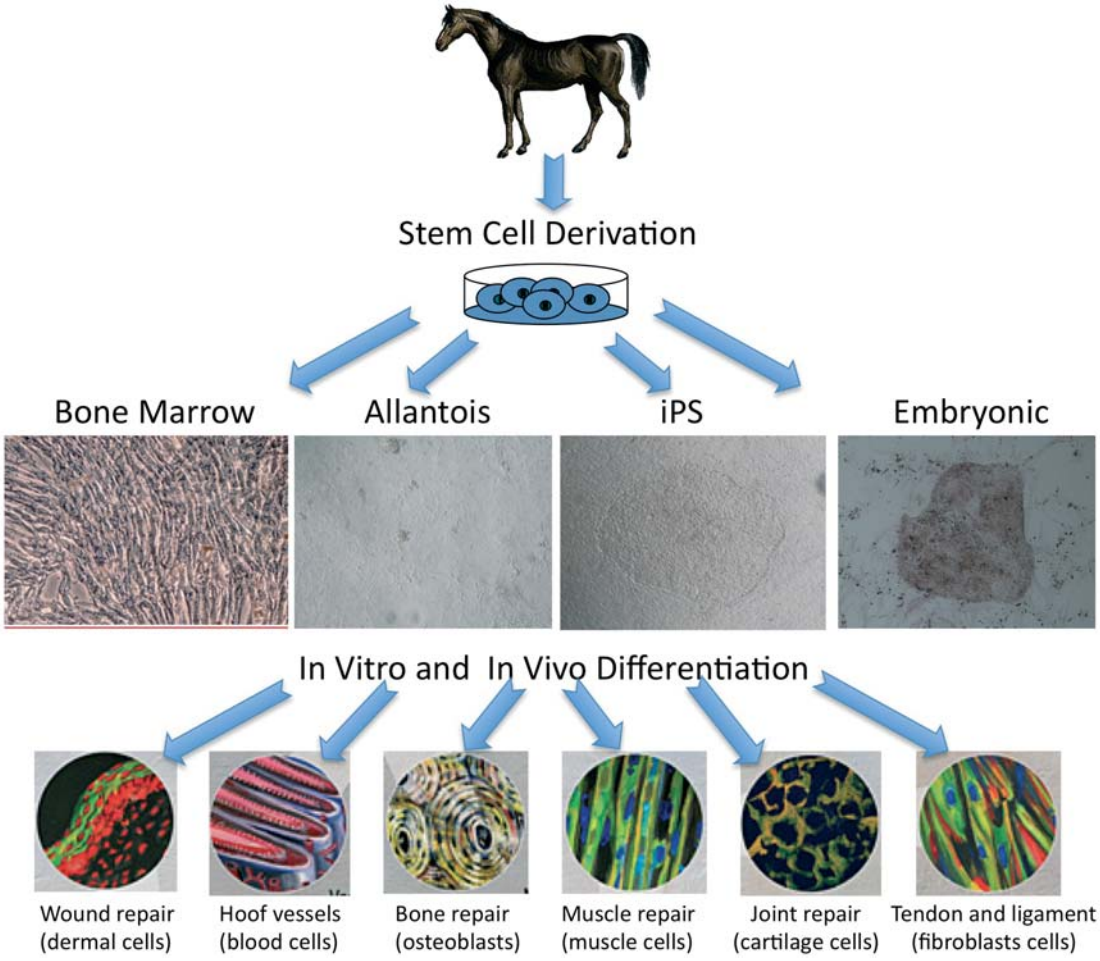

Figure 1. Overview of the pluripotent stem cells used in equine regenerative medicine. Examples of adult (bone marrow), extra-embryonic (allantois), embryonic (inner cell mass-derived) and induced pluripotent stem (iPS) cells show specific morphology *in vitro* and have multilineage differentiation potential for healing several injuries in horses.

II. ADULT STEM CELLS

Mesenchymal stem cells (MSCs) are an adult-derived stem cell population that can be isolated from multiple body tissues. These multipotent cells have the capacity to differentiate into lineages of mesenchymal origins, including osteoblasts (bone), chondrocytes (cartilage) and adipocytes (adipose tissue) [34]. Some prefer to refer to MSCs as multipotent stromal cells or mesenchymal progenitor cells, observing that the term "stem" might attribute more biological properties than the MSCs actually hold [9]. Only cells that have shown self-renewal ability, in-vivo long-term survival, and tissue repopulation with multilineage differentiation should be identified as MSCs [16].

Equine MSCs are of particular interest both for basic research and for the therapeutic approach to musculoskeletal diseases in the horse. Their multilineage differentiation potential gives them the capability to contribute to the repair of tendon, ligament and bone damage. Yet, enthusiasm for the use of MSCs for therapeutic use is tempered by their age-dependent decline in absolute numbers and the invasive nature of their harvest [28].

In humans, adult MSCs have been detected in various tissues such as the dermis, blood, muscle and the trabecular bone [35]. The cell population from some of these sources may have a more limited capacity for differentiation, containing monopotent or bipotent cells that have differentiation potentials developmentally adapted and restricted to the tissues in which they were found. This could lead to issues involving ease of isolation, cell yield, and donor site complications, suggesting that certain sources may be more suitable than others [3].

In equine medicine, which centers generally on musculoskeletal repair, the bone marrow is the most common source for isolation of multipotent MSCs and adipose tissues as well, due to the low morbidity associated with their harvest and their renewable nature. Herein we compare the advances made in both domains.

2.1 Adult adipose tissue-derived stem cells

Adult adipose tissue (AT) originates from the embryonic mesenchyme and consists mainly of adipocytes and a supportive stroma, composed of fibroblast-like precursor cells known as preadipocytes [5]. The latter were initially believed only be able to differentiate into cells of its tissue of origin, but recent studies have shown that these stromal cells are actually capable of differentiation into multiple other cell-lines [41]. This supportive stroma represents an important source of adult MSCs since its cells can be easily isolated in large quantities. AT-MSC can be isolated from its tissue when digested in collagenase type I [7], expanded *in vitro* and then inoculated into the damaged tissue.

There are many advantages to using adipose tissue as a source of MSCs in equine regenerative medicine. First, the presence of adipose tissue in horses is quite substantial and its harvest is much simpler and less prone to complications than bone marrow extraction [10]. Most horses have enough fat around their tail head to obtain the required amount for stem cell injection into their damaged tissue. The adipose tissue is collected either under sedation and local anesthesia or under a quick general anesthesia. In humans it has been reported that AT-MSCs can be quickly isolated from adipose tissue and the resulting stromal vascular cell fraction (SVF) contains a greater proportion of stromal/stem cells per unit volume in comparison to bone marrow [14]. In horses, it has been shown that for the same quantity of tissue sample, the total quantity of AT-MSCs attained after 21 days in culture is significantly larger that for bone marrow MSCs [36]. This represents an important advantage since tissue lesions in horses will usually require a large quantity of cells to ensure successful and long lasting therapeutic repair. Adipose tissue seems to be an accessible and abundant source of adult derived stem cells.

Some disadvantages of AT-MSCs are the slightly lower osteogenic capability than that of BM-MSCs, the non-sterile conditions and risk of pathogen agents at the collection site and the difficulty to obtain fat from highly fit athletes [3]. Also, although the use of autologous AT-MSCs allows a lower risk of immunosuppression, it also requires a longer wait before treatment, due to time period required for tissue collection, stem cell isolation, culture and characterization. Although there is a higher possibility of rejection and risk of disease transmission, allogenic AT-MSCs present certain advantages as well for

allowing rapid treatment to injured horses using cells with optimal proliferation and differentiation potential. Moreover, the treated animal will also be exempt of the anesthesia and surgery required for AT-MSC harvesting [6].

2.2 Bone marrow-derived stem cells

The bone marrow (BM) stroma is formed of hematopoietic, for the most part, and mesenchymal multipotent stem cells. It has been reported that MSCs populate 0.001 to 0.1% of the total population of the human bone marrow [26], and a similar assumption may be valid for the equine species. Currently, equine BM-MSC are harvested from the horse's sternum and isolated by Percoll density gradient separation [2] followed by *in vitro* proliferation and characterization by adipogenic, osteogenic, and chondrogenic *in vitro* differentiation analysis [1] and finally grafted in the damaged tissue. Since the preparations of the BM-MSCs takes 2-4 weeks, the wounded animals' bone marrow is aspirated as promptly as possible. Optimally, stem cell clinical implantation is therefore performed within one to two months of the injury. The cells are supported by the granulation bed formed and strategy avoids substantial fibrosis of the site.

BM-MSCs are the most commonly chosen source of stem cells because of their easy accessibility and for their capacity to produce large numbers of MSCs. Much like AT-MSCs, an important advantage linked to BM-MSCs is that when recovered from the injured animal itself they avoid the risks of immune rejection. Osteogenic gene expression and mineral deposition of the BM-MSCs, before and after induction with osteogenic culture conditions, show that the bone marrow contains the largest quantity of osteoprogenitors and these cells possess the highest osteogenic potential *in vitro* [3].

The presence of these precursor cells, however, show a lower cell plasticity for the BM-MSCs, making differentiation into other cell lines more difficult. Also, the production of large numbers of autologous BM-MSCs is lengthy and costly, requiring bone marrow extraction, MSC isolation and expansion, testing for contaminants and, finally, transplantation. The bone marrow collection method commonly used exposes the horses to possible complications such as pneumothorax and pneumopericadium. [10]

Currently the most utilized source of stem cells for clinical purposes, BM-MSCs are used to treat bone, ligament, tendon and cartilage lesions. More specifically, BM-MSCs have been reported to successfully repair superficial digital flexor tendon lesions as well as soft palate defects in horses [32]. Equine MSCs improve the early healing response in articular cartilage lesions, but long-term tissue repair not does seem to be enhanced.

III. EXTRA-EMBRYONIC STEM CELLS

In addition to stem cells derived from adult tissues, stem cells can be found in extra-embryonic tissues including umbilical cord blood, umbilical cord matrix, and amnioticuid. Stem cells from extra-embryonic sources have the advantages of being obtained by non-invasive procedures and allowing treatment with low immunogenicity. The three sources of extra-embryonic tissue are harvested immediately after foaling and stored frozen for potential future transplantation; thereby enabling the horse to have a supply of autologous stem cells in case of future injuries or disease.

3.1 Umbilical cord blood

A few ml of cord blood can be collected from intact umbilicus at birth without complication to the foal or the mare. The umbilical cord blood (UCB) stem cells have a fibroblast-like morphology and have been found to express stem cell markers such as Oct-4, Tra1-60 and Tra1-81 and SSEA-1 [28]. An important factor is that the stem cells isolated from UCB have better proliferative and plasticity potency than that of adult derived MSCs, but not as good as embryonic stem cells, suggesting UCB-MSCs are the primitive cell type of the two. Adult-derived MSCs show better differentiation capacity when limited to their tissue of origin cell line. Predominance for the chondrogenic and osteogenic pathways is observed for BM-MSCs and the adipogenic pathway for AT-MSCs. However, once in tissue-specific culture with presence of growth factors, UCB stem cells are capable of differentiation towards osteogenic, chondrogenic and adipogenic pathways as well as cell types of hepatocytes and endodermal origin [3]. Equine UCB-MSCs have superior immune tolerance, proliferative potential and less senescence occurrence in later passages than other adult-derived equine MSCs [19].

3.2 Umbilical cord matrix

The umbilical cord matrix (UCM) is a gelatinous connective tissue composed of myo?broblast-like stromal cells, collagen ?bers, and proteoglycans [18]. It is reported to be rich in young MSCs with high proliferation ability. The UCM are harvested identically as UCB, except that it is the blood vessel free umbilical cord tissue that is collected. UCM stem cells are isolated by collagenase digestion of the cord tissue and expanded in culture until an abundant quantity of cells becomes available for characterization and di?erentiation procedures [17]. Much like UCB stem cells, UCM stem cells have the capacity to differentiate into the three major mesenchymal cell lineages (bone, cartilage and fat). They also express the embryonic markers Oct-4 and SSEA-4 and can adopt neuron-like morphology, implying they are also situated between adult-derived MSCs and embryonic stem cells in pluripotency [25]. These characteristics play an important role in successful cell-based therapies in the horse. One downside for both UCB and UBM MSCs is the lack of sterilization of the tissue and the environment during the harvest, causing higher risks of contamination. In cell culture, the presence of larger amounts of antibiotics is a means to reduce this risks in clinical experiments.

Experiments with equine amniotic membranes showed possible presence of stem-like cells, due to pluripotency marker expression and osteogenic differenciation potential, giving rise to a plausible new source of MSCs. However, immunohistochemical studies, preclinical experimentation, and immunological evaluation must be performed before more can be said about this potential new source of pluripotent stem cells [20].

IV. EMBRYO-DERIVED STEM CELLS

Before implantation in the uterus, embryos are at the blastocyst stage and are composed of an outer layer of cells called the trophectoderm, a fluid-filled cavity named the blastocoele and finally the inner cell mass (ICM), also called epiblast. The trophectoderm cells contribute to the placental chorion, whereas the ICM contains pluripotent embryonic stem (ES) cells that possess the ability to develop into any cell type of the organism [4]. It was found that, when cultured in a media containing leukemia inhibitory factor (LIF) or in presence of embryonic fibroblast as feeder cells, ES cells proliferate, replicate and can be maintained in an undifferentiated pluripotent state providing a potentially unlimited source of stem cells. When withdrawn from these culture conditions, ES cells will spontaneously differentiate into cells of the three germ layers; ectoderm, mesoderm and endoderm [31]. It is thus possible to control the differentiation pathway taken by the stem cells by adjusting the culture conditions and generating tissue-specific precursors [38]. Unlike adult or extra-embryonic derived MSCs that reach senescence after a certain number of passages, the ES cells ability to remain pluripotent throughout extended culture periods is an important advantage in choosing blastocyst derived stem cells for therapeutic and research applications. In contrast, adult stem cells replicative life span is influenced by the cell type, donor age and donor species [12].

Equine ES cells could represent a significant source of stem cells in the field of regenerative medicine. Using the somatic cell nuclear transfer (SCNT), an enucleated oocyte can be reconstructed with any cell provided by the injured animal to produce an embryo. The oocyte's cytoplasm is capable of reprogramming the donor cell's nucleus to re-establish embryonic gene expression patterns. Nuclear transfer embryonic stem (NTES) cells can then be isolated from the inner cell mass (ICM) once the embryo has developed to the blastocyst stage [8][22]. Such NTES cell line differentiation would then be induced to produce the desired cell type for transplantation in the injured horse (Fig. 2). These autologous cells, except for the mtDNA inherited from the oocyte, would avoid the risk of immune rejection often caused by use of allogenic MSCs. ES cells also have a higher plasticity than MSCs, which could allow better long-term repair in damaged tissues.

Unfortunately, a large number of oocytes is necessary for equine NTES cell production, since both SCNT and NTES cell line isolation have low efficiencies. Horses are seasonal breeders and single ovulatory species and slaughterhouses are scarce, making the harvest of multiple oocytes complicated [13]. Another potential obstacle when using ES cells for therapeutic treatments is their potential for uncontrolled proliferation and predisposition towards teratoma formation *in vivo*.

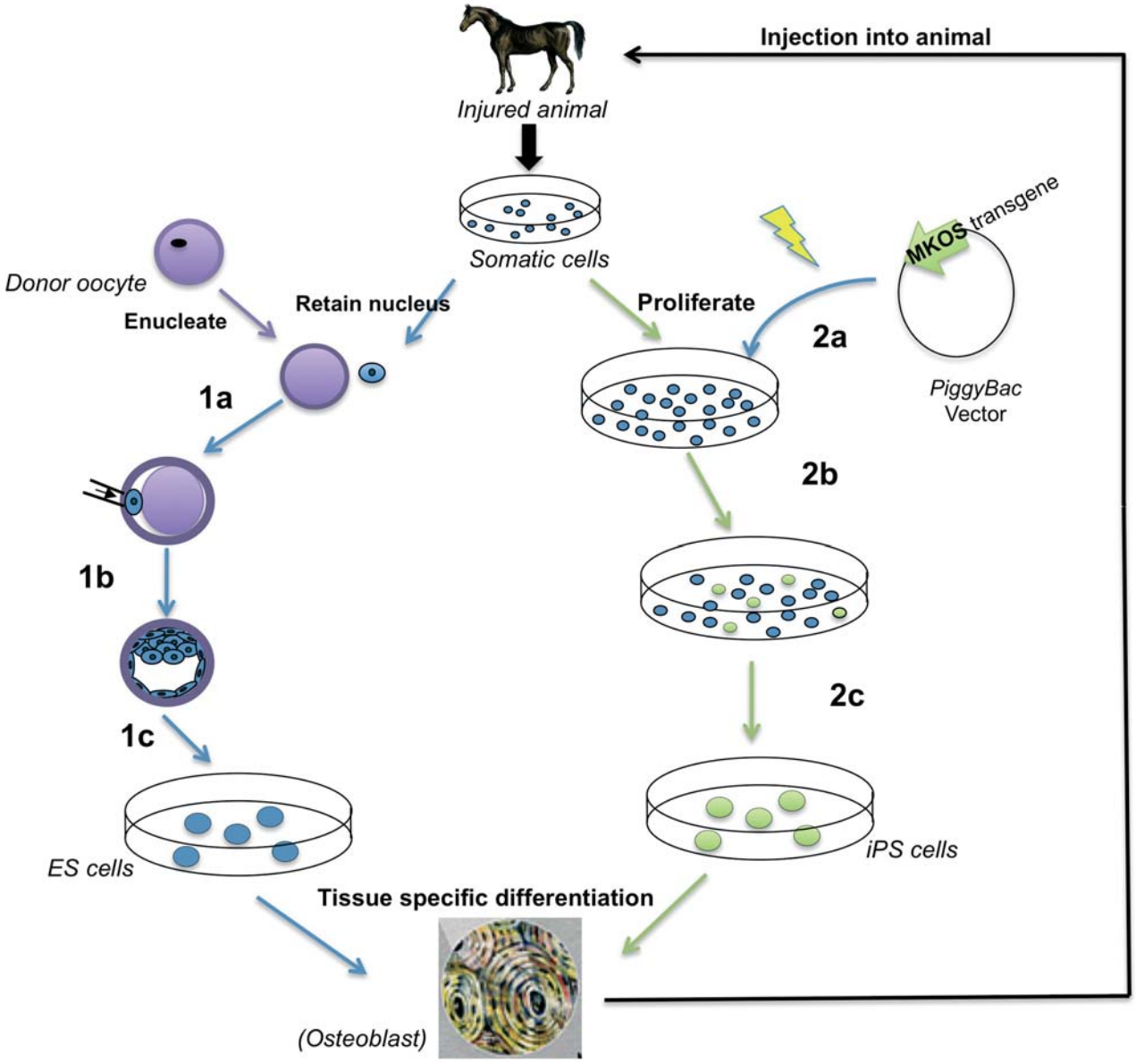

Figure 2. Comparison of embryonic stem (ES) and induced pluripotent stem (iPS) cell production for regenerative medicine in horses. Somatic cells harvested from an injured horse are fused to an enucleated oocyte (1a) and activated to develop to the blastocyst stage (1b). The inner cell mass (ICM) is isolated to form immune-compatible ES cells (1c). In contrast, to produce iPS cells fibroblasts are transfected (2a) with a *PiggyBac* transposon that contains the reprogramming factors c-Myc, Klf-4, Oct-4 and Sox-2 that proliferate (2b) and eventually become iPS colonies (2c). The pluripotent and autologous nature of ES and iPS cells enable tissue specific differentiation and grafting to heal the original injured horse.

However, unlike mouse and human ES derivation, previous attempts to derive equine ES line have been unable to confirm true pluripotency. The first ES-like cells produced, using *in vivo* derived equine embryos, expressed a few characteristic murine and human pluripotency cell markers, and were capable of *in vitro* differentiation *in vitro* into hematopoietic and neural precursor cells [30]. Another study showed the successful spontaneous *in vitro* differentiation of ES-like cells into the three germ layer once removed from the feeder layer [21]. Parthenogenic-derived equine embryos have been successfully used to obtain ES-like lines, indicating that *in vitro* culture conditions are not detrimental to the quality of the ICM cells utilized for ES cell line derivation [8]. These ES-like cells could not, however, form teratomas when injected *in vivo*, nor produce chimeric horses, two important ES cell line chara-

cterization tests. On the other hand, this inability to form teratomas may be advantageous for therapeutic use by lowering the risk of uncontrolled differentiation once the ES-like cells are injected in the horse's injury site. There has yet to be a clinical experiment done on horses to see if these cells would help regenerate cells on a damaged tissue without tumor formation.

V. INDUCED REPROGRAMMING OF ADULT CELLS

A landmark in stem cell research was the establishment of a protocol to reprogram differentiated cells back to their initial stage of pluripotency. Although it was possible to reprogram differentiated cells to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with ES cells, little was known about the factors that induce reprogramming (Figure 2). Induced pluripotent stem (iPS) cells were generated by the expression of a set of typical stem cell transcription factors (Oct4, Sox2, Klf4, c-Myc, NANOG and Lin28) in adult somatic cells [34]. These iPS cells show great potential for regenerative therapy as they can differentiate into all three embryonic germ layers and can be expanded to large quantities *in vitro*. Apart from murine rodents, iPS cells have been produced in a few species such as humans [40] and porcine [39] and most recently in equine [24]. The later was achieved by incorporating via *piggyBac* transposon-based method that, once the transposon electroporated into the equine fetal fibroblast, delivers transient transgenes containing the reprogramming factors Oct4, Sox2, Klf4 and c-Myc. The ectopic expression of these factors is doxycycline-dependant and the presence of this antibiotic in the culture media initiates the somatic cells' reprogramming. Equine iPS cells show a stable karyotype after extended culture, express pluripotent properties, and are able to form teratomas composed of all three embryonic germ layers in immunodeficient mice [24]. Once iPS cells are confirmed to be safe for therapeutic application, they could have a major impact in regenerative medicine especially since they can be produced for specific patients without raising the ethical issue that ES cells tend to.

This new discovery represents a new way of approaching musculoskeletal injury treatment in horses, providing stem cells with control over the expression of the pluripotent factors. Injecting these cells in the injury site of a horse being treated with doxycyclin allows the cells to proliferate and expand in the damaged tissue. Once the animal's doxycyclin treatment is withdrawn, the iPS cells will differentiate into the intended tissue that requires repair. Cell specification could either happen naturally, because of the tissue surrounding factors inducing the right differentiation, or by outside influence via incorporation of specific factors for the iPS cells. This control over the expression of the pluripotency markers could also reduce the chances of unwanted teratoma formation. iPS cells could represent a better source for long-term tissue repair because of its higher pluripotent state than MSCs and, for equine, ES cells. Their capacity to expand in large quantities *in vitro* is an advantage towards ES cells, and their painless and easy accessibility, requires only a skin biopsy, is more appealing than the methods used for adult MSC harvest.

To date only fetal fibroblasts have successfully been used to derive equine iPS, which is somewhat disadvantageous for clinical applications. Allogenic iPS cells could have a higher risk of rejection if implanted in an injured horse. Ideally, one would use the injured animal's own adult fibroblast to produce and proliferate iPS cells that would then be transplanted to the damaged tissue. However, adult cells might be a bigger challenge to derive, their reprogramming efficiency being affected by age, origin and cell type [15], which will most likely also have an impact on the resulting iPS cell quality.

VI. CONCLUSION

Presently, equine MSCs are more commonly used in regenerative therapies due to their autologous and tissue specific differentiation properties. For musculoskeletal damages, bone marrow MSCs seem to be the most effective cells to improve early healing response, but their low plasticity hinders long-term tissue restoration, which can lead to facilitated re-injuries. Extra-embryonic stem cells could be a valid option of stem cell source because of their wider plasticity and their less advanced cell state, compared to adult-derived stem cells. Studies have recently shown that UCB-MSC transplantation into fractured mouse femurs allowed the accelerated the repair of the tissues and bone substitution [23]. However, no

clinical applications have yet been conducted on the horse.

It is also possible to derive autologous pluripotent stem cells in horses by using both ES and iPS cell-derived approaches. Although ES cell lines are generally the main source of stem cells used for regenerative medicine in other species, due to its pluripotency and easy proliferation, a real equine ES cell line has yet to be derived. The risk of uncontrollable division once implanted in the animal is also an important factor against ES cells for clinical applications. Alternatively, equine iPS cells have effectively been produced and show excellent stability during prolonged *in vitro* culture. They additionally have the ability to differentiate into the three germ layers *in vivo*, suggesting that they could soon be used in pre-clinical trials. Since they are antibiotic dependent, it is possible that their proliferative power could be controlled and unwanted tumor formation avoided. Although further studies need to be performed to assess their regenerative properties, iPS cells will most possibly become one of the most important sources of stem cells for future clinical applications.

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