Review Article

Current Status of Canine Umbilical Cord Blood-Derived Mesenchymal Stem Cells in Veterinary Medicine

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Stem cell therapy has prompted the expansion of veterinary medicine both experimentally and clinically, with the potential to contribute to contemporary treatment strategies for various diseases and conditions for which limited or no therapeutic options are presently available. Although the application of various types of stem cells, such as bone marrow-derived mesenchymal stem cells (BM-MSCs), adipose tissue-derived mesenchymal stem cells (AT-MSCs), and umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs), has promising potential to improve the health of different species, it is crucial that the benefits and drawbacks are completely evaluated before use. Umbilical cord blood (UCB) is a rich source of stem cells; nonetheless, isolation of mesenchymal stem cells (MSCs) from UCB presents technical challenges. Although MSCs have been isolated from UCB of diverse species such as human, equine, sheep, goat, and canine, there are inherent limitations of using UCB from these species for the expansion of MSCs. In this review, we investigated canine UCB (cUCB) and compared it with UCB from other species by reviewing recent articles published from February 2003 to June 2017 to gain an understanding of the limitations of cUCB in the acquisition of MSCs and to determine other suitable sources for the isolation of MSCs from canine. Our review indicates that cUCB is not an ideal source of MSCs because of insufficient volume and ethical issues. However, canine reproductive organs discarded during neutering may help broaden our understanding of effective isolation of MSCs. We recommend exploring canine reproductive and adipose tissue rather than UCB to fulfill the current need in veterinary medicine for the well-designed and ethically approved source of MSCs.

1. Introduction

In the last 20 years, stem cells have received ample attention from researchers in both human and veterinary medicine for their functional characteristics and therapeutic potential in different applications [1–4]. The number of animals previously treated in veterinary medicine provides a consequential basis for estimating the effectiveness of stem cell therapy in the treatment of different diseases [5, 6]. Nearly all types of animal tissues can be repaired or regenerated by the explicit action of stem cells [7], which exhibit high potential for propagation and differentiation [8]. Moreover, animal models are extensively used to examine the properties and promising potential of stem cells for reasonable application in human medicine in the future. Consequently, human and veterinary medicine are intertwined in the emerging field of stem cell research. Pioneering innovations in stem cell research have been accomplished by the collaboration of clinical and veterinary scientists.

For instance, adult stem cells isolated from various sources, mainly bone marrow- (BM-) and adipose tissue- (AT-) derived stem cells, have been widely used for the treatment of different animal diseases [9, 10]. As in human medicine, adult mesenchymal stem cells (MSCs) play an
important role in veterinary medicine for the treatment of acute injury and chronic disorders. In brief, MSCs, also known as marrow stromal cells [11] or mesenchymal progenitor cells, are considered the most heavily utilized stem cells in the field of regenerative medicine and tissue engineering [12, 13] to overcome the complications and limitations of gene-based therapies. Currently, MSCs are used in clinical cell therapies and trials in many countries [14] for their in vitro expansion, notable multilineage differentiation potential [15, 16], capability to treat tissue injury [17, 18], viability after long-term storage by cryopreservation [19], support of hematopoietic stem cell (HSC) expansion as feeder cells [20], and immunomodulatory properties [21, 22]. These extensively applied cells were first depicted by Friedenstein et al. as a cell population analogous to fibroblasts [23]. They have the potential to differentiate into numerous cell types such as osteoblasts, adipocytes, cardiomyocytes, chondrocytes, hepatocytes, and brain cells [24–35]. These cells can be isolated from BM, AT, peripheral blood, skeletal muscle, connective tissue of the dermis, and Wharton’s jelly (WJ) as well as umbilical cord blood (UCB) [30, 36–39].

Although BM represents an abundant source of MSCs [33, 40] in the field of tissue engineering and cell-based therapy, harvesting of cells is invasive with a stringent donor age requirement and increased donor site morbidity [41–46]. Therefore, UCB has been identified as an ideal alternative source in terms of ease of accessibility as well as reduced morbidity. UCB carries a large number of MSCs per volume, which are more flexible and pluripotent than bone marrow-derived mesenchymal stem cells (BM-MSCs) [38, 47]. Additionally, it has been proposed that umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) are not as mature as other stem cells and may not induce alloreactive responses that harmonize the immune system [32, 48, 49] and have the lower carcinogenic potential [50].

Nevertheless, although the presence of HSCs and their isolation from UCB are well established [51–54], the statistics concerning the existence of UCB-MSCs are contentious and require further evaluation. Earlier experiments to isolate UCB-MSCs from different species have either been aborted, have been time-consuming and onerous [55–57], or have been only 30–60% effective under suitable conditions [38, 39, 58–63].

UCB-MSCs can be isolated from different species such as human [37, 58, 59, 64–74], sheep [75, 76], equine [77–79], canine [80–84], and goat [85]. Human UCB-MSCs (hUCB-MSCs) are collected noninvasively at the time of delivery, whereas sheep UCB is collected intrusively by the surgical intrauterine approach [75, 76]. Equine UCB represents a noninvasively and nontraumatically retrieved source of stem cells, with potentially excellent cellular characteristics including proliferation capacity, immune tolerance, and differentiation potency [78]. It is reported that UCB from more than 100 mares and foals has been collected safely and efficaciously. The enrichment rate of MSCs from equine UCB is very high compared with that of other species [86].

Canine and feline are mainly considered companion animals rather than laboratory animals. Generally, pet owners prefer their pet (canine and feline) to be neutered (spayed or castrated) for the purpose of sterilization. After spay, canine UCB (cUCB) is impossible to collect, as the females lose their reproductive capability. In this view, cUCB raises the problem of limited availability. Yet, there are a few studies on the successful isolation and application of canine UCB-MSCs (cUCB-MSCs).

Even though UCB-MSCs are the latest tool and have already been commercialized for human medicine, the progress of UCB-MSC research in veterinary medicine is at a standstill. Therefore, veterinary medicine must overcome the major challenges of UCB-MSCs. We know that cUCB-MSCs have a great impact on veterinary medicine. Thus, cUCB-MSCs should be further explored to boost the supply thereof. In this respect, this is the first review that highlights the limitations of cUCB for the isolation of MSCs and suggests another significant source of MSCs in canine.

2. Umbilical Cord Blood and Mesenchymal Stem Cells

The umbilical cord, also known as the navel-string or birth cord, is the channel between the growing placenta and fetus. In the course of prenatal development, the umbilical cord is a physiological and inherent part of the fetus. Typically, the umbilical cord consists of two arteries (the umbilical arteries) as well as one vein (the umbilical vein) buried in WJ, a gelatinous element composed largely of mucopolysaccharides. The two umbilical arteries transfer deoxygenated, nutrient-diminished blood away, whereas the umbilical vein carries oxygenated, nutrient-boosted blood to the fetus. The umbilical cord is covered by an epithelium obtained from the enveloping amnion.

UCB has been utilized as a rich and readily available alternative source of primitive and unspecialized stem cells since 1988 [87]. The blood remaining in the umbilical vein after birth is an abundant source of hematopoietic stem and progenitor cells. This criterion makes UCB an allogenic donor source that can be applied in a variety of hematologic, pediatric, genetic, oncologic, and immunologic disorders [53, 88–90]. Fresh UCB is also an auspicious source of non-HSCs such as MSCs, endothelial cells, and unrestricted somatic stem cells [30, 91–93]. Although several previously published articles reported the identification of MSCs in UCB [38, 39], some authors debated the existence of MSCs in UCB and declared that only HSCs are present therein [56, 94]. The origin of MSCs in UCB is unrecognized, but it is possible that the cells are discharged from the fetal liver or bone marrow into the fetal circulation [65]. Presently, the isolation of MSCs from UCB has a lower success rate compared with BM-MSCs (63% versus 100%) [67]. AT is another alternative source that can be accessed less invasively and repeatedly with an easy procedure, resulting in larger quantities of fresh MSCs [95].

3. Process of Article Selection

We utilized the search engines PubMed and Google Scholar to gather a list of publications and manuscripts detailing
research, employment, or isolation of MSCs from human and animal subjects from February 2003 to June 2017. For a report to be included in this survey, it must have contained “Umbilical Cord Blood-derived Mesenchymal Stem Cells (UCB-MSCs)” in either the heading or the abstract. The keyword “Umbilical Cord Blood-derived Mesenchymal Stem Cells (UCB-MSCs)” combined with “human,” “equine,” “sheep,” “goat,” and “canine” was used to generate the list. We did not include any review articles in our survey. Highly relevant articles were initially determined by the heading and abstract, followed by a further examination to confirm whether the collection of UCB was from human or animal subjects. We accept that this search technique was not encyclopedic, as there are many more articles in journals that are not included in PubMed or Google Scholar. We evaluated the listed studies by different characteristics such as the weight of the species, final blood volume, and enrichment rate of MSCs. For studies in which UCB-MSCs were isolated from humans, the key areas recognized were ethics (informed consent was obtained), route of delivery, UCB unit and volume, and MSC success rate. For studies in which UCB-MSCs were isolated from animals, the key areas noted were ethics (reporting of omission and approval of the study by the Animal Care and Use Committee), study design (distribution of groups and sample number and volume), route of delivery, and experimental animals (species, breed, and weight).

4. UCB-MSC Articles Entailing Human and Animal Sources

The electronic search singled out 130 articles. A total of 20 articles based on human and animal sources of UCB-MSCs were considered based on the heading, abstract, and content (species, UCB unit and volume, and MSC success rate). Review articles, duplicates, and irrelevant papers were removed. In total, 55% (11/20) of articles reported human subjects, and 45% (9/20) of papers reported animal subjects, such as equine, sheep, goat, and canine (Table 1). Nine articles regarding human subjects stated that UCB was collected with the informed consent of the mothers, while four studies detailing animal subjects received approval from an animal ethics committee (Table 2).

5. Success Rate of MSC Isolation from UCB

Some researchers successfully isolated 33.3%–60% of hUCB-MSCs under different cell culture conditions [69, 71]. Bieback et al. reported that a net volume of more than 33 mL of UCB and a mononucleated cell (MNC) count higher than 1 × 10^8 are difficult to achieve for the isolation of UCB-MSCs from human subjects. However, they were capable of increasing the success rate from 29% to 63% [37]. Jin et al. reported the isolation of 50% of UCB-MSCs from 24 pregnant women [67]. When the blood volume exceeds 90 mL, 90% of MSCs from UCB can be isolated [66, 70]. Liu et al. stated that the efficacy of MSC isolation from UCB can reach 75% (n = 144) [72]. Sibov et al. and Johannes et al. demonstrated that more than 70 mL of UCB is required for the successful isolation of MSCs [70, 73]. On the other hand, Thomas et al. isolated equine UCB-MSCs from a volume of 42 mL at a 100% success rate with PrepaCyte-EQ (PEQ) medium. The same authors isolated MSCs with a success rate of 57% from a volume of 65–250 mL of equine UCB [77, 78]. Different authors have investigated the isolation of MSCs from UCB of goat and sheep (Table 3) [76, 85].

Although Kang et al. and Jang et al. separately continued their experiments with cUCB-MSCs, they did not explicitly provide any information about the blood volume and number of canine subjects they used [80, 83]. Lim et al. successfully isolated MSCs from an exceedingly low UCB volume of 8 mL [84]. As the UCB volume of canine is insufficient to isolate MSCs, another author utilized the blood of canine fetus heart along with UCB [82] (Tables 4 and 5).

### 6. MSCs in Human and Veterinary Medicine

The application of stem cells in cell-based therapies and tissue engineering is increasing to overcome the complications and hurdles of gene therapy. In both animals and human, stem cell implantation can serve as an advanced treatment for some incurable conditions such as bone fracture malignancies [96, 97], spinal cord injuries [98], and genetic disorders [99]. Tables 6 and 7 summarize the application of MSCs in the treatment of a wide range of diseases in preclinical studies of experimental animal models and veterinary clinical studies of animals with naturally occurring diseases. In human medicine, MSC products have already been developed and approved in South Korea. Cartistem® (Medipost), the world’s first allogenic hUCB-MSC drug, has gained popularity in South Korea since January 2012 for the treatment of cartilage injury and osteoarthritis, and efforts are presently being made to broaden its indications for different target diseases. However, the isolation and characterization of stem cells obtained from numerous tissues and sources have led to highly detracting arguments regarding stem cell therapy [100–102].

Stem cell researchers worldwide are endeavoring to acquire the ideal or optimal autologous or allogenic MSCs, not only from human but also from equine, canine, and feline, to treat diseases such as musculoskeletal diseases, degenerative arthritis, atopic dermatitis, myocardial disease, chronic renal failure, and nerve damage [103]. Based on the concept of “One Health,” new synergic therapies such as stem cell therapies for human and veterinary medicine are in increasing demand and developed in collaboration—the time for focus on human and animal health is now [104].

<table>
<thead>
<tr>
<th>Sources of UCB</th>
<th>Reference</th>
<th>Number of articles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>[75–78, 80, 82–85]</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1: Articles included in this survey regarding UCB-MSCs from different species.
MSCs, specifically multipotent stem cells, have the capability to generate adipogenic, chondrogenic, osteogenic, and myogenic as well as endothelial cell lineages [14, 37]. Because of these possibilities, there has been an increased focus on the isolation of MSCs from different sources for transplantation and tissue engineering. MSCs have been isolated from diverse adult-derived sources such as BM, AT, lung, heart, peripheral blood, synovium skeletal muscle, dermis, and dental pulp as well as from fetal or neonatal tissues such as amniotic fluid and membrane, placenta, UCB, cord vein, WJ, and umbilical cord [59, 105–108]. BM is a bountiful source of MSCs; however, AT is a reliable source of MSCs with the best frequency, and UCB seems to be a remarkable alternative source that allows expansion for a greater number of MSCs [59, 64, 67].
Table 6: Preclinical studies with MSCs.

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Cell type</th>
<th>Pathology</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Human AD-MSC</td>
<td>Peripheral nerve injury</td>
<td>Repair of nerve</td>
<td>[119]</td>
</tr>
<tr>
<td>Rat</td>
<td>Human AD-MSC</td>
<td>Cerebral ischemia</td>
<td>Repair of nerve</td>
<td>[120]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Human UCB-MSC</td>
<td>Hind limb ischemia</td>
<td>Repair of artery</td>
<td>[121]</td>
</tr>
<tr>
<td>Rat</td>
<td>Human UCB-MSC</td>
<td>Cavernosal nerve injury</td>
<td>Improved function</td>
<td>[122]</td>
</tr>
<tr>
<td>Rat</td>
<td>Allogenic AD-MSC</td>
<td>Peripheral nerve injury</td>
<td>Repair of nerve</td>
<td>[123]</td>
</tr>
<tr>
<td>Rat</td>
<td>Allogenic BM-MSC</td>
<td>Skin wound</td>
<td>Repair of skin</td>
<td>[124]</td>
</tr>
<tr>
<td>Rat</td>
<td>Autologous BM-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[125]</td>
</tr>
<tr>
<td>Rat</td>
<td>Autologous AD-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[126]</td>
</tr>
<tr>
<td>Rat</td>
<td>Mouse SC-MSC</td>
<td>Erectile dysfunction</td>
<td>Improved function</td>
<td>[127]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Human AD-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[128]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Human UCWJ-MSC</td>
<td>Normal</td>
<td>No immune rejection</td>
<td>[129]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Porcine S-MSC</td>
<td>Osteochondral defect</td>
<td>Immune rejection</td>
<td>[130]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Allogenic S-MSC</td>
<td>Articular cartilage defect</td>
<td>Repair of defect</td>
<td>[131]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Allogenic BM-MSC</td>
<td>Articular cartilage defect</td>
<td>Repair of defect</td>
<td>[132]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Allogenic/autologous BM-MSC</td>
<td>Bone defect</td>
<td>Repair of defect</td>
<td>[133]</td>
</tr>
<tr>
<td>Porcine</td>
<td>Autologous BM-MSC</td>
<td>Articular cartilage defect</td>
<td>Repair of defect</td>
<td>[134]</td>
</tr>
<tr>
<td>Porcine</td>
<td>Mouse BM-CMSC</td>
<td>Vocal fold wound</td>
<td>Repair of vocal fold</td>
<td>[135]</td>
</tr>
<tr>
<td>Porcine</td>
<td>Allogenic S-MSC</td>
<td>Articular cartilage defect</td>
<td>Repair of defect</td>
<td>[136]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic BM-MSC</td>
<td>Bone defect</td>
<td>Repair of defect</td>
<td>[137]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic BM-MSC</td>
<td>Cardiac ischemia</td>
<td>Improved function</td>
<td>[138]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic BM-MSC</td>
<td>Myocardial infarction</td>
<td>Improved function</td>
<td>[139]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic BM-MSC</td>
<td>Skin wound</td>
<td>Repair of skin</td>
<td>[140]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic BM-MSC</td>
<td>Normal</td>
<td>Inflammation, tubercular necrosis, mineralization, and fibrosis of kidney</td>
<td>[141]</td>
</tr>
<tr>
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<td>Allogenic AD-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[80]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic UCB-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[84]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Disc degeneration</td>
<td>Repair of nerve</td>
<td>[142, 143]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Bone defect</td>
<td>Repair of defect</td>
<td>[144]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Static nerve injury</td>
<td>Repair of nerve</td>
<td>[145]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Osteochondral defect</td>
<td>Repair of defect</td>
<td>[146]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Myocardial infarction</td>
<td>Improved function</td>
<td>[147]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Osteonecrosis of the femoral head</td>
<td>Repair of blood vessel</td>
<td>[148]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Oral ulcer</td>
<td>Repair of ulcer</td>
<td>[149]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Diabetes</td>
<td>Improved function</td>
<td>[150]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Vocal fold wound</td>
<td>Repair of vocal fold</td>
<td>[151, 152]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Periodontal defect</td>
<td>Repair of defect</td>
<td>[153]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Periodontal class II furcation defect</td>
<td>Repair of defect</td>
<td>[154]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic/autologous BM-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[155]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous AD-MSC</td>
<td>Spinal cord injury</td>
<td>Repair of nerve</td>
<td>[156]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous MSC</td>
<td>Chondral defect</td>
<td>Repair of defect</td>
<td>[157]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic AD-MSC</td>
<td>Thoracolumbar intervertebral disc disease</td>
<td>Improved clinical sign</td>
<td>[158]</td>
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<tr>
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<td>Allogenic AD-MSC</td>
<td>Superficial digital flexor tendonitis</td>
<td>Repair of tendonitis</td>
<td>[159]</td>
</tr>
<tr>
<td>Equine</td>
<td>Autologous BM-MSC</td>
<td>Superficial digital flexor tendonitis</td>
<td>Repair of tendonitis</td>
<td>[160]</td>
</tr>
<tr>
<td>Equine</td>
<td>Autologous BM-MSC</td>
<td>Osteoarthritis</td>
<td>Repair of osteoarthritis</td>
<td>[161]</td>
</tr>
<tr>
<td>Equine</td>
<td>Allogenic/autologous BM-MSC</td>
<td>Normal</td>
<td>Enhancement of MSC</td>
<td>[162]</td>
</tr>
<tr>
<td>Dolphin</td>
<td>Autologous AD-MSC</td>
<td>Normal</td>
<td>Cell collection success</td>
<td>[163]</td>
</tr>
<tr>
<td>Caprine</td>
<td>Autologous BM-MSC</td>
<td>Osteoarthritis</td>
<td>Repair of osteoarthritis</td>
<td>[164]</td>
</tr>
</tbody>
</table>

AT-MSC: adipose tissue-derived mesenchymal stem cells; UCB-MSC: umbilical cord blood-derived mesenchymal stem cells; BM-MSC: bone marrow-derived mesenchymal stem cells; SC-MSC: skeletal muscle-derived mesenchymal stem cells; S-MSC: synovium-derived mesenchymal stem cells; UCWJ-MSC: umbilical cord Wharton’s jelly-derived mesenchymal stem cells.
8. UCB-MSCs from Human Sources

The frequency of MSCs in hUCB is a point of controversy among researchers [56, 57, 107]. This is attributed to the persistent difficulties in the isolation of UCB-MSCs. Most researchers have adopted suitable conditions from the literature to enrich the recovery of UCB-MSCs, including choosing full-term UCB units, storing for no more than 15 h, and obtaining an MNC count above $1 \times 10^8$ as the selection criterion for UCB units [37]. While Rebelatto et al. declared that sample volume did not correlate with MSC isolation [62], others have suggested that sample volume (minimum 33 mL [37] or 45 mL [109]) is a critical parameter. The enrichment rate of MSCs has varied broadly from 10% to 60% [110–112]. Namely, of 644 UCB units were subjected to MSC isolation in diverse studies, only 167 successful outgrowths have been recorded (26% success rate) [38, 58–60, 62, 113]. A high enrichment rate of 90% MSCs was obtained when the UCB volume was more than 80 mL [66, 70].

Many groups have reported an isolation efficiency of 65% using numerous culture methods such as reduction of monocytes and lymphocytes from MNCs before cell seeding, development of cells under hypoxia, and the inclusion of cytokines, platelet lysate, or medium supplements [64, 66, 114, 115]. Other published studies accomplished isolation of MSCs from human UCB with up to 40–90% efficiency by adopting different parameters [20, 37, 65–67, 69, 72]. Table 8 represents the yield rate of UCB-MSCs isolated from human and animal sources fulfilling special criteria.

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Cell type</th>
<th>Pathology</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Gastrocnemius tendon injury</td>
<td>Repair of tendon</td>
<td>[165]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous BM-MSC</td>
<td>Chronic Chagas cardiomyopathy</td>
<td>Improved function</td>
<td>[166]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous AD-MSC</td>
<td>Chronic anconitis</td>
<td>Repair of anconitis</td>
<td>[95]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous AD-MSC</td>
<td>Arthritis, patella luxation</td>
<td>Repair of arthritis</td>
<td>[167]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous AD-MSC</td>
<td>Chronic arthritis of hip joint</td>
<td>Repair of arthritis</td>
<td>[168]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous AD-MSC</td>
<td>Atopic dermatitis</td>
<td>Repair of dermatitis</td>
<td>[169]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic AD-MSC</td>
<td>Lumber herniated intervertebral disc</td>
<td>Repair of nerve</td>
<td>[170]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic AD-MSC</td>
<td>Hip dysplasia</td>
<td>Improved function</td>
<td>[9]</td>
</tr>
<tr>
<td>Canine</td>
<td>Allogenic AD-MSC</td>
<td>Chronic osteoarthritis related to hip dysplasia</td>
<td>Reduced lameness</td>
<td>[171]</td>
</tr>
<tr>
<td>Canine</td>
<td>Autologous AD-MSC</td>
<td>Severe osteoarthritis</td>
<td>Reduced lameness</td>
<td>[172]</td>
</tr>
<tr>
<td>Canine</td>
<td>Human ESC-MSC</td>
<td>Anal furunculosis</td>
<td>Recovery of fistulas</td>
<td>[173]</td>
</tr>
<tr>
<td>Feline</td>
<td>Autologous AD-MSC</td>
<td>Chronic kidney disease</td>
<td>Moderate improvement in GFR</td>
<td>[174]</td>
</tr>
<tr>
<td>Feline</td>
<td>Allogenic AD-MSC</td>
<td>Chronic kidney disease</td>
<td>Modest improvement in renal function</td>
<td>[175]</td>
</tr>
<tr>
<td>Feline</td>
<td>Allogenic AD-MSC</td>
<td>Chronic kidney disease</td>
<td>No adverse effect</td>
<td>[176]</td>
</tr>
<tr>
<td>Feline</td>
<td>Allogenic AD-MSC</td>
<td>Chronic gingivostomatitis</td>
<td>Reduction in the severity of clinical disease</td>
<td>[177]</td>
</tr>
<tr>
<td>Equine</td>
<td>Allogenic UCB-MSC</td>
<td>Chronic laminitis</td>
<td>Repair of laminitis</td>
<td>[178]</td>
</tr>
<tr>
<td>Equine</td>
<td>Autologous BM-MSC</td>
<td>Tendon injury</td>
<td>Repair of tendon</td>
<td>[179]</td>
</tr>
<tr>
<td>Equine</td>
<td>Autologous BM-MSC</td>
<td>Superficial digital flexor tendon</td>
<td>Repair of tendon</td>
<td>[180]</td>
</tr>
<tr>
<td>Equine</td>
<td>Autologous BM-MSC</td>
<td>Tendinitis, desmitis</td>
<td>Repair of inflammation</td>
<td>[181]</td>
</tr>
<tr>
<td>Equine</td>
<td>Autologous AD-MSC</td>
<td>Bone spavin</td>
<td>Improvement in inflammatory reaction and clinical conditions</td>
<td>[182]</td>
</tr>
<tr>
<td>Equine</td>
<td>Allogenic AD-MSC</td>
<td>Endometriosis</td>
<td>Positive remodeling of endometrial tissue</td>
<td>[183]</td>
</tr>
<tr>
<td>Equine</td>
<td>Allogenic AD-MSC and PRP</td>
<td>Superficial digital flexor tendon</td>
<td>Repair of tendonitis</td>
<td>[184]</td>
</tr>
<tr>
<td>Equine</td>
<td>Allogenic AD-MSC and PRP</td>
<td>Tendonitis</td>
<td>Improved function</td>
<td>[185]</td>
</tr>
<tr>
<td>Dolphin</td>
<td>Autologous BM/AD-MSC</td>
<td>Skin wound</td>
<td>Repair of skin</td>
<td>[186]</td>
</tr>
</tbody>
</table>

AT-MSC: adipose tissue-derived mesenchymal stem cells; UCB-MSC: umbilical cord blood-derived mesenchymal stem cells; BM-MSC: bone marrow-derived mesenchymal stem cells; ESC-MSC: embryonic stem cell-derived mesenchymal stem cells; GFR: glomerular filtration rate; PRP: platelet-rich plasma.

9. UCB-MSCs from Animal Sources

Successful isolation of MSCs (up to 100%) from equine UCB has been reported with the use of special culture media with a large volume of 42–250 mL [77, 78]. Although the isolation of MSCs from sheep and goat was successful, the researchers did not reveal any specific parameters that resulted in an effective number of MSCs [75, 76, 85]. Based on our literature survey, we have demonstrated that a large volume of UCB with some other special determinants is a principal factor to achieve high MSC yield. As the blood volume of cUCB is too low to yield an efficient number of MSCs, cUCB may be recognized as an unsuitable source of MSCs. Lim et al.
whole blood. FD medium 20

Table 8: Yield rate of UCB-MSC isolation from human and equine fulfilling special criteria.

<table>
<thead>
<tr>
<th>Source of UCB</th>
<th>Parameters</th>
<th>Yield rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Explant method</td>
<td>40</td>
<td>[69]</td>
</tr>
<tr>
<td>Human</td>
<td>Large BV with HPL medium</td>
<td>46</td>
<td>[20]</td>
</tr>
<tr>
<td>Human</td>
<td>Large number of UCB units</td>
<td>50</td>
<td>[67]</td>
</tr>
<tr>
<td>Human</td>
<td>Large BV with MesenCult Proliferation kit</td>
<td>60</td>
<td>[71]</td>
</tr>
<tr>
<td>Human</td>
<td>ST ≤ 15 h, BV ≥ 33 mL, ≥10⁸ MNC count</td>
<td>63</td>
<td>[37]</td>
</tr>
<tr>
<td>Human</td>
<td>Large number of UCB units with serum-free culture medium</td>
<td>75</td>
<td>[72]</td>
</tr>
<tr>
<td>Human</td>
<td>Factorial experiment with large BV</td>
<td>≥90</td>
<td>[66]</td>
</tr>
<tr>
<td>Human</td>
<td>ST ≤ 2 h, V ≥ 90</td>
<td>90.9</td>
<td>[65]</td>
</tr>
</tbody>
</table>
| Human         | DP ≤ 37 weeks, 
BV ≥ 80 mL, ST ≤ 6 h, 
PEQ medium | 90             | [70]      |
| Equine        | BV 42 mL, FUD medium | 60             | [77]      |
| Equine        | Large BV | 57             | [78]      |


worked with a 58 kg mongrel dog, which very rarely have more than 8 mL of UCB [84]. In two independent studies by Kang et al. and Jang et al., UCB from multiple dogs was collected to obtain the desired number of MSCs [80, 83]. As the amount of UCB from canine cannot produce a sufficient number of MSCs, another researcher collected blood from the canine fetal heart and successfully obtained MSCs [82], which creates an inconsistency and ethical problems in the pursuit of animal experiments. Our attempt to isolate MSCs from the umbilical cord (Figure 1) [116] of three healthy dogs resulted in an average sample volume of 1 mL and a yield rate of 66% (unpublished data).

10. UCB from Companion Animals

Nowadays, canine cesarean sections (C-sections) are decreasing because of the increase in spaying or castration to prevent the birth of unwanted litters, extend longevity and promote pet health, and possibly mitigate undesirable behaviors. More precisely, spaying can help prevent uterine infections and breast tumors. Concurrently, castration of male companion animals precludes testicular cancer as well as some prostate problems. The traditional time for neutering is before the onset of sexual maturity, as long as the animal is healthy. Spaying involves abdominal surgery to remove the ovaries and uterus, whereas castration involves elimination of the testes, resulting in infertility. Under these circumstances, while acquiring cUCB is normally challenging, these removed reproductive organs are frequently accessible in veterinary clinics. Specifically, the ovaries and testes are often discarded as medical waste but are proven to be potent and reliable sources of MSCs. MSCs from these reproductive organs (testis and ovary with adjacent portions) with high differentiation potentiality have already gained popularity.

These MSCs are able to differentiate into three mesodermal cell types, including adipogenic, osteogenic, and chondrogenic, indicating multipotent properties resembling those of AT-MSCs [117]. We can thoroughly examine the presence of MSCs in canine reproductive organs and surrounding tissues (fat and connective tissue) discarded during neutering operations; these MSCs also exhibit growth characteristics indistinguishable from those of AT-MSCs. Therefore, further investigation of canine reproductive organs and tissues with the assistance of new preservation technology [118] will improve our understanding of reproductive stem cell biology to potentially augment cell-based therapies and regenerative medicine.

Unlike in humans, it is almost impossible to obtain UCB from canine immediately after natural birth, as the dam’s instinct prompts her to ingest the puppy’s placenta and amniotic sac. From this perspective, we have made many efforts (unpublished data) to collect cUCB from Korean veterinary clinics. However, this was difficult, as many pet owners tend to prevent their pet from breeding. Conversely, if the canine pet is pregnant, the owner generally prefers natural birth unless a medical reason necessitates C-section. Therefore, the reduction in canine C-sections in veterinary hospitals, the introduction of specialized breeders, and changes in the supply of canine make it almost impossible to obtain UCB from canine. Conducting a surgical procedure or C-section in canine solely to obtain UCB for laboratory purposes gives rise to the question of legitimacy. To solve this problem, we can use MSCs from canine AT [95] along with reproductive tissue. It has been demonstrated that MSCs from AT have higher frequency and potentiality compared with UCB-MSCs [59, 67].
11. Conclusions

One challenge in cellular therapy and regenerative medicine is the availability of alternative stem cell sources. Although cUCB-MSCs can proliferate and differentiate as stem cells, cUCB is not an ideal source because of low volume and ethical considerations of using a companion animal. In this regard, we suggest broadening the investigation of canine reproductive tissue and AT, instead of UCB, as the preferred source of MSCs.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

Tania Sultana and Soojung Lee contributed equally to this work and should be considered co-first authors.

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