How to Harvest Bone Marrow Derived Mesenchymal Stem Cells for Expansion and Injection

Laurie R. Goodrich, DVM, MS, PhD, Diplomate ACVS; David D. Frisbie, DVM, PhD, Diplomate ACVS; and John D. Kisiday, PhD

Authors' address: Colorado State University, College of Veterinary Medicine, 300 West Drake Road, Fort Collins, CO 80523; e-mail: laurie.goodrich@colostate.edu.

1. Introduction

The use of mesenchymal stem cells or progenitor cells has been advocated for treatment of many musculoskeletal diseases in the equine. Specifically, these cells have been identified as useful therapies for tendonitis, desmitis, and joint disease.[1–4] Bone marrow and adipose tissue have emerged as excellent sources of progenitor cells. Both sources have low morbidity associated with tissue harvest, and they are not associated with detrimental effects on donor sites of collection because of the renewable nature of these tissues. Bone marrow derived mesenchymal stem cells (BMDMSCs) have been compared with adipose derived progenitor cells (ADPCs) in many different studies, and they show superiority in their ability to heal the various target tissues of the musculoskeletal system.[5–9] Although many practitioners feel comfortable harvesting fat for ADPC collection, there may be a decreased comfort level with harvesting bone marrow because of a lack of familiarity of the anatomic structures and technique. This may result in clinicians opting out of pursuing this choice of stem cell therapy.

The objective of this presentation is to familiarize practitioners with the anatomic structures most commonly used for BMDMSC collection. Additionally, it will explain the techniques of collecting, packaging, and transporting bone marrow aspirates for collection, expansion, and 150 injections into diseased tissues.

2. Methods

BMDMSC Harvest From the Ilium

A thorough clinical examination should first be performed to note any past trauma to the tuber coxae region of the right and left side of the horse. If any previous trauma has occurred, the contralateral side should be chosen for collection. Sedation should be performed using detomidine hydrochloride at 0.02 mg/kg.[a] The tuber coxae should then be aseptically prepared. The tuber coxae should be palpated to determine the dorsal, ventral, cranial, and caudal borders. The authors feel that it is important to aspirate bone marrow at the region of the tuber coxae located one-half of the way between the dorsal, ventral, and cranial border and caudal border. Figure 1 shows a skeletal example of the tuber coxae

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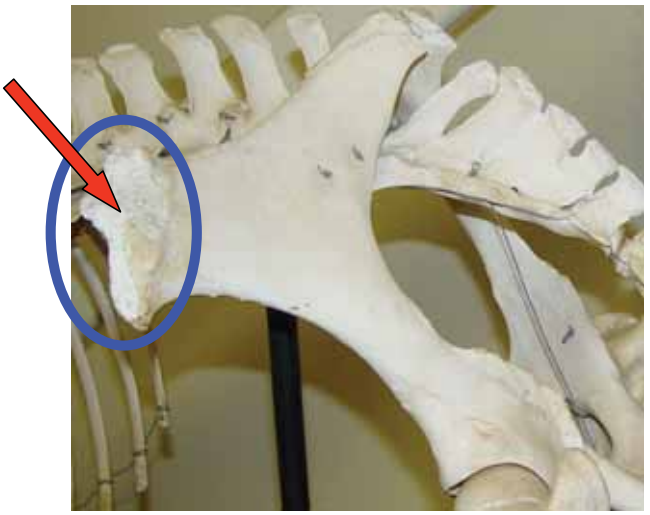

Fig. 1. A gross image of the tuber coxae and the ilium of a horse. Note the shape of the tuber coxae (circled in blue). The red arrow points to the site where the needle should puncture the tuber coxae.

and ilial wing. When this region is identified, 5 ml of mepivicaine hydrochloride[b] should be injected to locally anesthetize the area (Fig. 2). The center of the tuber coxae has been observed to be the most "stem cell rich" on dissection of this area (Fig. 3).[c] A 15 blade is then used to make a stab incision down to the periosteum (Fig. 4). A Jamshidi needle[d] is inserted with a moderate amount of force in a corkscrew-turning motion until the end of the needle extends ~4–5 cm into the ilium (Fig. 5). The obturator within the Jamshidi needle should then be removed, and a 60-ml syringe filled with 10,000,000 IU of heparin sulfate should be attached to the needle. The plunger should be withdrawn the length of the syringe (Fig. 6). It is necessary to apply this much force to the plunger to create negative pressure for aspiration of the thick bone marrow aspirate. It may take 1–2 min of constant pressure to begin to retrieve the aspirate. After the syringe is filled with 10 ml of bone marrow aspirate, it is detached from the needle, and the obturator is reinserted. The needle should be withdrawn 2 cm and then redirected (continuing to stay in the general center of the ilium). Another 5–10 ml aliquot (heparinized with 10,000,000 IU) is withdrawn. The obturator of the Jamshidi needle is then replaced, and the needle is withdrawn using a reverse corkscrew motion. The skin is closed using a skin staple. The syringes of bone marrow aspirate should be capped with a needle or syringe cap and placed in a Styrofoam box with cold packs. They should be transported overnight to the company performing the expansion.

Fig. 2. The area surrounding the tuber coxae is clipped and aseptically prepared.

BMDMSC Harvest From the Sternum

The horse is sedated as above. The sternum should be palpated from the point between the forelimbs to the caudal aspect ~16 cm caudal to the point located directly between the forelimbs. Although there is muscle coverage over the ventral sternum, it can be palpated well when a forefinger applies pressure on the midline. At the caudal aspect of sternum, the body wall attaches to the caudal sternum, and the pliability of the musculature changes where the sternum ends. The sternum should be clipped and aseptically prepared from directly between the forelimbs and extending 8–10 cm caudally and 2 cm on either side of the midline (Fig. 7). An area ~8 cm cranial to the caudal most aspect of the ilium should be blocked with 6 ml of mepivicaine hydrochloride. Care is taken to locally anesthetize SC tissue as well as the deep muscle and periosteal layer of the sternum. This area of the sternum is chosen because of the close approximation of sternebrae as shown in Figure 8. A stab incision through the skin and deeper SC tissue is made with a #15 blade to the ventral aspect of the sternum. A Jamshidi needle is then inserted through the skin portal to the ventral sternum. At this location of the sternum, there is usually an approximate distance of 2 cm between the skin and ventral sternum. The needle end should be carefully rocked cranially and caudally to confirm that the needle is in contact with the ventral sternum so as to accurately judge the correct distance to force the needle through the sternum (Fig. 9). After the ventral sternum is felt (through the needle), gentle rotation of the Jamshidi needle with mild, controlled force should continue until the needle extends ~3 cm into the sternum. As can be seen from Figure 8, the more caudal that the Jamshidi needle is placed in the sternum, the less distance exists between the ventral sternum and the dorsal sternum. It is important to avoid puncture

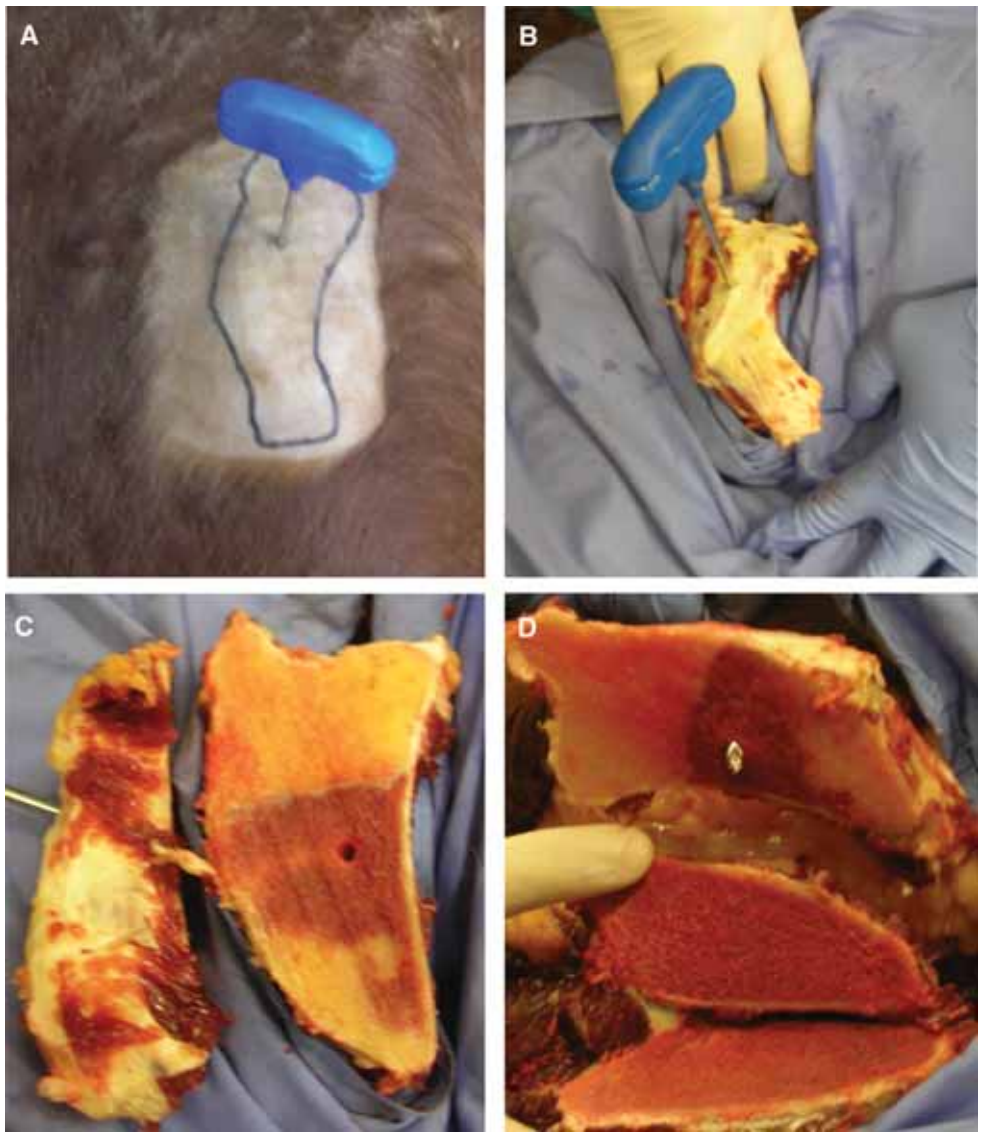

Fig. 3. The tuber coxae has been (A) outlined and (B) dissected, and a Jamshidi needle has been placed through the crest into the ilium. After placing the needle ~5 cm into the ilium, the ilium was sagittally cut to reveal the most bone marrow "rich" area of the ilium to be located in the (C) center. As further sagittal sections were made deeper into the illial wing, (D) more bone marrow "rich" areas were seen.

of the dorsal aspect of the sternum, because there are large blood-filled structures located immediately dorsal to the sternum. It is easy to avoid puncturing through the dorsal sternum by judging the distance the needle extends into the sternum and not extending past 3 cm. The obturator is then removed, and the heparinized syringe (prepared as above) should be connected to the needle. The plunger is withdrawn, and 10 ml of bone marrow aspirate is collected. The obturator is then replaced. The Jamshidi needle is redirected in a cranial or caudal location, or alternatively, another location cranially or caudally may be locally anesthetized and aspirated similar to the first draw. A skin staple is then placed in the skin to close the stab incision, and the bone marrow aspirate is placed in a transport vehicle as described above.

3. Results

Approximately 125 bone marrow collections/expansions have been performed at the Orthopaedic Research Center at Colorado State University Veterinary Teaching Hospital and various equine practices in which we collaborate. Most aspirates are performed at the ilium because of the ease of collection. Collection at the ilium also obviates the need to be underneath the horse at an awkward position. On several occasions, bone marrow collections from the ilium were not successful, which was likely caused by a low nucleated cell count. In such

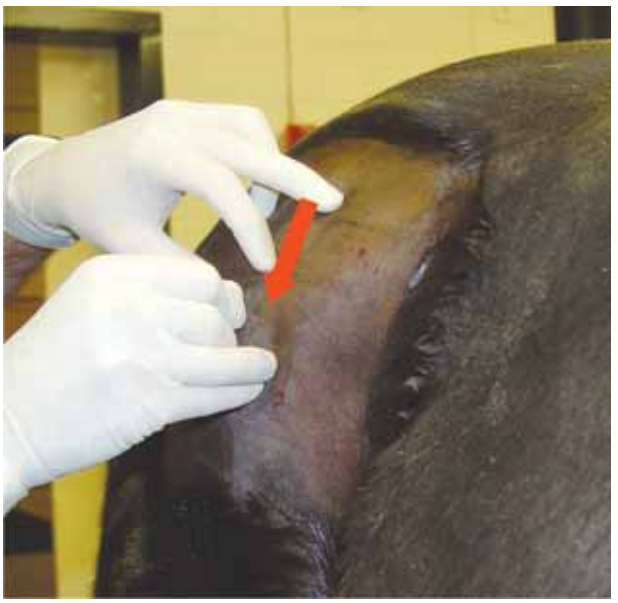

Fig. 4. A stab incision is made in the center of the tuber coxae with a #15 blade.

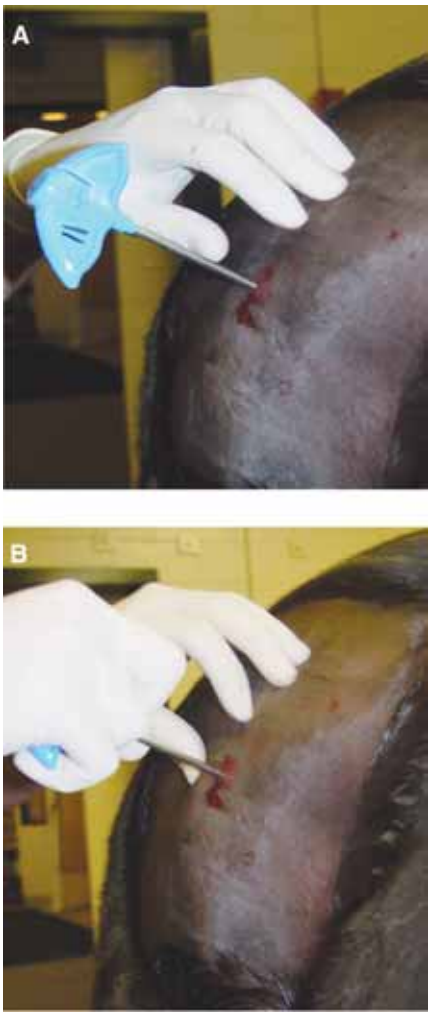

Fig. 5. A Jamshidi needle is (A) placed through the skin incision and (B) rotated with moderate force to puncture into the ilium.

Fig. 6. The plunger of the 60-ml heparinized syringe is withdrawn the length of the syringe to create adequate negative pressure to aspirate the thick bone marrow.

cases, stem cell cultures were established from sternal aspirates. Therefore, we have not had any horse in which BMDMSCs have not been successfully collected from either the ilium or sternum (when the ilium was unsuccessful). Most aspirates yield 5,000,000–30,000,000 cells after 2–3 wk of culture expansion. These cells are then placed in vials, frozen, and shipped back to the clinician overnight. Injection proceeds on receipt of the frozen vials. They should be quickly thawed in a warm water bath, aspirated in a volume of saline that is appropriate for the lesion, and injected (using ultrasound guidance to visualize lesions, if needed).

4. Discussion

Based on previous studies as well as our clinical impression, the authors feel that BMDMSCs are

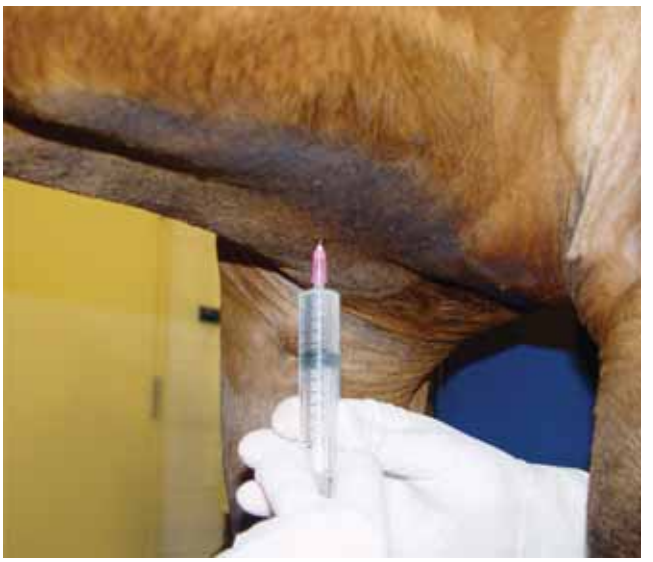

Fig. 7. The area clipped should extend from between the forelimbs to the caudal aspect of the sternum.

Fig. 8. A midsagittal section of an equine sternum reveals the areas rich in stem cells.

superior to ADPC. The technique of bone marrow harvest described above allows easy collection and low tissue morbidity.[5–7] When ADPCs are collected, a sizable incision and scar result. In many equine athletes, this tissue morbidity is inconvenient and unacceptable. Furthermore, when BMDMSCs are expanded, the number of mesenchymal stem cells is often 100 times the number of cells harvested from ADPC when adipose tissue is harvested, progenitor cells collected, and sent back in 48 h.[e] However, ADPC numbers could likewise be amplified with culture expansion. Although the question still exists as to whether or not "more is better" in terms of cell numbers, it would seem logical that large soft tissue lesions may need greater numbers of cells to heal.

The amount of bone marrow withdrawn is important. Comparisons have been made in our laboratory[f] that suggest that withdrawing >10 ml of bone marrow decreases the successful yield of stem cells. It has been noted through culturing various volumes of bone marrow aspirates that after ~10 ml of marrow has been withdrawn, blood rather than bone marrow fills the syringe. This results in a "diluted" nucleated cell number, increasing the number of cell culture flasks needed and causing reduced growth and expansion of BMDMSCs. This finding is in agreement of others.[10]

The authors feel that this therapy has been very successful in various cases of tendon, ligament, and joint disease. A multicenter clinical study is ongoing to observe the outcome of the treatment. Early results seem promising; however, long-term trials will help distinguish the cases that will benefit the most from this therapy.

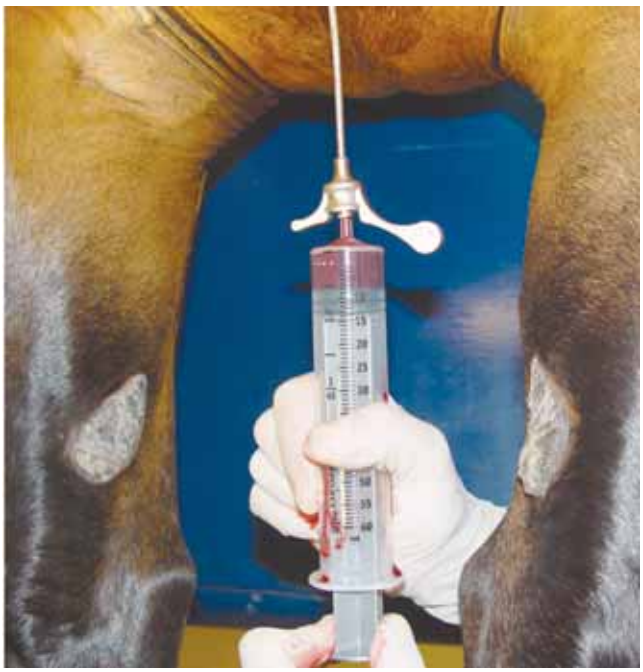

Fig. 9. Collection of bone marrow aspirate from the sternum through a Jamshidi needle.

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