Procedures

Aspirating from the Sternum

Universal Transport Kit Contents:

1 x Transport box:
- Box is insulated styrofoam
- Large Ice Pack Frozen

1 x Aspiration kit:
- 11G x 4" BM aspiration needle with trocar, needle rest and cap
- #11 scalpel blade
- 2 x 30mL syringes with pre-loaded Heparin (1000u/mL) + luer lock caps
- 4 x 5ml syringes
- 2 x 4ml Na Citrate vacutainers (blue top for bone marrow aspirate)
- 1 x resealable plastic bag
- 1 x sterile drape
- 4 x sterile swabs
- 2 x 21G x 1/5" needles
- Multiple strips of parafilm in resealable zip lock bags
- 1 x 500mL blood collection bag w/CPDA
- Aspiration & Implantation instructions
- Bubble Wrap to prevent breakage
- Submission form (also available online at www.equinepartnersamerica.com)

(Transport Boxs & Aspiration Kits are available from Equine Partners America @ 800-752-8538)

You will also need:
- Local anaesthetic (eg. Mepivicaine)
- Sedative (eg. Alpha 2 agonist and opiate)
- Ultrasound machine & clippers
- Cotton wool to stabilize tubes during transport to the lab (or use bubble wrap supplied in kit)
- For larger lesions (see submission form guidelines) you will aslo need more 10ml syringes and more 5ml NaCit vacutainers.

How to take a bone marrow aspirate from the sternum

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of tendon and ligament injuries. The protocol for bone marrow aspiration is shown here. The tuber coxa is an alternative site.

Indications

- We recommend using bone marrow mesenchymal stem cells for regenerative therapy of ligament and tendon injuries as well as joint inflammation and injury, especially when associated with ligament injuries.
- Tendon and ligament stem cell treatment can be used in conjunction with platelet rich plasma therapy.
- It is the clinician's choice whether we suspend the cells in Platelet Rich Plasma, Autologous Conditioned Serum, or Bone Marrow Aspirate Supernatant.

Technique

As the bone marrow is delivered to the laboratory the day after it is obtained, aspirations should only be carried out Monday–Thursday. The horse is first sedated with a combination of Alpha 2 agonist and opiate (e.g. detomidine HCl and butorphanol). A 10cm wide band overlying the sternum is clipped and scrubbed with surgical scrub (e.g. chlorhexidine) and surgical spirit. The sternum is examined ultrasonographically to identify the three most caudal sternebrae by the appearance of their intersternebral spaces (figures 1 and 2).

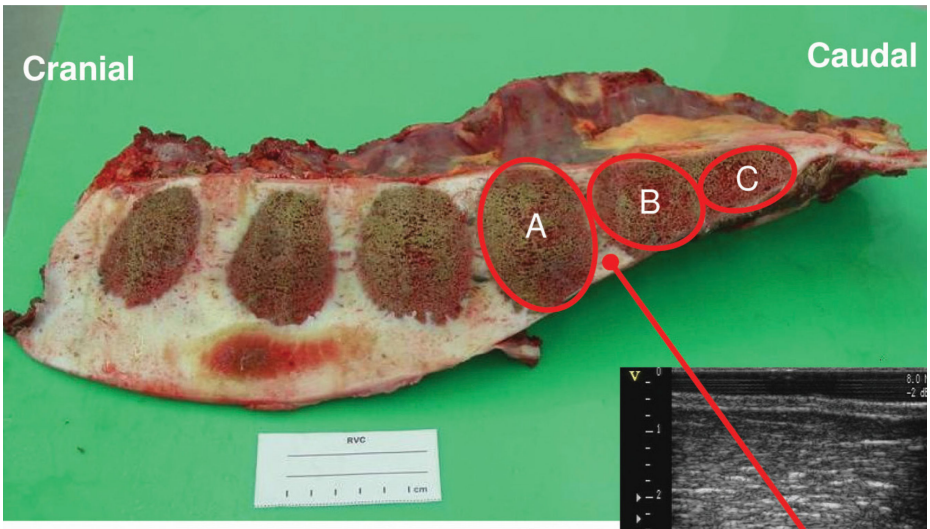

Figure 1
Longitudinal sternal section. There is a prominent intersternebral space, seen ultrasonographically as a V-shaped deficit between the routinely aspirated sternebrae (A and B). There is a small, and occasionally difficult to identify, intersternebral space between the most caudal sternebrae (B and C) and an asymmetrical space cranial to Sternebra A. The most caudal sternebra (C) is thin and there is an increased risk of penetration of the thoracic cavity; the more cranial sternebrae are covered by a bony prominence, therefore sternebrae A and B are routinely aspirated.

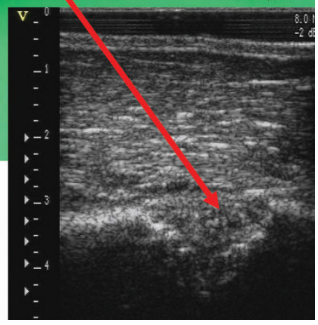

Figure 2
The ultrasonographic appearance of the intersternebral space between sternebrae A and B. This space is usually level with the caudal aspect of the elbow.

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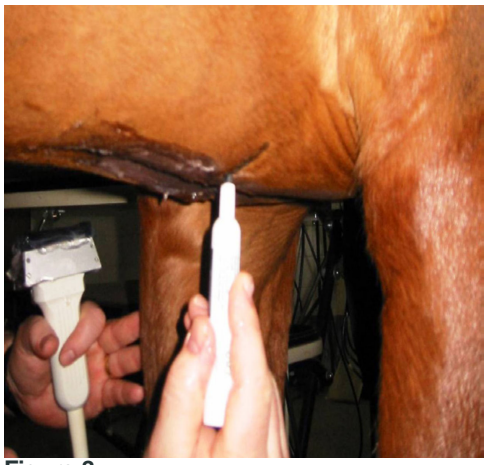

Figure 3
Marking the intersternebral space between the two sternebrae to be aspirated

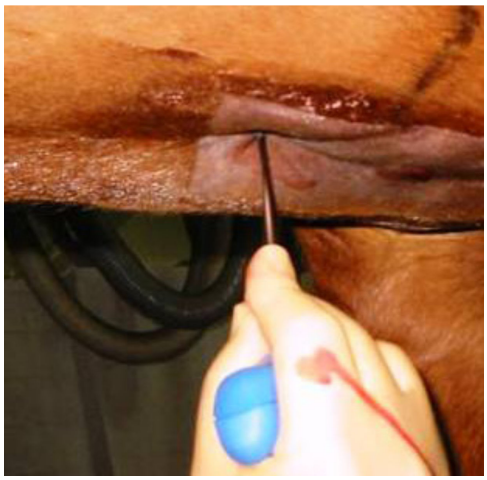

Figure 4
Insertion of Jamshidi needle. Once the Jamshidi needle makes contact with the ventral surface of the bone the index finger should be placed 1-2cm from the skin surface. This facilitates insertion of the needle to the correct depth.

Figure 5
Successful aspiration of bone marrow

- The position of the intersternebral spaces is marked on the adjacent hair with a marker pen (figure 3).
- The area of the sternum is prepared aseptically and 5 ml local anesthetic placed along the predicted aspiration entry points (midway between each mark / intersternebral space).
- The area is then scrubbed a final time before a small stab incision is made through the skin at the more cranial site with a No. 11 scalpel blade, in a sterile fashion. If the horse has moved then the mark on the skin may have moved position. Ensure the horse is in the same position as when marked.
- The Jamshidi needle is introduced through the stab incision and advanced until it contacts the ventral surface of the sternebra in the mid-line, (figure 4). This varies between breeds but should be around 2cm.
- The index finger is placed 1–2cm from the skin surface on the needle shaft and the needle gradually advanced using rotating movements until the index finger is against the skin surface. This ensures the needle does not penetrate the deep surface of the sternebrae.
- The central trocar is removed from the Jamshidi needle. Bone marrow does not initially flow spontaneously from the needle: gentle aspiration with an attached syringe is required. This is occasionally, but only initially, associated with a small amount of discomfort to the horse, usually manifested by a slight guarding of the abdomen. Thereafter bone marrow lows easily into the syringe (figure 5) and is spontaneously shed from the needle when the needle is disconnected.
- The first pre-loaded 30ml syringe is attached to the Jamshidi needle, 24ml of bone marrow is gently aspirated (making up a total volume of 30ml); Place sterile cap on the syringe and label with patient informaton. Place syringe into the sterile Instant-Sealing Sterilization Pouch provided.
- The bone marrow sample is gently agitated in the syringe to ensure adequate mixing of the anticoagulant with the marrow (bone marrow clots extremely quickly).
- **NOTE:** This step is only necessary if you do not want to suspend the cells in Platelet Rich Plasma. Two or more 4ml samples are then taken (into plain 5ml syringes) and transferred immediately to sodium citrate glass blood tubes by needle injection. These samples are used to derive the bone marrow supernatant used to re-suspend mesenchymal stem cells for implantation. For very large lesions more than two such samples should be taken.
- The Jamshidi needle is then withdrawn completely before being inserted, in the same fashion, into the second, more caudal, sternebra. 26ml bone marrow is aspirated into the remaining pre-loaded 30ml syringe (again making up a total volume of 30ml) and the sample is capped and placed into a sterile Instant-Sealing Sterilization Pouch.
- Once the Jamshidi needle is withdrawn, the portals can continue to bleed but pressure is usually all that is necessary to stop this hemorrhage. Closure is unnecessary.

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Joint Injections

- When using BM-MSC's for intra-articular injections an additional 40mL of venous blood is required for processing.
- Using sterile technique, aseptically collect a minimum of 40mL of venous blood into 4x plain, red top tubes (10mL in each tube).
- Label the blood samples with the patient's name, and ship alongside the heparinised and citrated bone marrow samples.
- This updated protocol is required for all stem cell injections used for treating joint problems

Blood Collection: Platelet Rich Plasma and Autologous Serum Scheduling

- The following protocol is for blood collection using the 500ml blood collection bag with CPDA. Blood will be processed into PRP for stem cell suspension (if requested), and Autologous Serum for use in culture of stem cells prior to injection. If you choose to use Bone Marrow Aspirate as a substitute for PRP you must collect blood for Autologous Serum AND Bone Marrow Aspirate and transfer to the Na Citrate Vacutainers. Please reference the "Bone Marrow Collection" protocol (Sternum Page 2 of 3) for more details.
- Sedate the patient and prepare the vein as for a sterile procedure. Clip, clean, and scrub the area thoroughly, then block the site for venipuncture.
- While wearing sterile gloves, puncture the prepared vein with the blood collection bag needle. The blood bag should be gently rocked as the blood is being collected, but do not shake vigorously.
- Clamp the blood collection bag close to the bag opening, and tie the middle of the tubing in a knot and cut off the needle. Date and label the blood collection bag with the patient's name and practice/RDVM name.
- Put into zip lock bag and seal.

Scheduling

Collect BM aspirate and blood on a Monday, Tuesday, Wedneday, or Thursday, and ship overnight express for morning arrival. **Do not freeze the biological materials!** The cells will be ready in 3-4 weeks. We will call in 5 to 7 business days before the cells will be sent so that scheduling of treatment can be confirmed. We will ship the cells the afternoon before the treatment appointment, and the cells will arrive at your hospital by 11am Tuesday, Wednesday, Thursday or Friday. **Do not freeze the cells!** Rather keep them refrigerated and clean. The lesion should be treated by the end of the day.

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Fig 1: Label the containers and tubes

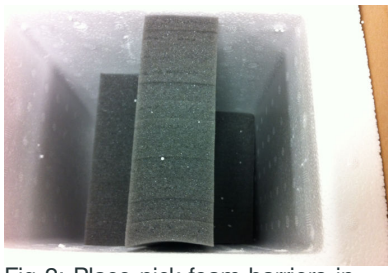

Fig 2: Place pick-foam barriers in proper orientation

Fig 3: Place the pre-frozen pack and the bone marrow syringes in the transport box

Fig 4: Place the blood collection bag in the transport box

Fig 5: Seal the transport box and label correctly

Transportation

- ☐ Label bone marrow syringes, citrate tubes, and/or blood colelction bag with the date and horse's details with the completed sample submission form. (Figure 1)
- ☐ Place the pick-foam barriers into the shipping box as seen in Figure 2.
- ☐ Pick-foam barriers should be placed in the following order from left to right: Medium, Large, Small. Place the pre-frozen, glacier ice pack vertically inbetween the small and large pick foam barriers. (Figure 3)
- ☐ Ensure that the bone marrow syringes, citrate tubes, and blood collection bag are properly closed and correctly identified. Then place the samples into a sealable plastic bag. Place the bone marrow syinges vertically inbetween the medium pick foam and the wall of the insulated shipping box (Figure 3). Place the blood collection bag horizontally on top of the bone marrow syringes (Figure 4). Close the insulated shipping box.
- ☐ Put the completed sample submission form into a plastic bag/ "documents enclosed" envelope and place in the transport box. (Figure 5)
- ☐ If the courier is not coming immediately then please refrigerate the sample at 4°C while you wait for it to be collected.
- ☐ Seal the transport box and send via FedEx or UPS (guaranteed next day delivery (by 1pm) with $1000 consequential loss cover) to the Laboratory at the address below.
- ☐ It is very important that you complete the air waybill with description of contents like this: 'Bone Marrow, not restricted, as per IATA Exemption Ref 3.6.2.2.3.2..

Laboratory Address

Regenerative Medicine Service, MDS EMC
Virginia Tech
17690 Old Waterford Road
Leesburg, Virginia 20176

- ☐ Finally, inform Equine Partners America of the expected arrival date via info@equinepartnersamerica.com or 800-752-8538

Arranging for Implantation

Equine Partners America will contact the practice about 1 week after receipt to confirm that the submitted sample contained viable cells. Equine Partners America will liaise with the practice to arrange a suitable date for delivery of cells. (2-4 weeks depending on growth rate). Delivery is normally pre-1pm (pre-9am delivery is available at a small premium).