Aspirating from the Tuber Coxae

Stem Cell Therapy Procedures

How to: Take a bone marrow aspirate from the Tuber Coxae

Equipment required

1 x Transport box (available from Biobest, Tel: +44 (0)131 440 2628)

Transport box includes:

- 1 x styrofoam insulated box validated to keep the sample at +2-8°C for 48 hours
- 2 x gel cooling packs **(one to be pre-frozen for at least 24hrs before aspiration, one to be chilled at +4°C)**
- 1 x insulating foam insert to protect and stabilise tubes during transport

1 x Aspiration kit (available from Biobest, Tel: +44 (0)131 440 2628)

Aspiration kit includes:

- 1 x 11 Gauge 4" Jamshidi Biopsy Needle (also available separately from Biobest)
- 1ml Heparin (5000iu/ml)
- 2 x 30ml plastic sterile Universal Containers (white) – for heparinised bone marrow
- 4 x 5ml NaCit vacutainers (blue) (more for larger lesions – see submission form for details)
- 1 x 50ml syringe
- 2 x 10ml syringes
- 1 x No. 11 scalpel blade
- 2 x 21G 1.5" needles
- 2 x packs of 5 sterile swabs
- 1 x polythene sterile drape (60 x 90cm)
- 1 x resealable plastic bag (to surround foam insert)
- Sample submission form (also available at www.biobest.co.uk)

You will also need:

- Local anaesthetic (eg. mepivicaine)
- Sedative (eg. α2 agonist and opiate)
- Ultrasound machine (7.5-10 MHz)
- Clippers
- For larger lesions (see submission form guidelines) you will aslo need:
  - more 10ml syringes
  - more 5ml NaCit vacutainers
- Aspiration & Implantation instructions
- 2 x 30ml syringes may be required to attain sufficient suction

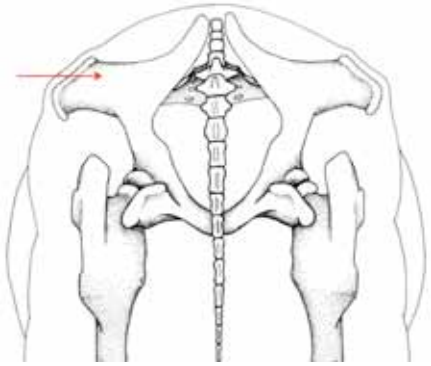


Figure 2
Caudal view of the tuber coxae indicating the points of palpation and site of entry for the Jamshidi needle

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of equine orthopeadic injuries. The recovery and expansion of stem cells is a service offered to veterinary surgeons who have been trained in the technology in accordance with the Veterinary Medicines Directorate (VMD). The use of stem cells in the UK is governed by the VMD and only a VMD authorised laboratory should be used.

The protocol for bone marrow aspiration from the tuber coxae is shown here. The sternum is an alternative site for bone marrow aspiration (instructions for this can be found on page 3).

Technique

N.B. 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 α2 agonist and opiate (e.g. detomidine HCl and butorphanol).
- A 10cm square area overlying the tuber coxae is clipped (figure 1).

In an average sized horse the aspiration site is a point 2cm caudal to the ridge running from craniodorsal to caudoventral and approximately one quarter of the way down from the top (figure 2).

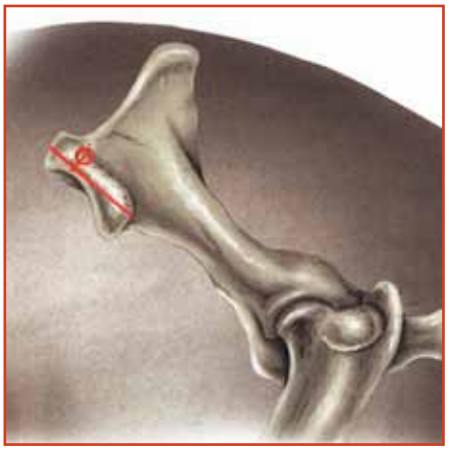
- The area is prepared aseptically and 5ml local anaesthetic is placed along the aspiration path and over the periosteum.

Warning
This process involves the preparation of a substance intended for administration to a patient. Sterility and aseptic technique is paramount. Nupsala cannot be held responsible for operator error.

Figure 1
Clipped area indicating the position of the tuber coxae

Aspirating from the Tuber Coxae

Figure 3 & 4
Insertion of Jamshidi needle. Once the Jamshidi needle makes contact with the caudolateral surface of the bone, advance the needle 6 cm, making sure the needle remains horizontal and square to the horse at all times.



- Prior to aspiration, the 50ml syringe is pre-loaded with 1ml of 5,000iu/ml heparin. A larger syringe can be used to increase negative pressure.
- The area is scrubbed a final time before a small stab incision is made through the skin at the aspiration site with a No. 11 scalpel blade.
- The Jamshidi needle is introduced (horizontally) perpendicular to the horse's sagittal plane through the stab incision and advanced until it contacts the surface of the tuber coxae.
- The needle is gradually advanced 6cm into the bone using rotating movements, always ensuring that the needle remains horizontal and square to the horse (figures 3 & 4).
- The central trocar is removed from the Jamshidi needle. Bone marrow does not initially flow spontaneously from the needle: aspiration with an attached syringe is required.
- The pre-loaded 50ml syringe is attached to the Jamshidi needle and full negative pressure is applied in order to aspirate 19ml bone marrow (making up a total volume of 20ml).
- 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). The sample is then transferred into the two universal plastic containers provided, divided into two 10ml aliquots. Continue to gently mix the samples once in these containers.
- A further sample, without heparin, is then taken into a plain 20ml syringe. This sample is transferred immediately to two or more sodium citrate glass blood tubes (4ml bone marrow into each tube). The tubes should be vigorously agitated to prevent clotting, with thumbs over the lids.
- For very large lesions (over 30% cross-sectional area) more than two sodium citrate tubes should be submitted (two more per 10 million additional cells required). These samples are used to derive the bone marrow supernatant used to re-suspend mesenchymal stem cells for implantation. Care should be taken when transferring bone marrow to citrated tubes or universal containers to avoid the possibility of contamination.
- Once the Jamshidi needle is withdrawn, the portal can continue to bleed but pressure is usually all that is necessary to stop this haemorrhage. Closure is unnecessary.

Please turn to page 5 for the packaging and transportation instructions

Nupsala ***offers training and advice to vets using the stem cell treatment for the first time. Please ask for more details.***

Aspirating from the Sternum

Equipment required

1 x Transport box (available from Biobest, Tel: +44 (0)131 440 2628)

Transport box includes:

- 1 x styrofoam insulated box validated to keep the sample at +2-8°C for 48 hours
- 2 x gel cooling packs **(one to be pre-frozen for at least 24hrs before aspiration, one to be chilled at +4°C)**
- 1 x insulating foam insert to protect and stabilise tubes during transport

1 x Aspiration kit (available from Biobest, Tel: +44 (0)131 440 2628)

Aspiration kit includes:

- 1 x 11 Gauge 4" Jamshidi Biopsy Needle (also available separately from Biobest)
- 1ml Heparin (5000iu/ml)
- 2 x 30ml plastic sterile Universal Containers (white) – for heparinised bone marrow
- 4 x 5ml NaCit vacutainers (blue) (more for larger lesions – see submission form for details)
- 2 x 20ml syringes
- 2 x 10ml syringes
- 1 x No. 11 scalpel blade
- 2 x 21G 1.5" needles
- 2 x packs of 5 sterile swabs
- 1 x polythene sterile drape (60 x 90cm)
- 1 x resealable plastic bag (to surround foam insert)
- Sample submission form (also available at www.biobest.co.uk)

You will also need:

- Local anaesthetic (eg. mepivicaine)
- Sedative (eg. α2 agonist and opiate)
- Ultrasound machine (7.5-10 MHz)
- Clippers
- For larger lesions (see submission form guidelines) you will aslo need:
  - more 10ml syringes
  - more 5ml NaCit vacutainers
- Aspiration & Implantation instructions

How to: Take a bone marrow aspirate from the Sternum

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of equine orthopeadic injuries. The recovery and expansion of stem cells is a service offered to veterinary surgeons who have been trained in the technology in accordance with the Veterinary Medicines Directorate (VMD). The use of stem cells in the UK is governed by the VMD and only a VMD authorised laboratory should be used.

The protocol for bone marrow aspiration from the sternum is shown here. The tuber coxae is an alternative site for bone marrow aspiration (instructions for this can be found on page 1).

Technique

N.B. 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 α2 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).

Warning
This process involves the preparation of a substance intended for administration to a patient. Sterility and aseptic technique is paramount. Nupsala cannot be held responsible for operator error.

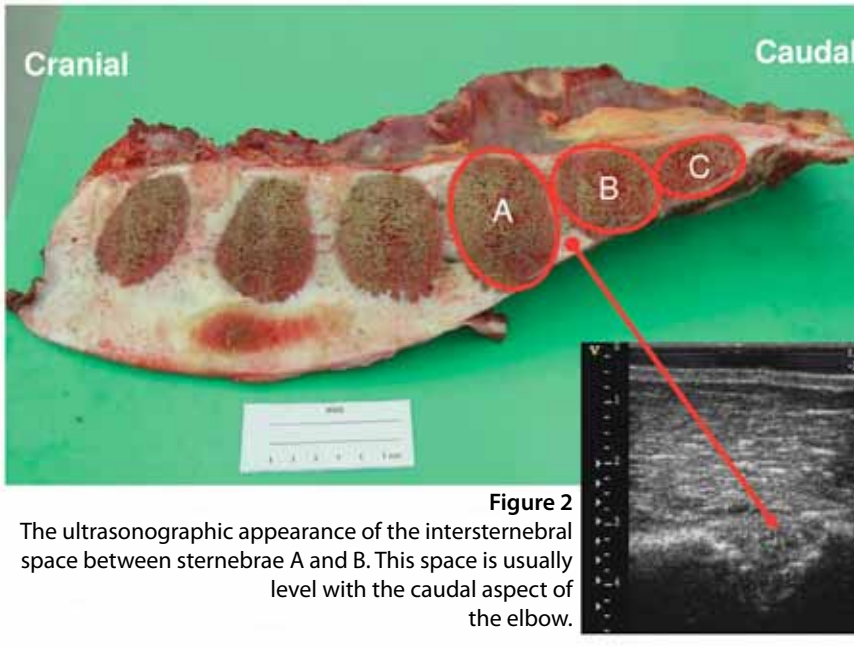


Figure 1
Longitudinal sternal section. There is a prominent intersternebral space, seen ultrasonographically as a V-shaped deficit between the routinely aspirated sternebrae (A and B). There is a small, and occasionally difficult to identify, intersternebral space between the most caudal sternebrae (B 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.

Aspirating from the Sternum

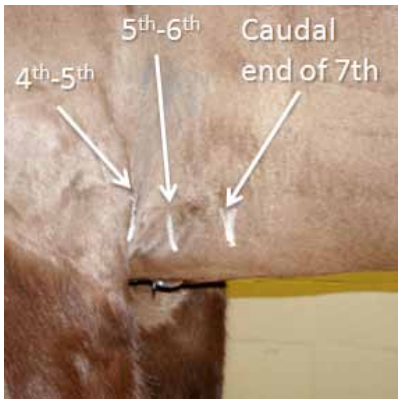


Figure 3
Marking the intersternebral space between the two sternebrae to be aspirated

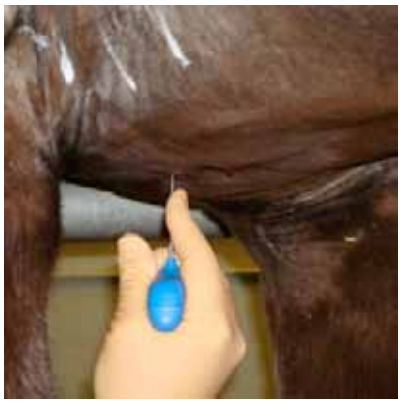


Figure 4
Insertion of Jamshidi needle. Once the Jamshidi needle makes contact with the ventral surface of the bone the index finger should be placed 1-2cm from the skin surface. This facilitates insertion of the needle to the correct depth.

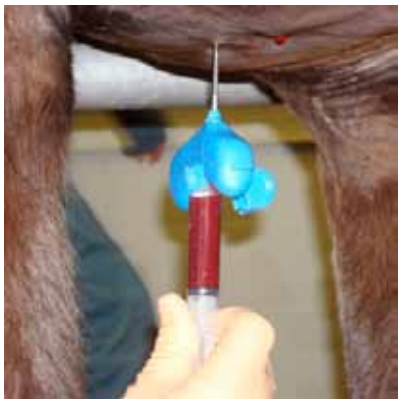


Figure 5
Successful aspiration of bone marrow

- Ultrasonographically, locate the intersternebral space between the 5th/A and 6th/B sternebrae. Keeping the transducer longitudinal on the midline and the center of the Intersternebral space center to the transducer/ screen, place a skin staple at the cranial end of the transducer. This will mark the region of the 5th/A sternebrae (figure 3).
- The area of the sternum is prepared aseptically and 5ml of local anesthetic placed at the location of the skin staple ensuring the local anesthetic is placed deep and in contact with the sternum.
- The area is then scrubbed a final time before a small stab incision is made through the skin at the location of the skin staple in a sterile fashion.
- Prior to aspiration, 1 x 20ml syringe is pre-loaded with **1ml** of 5,000iu/ml heparin. It is very important that the correct amount of heparin is used.
- 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. Ensure the needle is midline and straight.
- The needle is advanced into the 5th sternebrae. This is the only sternebrae from which bone marrow is collected.
- 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 pre loaded 20ml 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 flows easily into the syringe (figure 5) and is spontaneously shed from the needle when the needle is disconnected.
- The pre-loaded syringe is attached to the Jamshidi needle and 19ml bone marrow is drawn into it (making up a total volume of 20ml) and the sample is transferred to a sterile plastic Universal Container.
- The bone marrow sample is gently agitated in the container to ensure adequate mixing of the anticoagulant with the marrow (bone marrow clots extremely quickly).
- Two or more 4ml samples, as required, are then taken (into plain 10ml 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 (two more per additional 10 million cells required).
- Care should be taken when transferring bone marrow to citrated tubes and universal containers to avoid the possibility of contamination.
- Once the Jamshidi needle is withdrawn, the portals can continue to bleed but pressure is usually all that is necessary to stop this haemorrhage. Closure is unnecessary.

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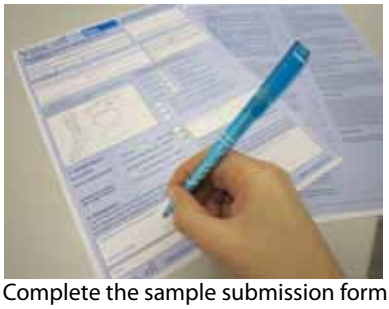
Packaging and Transportation

Label the containers and tubes



Complete the sample submission form and read the terms and conditions

Place the pre-frozen and chilled packs in the transport box as shown

Pack the bagged samples into the transport box

Seal the transport box and label correctly

Packaging & Transportation

Label Universal Containers and citrate tubes with the date and horse's details and complete the sample submission form.

- Put the pre-frozen cool pack in the bottom of the transport box and place second (chilled at 4°C, not frozen) cool pack above it to stop the samples coming into contact with the frozen pack. It is very important the bone marrow does not freeze as this will kill any cells in the sample. Do not allow the sample to contact a frozen pack directly.

Ensure that the sample containers and citrate tubes are properly closed and correctly identified. Then place the samples into the holes in the foam insert provided and place the foam inside a large plastic bag with absorbent material (e.g. cotton wool). Place the bag containing the foam insert into the cooled transport box provided. If for any reason a foam insert is not available, the tubes containing the samples should be well insulated using another material (e.g. paper towels) and enclosed in the plastic bag with absorbant material in case the sample leaks in transit.

- Put the completed sample submission form into a plastic bag/'documents enclosed' envelope and place in/stick on the transport box.
- If you are based **in the UK**, seal the transport box and send via Royal Mail (guaranteed next day delivery (by 1pm) with £1000 consequential loss cover) to Biobest at the address below.
- If you are based **outside the UK**, please book a courier to deliver within 24hrs to the Biobest 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

Biobest Laboratories Ltd
6 Charles Darwin House
The Edinburgh Technopole
Milton Bridge
Near Penicuik
EH26 0PY
United Kingdom

Finally, inform Biobest of the expected arrival date via enquiry@biobest.co.uk or +44 (0)131 440 2628.

Arranging for Implantation

Biobest will contact you to confirm that the sample has arrived safely. Biobest 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). If you would like further updates on the progress of the cells please telephone Biobest on +44 (0)131 440 2628.

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