NUPSALA
Veterinary Services

Leading veterinary orthopaedic treatments

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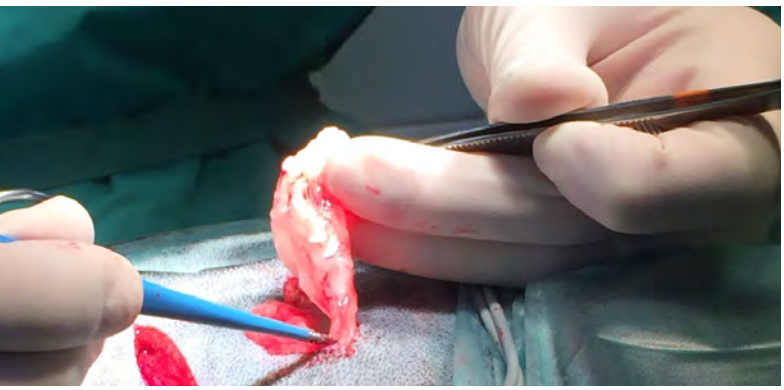
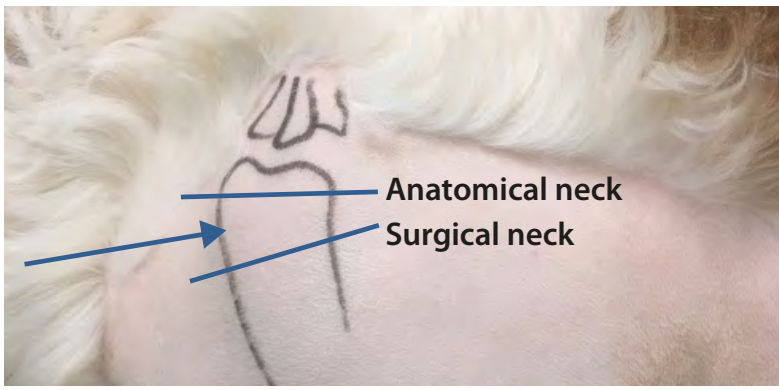
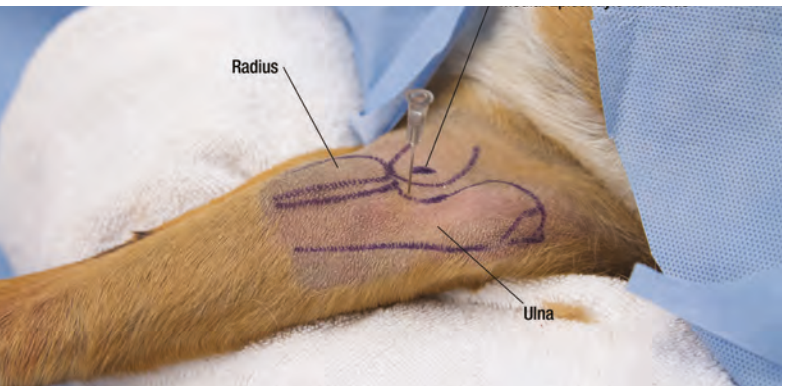
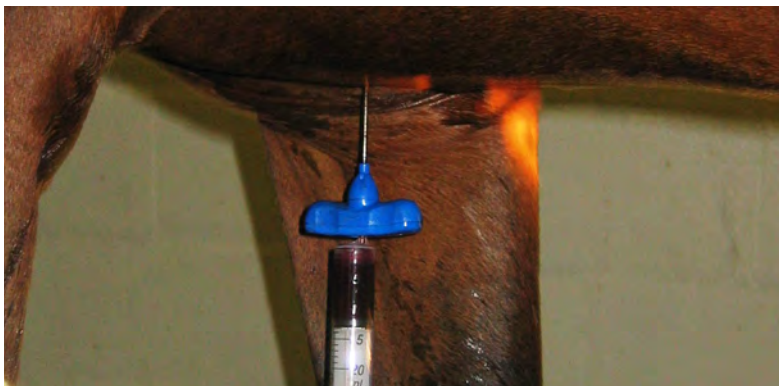
Nupsala Canine & Equine Procedures Handbook

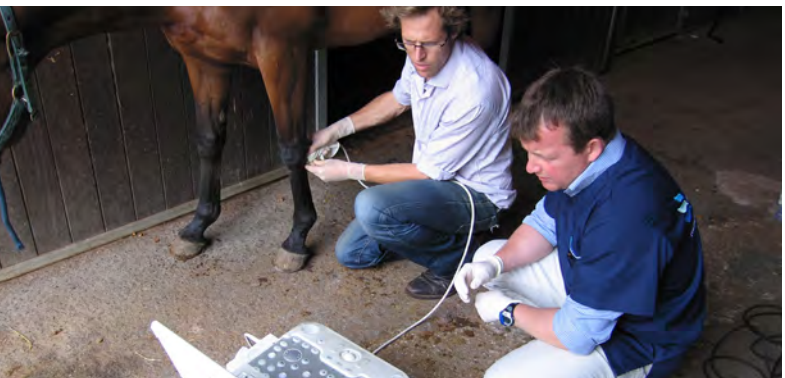
Guidelines for clinicians

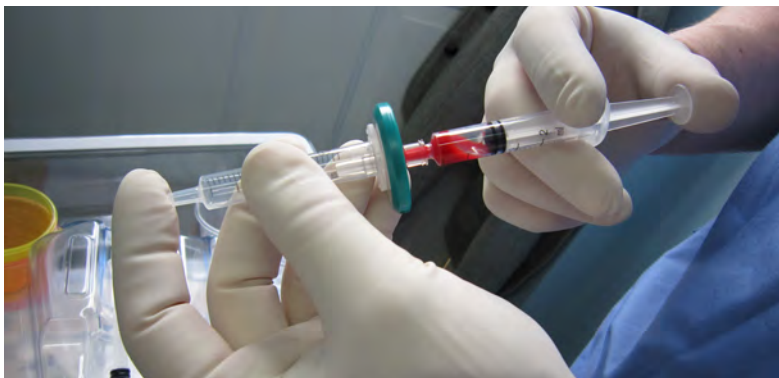












Contents

Nupsala offers training and advice to vets in all aspects of these procedures. Please ask for more details:

info@nupsala.com
+44(0)1865 922227

There are also further instructions and videos on the Nupsala website:
www.nupsala.com

Warning
These procedures involve the harvesting, procurement and preparation of substances intended for administration to a patient. Correct clinical procedure, sterility and aseptic technique is paramount. Nupsala cannot be held responsible for operator error.

Harvesting Canine Adipose Tissue

Stem Cell Therapy Procedures

How to: Harvest canine adipose tissue

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 Harvesting kit (available from Biobest, Tel: +44 (0)131 440 2628)

Harvesting kit includes:

- 2 x 50ml plastic sterile universal containers (white) containing transport medium
- 2 x 8ml Serum Tubes
- 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)
- Stitch kit
- Sedative
- Clippers
- Harvesting instructions

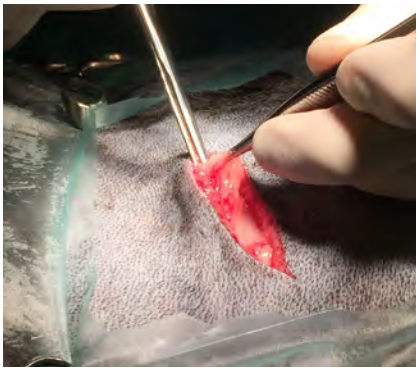


Figure 1 - Sterile incision site

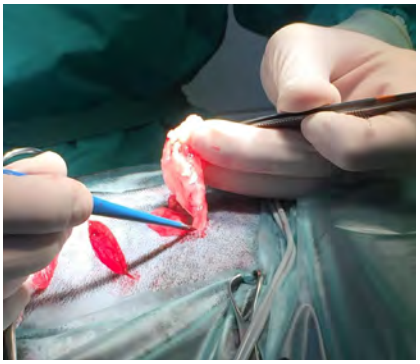


Figure 2 - Extraction of adipose tissue

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.

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of canine orthopaedic 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 harvesting of adipose tissue is shown here.

Technique

N.B. As the adipose tissue is delivered to the laboratory the day after it is obtained aspirations should only be carried out Monday-Thursday. We can offer a weekend service where possible, for an additional cost.

- Sedate or anaesthetise the dog and place it in lateral recumbancy.
- Clip a 10cm^2 area from where the specimen will be taken (just behind the scapula). You should be able to palpate the subcutaneous fat.
- Block the area for harvesting with local anaesthetic.
- Aseptically prepare the area.
- Under sterile conditions make a small incision (figure 1).
- Lift the skin to expose the adipose tissue beneath and extract approximately two tablespoons (figure two). A second incision can be made to ensure sufficient tissue is obtained.
- The harvested tissue is placed into the sterile conical tubes for processing (figure 3).
- The incision sites are closed and dressed.
- Two vials of 8ml of blood are also collected in the serum tubes (fig. 4).

Once you have collected the adipose tissue please refer to our 'Packaging & Transportation' procedures to send the material to Biobest's laboratory for stem cell culture.

Figure 3 - Adipose tissue in conical tube for processing

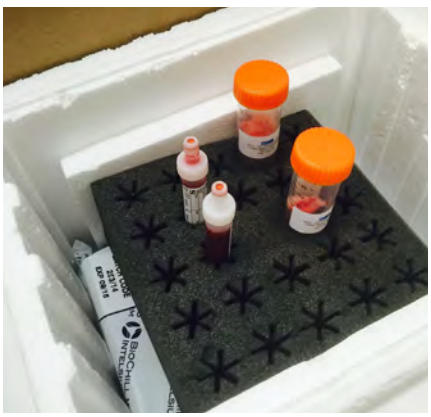


Figure 4 - Blood and adipose tissue post collection

Aspirating Canine Bone Marrow

Stem Cell Therapy

How to: Aspirate canine bone marrow

Equipment required

2 x 30 ml Syringes (1 spare)
1 x 11Ga Jamshidi Needle with blunt stylet
2.5ml heparin (5000iu/ml)

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.

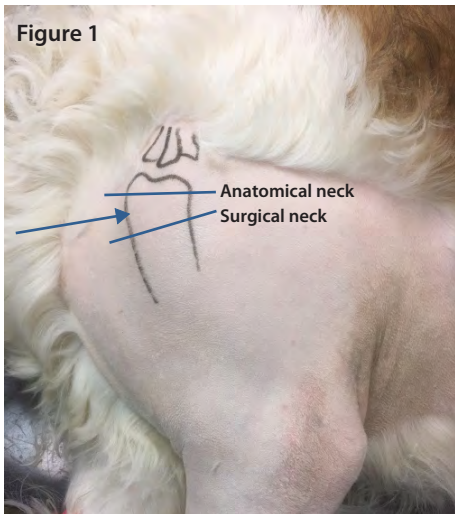
Introduction

These procedures describe the way to aspirate canine bone marrow in order to harvest mesenchymal stem cells and growth factors from the patient's own bone marrow. The bone marrow can be processed patient-side using a specialist centrifuge or sent to Biobest Laboratories for stem cell culture.

Technique

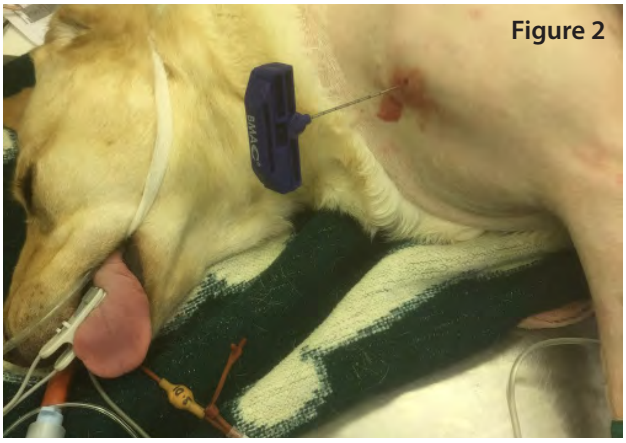
The dog is first anaesthetised and placed in lateral recumbency. An area overlying the shoulder is clipped, exposing the aspiration site. The site for aspiration is the proximal aspect of the humerus mid way between the surgical neck and the anatomical neck (Figure 1). The area is prepared aseptically and 5ml local anaesthetic is placed along the aspiration path and over the periosteum.

Figure 1



Prior to aspiration, the 30ml syringe is pre-loaded with 1.5ml of 5,000iu/ ml heparin. 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 dog's sagittal plane through the stab incision and advanced until it contacts the surface of the bone.

Figure 2



The needle is gradually advanced 1-2cm into the bone using rotating movements, always ensuring that the needle remains horizontal and square to the dog (Figure 2). 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 (Figure 3). The pre-loaded 60ml syringe is attached to the Jamshidi needle and full negative pressure is applied in order to aspirate 28.5ml bone marrow (making up a total volume of 30ml). 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).

Once you have collected the bone marrow please refer to our 'Packaging & Transportation' procedures if you are sending the material to Biobest's laboratory for stem cell culture. If you are processing it patient-side using a centrifuge system please refer to the manufacturer's instructions.

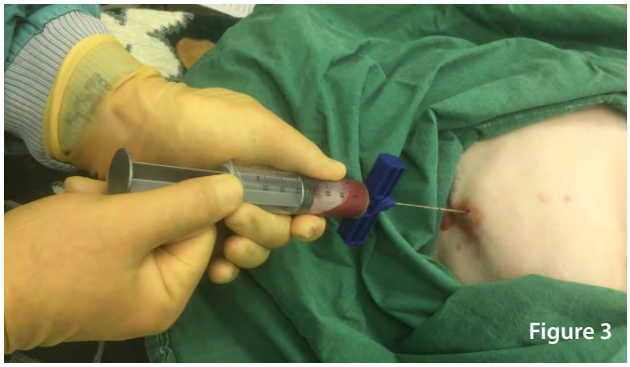


Figure 3

Harvesting Equine Adipose Tissue

Stem Cell Therapy Procedures

How to: Harvest equine adipose tissue

Equipment required

11 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 Harvesting kit (available from Biobest, Tel: +44 (0)131 440 2628)
Harvesting kit includes:
• 2 x 50ml plastic sterile universal containers (white)
• 2 x 8ml Serum Tubes
• 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)
• Stitch kit
• Sedative
• Clippers
• Harvesting instructions

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of equine orthopaedic 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 harvesting of adipose tissue is shown here. N.B. As the adipose tissue is delivered to the laboratory the day after it is obtained aspirations should only be carried out Monday-Thursday. We can offer a weekend service where possible, for an additional cost.

Technique

- Sedate the horse.
- Clip a 10cm^2 area from where the specimen will be taken.
- The area next to the tail head is blocked using a local anaesthetic
- Aseptically prepare the area.
- Under sterile conditions make a small vertical incision (figure 1).
- Lift the skin to expose the adipose tissue beneath and extract approximately two tablespoons (figure 2).
- The harvested tissue is placed into the sterile conical tubes for processing (figure 2).
- The incision sites are closed and dressed using a stent bandage (fig. 3).
- Two vials of 8ml of blood are also collected in the serum tubes (fig. 4).

Once you have collected the adipose tissue please refer to our 'Packaging & Transportation' procedures if you are sending the material to Biobest's laboratory for stem cell culture. If you are processing it patient-side using a centrifuge system please refer to the manufacturer's instructions.

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.

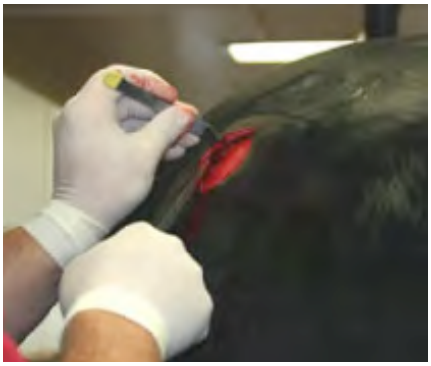


Figure 1 - Sterile incision site

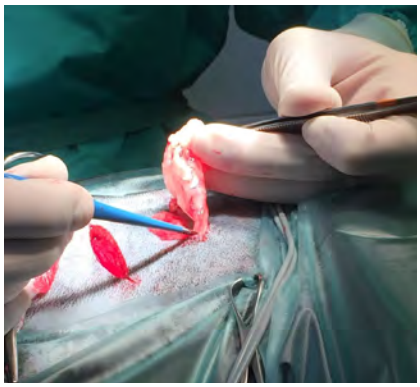


Figure 2 - Extraction of adipose tissue

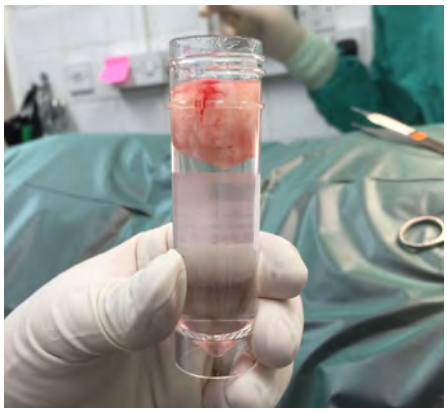


Figure 3 - Adipose tissue in conical tube for processing

Figure 4 - Blood and adipose tissue post collection

Aspirating from the Equine Sternum

Stem Cell Therapy Procedures

How to: Take an equine bone marrow aspirate 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 injuries – 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 injuries (see submission form guidelines) you will also need:
  - more 10ml syringes
  - more 5ml NaCit vacutainers

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of equine orthopaedic 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 equine bone marrow aspiration from the sternum is shown here. The tuber coxae is an alternative site for bone marrow aspiration (please refer to Nupsala's procedures "Aspirating from the Tuber Coxae").

Once you have collected the bone marrow please refer to our 'Packaging & Transportation' procedures if you are sending the material to Biobest's laboratory for stem cell culture. If you are processing it patient-side using a centrifuge system please refer to the manufacturer's instructions.

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).

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 sternebra A is recommended for aspiration.

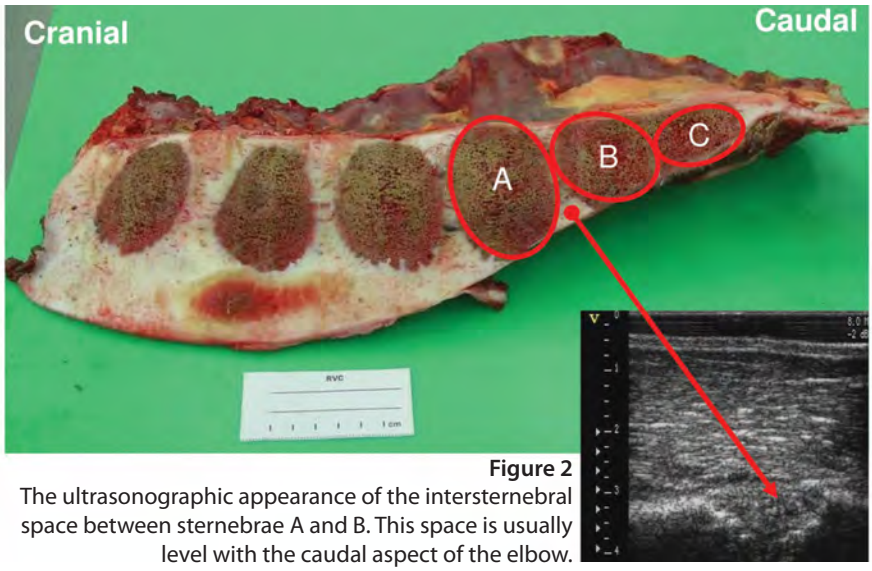


Figure 2
The ultrasonographic appearance of the intersternebral space between sternebrae A and B. This space is usually level with the caudal aspect of the elbow.

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.

Aspirating from the Equine Sternum

Figure 3
Marking with a staple the site of aspiration at the 5th sternebra

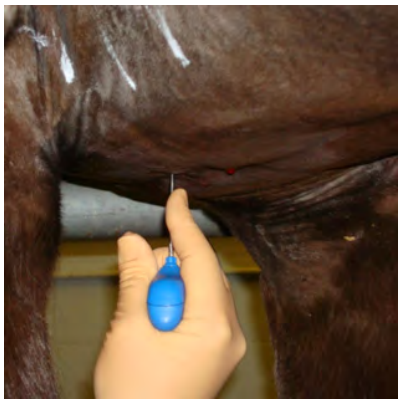


Figure 4
Insertion of Jamshidi needle. Once the Jamshidi needle makes contact with the ventral surface of the bone the index finger should be placed 1-2cm from the skin surface. This facilitates insertion of the needle to the correct depth.

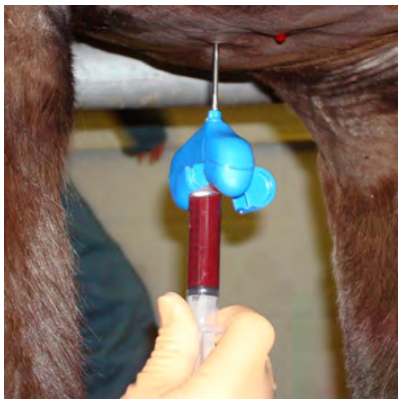


Figure 5
Successful aspiration of bone marrow

- 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 aneasthetic 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 midline, (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 injuries 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.

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

Aspirating from the Equine 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 injuries – 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 injuries (see submission form guidelines) you will also need:
  - more 10ml syringes
  - more 5ml NaCit vacutainers
- Aspiration & Implantation instructions
- 2 x 30ml syringes may be required to attain sufficient suction

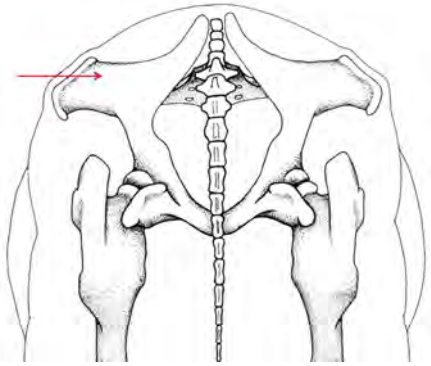


Figure 2
Caudal view of the tuber coxae indicating the points of palpation and site of entry for the Jamshidi needle

Stem Cell Therapy Procedures

How to: Take an equine bone marrow aspirate from the tuber coxae

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of equine orthopaedic 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 equine bone marrow aspiration from the tuber coxae is shown here. The sternum is an alternative site for bone marrow aspiration (please refer to Nupsala's procedures "Aspirating from the sternum").

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.

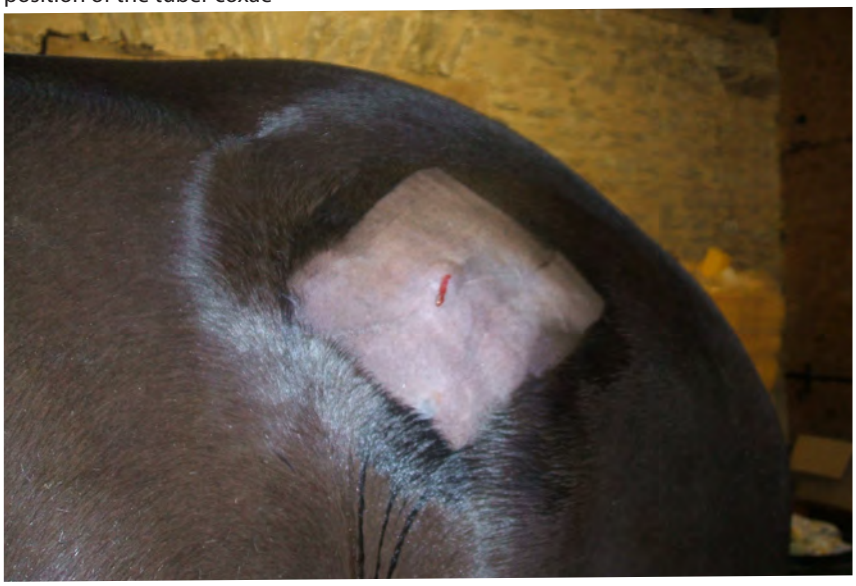
- The horse is first sedated with a combination of α2 agonist and opiate (e.g. detomidine HCl and butorphanol).
- A 10cm square area overlying the tuber coxae is clipped (figure 1).

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 Equine Tuber Coxae

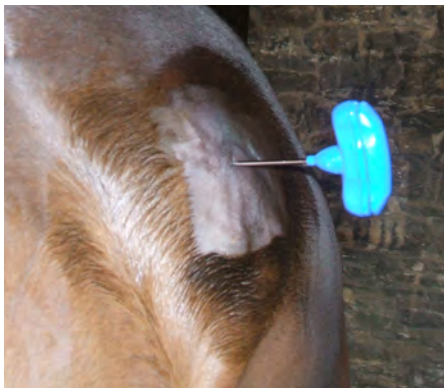
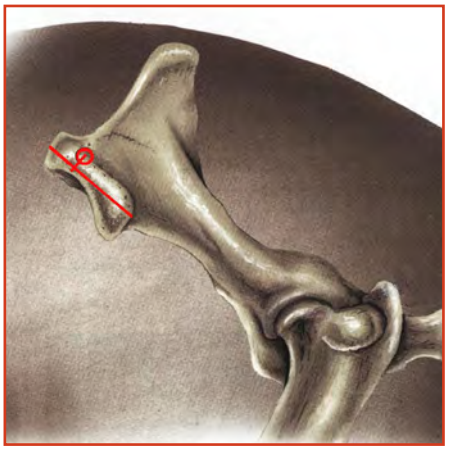


Figure 3 & 4
Insertion of Jamshidi needle. Once the Jamshidi needle makes contact with the caudolateral surface of the bone, advance the needle 6 cm, making sure the needle remains horizontal and square to the horse at all times.



- 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 injuries (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.

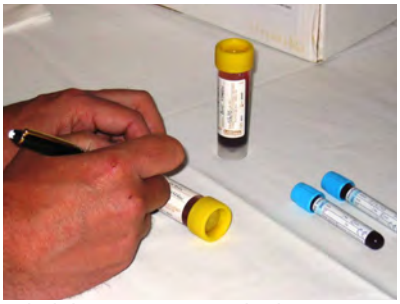
Once you have collected the bone marrow please refer to our 'Packaging & Transportation' procedures if you are sending the material to Biobest's laboratory for stem cell culture. If you are processing it patient-side using a centrifuge system please refer to the manufacturer's instructions.

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

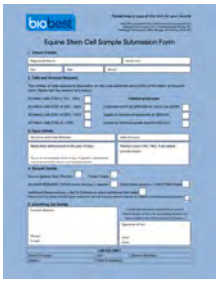
NUPSALA Veterinary Services

Your partner in regenerative medicine

Packaging & 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

Stem Cell Therapy Procedures

Packaging & transportation of material for lab processing (equine & canine)

Label universal containers and citrate tubes with the date and dog or 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 or '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

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

N.B. Please 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.

Canine Joint Injection Guidelines

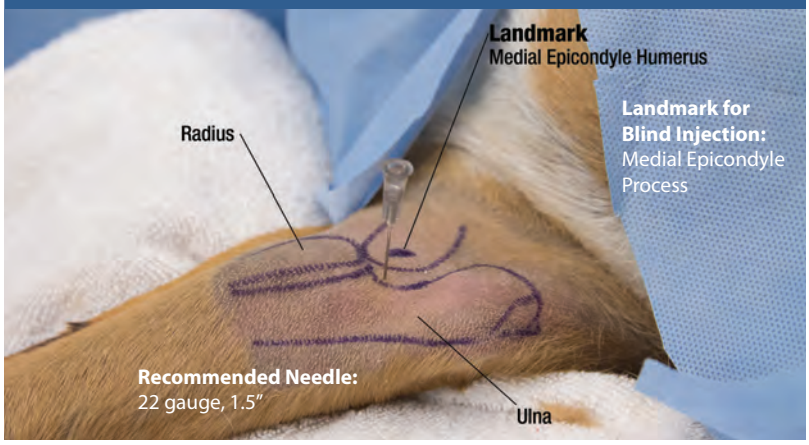
Stem Cell Therapy Procedures

Guidelines for canine joint injection

Elbow

• Osteoarthritis

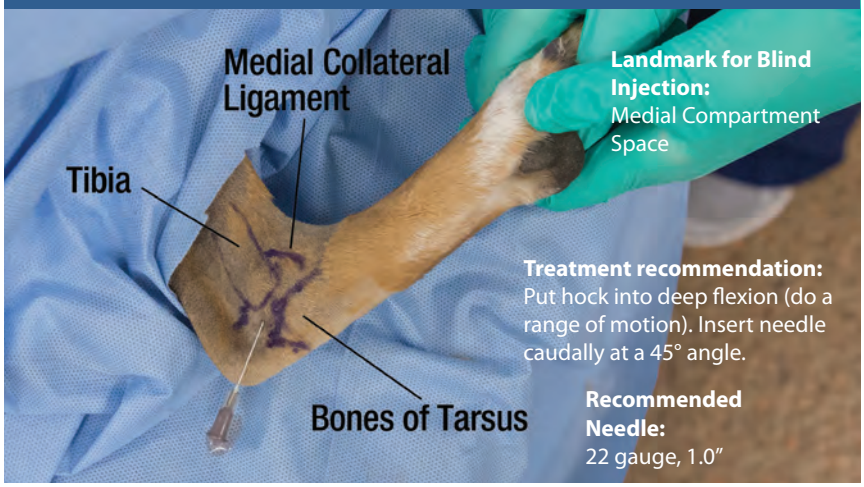
Total Injection Volume For Dog Size	
Miniature <5kg	0.25 - 0.5 ml
Small 5-12kg	0.5 - 1 ml
Medium 12-25kg	1 - 1.5 ml
Large 25-50kg	1.5 - 2 ml
Giant >50kg	2 - 2.5 ml



Hock

• Osteoarthritis

Total Injection Volume For Dog Size	
Miniature <5kg	0.25 ml
Small 5-12kg	0.25 - 0.5 ml
Medium 12-25kg	0.25 - 1 ml
Large 25-50kg	0.75 - 1 ml
Giant >50kg	1 ml



Images and information courtesy of Companion Regenerative Therapies

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

Canine Joint Injection Guidelines

Carpus

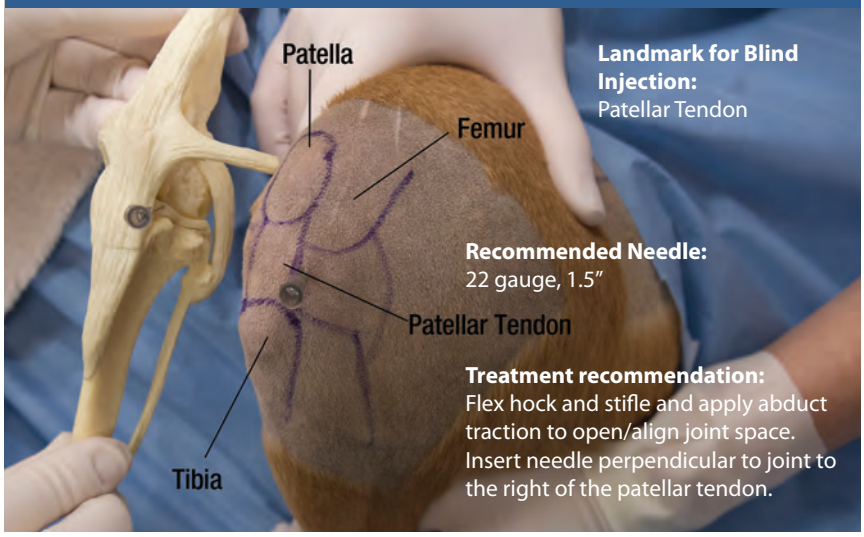
- Osteoarthritis

Total Injection Volume For Dog Size	
Miniature <5kg	<0.25 ml
Small 5-12kg	0.25 - 0.5 ml
Medium 12-25kg	0.25 - 1 ml
Large 25-50kg	0.75 - 1 ml
Giant >50kg	1 ml

Stifle

- Osteoarthritis
- Meniscal Injury
- Early Partial Tears

Total Injection Volume For Dog Size	
Miniature <5kg	0.5 ml
Small 5-12kg	0.5 - 1 ml
Medium 12-25kg	1 - 1.5 ml
Large 25-50kg	1.5 - 2 ml
Giant >50kg	2 - 3 ml

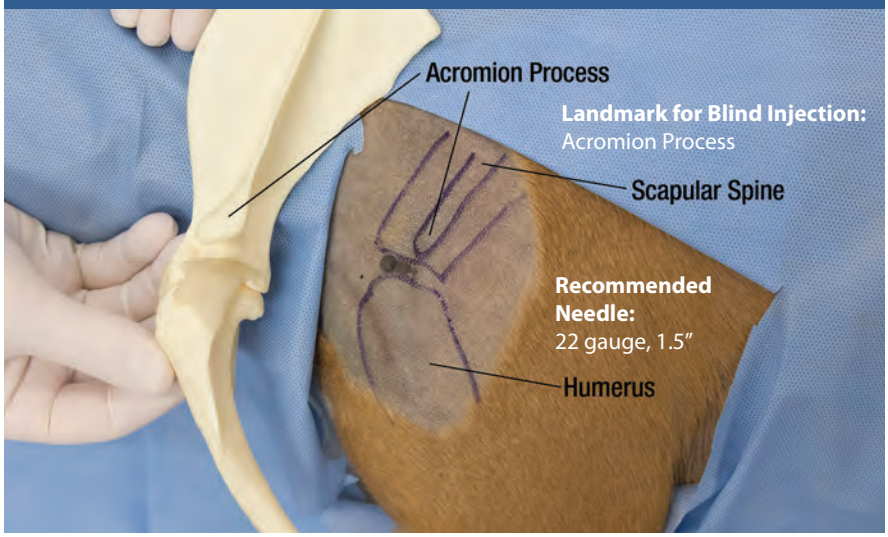


Canine Joint Injection Guidelines

Shoulder

- Osteoarthritis
- OCD
- Medial Shoulder Syndrome
- Bicep Tenosynovitis

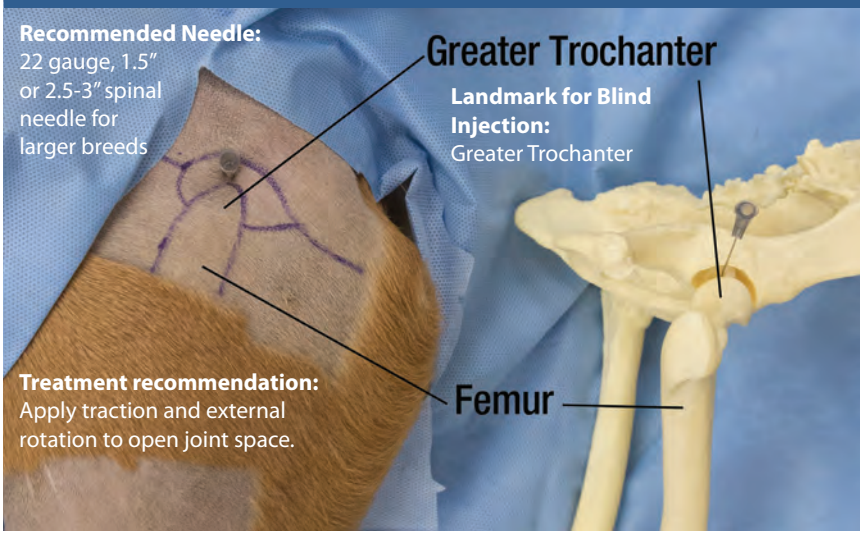
Total Injection Volume For Dog Size	
Miniature <5kg	0.5 ml
Small 5-12kg	0.5 - 1 ml
Medium 12-25kg	1 - 1.5 ml
Large 25-50kg	1.5 - 2 ml
Giant >50kg	2 - 3 ml



Hip

- Osteoarthritis

Total Injection Volume For Dog Size	
Miniature <5kg	0.5 ml
Small 5-12kg	0.5 - 1 ml
Medium 12-25kg	1 - 1.5 ml
Large 25-50kg	1.5 - 2 ml
Giant >50kg	2 - 3 ml



Equine Stem Cell Implantation

Stem Cell Therapy

How to: Implant stem cells into an equine tendon/ligament

Equipment required

1 x sterile drape 2 x 2ml syringes (for stem cells)
4 x 2ml syringes (for local analgesia)
1 x sterile arthroscopic camera sleeve (or similar)
1 x intrasite gel (or other sterile gel)
2 x 21G – 2 inch (50mm) needles 2 x 23G – 1 inch (25mm) needles
Sterile swabs
Local anaesthetic (LA mepivicaine)
Sedative (α2 agonist and opiate)
Neomycin / Penicillin (30ml sid x 3dd)
Stable support bandage material
Ultrasound
Clippers

Figure 1
The site for analgesia of the palmar and subcutaneous nerves at the subcarpal site

Figure 2
Localising needle in core lesion allows good spread of the cells throughout the lesion after implantation.

Introduction

Autologous mesenchymal stem cells are implanted as a treatment of tendon and ligament 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 VWD and only a VWD authorised laboratory should be used. The protocol for implantation of stem cells into a damaged tendon or ligament is described here.

Technique

The cells should be implanted as soon as possible after arrival and therefore implantation should normally be carried out on Tuesday–Friday (to avoid delayed transit of the cells over a weekend).

- Restrain and sedate the patient with a combination of α2 agonist and opiate (e.g. detomidine HCl and butorphanol).
- Clip the leg to be implanted to include subcarpal local anaesthetic sites
- Clean the site with surgical scrub (eg. chlorhexidine) and surgical spirit.
- Perform an ultrasonographic examination to identify the core lesion, its extent and the appropriate sites for stem cell implantation.
- Aseptically prepare the site for local analgesia.
- To ensure complete desensitisation of the skin overlying the tendon and superficial digital flexor tendon, both the palmar nerves deep to the metacarpal fascia and the subcutaneous nerve supply superficial to the fascia have to be 'blocked' on either side of the limb at the subcarpal site (figure 1). If the suspensory ligament is being treated, the palmar metacarpal nerves should also be 'blocked'.
- The palmar metacarpal region should then be prepared aseptically.
- In a sterile fashion, load 2 x 2ml syringes with each of the 1ml stem cell aliquots.
- Place the ultrasound transducer in a sterile sleeve (a sterile arthroscopic camera sleeve with the end sealed can be used for this purpose). Contact between the transducer and the skin is optimised with the use of scanning gel within the sleeve and intrasite gel on the outside.
- Using triangulation of a 21G – 2 inch needle and the ultrasound transducer longitudinally 'in line' with the needle (figure 2) the needle can be visualised entering the tendon (figure 3). Care should be taken to ensure the tip of the needle is visible ultrasonographically so that the end does not penetrate the deep surface of the tendon.

Equine Stem Cell Implantation

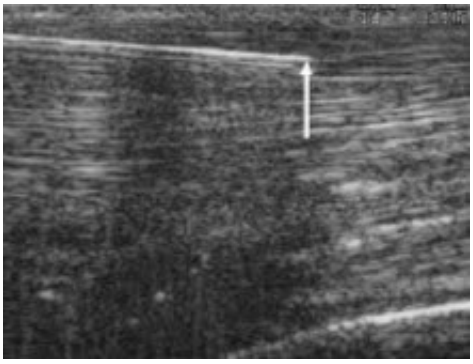


Figure 3
Visualisation of the needle entering the tendon

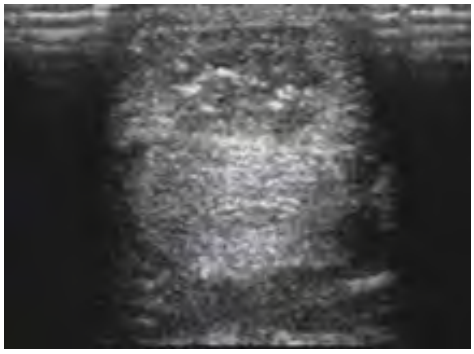


Figure 4
After injection air bubbles from the injection are present within the core lesion

- The first site of injection is usually the mid-point of the lesion as this often allows good spread of the cells throughout the lesion after implantation.
- Inject the stem cells into 1–3 sites depending on the nature of the core lesion (more advanced healing requires more injection sites due to less spread). Accurate placement is confirmed by the presence of air bubbles within (and only within) the core lesion (figure 4) which will also indicate the degree of spread.
- Bandage the limb immediately to minimise subcutaneous haemorrhage and loss of injected cells from the tendon.
- Administer intramuscular neomycin/penicillin and provide sufficient for a three day course.
- Discuss the post-operative exercise regime with the owner (please refer to Nupsala's "Equine Rehabilitation Instructions" which offers guidelines for this).

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.

NUPSALA Veterinary Services

Leading veterinary orthopaedic treatments

Canine Physiotherapy & Rehabilitation

Canine Rehabilitation Guidelines

Post implantation of stem cells, BMAC or PRP

The following rehabilitation instructions are a guide. Consideration should be given to the extent of the pathology, co-existing pathologies and the structure treated. The dog should be rested for an initial 24-hour period, taken for toileting on a lead. Post suture removal commence input 1-2 times per week. This should be guided by an ACPAT registered Veterinary Physiotherapist. Commence Underwater Treadmill Therapy- commence at 8 weeks (post vet review). This should be 1-2 times per week until 12 weeks or the patient has equal muscle mass.

DO NOT INCLUDE:
- Ice to the affected joint
- Shockwave therapy
- NSAIDs
- Class 4 Laser
- Therapeutic Ultrasound
- NMES

Treatment can consist of:

- Manual Physiotherapy including isometric exercises, proprioception exercises and PROM
- Facilitation of early and equal weight bearing
- Class 3b Cold Laser

Physiotherapy should continue until full ROM (range of motion), equal weight bearing and normal exercise has resumed.

The dog should be walked on a lead until he is fully weight bearing. The duration of this walk exercise should be increased by 10 minutes every week. Steps, inclines and jumping should be avoided until he is fully weight bearing

Owner's Home Plan

Rehabilitation after your dogs treatment can be incorporated into your normal day and exercise regime. Below are 5 simple things you can do to help your dog gain movement and strength. It is also recommended that you work with an ACPAT Veterinary Physiotherapist to help your dog resume full activity as quickly as possible.

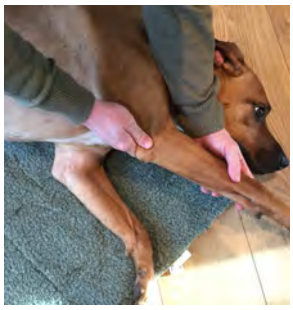
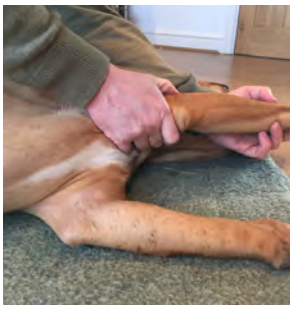
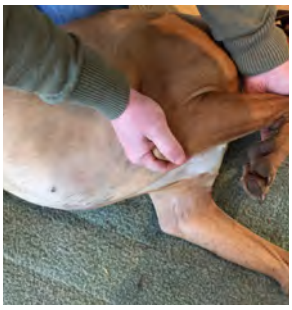
1. Sit to stand

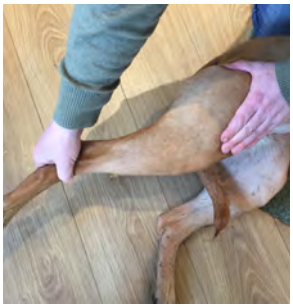
Aim to do 3-5 times. Use a treat, ask your dog to sit, then stand,

Canine Physiotherapy & Rehabilitation

2. Range of movement

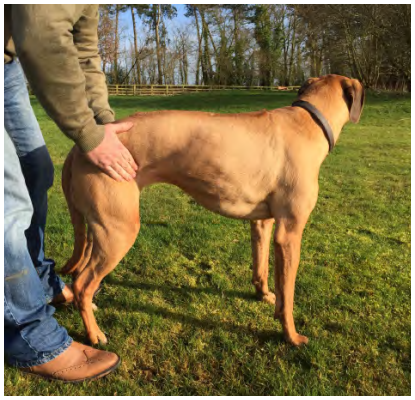
During your "down time" with your dog slowly and carefully move the leg through its whole range of movement as demonstrated to you by your vet. It is very important you move with your dog, not against any resistance. Repeat up to 5 times.

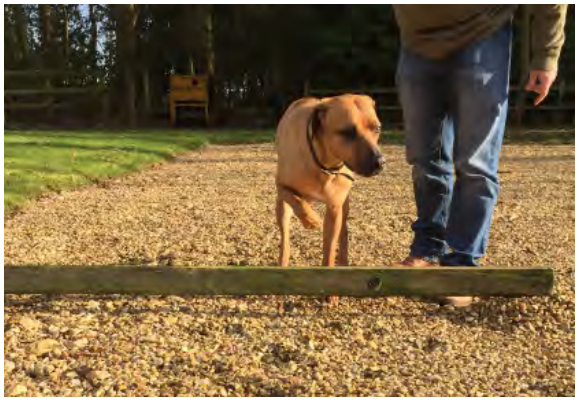

3. Weight shift

Stand the dog squarely and shift his weight forwards, backwards and side to side using your hands on his body or pelvis. This can be done whilst he's eating, but we recommend using a raised bowl or placing it on a step. Again repeat 5 times. If your dog takes a step you have moved him too far.

4. Obstacles

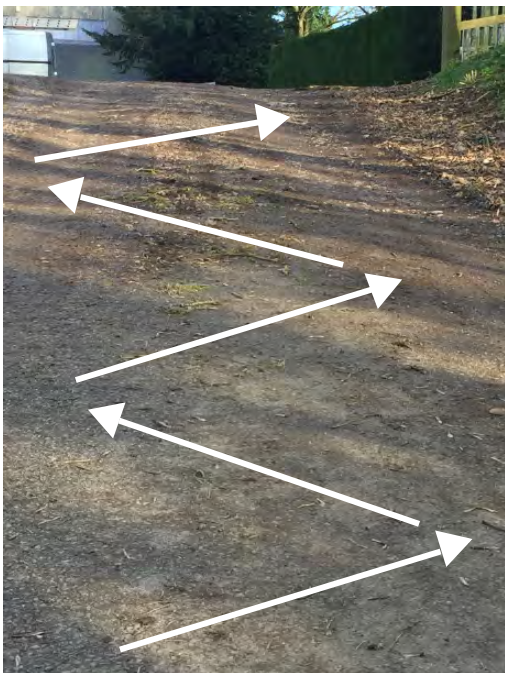
Step over poles or sticks laid in the garden or obstacles you come across on your walks. Sticks should be small - just enough for your dog to have to take a larger than normal step. You will find lots of small obstacles whilst you are out on your daily walk.



Canine Physiotherapy & Rehabilitation

5. Zig-zags

Step up and down the curbs of the pavement in a zig-zag whilst you're walking and zig-zag your way up and down hills or inclines. It is important to not use really steep hills.



Monitor Carefully

With all of these exercises it is important to monitor your dog's behaviour, response and progress. They shouldn't find the exercises too difficult or tiring. If they look stiff or sore afterwards or the next day you have most likely worked them slightly too hard. They should also not resent you doing the exercises either. If in any doubt contact your vet or physiotherapist for advice.

This is a general guide to some basic initial rehabilitation provided by our ACPAT Chartered Physiotherapist (Helen Millward SRP, BSc Hons Physiotherapy, MSc Veterinary Physiotherapy, ACPAT Cat A). We recommend that further rehabilitation is guided by your local fully qualified practitioner or contact Helen via the Nupsala office.

Millwards Veterinary Physiotherapy

Equine Rehabilitation Instructions

Equine Rehabilitation Instructions
Post implantation of stem cells, BMAC or PRP

The following rehabilitation instructions are a guide, giving controlled ascending workload. Consideration should be given to the extent of the injury, pathology elsewhere or within the opposite leg and the structure treated. In addition to the protocol below the following information can help you tailor the rehabilitation:

- Record the cross sectional areas of the treated and non-treated tendon or ligament. Do this for both fore or hind limbs depending on whether it is a forelimb or hindlimb injury. Any increase greater than 10% is indicative that the workload is too high and needs to be decreased.
- Try to examine the limbs ultrasonographically before increasing from walk to trot or from trot to canter or when introducing jumping or galloping.
- As a rule the workload should be increased by 10% duration or intensity per week, but not both. Therefore if you want to introduce trotting into the exercise you need to decrease the length of time at walk.
- Where possible avoid using horse walkers. Walk in hand or ride and lead.
- Turn out is not recommended until after 24 weeks and should be in a paddock or pen no larger than four stables in size for the first few weeks.

Phase 1

During Phase 1 it is important to restrict and control the workload on the treated and non-treated limbs to allow for tissue repair and growth. Optimal loading is essential to achieving good tissue regeneration, too much is deleterious. During colder months maintain stable bandages when the horse is not being exercised to keep the limbs warm, but do not bandage too tight. Ridden exercise should not begin until Phase 2 so Phase 1 must be done in hand.

Phase 2

At this point the intensity will increase with the addition of trotting. Ridden exercise can commence. If possible examine the limbs ultrasonographically, checking cross sectional area measurements and fibre alignment.

Phase 3

At this point intensity increases with the introduction of canter. If there is no intention to increase fitness, turn out at this point is feasible. Before doing either, examine the limbs ultrasonographically, checking cross sectional area measurements and fibre alignment.

Exercise from week 40 onwards should be tailored to the requirements of the individual use of the horse. Refrain from strenuous competition until 12 months post initial treatment. When possible examine the limbs ultrasonographically prior to any competition.

Week 1	*Initial box rest for 3-4 days following treatment, thereafter 5 minutes walk in hand*
Week 2 - 4	*Gradually increase the walk to 15 minutes by the end of week 4*
Week 4 - 8	*Gradually increase the walk to 30 minutes by the end of week 8*
Week 8 - 12	*Gradually increase the walk to 60 minutes by the end of week 12*
Week 12 - 16	*Introduce trotting at 5 minutes with 55 minutes of walk increasing to 10 minutes of trotting by the end of week 16*
Week 16 - 20	*Exercise at 45 minutes walk 15 minutes trotting, increasing to 20 minutes trotting by the end of week 20*
Week 20 - 24	*Exercise at 45 minutes walk 20 minutes trotting, increasing to 30 minutes trotting by the end of week 24*
Week 24 - 30	*Gradually increase the exercise to the end of week 30 to include 20 minutes trotting & 10 minutes canter*
Week 30 - 34	*Maintain workload at no greater than 20 minutes trotting & 10 minutes canter*
Week 34 - 40	*Increase intensity to 20 minutes trotting and 15 minutes canter*

Freeze-Thawing PRP & ACS

How to: Freeze-thaw PRP (platelet rich plasma) and ACS (autologous conditioned serum)

Equipment required

Tubes containing PRP or ACS
0.2-micron filters (2-4 required)
LL Neg connector
3-inch blunt cannula
20ml syringe
2ml syringe and syring cap
Transfer needle (any guage)
Freezer at -18 degrees

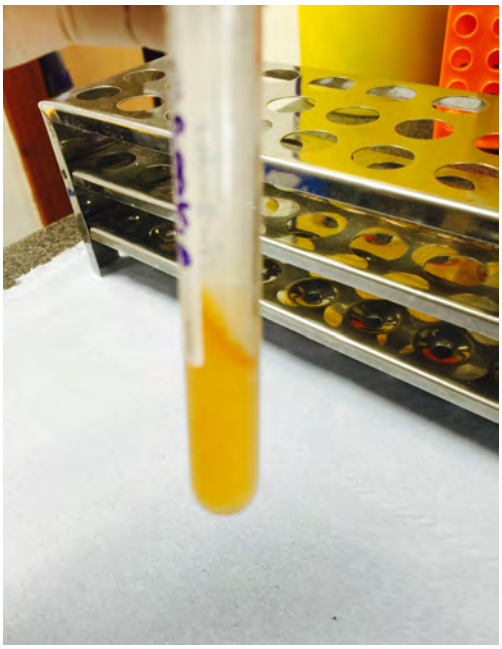


Figure 1
Frozen serum or PRP

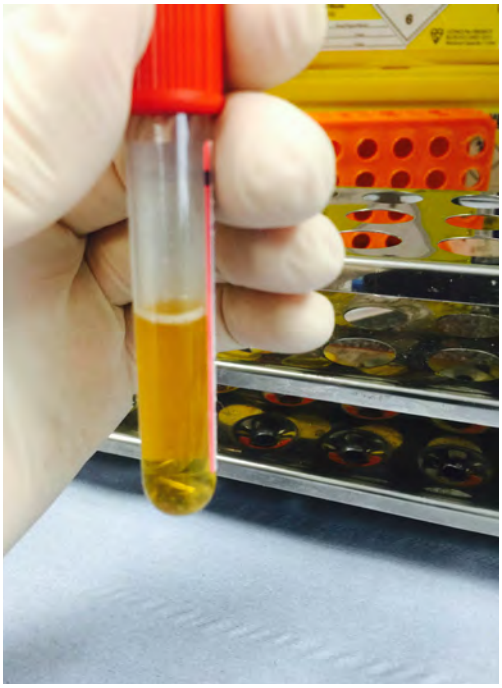


Figure 2
Thawed serum or PRP

Introduction

PRP and ACS can be frozen for leukocyte reduction ot to use as a treatment for joint tissue regeneration. The heterogeneous clinical outcome reported in the literature on PRP treatment for joint tissue regeneration reflects the lack of guidelines regarding the use of platelet concentrates, starting from their production up to their clinical application. The increasing awareness on the need for PRP standardisation is shown by the numerous biological studies investigating the role of each PRP variable on the healing potential of platelet concentrates.

Among these factors, the possibility to store PRP and the effects of freeze/thawing PRP remain a controversial point. Although some researchers avoid freeze/thawing, fearing deleterious effects on platelet function and GF release, others consider it to be a procedure that physically activates PRP and further leukocyte destruction as a result of freezing leads to reduction in Il-1β, pro inflammatory cytokine.

Roffi et al 2014, showed that growth factor release from platelets α-granules in frozen PRP both the immediate and at 7-day release were lower with respect to that of the fresh preparation, but without affecting the ability of PRP to induce proliferation and ECM production in chondrocyte and synoviocyte cultures. The only significant difference was detected for synoviocyte HGF expression, which was higher in the freeze/thawed PRP induced cells, thus suggesting that PRP cryopreservation is a safe procedure, which sufficiently preserves PRP quality and its biological activity.

Furthermore, with regard to pro-inflammatory cytokines, the immediate release of IL-1β does not significantly differ in fresh PRP with respect to that of frozen PRP. The absence of an increment of IL-1β detected in frozen PRP might be explained by the fact that freezing and thawing leads to leukocyte destruction (which can produce inflammatory cytokines directly and indirectly by platelet stimulation), resulting in a reduction in WBC number, preventing the "de novo" synthesis of this cytokine.

"PRP or ACS must be thawed thoroughly before processing to avoid clogging the filter"

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.

NUPSALA Veterinary Services

Leading veterinary orthopaedic treatments

Freeze-Thawing PRP & ACS

Technique

- Collect PRP or ACS via manufacturer's instruction and collect enough for the treatment. Excess product can remain frozen for future use;
- Transfer the PRP or ACS to a sterile vaccutainer with no added substance (red top) 4ml in each tube;
- Place the tube(s) in a zip lock bag or container to keep the tubes for that patient together;
- The tubes are then placed in the freezer for a minimum of 45 mins or until completely frozen (Figure 1);
- Long term storage of PRP or ACS should not exceed 6 months

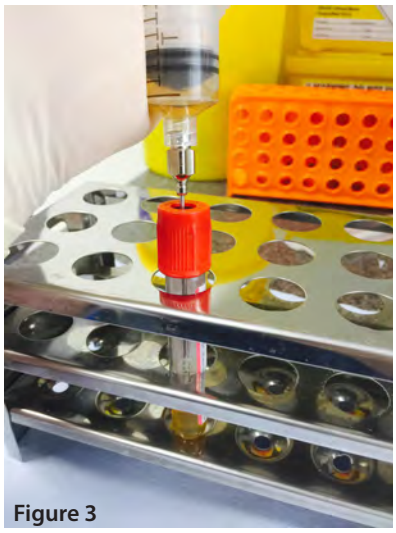


Figure 3

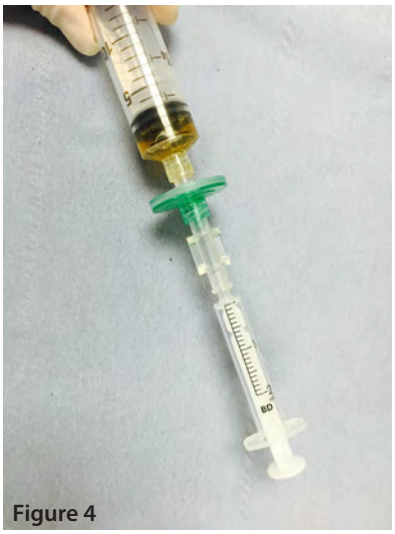


Figure 4

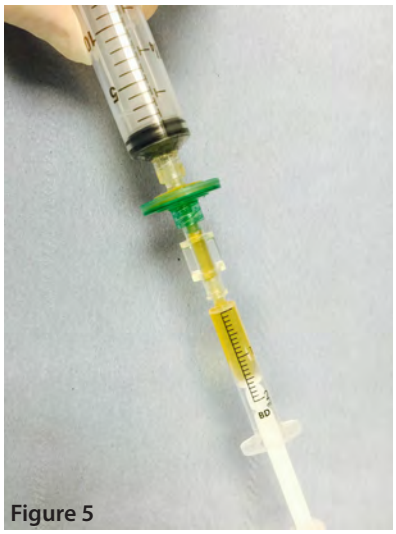


Figure 5

- Remove from the freezer. One tube will give approximately 2ml of final preparation.
- Thaw the frozen PRP completely – holding in your hand for 10mins is normally enough (Figure 2).
- Withdraw the PRP (Figure 3) and pass through a 0.2micron filter (Figure 4 & 5) to give the final preparation. If the filter becomes blocked, replace with new filter. Ice crystals can block the filter so ensure the tube is completely thawed.
- Place a cap or needle on the syringe (Figure 6)
- Once the final preparation is produced it should be used immediately.


Figure 6

References

C. T. FJORDBAKK, G. M. JOHANSEN†, A. C. LØVÅS, K. L. OPPEGÅRD and A. K. STORSET (2014) Surgical stress influences cytokine content in autologous conditioned serum Equine Vet J.

Hraha, T.H., Doremus, K.M., McIlwraith, C.W. and Frisbie, D.D. (2011) Autologous conditioned serum: the comparative cytokine profiles of two commercial methods (IRAP and IRAP II) using equine blood. Equine Vet. J. 43, 516-521.

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

Grading & Treatment of Canine OA - A

	Clinical and Imaging Observations	Joint Pathology
Grade 1	History of damage, dysplasia or surgery Intermittent lameness Little visible on radiographs	Early cartliage pathology with some surface disruption Early synovitis
Grade 2	Intermittent or mild lameness Some discomfort on manipulation Mild effusion Early marginal osteophytes Early subchondral sclerosis Synovial fluid less viscous	Cartilage focal fissuring Established mild patchy synovitis
Grade 3	Stiffness Some reduction of joint movement Crepitus / some pain Sclerosis and osteophytes X-Ray Synovial fluid physical changes	Cartilage with some focal erosions Chronic synovitis Osteophytes obvious at margins Some fibrous hypertrophy of joint capsule
Grade 4	Obvious thickening and restriction of joint Crepitus and pain Marked bony changes visible on X-Ray Effusion and synovial fluid changes possible	Cartilage loss and erosion Established chronic synovitis with thickened fibrotic synovium Peripheral nociceptor transformation Osteophytes obvious Thickening of fibrous joint capsule
Grade 5	Obvious pain on manipulation Reduced movement in enlarged joint Crepitus obvious Limited response to pain medication	Extensive cartilage loss and disruption Bone remodeling under cartilage with deformation of joint Chronic synovitis with extensive fibrosis Peripheral and central neuroperception increase with central plasticity Extensive marginal osteophytes Very thickened joint capsule

guideline for clinicians

Treatment Options	Strategy
NSAIDs	Inhibition of prostaglandins. Limit to 3 months and review after 7 days of non use
Corticosteroids IA injection e.g. Triamcinolone 1mg per joint	Inhibition of phospholipase, inflammatory cytokines and enzymes
ACS-IRAP IA injection 7 days apart for 3 or 4 treatments	Target inflammatory cytokines (IL-1)
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Platelet rich plasma (PRP)	Through growth factors released from the platelet: regulate collagen synthesis, stimulate cell proliferation, cell chemotaxis and angiogenesis. Growth and differentiation of chondrocytes.
Hyaluronic acid (HA) IA injection no less than 2 million Dalton	Viscosupplementation
Corticosteroids IA injection e.g. Triamcinolone 1mg per joint	Inhibition of phospholipase, inflammatory cytokines and enzymes
Platelet rich plasma (PRP)	Through growth factors released from the platelet: regulate collagen synthesis, stimulate cell proliferation, cell chemotaxis and angiogenesis. Growth and differentiation of chondrocytes.
Hyaluronic acid (HA) IA injection no less than 2 million Dalton	Viscosupplementation
Corticosteroids (IA injection e.g. Triamcinolone 1mg per joint)	Inhibition of phospholipase, inflammatory cytokines and enzymes
Platelet rich plasma (PRP)	Through growth factors released from the platelet: regulate collagen synthesis, stimulate cell proliferation, cell chemotaxis and angiogenesis. Growth and differentiation of chondrocytes.
Bone marrow aspirate concentrate (BMAC) **Stromal vascular fraction (SVF)**	Stem cells to modulate cell proliferation and differentiating young cells through the continuous synthesis of growth factors
Arthramid Vet	A visco-elastic implant with tissue integration to protect synovial tissue and provide joint lubrication
Bone marrow aspirate concentrate (BMAC) **Stromal vascular fraction (SVF)**	Stem cells to modulate cell proliferation and differentiating young cells through the continuous synthesis of growth factors
Arthramid Vet	A visco-elastic implant with tissue integration to protect synovial tissue and provide joint lubrication

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