

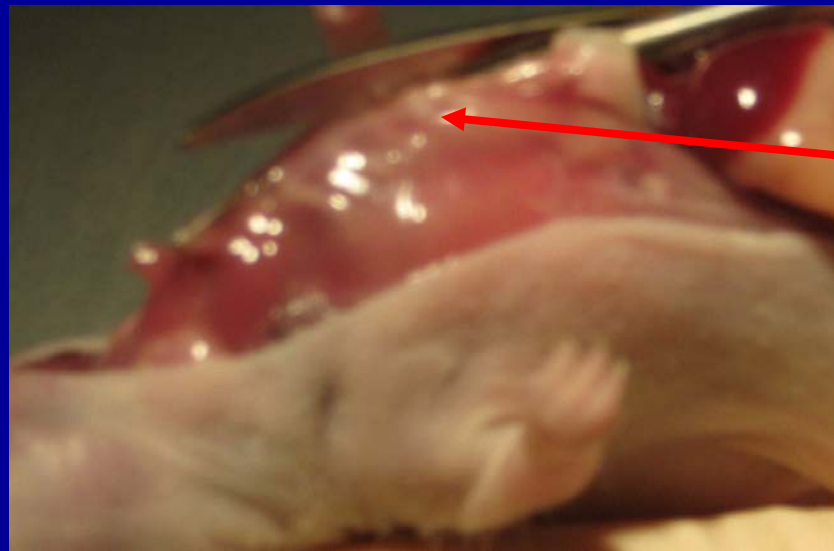
Perfusion Techniques

- Perfusion is done to fix tissues. In humans this is known as embalming.
- To reduce the amount of fixative needed and allow for good perfusions, you may be asked to do a “whole body” or a “target” perfusion.
- For a target perfusion, the brain is typically the tissue of interest.
- We will focus on the collection of brain, and mention differences in procedure to include whole body collection.

Perfusion Techniques

- **Euthanatize animal using CO₂.**
- **To ensure blood flow is not compromised, remove and perform cervical ASAP.**
- **Do not be over-zealous when performing cervical – it is essential that the jugulars and carotids remain intact!**

Perfusion Techniques.



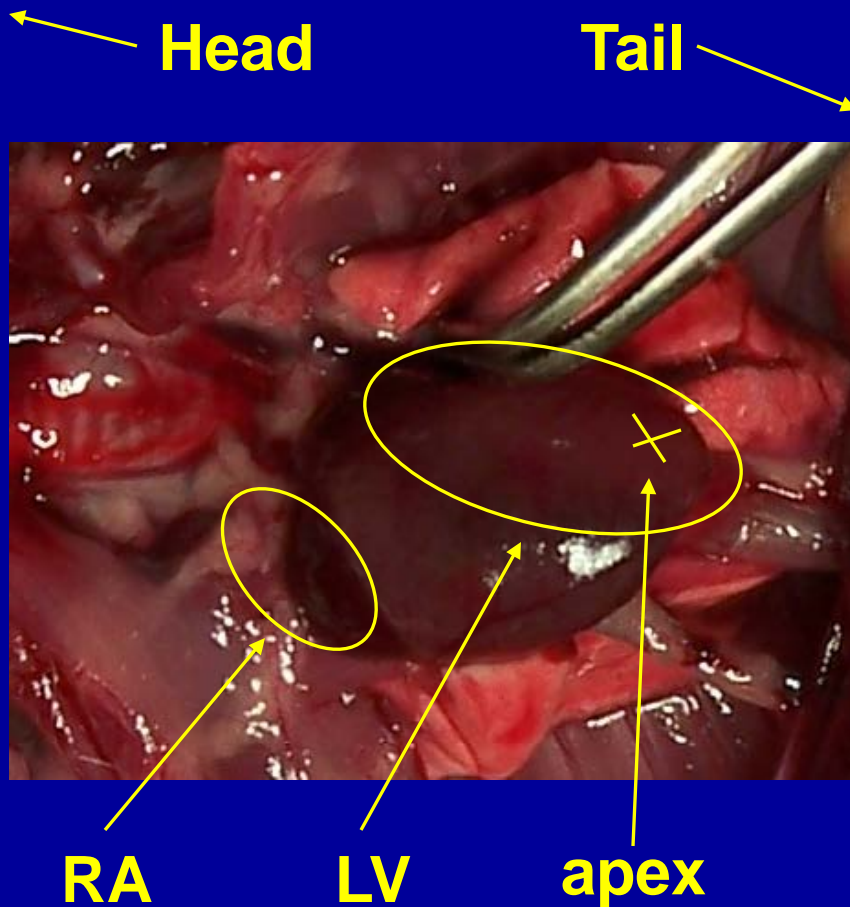
- Incise the skin from pelvis to chin.
- Incise abdominal muscle. Extend through thorax to the chin. Do not damage underlying organs (liver, heart, major vessels). Keep scissors tips **UP!**
- Where scissors pass from abdominal cavity to thoracic cavity, make sure tips are well **above** the heart.
- Split the sternum in one clean cut by sliding the lower blade all the way under the manubrium prior to closing the blades.

Perfusion Techniques

- Grasp the now separated rib cage halves. Gently 'roll' them back – you will hear some cracking as you open the area to better visualize the heart.
- Over zealous spreading or breaking of the ribs can result in them detaching or breaking behind the heart and being propelled into the ventricles. It can also result in tearing of the jugulars and carotids – **BE GENTLE!**



Perfusion Techniques



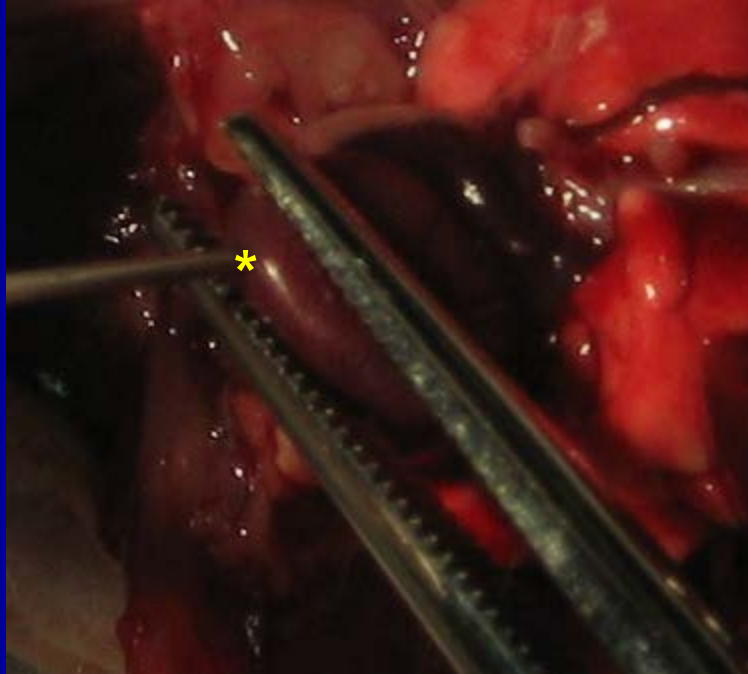
- Identify the left ventricle (LV), the apex of the heart, and the right atrium (RA).
- Position the mouse so that the head is towards the left and the tail to the right.
- In this position the LV will be the lighter colored, uppermost half of the heart, with the apex located at the tip of the LV.
- The RA will be located under/behind the lower, darker colored right ventricle.

Perfusion Techniques

- If collecting brain only, a pair of curved hemostats will be inserted (tips down) just above the liver and just below the lungs, and then clamped.
- This will occlude the major blood vessels (thoracic aorta and vena cava) to the lower body.
- If performing a whole body perfusion, skip this step.



Perfusion Techniques

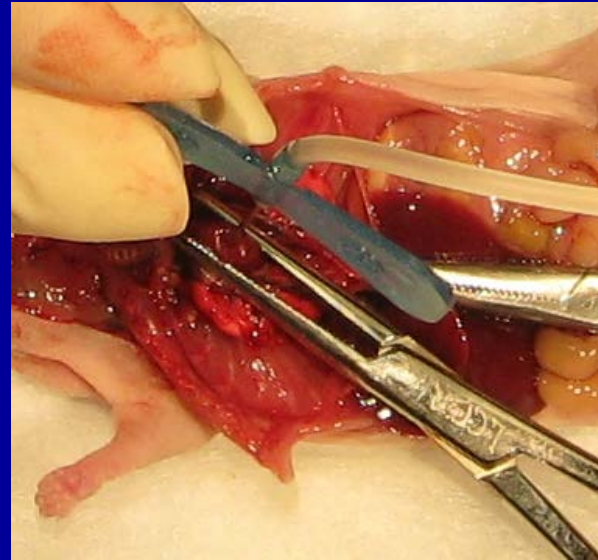


- Position a pair of straight tipped hemostats at the apex of the heart – the minimal amount of tissue necessary to hold the heart and clamp the perfusion needle is all that needs be grasped.
- Hold the heart upright by gently grasping – but not closing the instrument tips – so that a perfusion needle may be inserted perpendicularly through the tips and into the LV.

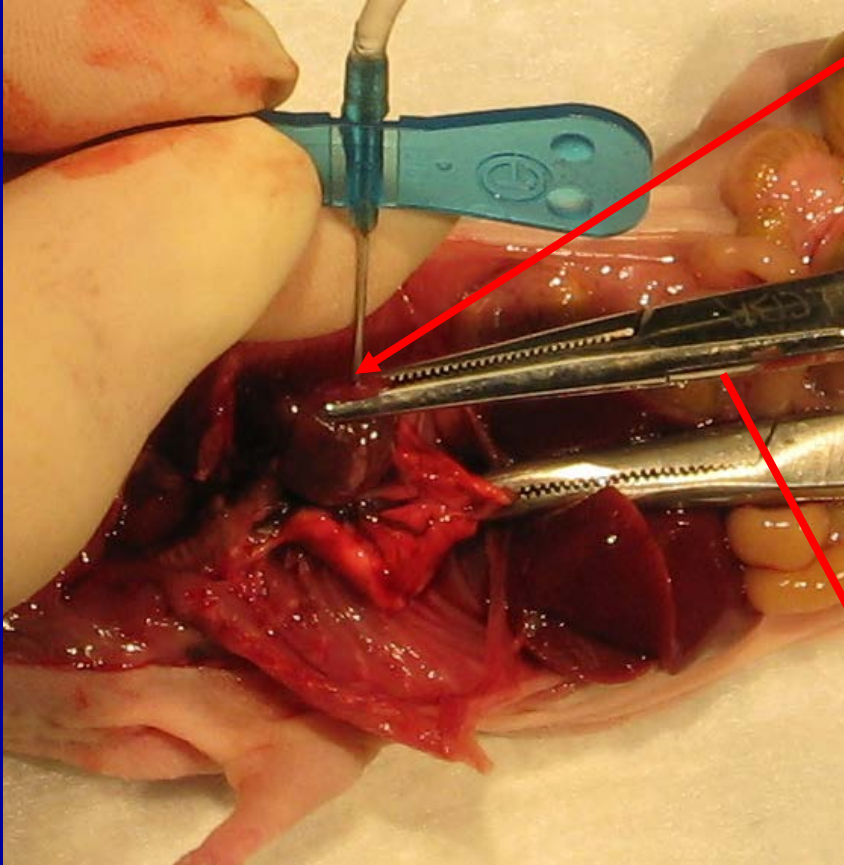
* Needle tip positioned at apex of LV

Perfusion Techniques

- The perfusion rig is generally a butterfly catheter with a 25g needle attached to a 5cc syringe (10 cc if whole body).
- The bevel of the needle is inserted into the LV, just through the instrument tips.
- Position the needle directly between the tips and insert straight down, with the bevel facing away. Clamp the needle.



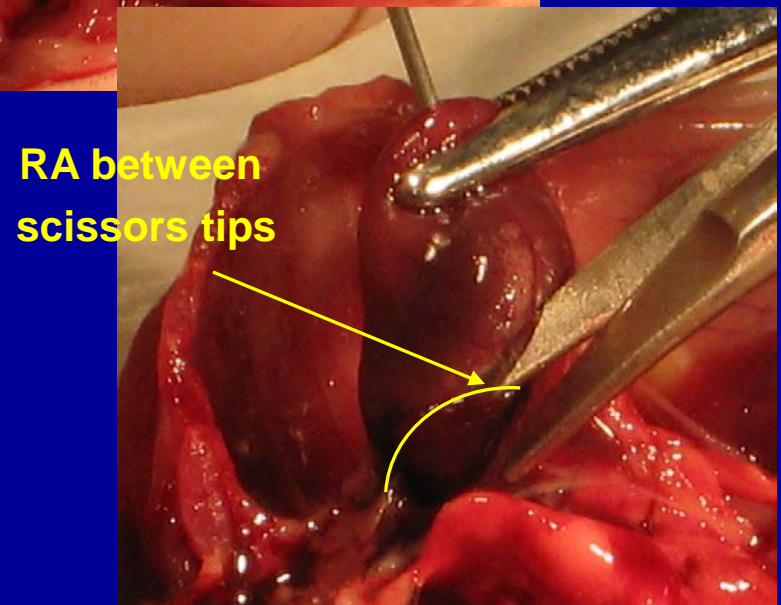
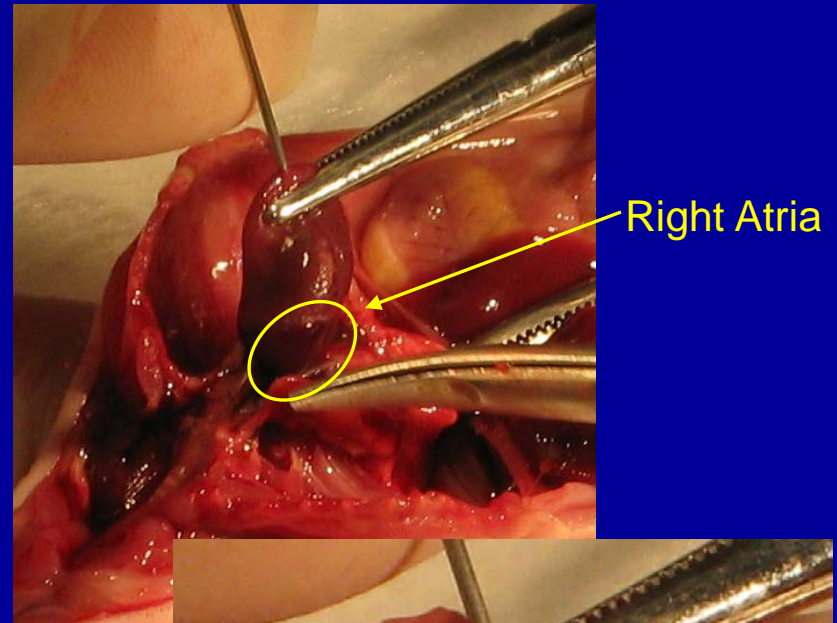
Perfusion Techniques



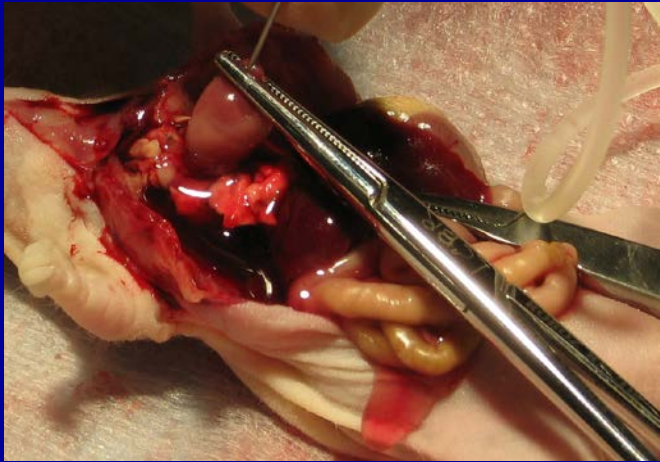
- Once the tip is through, the hemostats are clipped shut around the needle.
- **DO NOT LET GO OF THE BUTTERFLY CATHETER WING!**
- Continue to hold the catheter wing in a manner to ensure the needle tip does not pass through the backside of the ventricle.
- You may now gently rest the hemostats on the animal.

Perfusion Techniques

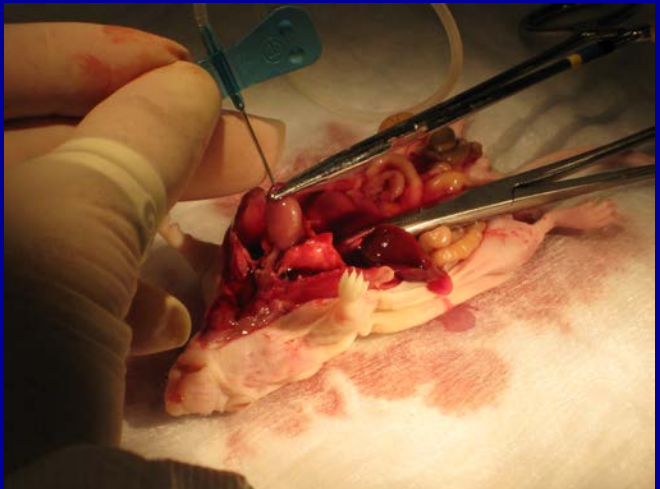
- While gently retracting the heart slightly upwards and tilted away from you, identify the RA.
- It is a dark, reddish-purple, flattened structure that wraps up next to the ventricle on that side.
- Using blunt tipped scissors, slide along the ventricle down towards the RA. The atrium will flip outwards. Clip.
- Dark venous blood flowing into the cavity = A successful clip.



Perfusion Techniques

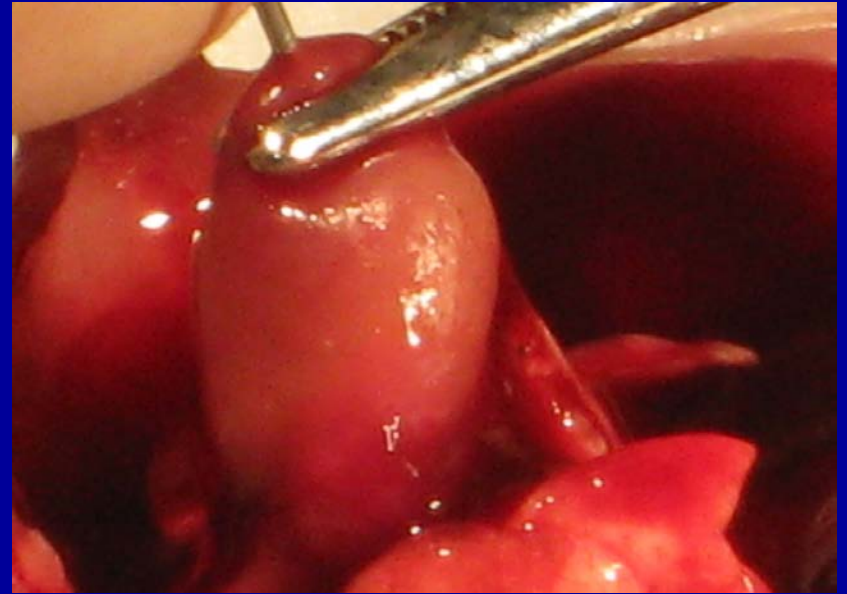


- **Begin perfusion; rate is slow and steady; 3-5 cc volume to get good brain perfusion; 8-10 for whole body.**
- **Successful perfusion is noted by concurrent 'twitching' as perfusate replaces blood within the vessels.**
- **Blood will flow out from the clipped atrium, turn gradually lighter, and finally become clear as perfusion continues and blood is replaced with perfusate material.**
- **When using formaldehyde perfusate, work in a hood.**



Perfusion Techniques

- Once perfusion is complete (heart will appear blanched), unclamp the hemostats, remove the needle, and begin brain collection.
- Remove the skin over the skull. The brain should appear visibly white through the bone, indicating blood has been flushed from the vessels.



Perfusion Techniques

- Remove the “skull cap” by first scoring the bone along the transverse suture, over the transverse sinus.
- One scissor blade is slid under the flap of bone, the blade then turned so it may be introduced in cutting position.
- The entire blade is slid under the bone, taking care to stay on midline, in the natural depression between the cerebral hemispheres. Cut.



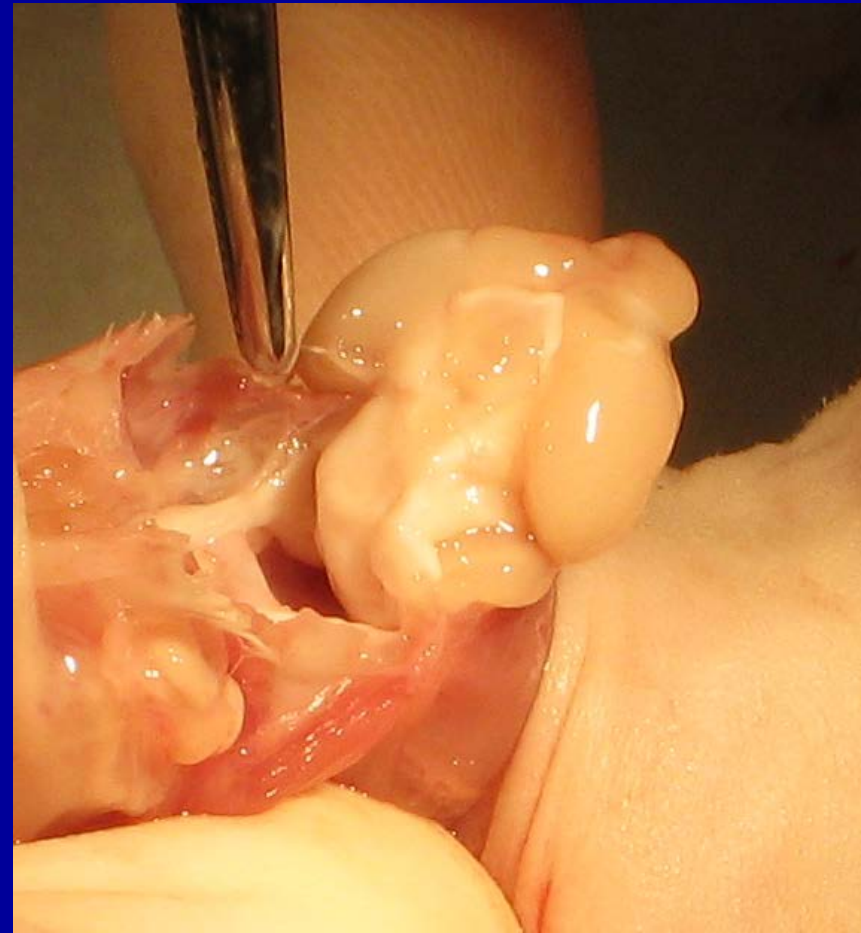
Perfusion Techniques

- Using blunt, fairly stout forceps or hemostats – slip one tip under the cut half-flap and lift up.
- DO NOT close the instrument tips – this will cause just the corner to break off. Keep the tips spread apart to lift the half-flap up/off the brain.

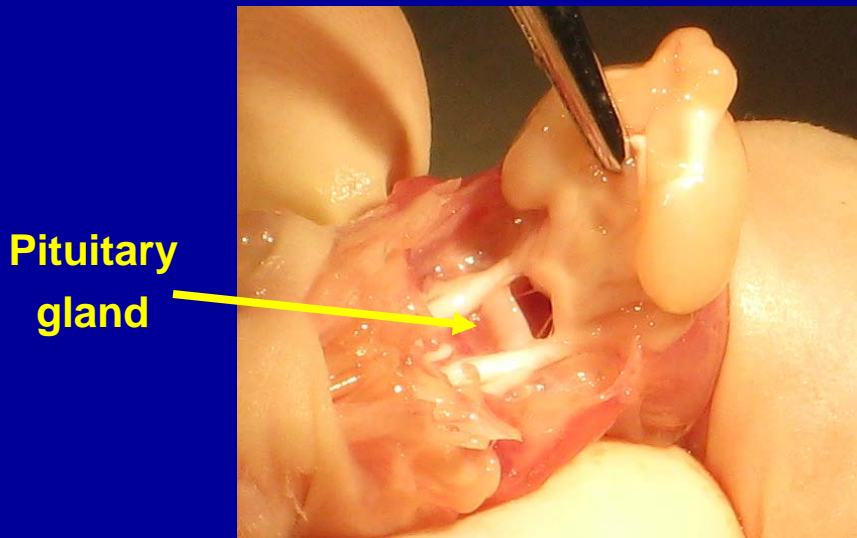
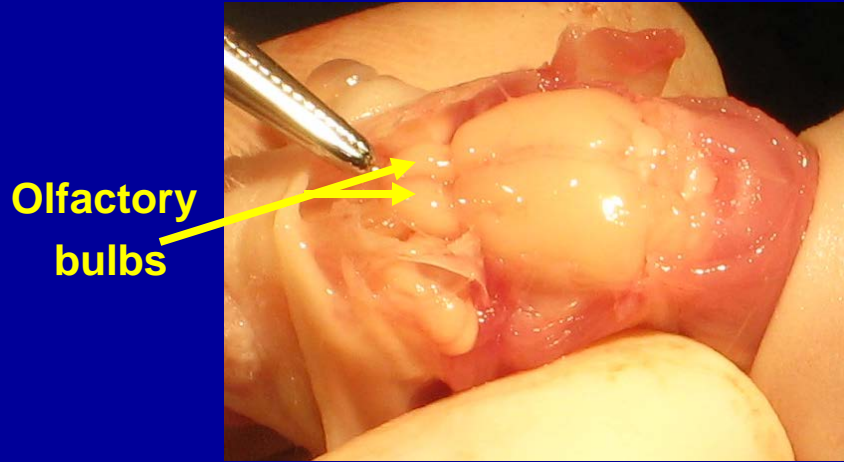


Perfusion Techniques

- **Blunt tipped forceps are gently pushed along the back curvature of the skull and then used to lift the brain forward.**



Perfusion Techniques



- If research requires olfactory bulb harvest, these are located just rostral (towards the nose) of the brain and appear as two small ovals.
- The pituitary is located between the optic nerves and may be gently blunt-dissected out.

Perfusion Techniques

- The entire brain should appear blanched and the vessels cleared of blood. It will feel firm and slightly rubbery.
- Cassetting the brain for processing can be done with just a plain cassette.
- If submitting the pituitary gland, a sponge must be included, with the sponge corner folded only over the gland.



Perfusion Techniques - Troubleshooting

If perfusate does not flow, check to ensure that the needle tip is not occluded by the hemostats.

If the lungs begin to inflate and perfusate discharges from the nose, reposition the needle. It is in the right ventricle, not the left, where it should be.

You may not get complete perfusion if in the right ventricle. Much of the perfusate will simply exit immediately through the clipped right atrium.

When doing a whole body perfusion, complete blanching of the liver to a beige/light tan is a strong indication of correct total body perfusion.