PROSECTION OF THE MOUSE

General Outline

University of South Florida

2011

Prepare Work Area

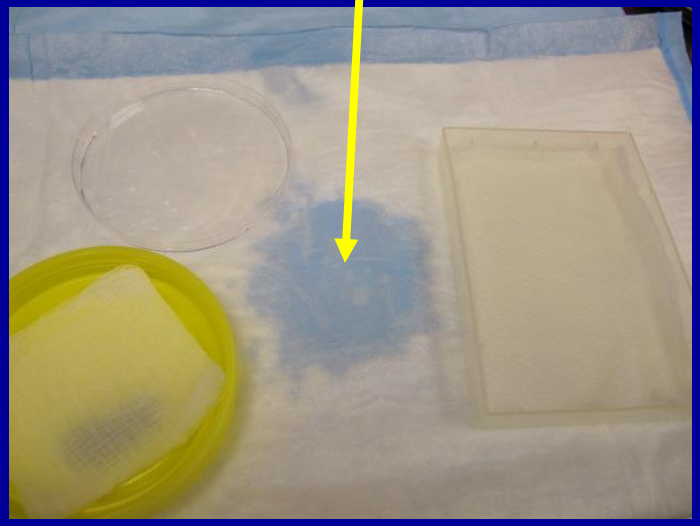
- All formalin, PBS/saline, alcohol* and/or media, as directed by researcher

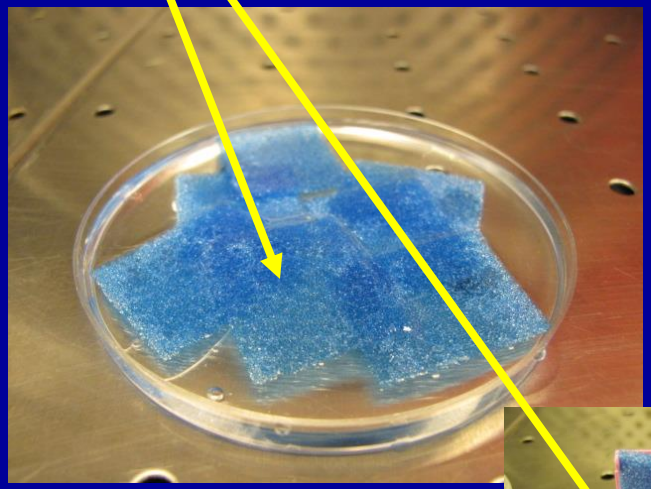
***Alcohol does NOT do much more than keep fur from flying!**

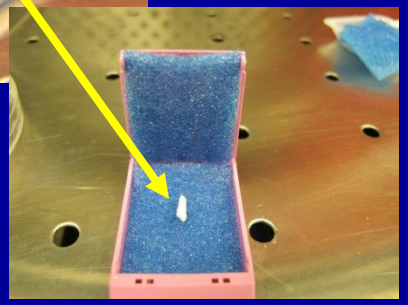
Prepare Work Area

PBS moistened work area(s)

PBS moistened sponges

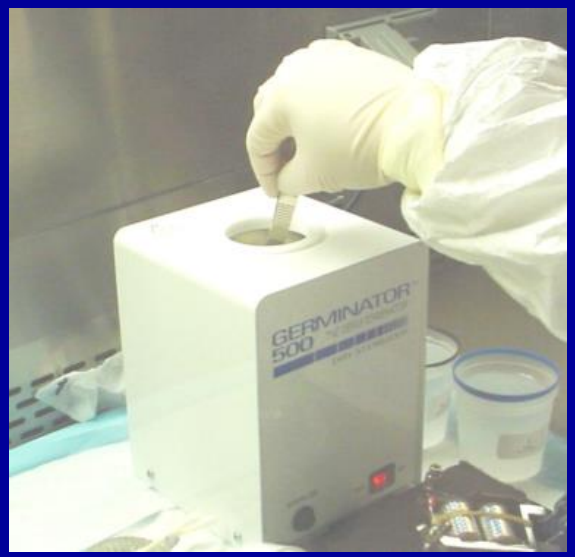






Prepare Work Area

- To prevent cross contamination when collecting tissue for phenotyping, use a bead sterilizer or other appropriate instrument sterilization method between animals!

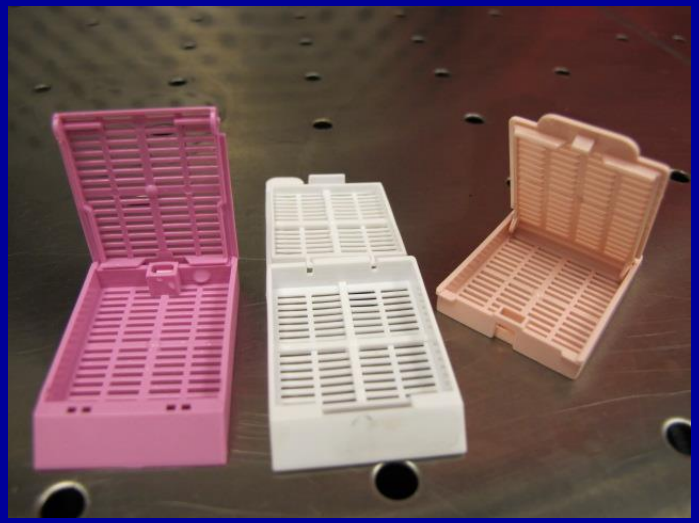


Selection of Cassette, Tube, Mold

- Based on PI instructions and intended "use" (e.g., how it will be processed)
  - Cassettes can be used in formalin or liquid nitrogen
  - Can simply 'free float' tissue in a tube (1:20 tissue:formalin) if only one tissue is taken
  - Tubes can be placed on wet ice or snap frozen in liquid nitrogen
  - Molds are used with OTC media, and are frozen in liquid nitrogen

Collection Options

Appropriate cassettes, tubes or molds and OTC



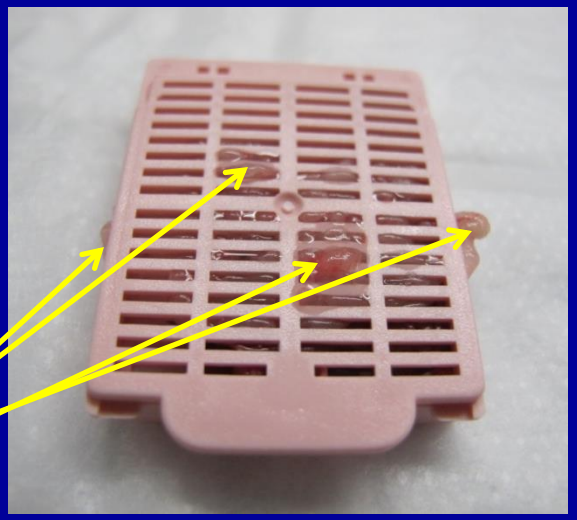
Different depth cassettes are dropped into formalin or liquid nitrogen

Mold used with OTC and dropped in liquid nitrogen

Cryo-tubes used with liquid nitrogen

Selection and Size

- Cassettes should be deep enough to contain the tissue **WITHOUT** compressing it.
  - Approximately the size of a nickel is the rule of thumb
  - Use larger cassettes for brain, whole liver, large tumors
- Mold should be of sufficient size to ensure tissue is **COMPLETELY** submerged in OTC media.
- Tubes should be large enough to allow easy placement of the tissue without forcing.



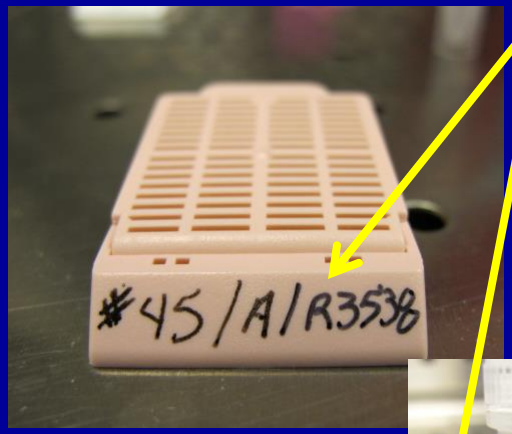
This is incorrect!

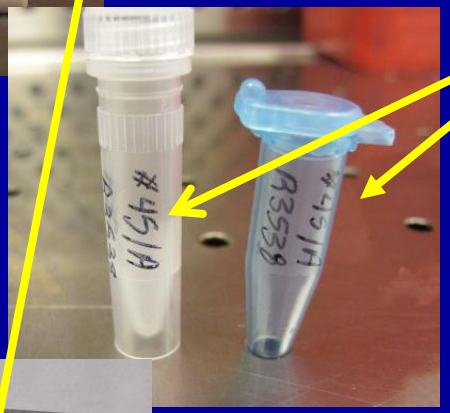
Label Properly!

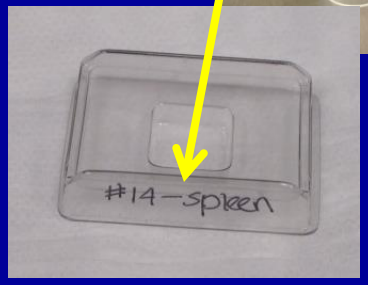
- Use only approved formalin-safe pens
  - You may use a pencil, but use care. They will smudge with handling
- LEGABLE!! Must be able to read it, or it's just an unidentified piece of tissue!
- Make sure the outer container has ALL the information, i.e., researcher, IACUC #, date, study number, animal ID(s), etc.
- If bone is present, label outside container to so indicate!
  - Bone is processed differently - needs to be removed from other tissues by histo/path staff

Primary Collection Vessel vs. Secondary (cassettes/tubes/molds)

ALL information present on main collection vessel/bag







Partial information or coding of secondary

Tissue Handling

- Ideally, you have 10-15 min to collect all samples
  - Place the animal on ice (wet ice covered by gauze so water doesn't contact tissues), or on an ice pack (slows decomposition)
  - Do NOT let tissues dry out! Use PBS to keep things moist – mist or "dunk" is fine
  - Do NOT place tissues on dry sponges or pads! Soak sponges/pads completely prior to use.

Contact & Fix Times

- OTC molds should be submerged for no longer than 15 sec – longer produces tissue fractures and poor quality slides
  - Media will turn from clear to opaque
  - Place in baggie. They sometimes pop out of mold – this will ensure proper ID of samples
- Direct “Snap” freezing of tissue w/forceps in liquid nitrogen is also ~15 sec; then place in a tube or baggie
- Both are stored on dry ice until moved to -80F

Contact & Fix Times

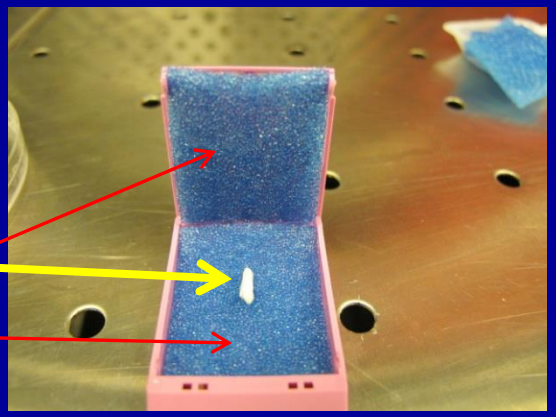
- Formalin fixation of intact organs
  - Brain, heart, kidney = 3 day minimum
    - Perfusion of brain and halving the kidneys will reduce this time to ~48hrs
  - Insufflated lungs need 3 days AND suture removed (don't cassette – let them float until suture is removed)
  - If lumen of gut is to be preserved, it must be flushed ("blown open") and not drained, then coiled immediately; float for one day
    - Typically requested for pathology (not histology)

Orientation

- “Interesting” or “target” areas should be placed with cut side/area of interest down
  - Otherwise, orient as it came out of the animal
- Kidneys can be cut longitudinally or cross wise; researcher’s direction; place cut side down
- Tumors can be cut!

Orientation – Use of Sponges

- Sponges are used to maintain position
- Use of one – on top of tissue
- Use of two – sandwiched between
  - Will prevent escape/loss of very small tissues
  - Will prevent curling of opened hollow organs (e.g., stomach)

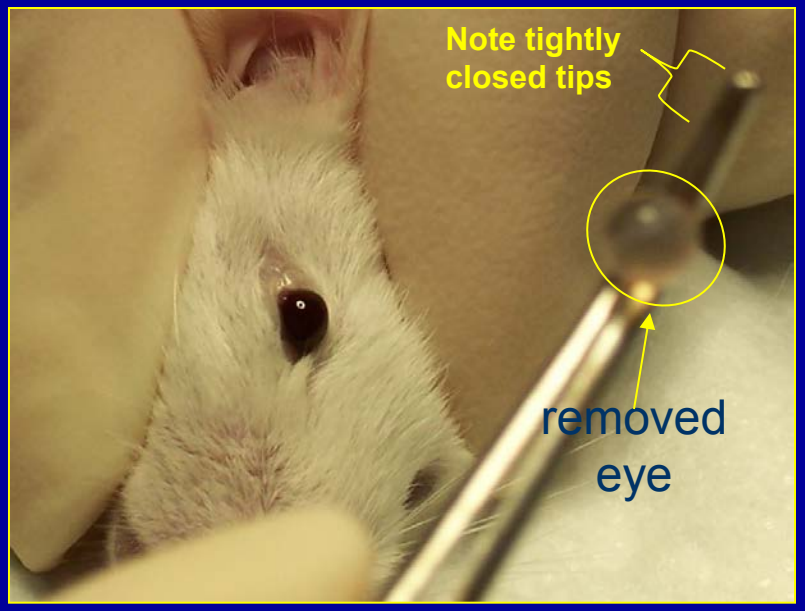


Fill Out Paperwork Accurately
Conduct an External Exam

- PI and IACUC Number
- Gross Examination
  - General activity, appearance/structural abnormalities
  - Fur & underlying skin appearance
  - Eyes, ears, anogenital openings

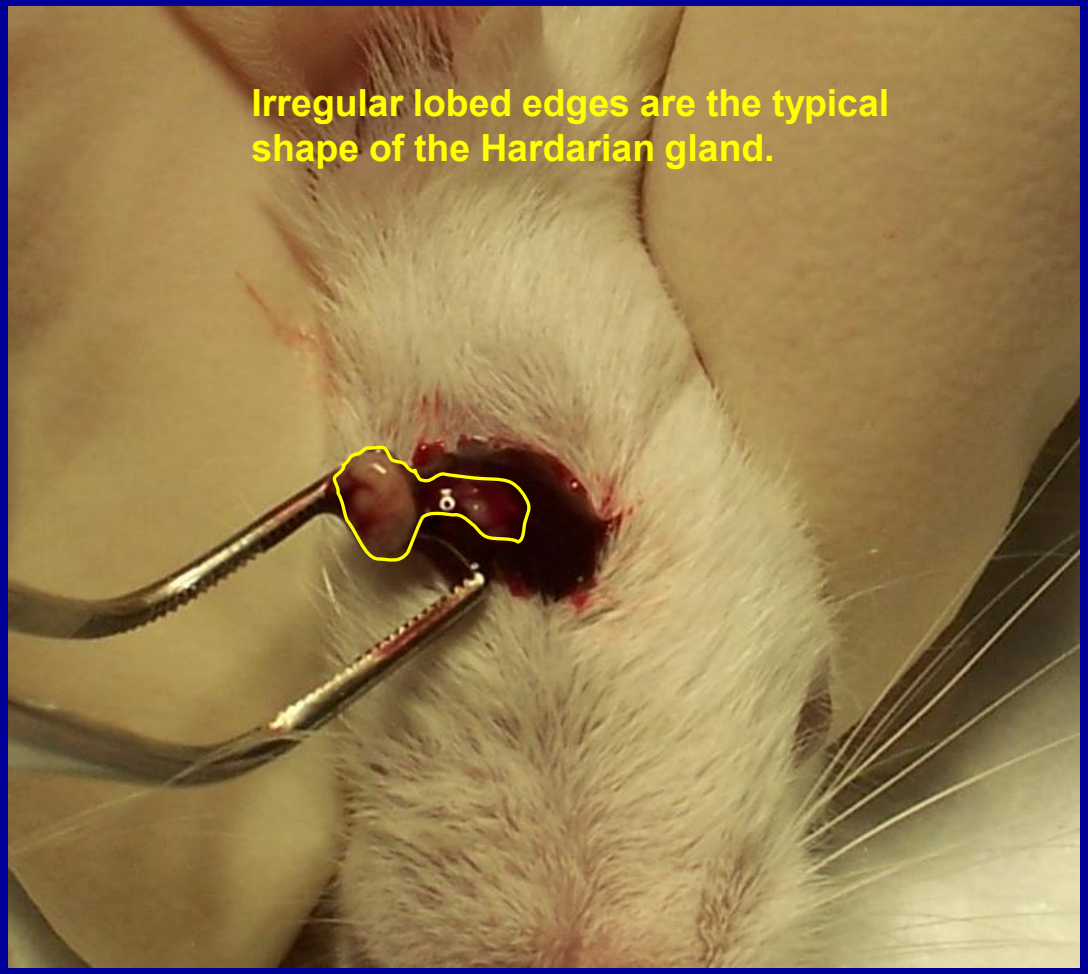
Removal of the eye

Press skin down in a manner so that the eye is proptosed. Place a pair of curved forceps (tips up) beneath and close them completely. If not closed tightly, tissue damage will occur. Pull straight up. This removes the eye and a section of the optic nerve.



Removal of the Hardarian gland

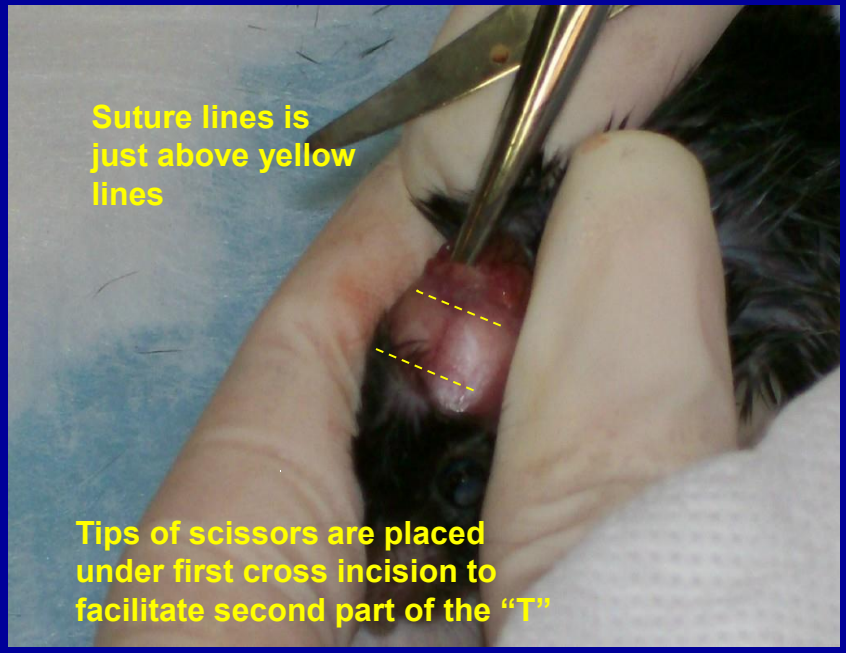
Hardarian gland removal is optional. Once eye has been removed, while still holding the skin taut, blot away blood. Use forceps to grip the tissue, slowly "wiggle" the gland forward and out. Color is normally grayish-tan; shape is irregular.



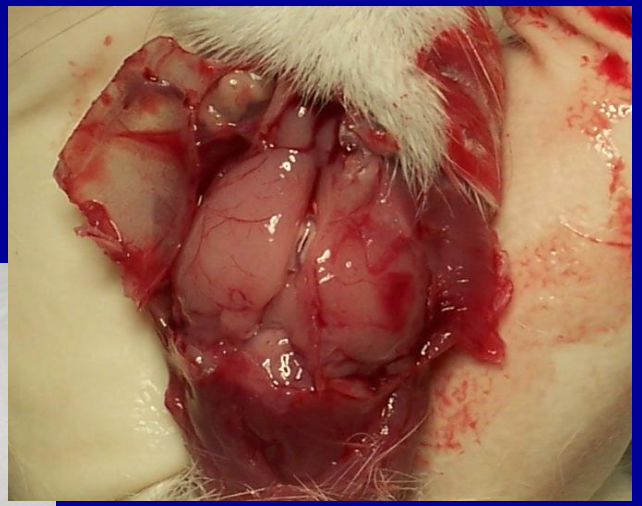
Brain Removal

Grasp the ears in such a way as to bring them down and tight against the side of the head. Make an incision just behind the base of the skull. A second cut is made midline, towards the nose.

Using ronguers or scissors, the same "T" type incision is made through the bone to expose the brain. Care is taken to follow "suture" lines, thereby avoid damaging underlying tissues. There are natural separations, or grooves, below these plates.

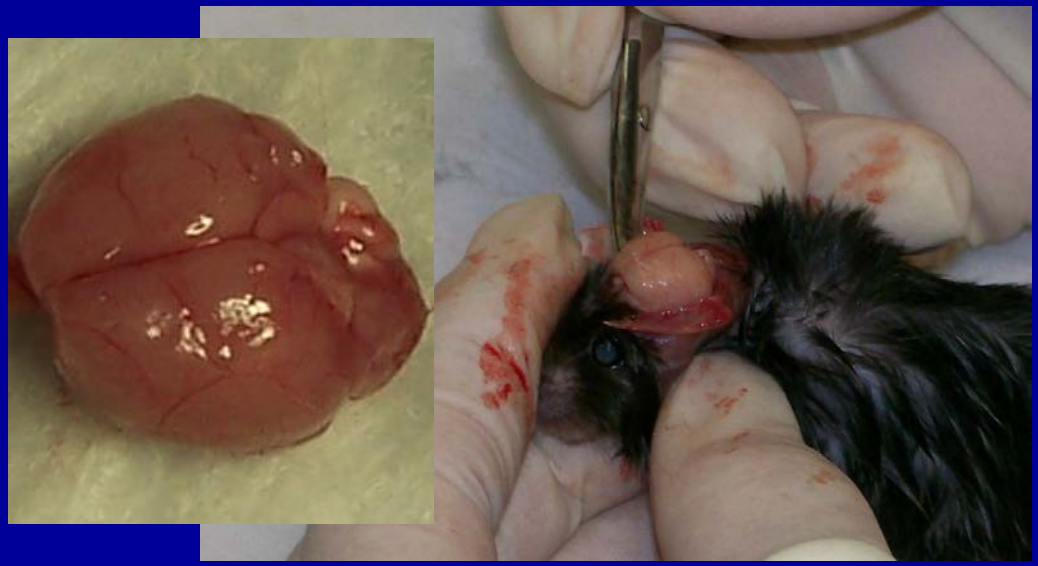


- **Pull/flip the bone flaps up/off with forceps. Place one tip of the forceps at the corner of the flap. W*ithout* closing the tips, use one tip to pop up the flap on that side. Prying with closed tips can result in a small corner-piece breaking off.**
- **Support either side of the skull (thumb + forefinger) to prevent the brain from splitting.**
- **DO NOT drive bone into the brain.**

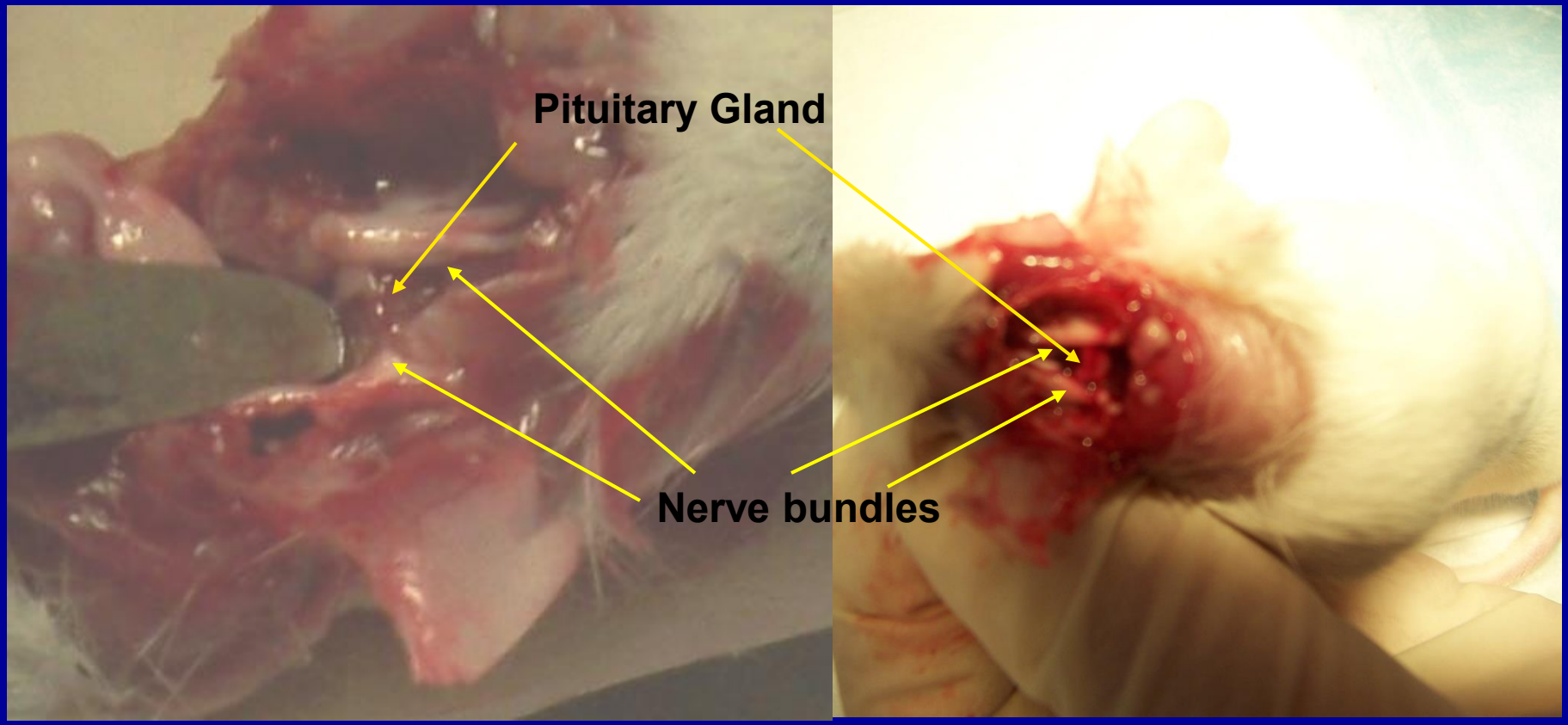


Collecting the Brain

- Use a blunt tipped instrument or spatula to lift the brain away from the skull.
- Start with instrument in dominant hand and introduce tip behind the brain at base of skull, directing the tip against the bone.
- Follow the curve up and around, levering the brain gently forward.
- Use a spatula or blunt instrument to cut the optic nerves.
- Brain will fall forward, once freed from the spinal cord.



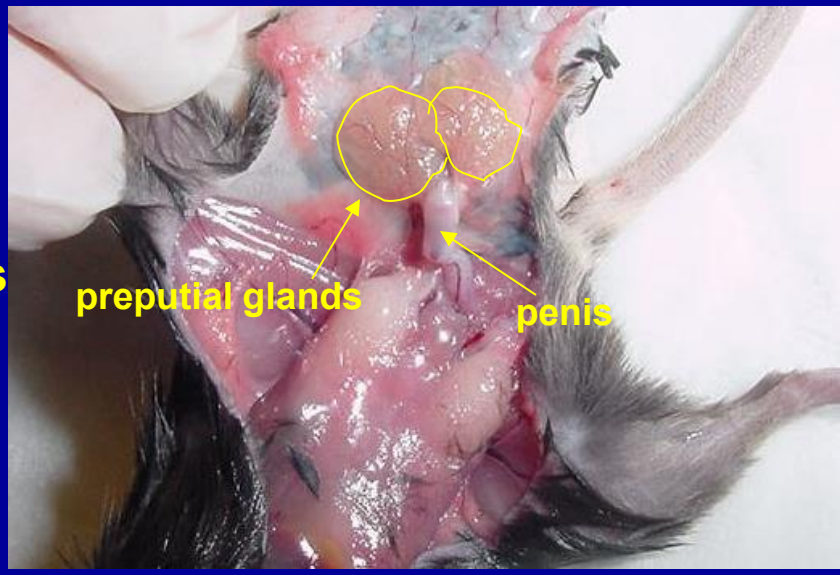
- Collection of the pituitary gland is optional.
- Once brain has been removed, the gland is easily identifiable as the organ between the two nerve bundles delineated below.
- The fascia around the gland is gently torn (with instrument tip or needle point) and the pituitary lifted out.



Begin skin incision at lower abdomen just above the prepuce in males, or vaginal opening in females.

Observe the underlying preputial glands in males – this is a common abscess site from fight wounds.

In photo to right, skin is reflected back via midline incision. The preputial glands are normal and would lie just in front of the penis

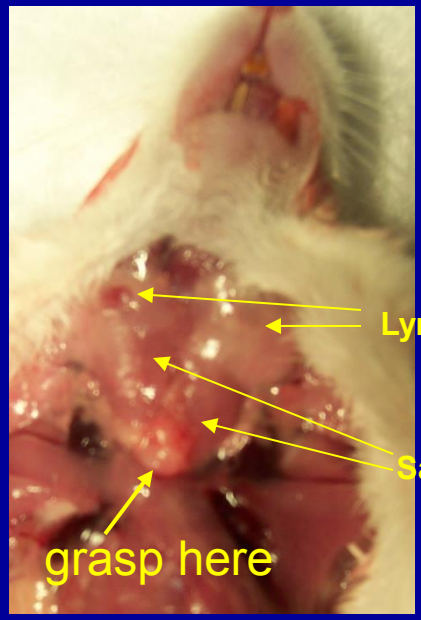


Extend incision upwards towards the chin. Place thumb over the submandibular area to prevent salivary glands from splitting apart from one another.

Skin along the length of incision is peeled back to allow examination of underlying structures.

Removal of the lymph nodes and salivary glands in the submandibular region is accomplished by gently grasping the fascia at the tip.

The glands, with the surrounding nodes, are gently teased upwards, then grasped just below the chin and pulled off as one unit.



The skin is peeled further back along the abdominal incision for visualization of the inguinal lymph nodes and mammary tissues.

Even with the knee, the skin is inverted over the technicians finger.

Note three blood vessels that intersect within the fat pad/mammary tissues. The lymph node is located at that intersection and appears as a hard, round opacity buried within the tissues.

Remove by pressing an instrument tip into the surrounding tissue, just alongside the node, and ripping a small hole.

The spread instrument tips are then pressed down along both sides of the node, then closed completely. Gentle upwards pressure will free the node and a very small amount of surrounding fat/tissues.

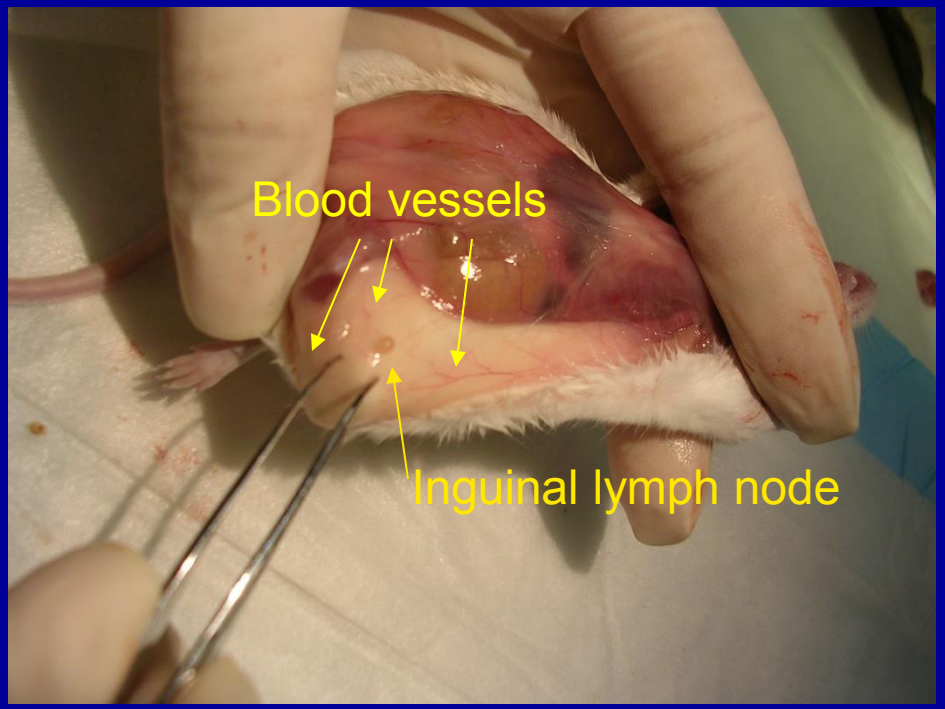
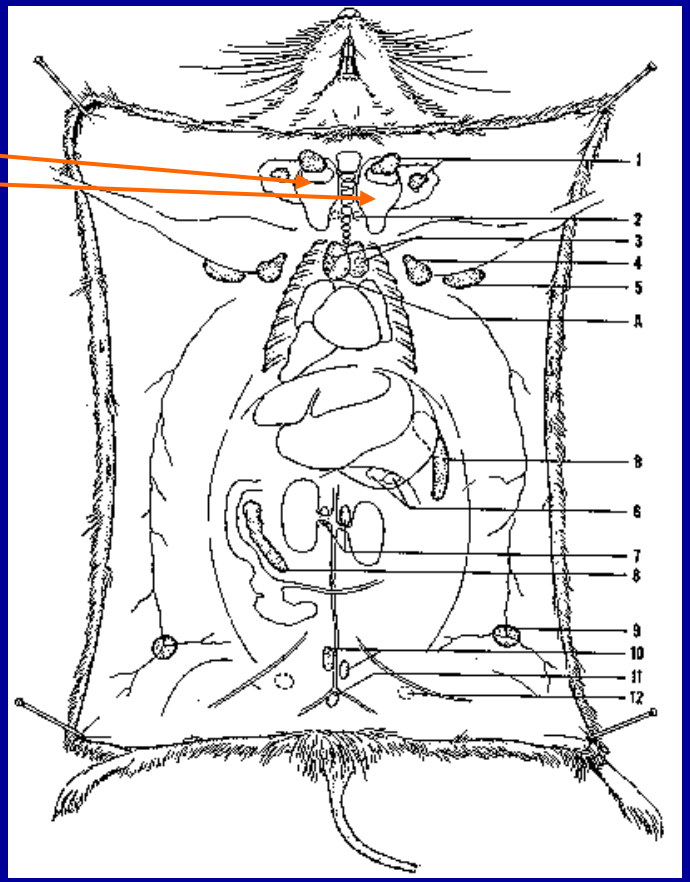


Diagram Key

- 1. **Superficial cervical nodes**
- 2. **Deep cervical nodes**
- 3. **Mediastinal nodes**
- 4. Axillary node
- 5. Brachial node
- A. Thymus
- B. Spleen
- 6. Pancreatic node
- 7. Renal nodes
- 8. Mesenteric node
- 9. **Inguinal node**
- 10. Lumbar nodes
- 11. Sacral Node
- 12. Sciatic node

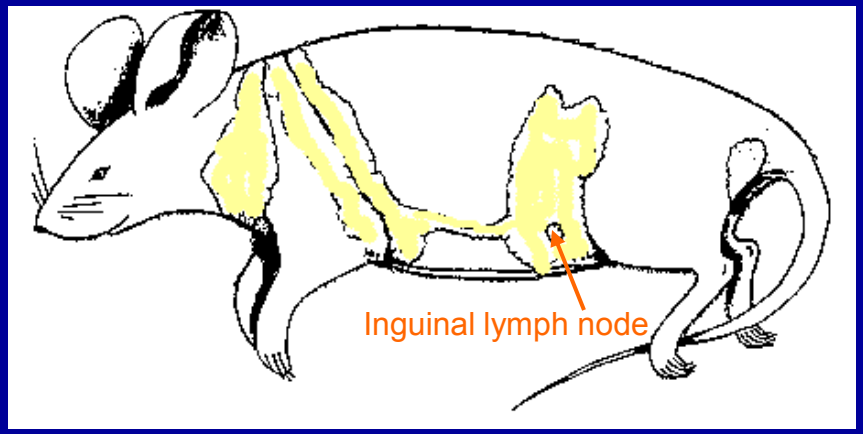
Salivary glands



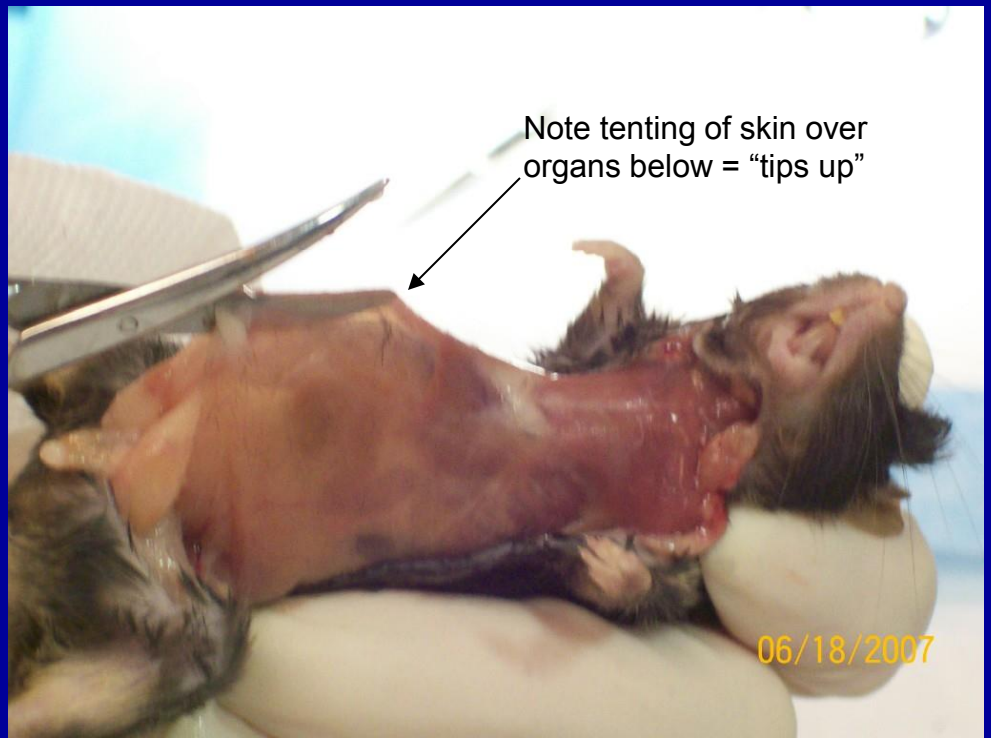
#1, 2, 3 are collected as the "submandibular lymph nodes"

9 is the inguinal lymph node. Note the intersection of the three blood vessels. In obese animals, vessel identification is difficult. Fat pads obscure view of the node.

- Mammary glands are depicted in the diagram to the right. Note location of the inguinal lymph node. Remove these glands by peeling back the skin and pulling the tissue away from its subcuticular connections via blunt dissection.



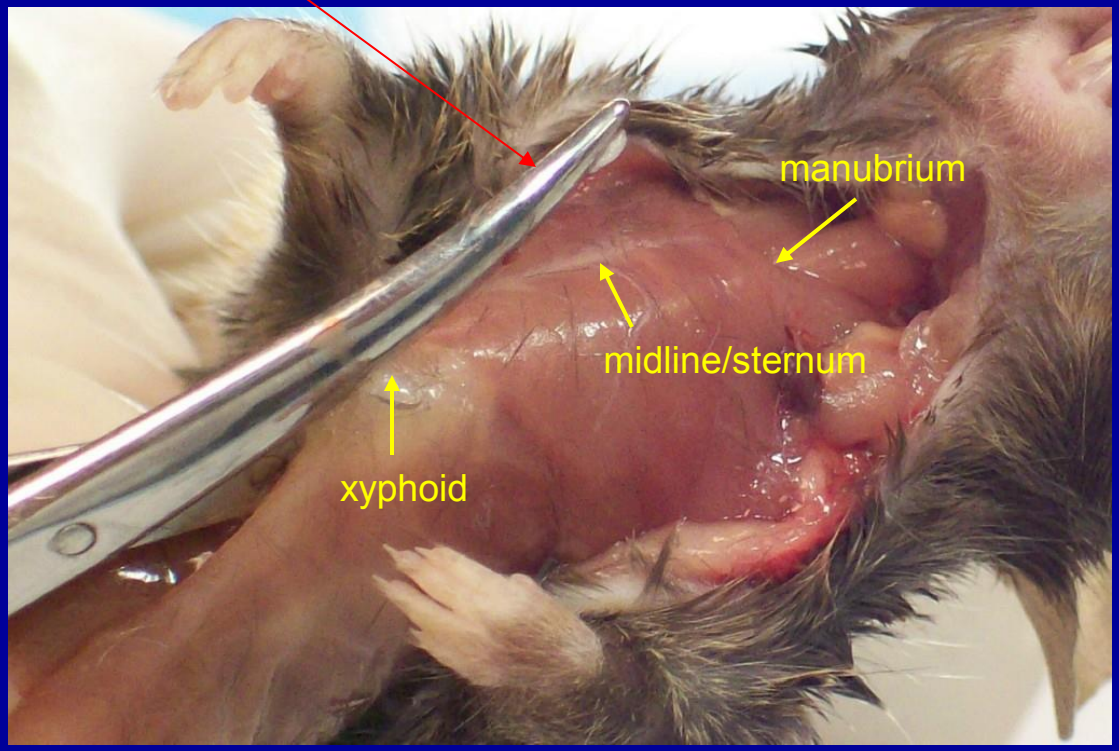
- Open the abdominal and thoracic cavities via one midline incision.
- Begin in the pelvic area and extend cranially towards the chin.
- Hold the animal in a “scruff’ to position for protecting the underlying tissues.
- Keep the abdominal muscle wall pulled taut with the scissors so that a “tent” forms. This will prevent accidental transection of underlying organs and intestines.



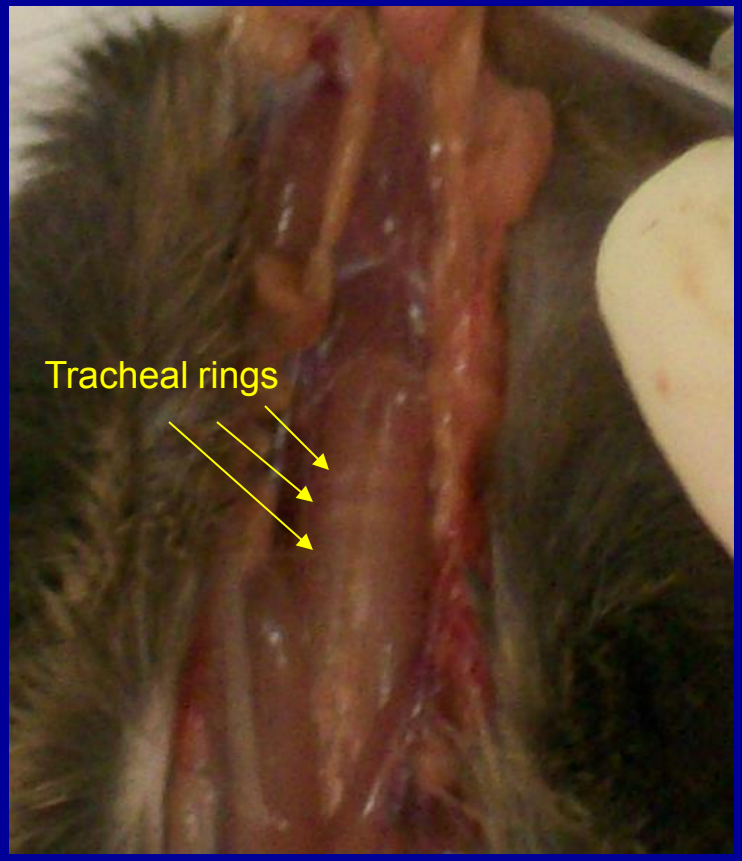
•At the transition between abdomen and thorax, the scissors are moved to one side of the xyphoid process/ sternum and advanced until tips extend past the manubrium (collar bone).

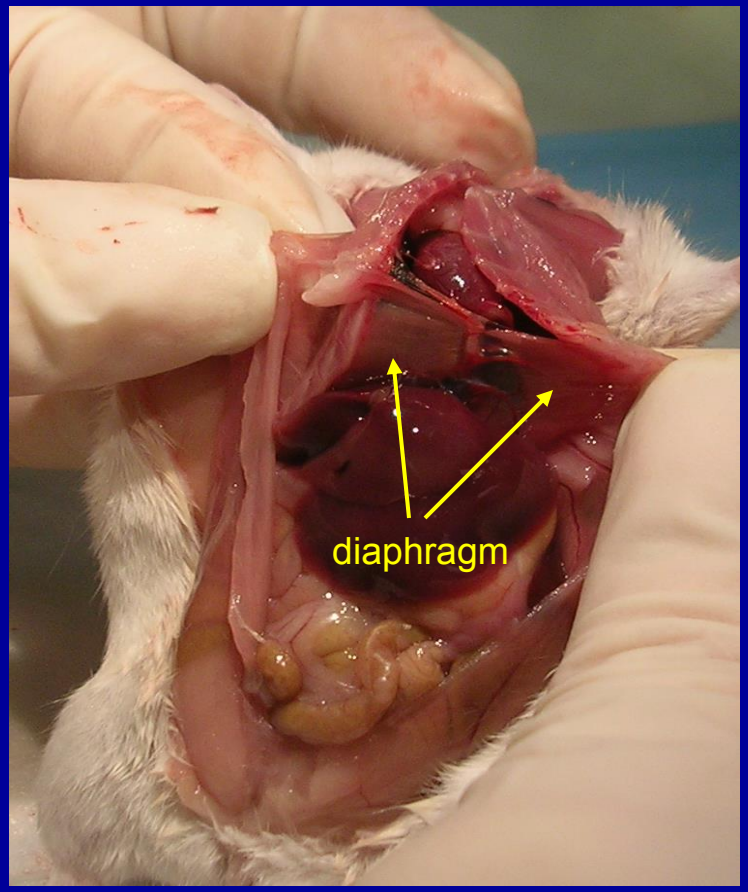
•Keep the tips "up." In the thorax, scisssor blade depth must remain shallow to prevent damage to thymus, heart, lungs, trachea and other underlying tissues.

•The lower blade should slide forward just under the ribs/sternum; <u>upper blade</u> should be the blade moving up and down.



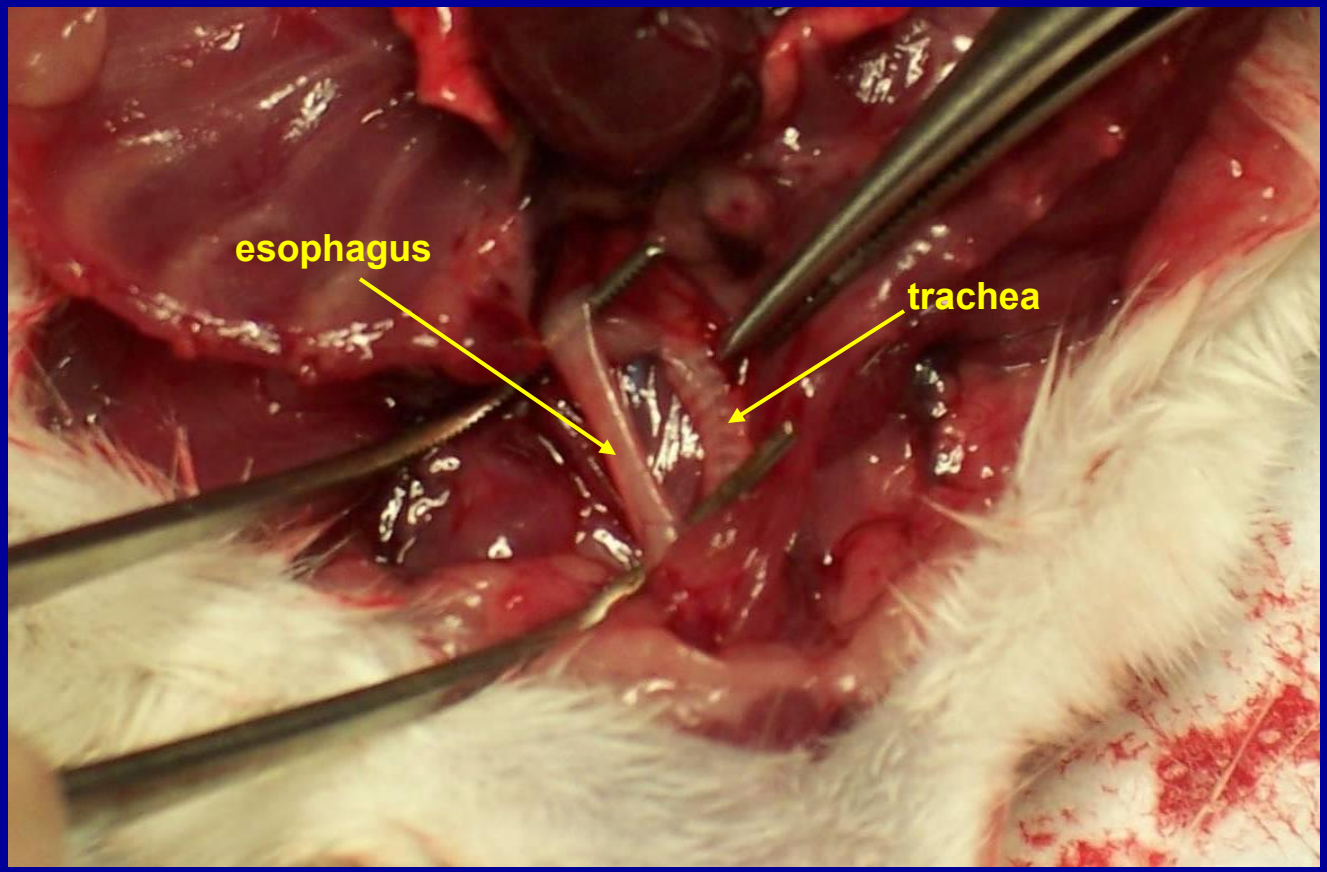
- Tracheal rings are visible just under the muscle.
- Muscle is best removed by simple blunt dissection.
- The esophagus lies <u>beneath</u> the trachea.



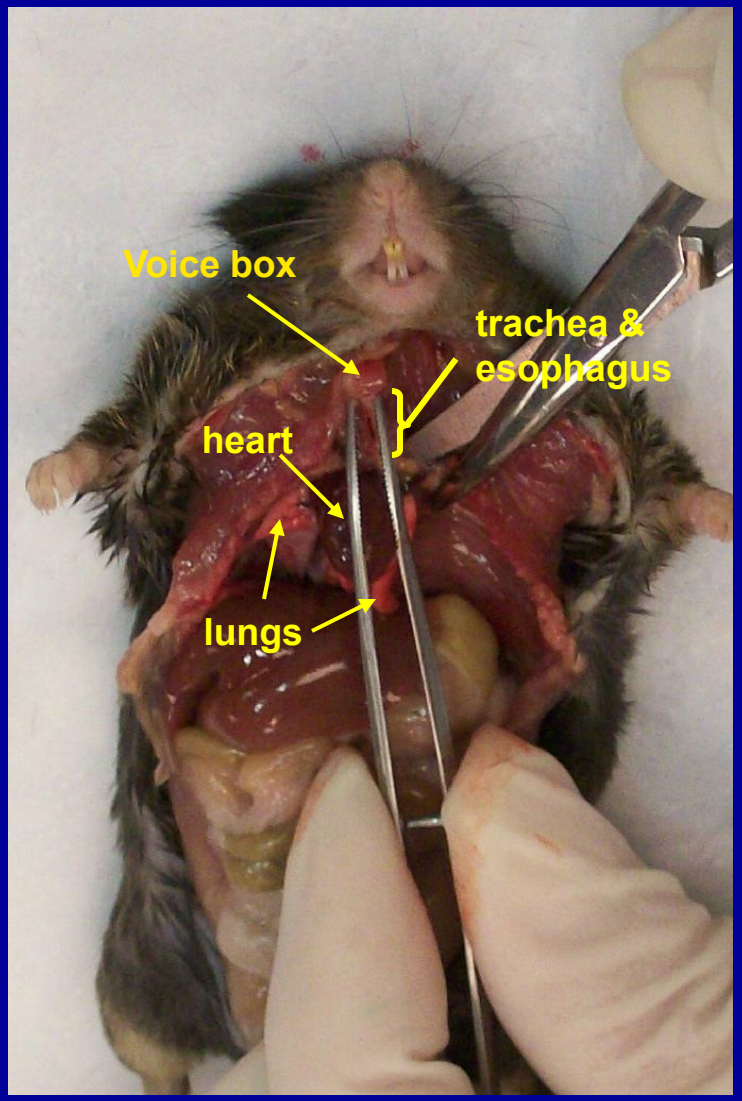


- Rib cage is sprung by grasping the lower ribs at the point where they meet the diaphragm and pulled apart.
- Further visualization is accomplished by breaking the rib tips, or cutting them away.
- Care is taken to not break them in a manner that pushes them up into the heart or lungs.

Blunt dissect to isolate the esophagus and trachea.



- Identify the larynx/voice box. The thyroid and parathyroid glands are located in muscles surrounding the voice box.
- Grasp the rings and cut the trachea free above the voice box. Lift up and place scissors beneath, in a tips down orientation. Trim towards the heart while exerting slight upward traction.
- Use care to ensure that blades stay beneath the trachea, thymus, heart and lungs. This collection of removed organs is known as the "pluck."
- Final removal of the pluck is via clipping of the esophagus at that point where it passes through the diaphragm.



- Collection of the lungs may be done with or without the heart attached. Heart removal is outlined in a subsequent slide.
- Inflation of the lungs may help with identification of aberrant structures and abnormalities, and/or aid in fixation of tissue.
- A blunt tipped 23-25g needle is threaded down the trachea. Trachea is pinched closed around the needle.

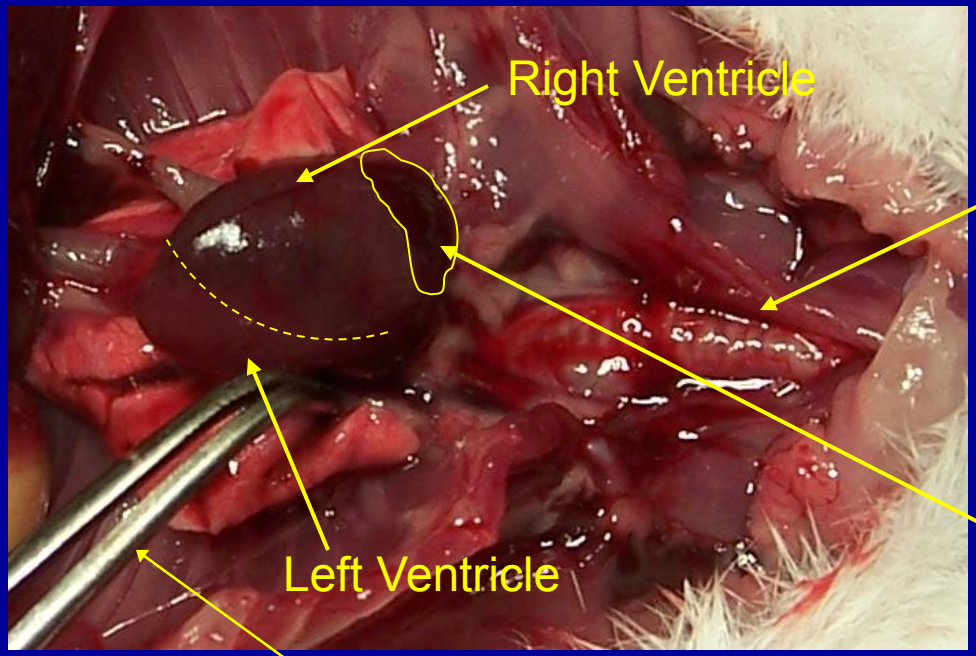
Saline, PBS, formalin or other material is gently pushed into the trachea until all lobes are fully inflated.

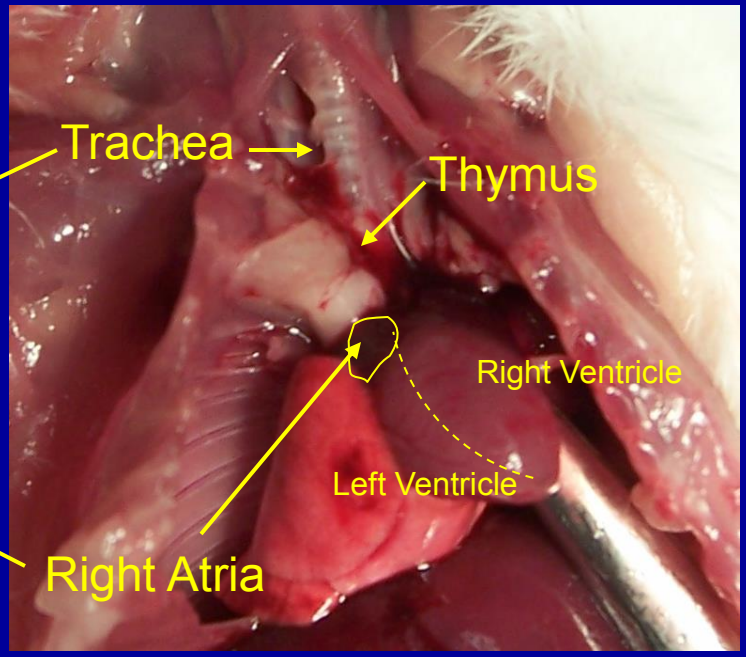
Normal color is coral pink; edges of each lobe are well defined/sharp.

Do not thread the needle too far into the trachea. This will place the needle tip into a single bronchi, resulting in incomplete total lung inflation.

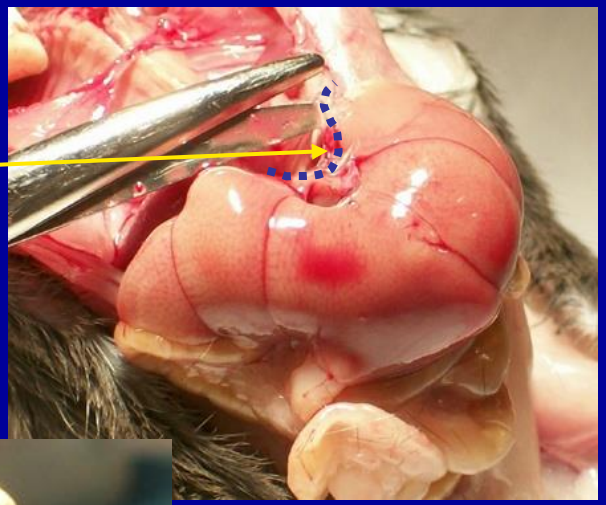
Transfix the tracheal stump with suture to prevent fluid from leaking back.

- Heart removal may occur before or after lung removal.
- Forceps are placed in “tips up” position, beneath the ventricles. Ensure that both atria are also between the open tips.
- Close forceps tightly. Lift straight up. This separates the heart from lungs without damage to the lungs.
- Thymus may be collected alone, with the heart, or as part of the pluck. It is a paired leaf-shaped structure. Using forceps, it is easily peeled from the heart.

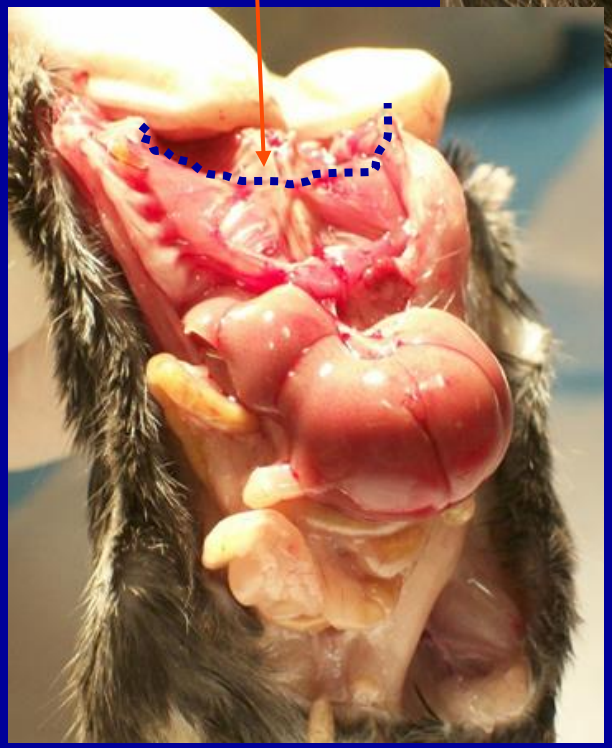




Forceps are TIPS DOWN for aid in visualization ONLY

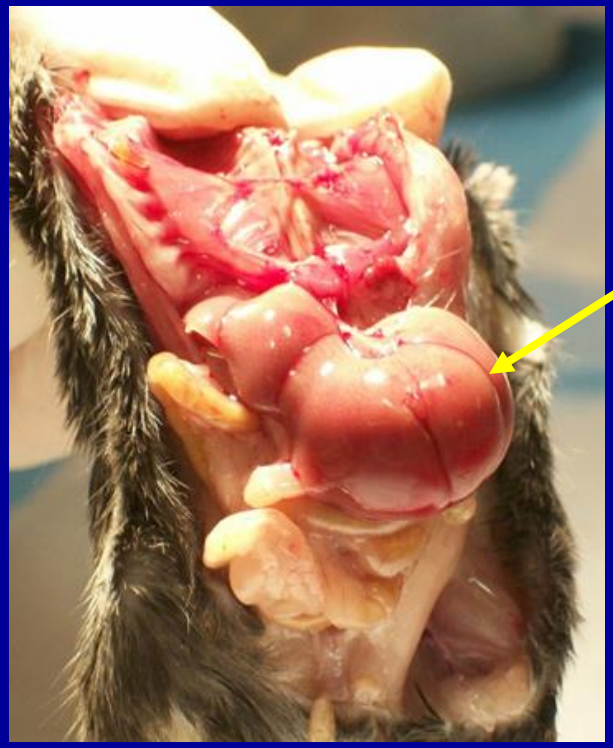


diaphragm



Diaphragm remnants should be gently cut away. Mesenteric attachments of the liver should be severed.

- **Caudate liver lobes wrap around the stomach. Gentleness must be used in removing them.**
- **Hold animal up and let the body "drape" over your hand (thumb and forefinger holding the thorax).**
- **Liver will fall away as mesenteric attachments are severed.**
- **GI tract/ Stomach should stay in position. They can be stabilized by placing the thumb over the stomach.**

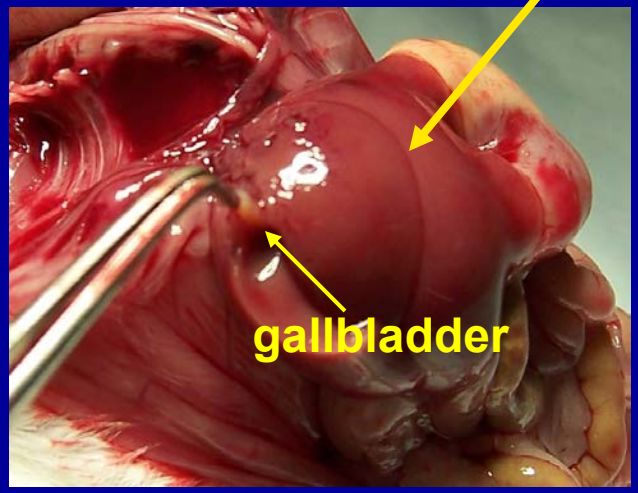


Note - this liver is pale and has petechia present

Normal liver edges are well defined and “sharp.”

Rounding of the edges may indicate inflammation.

Normal liver color and texture

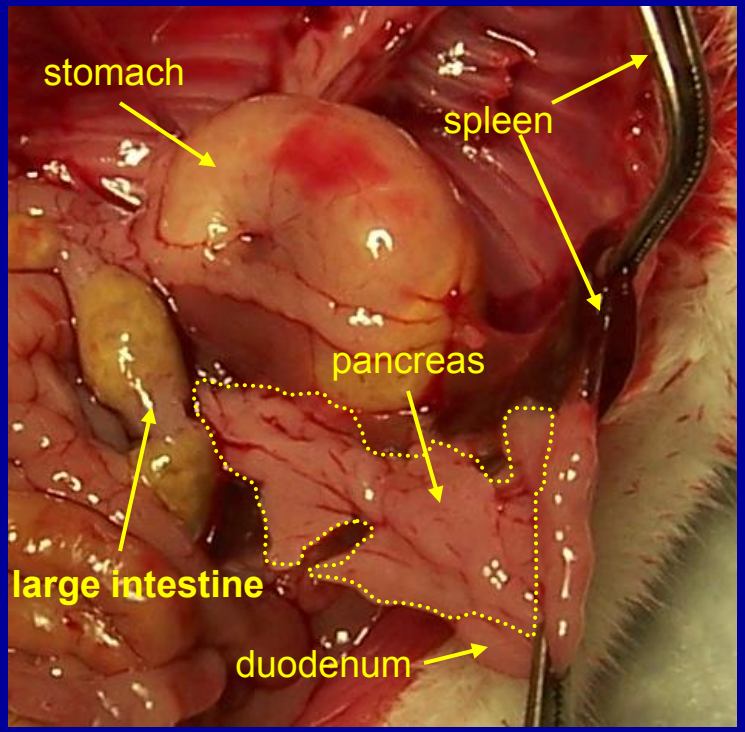


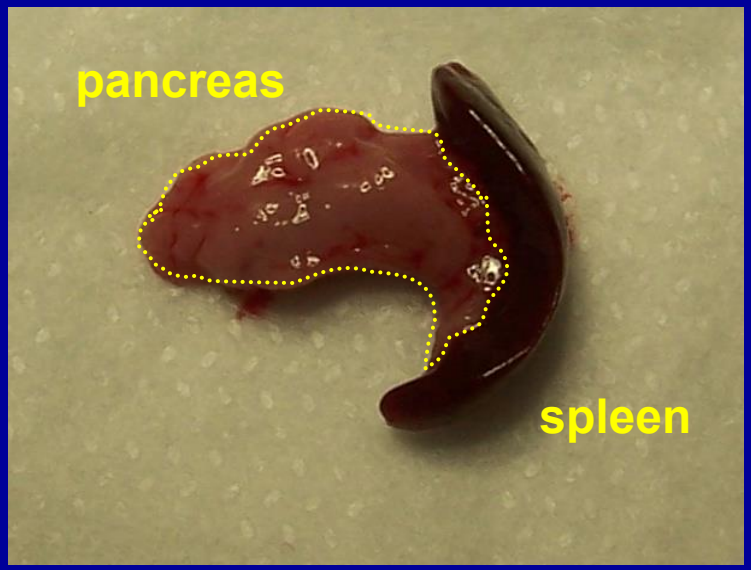
Gall bladder content should be clear yellow.

- Isolation of the pancreas is best accomplished by identification of the stomach, duodenum and large intestine. The pancreas is bordered on three sides by these organs.

- The spleen is easily identified as a “tongue” shaped organ, loosely attached to the pancreas and stomach. Spleen edges should be well defined. When cut on cross section, spleen shape is triangular.

Pancreas and spleen can be collected together or separately by breaking or cutting the mesenteric attachments between them and the surrounding organs.

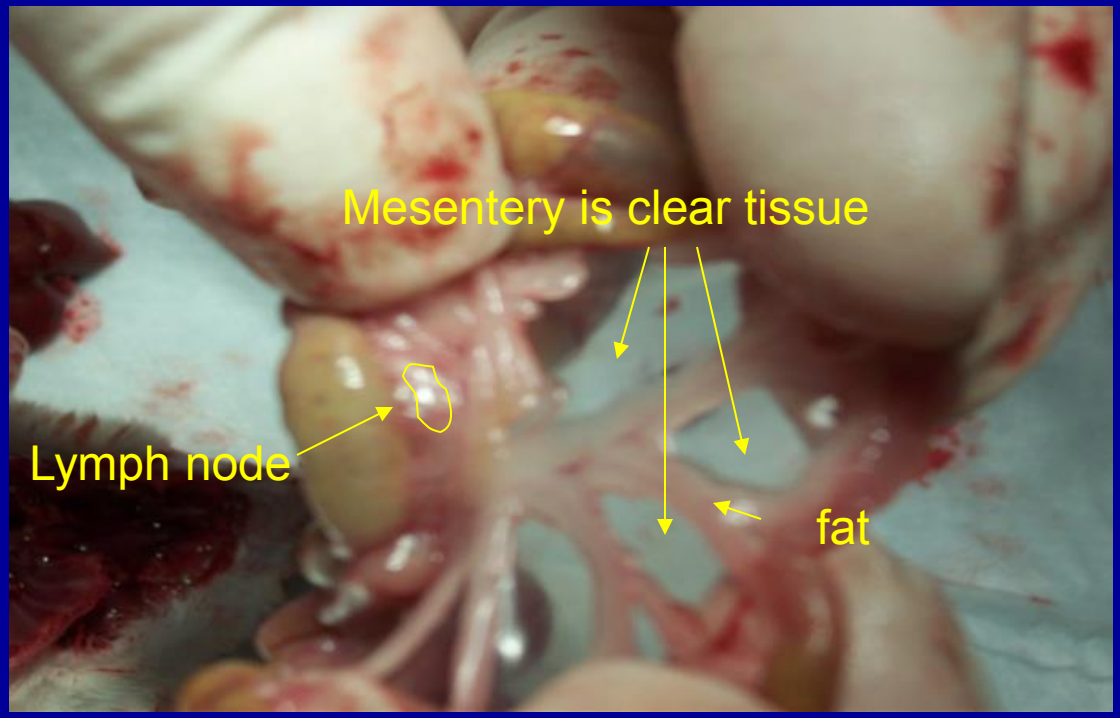




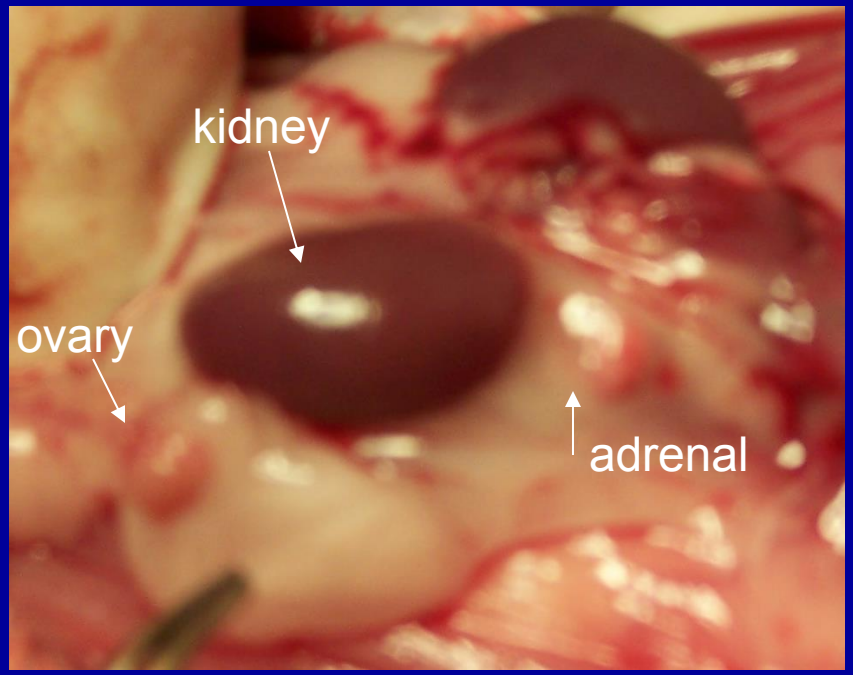
Pancreas is sometimes confused with fat. Fat is more uniform in consistency and whiter in color; pancreatic tissue is pinkish and organized in identifiable lobes.

Fat is an organ. A normal animal should have fat and fat pads. Fat is commonly found around the kidneys, sex organs, mesenteric tissues, and intestines. Normal mesenteric fat is illustrated below in the intestinal arcades. Absence of these stores is a recordable abnormality.

Note – mesenteric lymph nodes may be collected from this area.



- Collection of kidneys and/or adrenal glands is accomplished by first identifying the fat pad surrounding both.
- The pinkish adrenal sits just above the kidney.
- In female specimens, care must be taken to prevent damage to the ovary, which is buried within the fat pad just below the kidney.
- Forceps are placed around the venous and arterial supply and under the kidney itself, in a “tips up” position. If collecting the adrenal w/kidney, include it. The forceps are then closed completely. Scissors are used to free the organ(s).
- If collecting only the adrenal gland, forceps are introduced below the organ, and it is lifted enough to cut beneath.

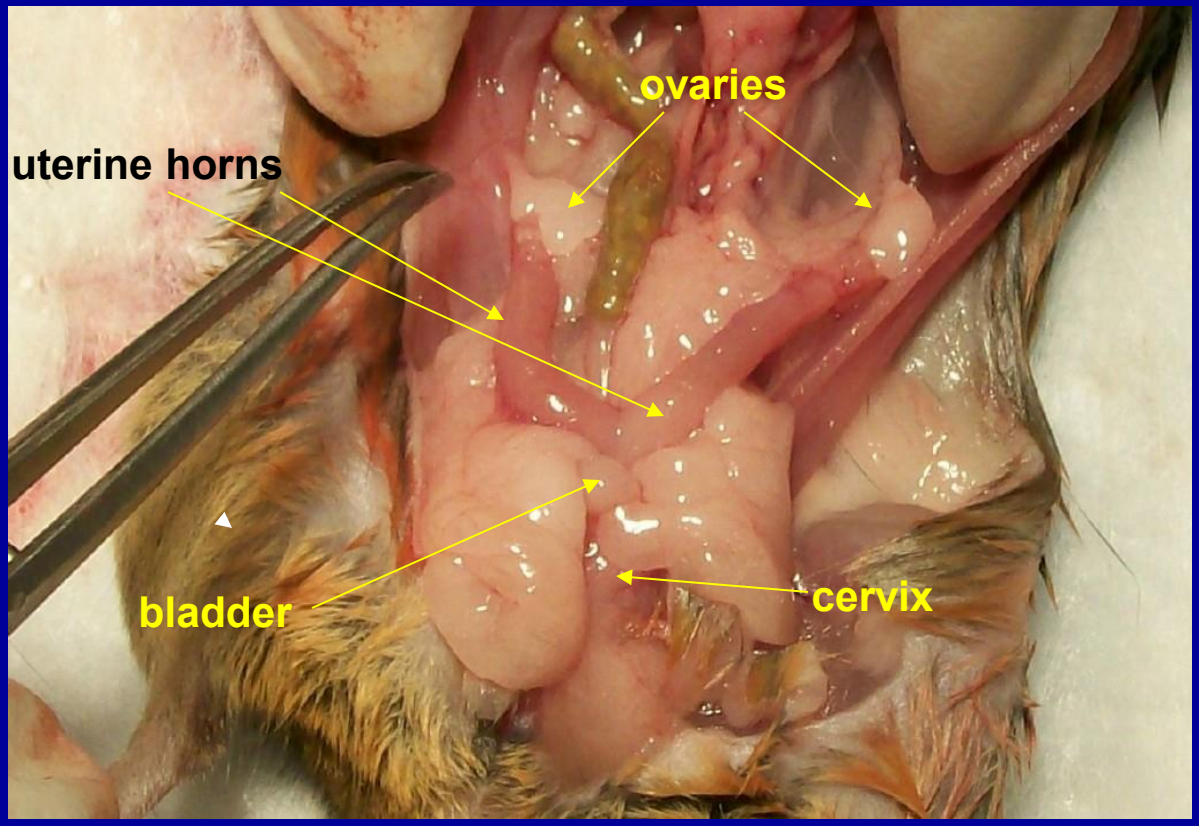


Uterus, ovaries, cervix and bladder are taken together.

Ovaries are identified within the fat pad below kidney.

Uterine horns are carefully cut away from surrounding fat.

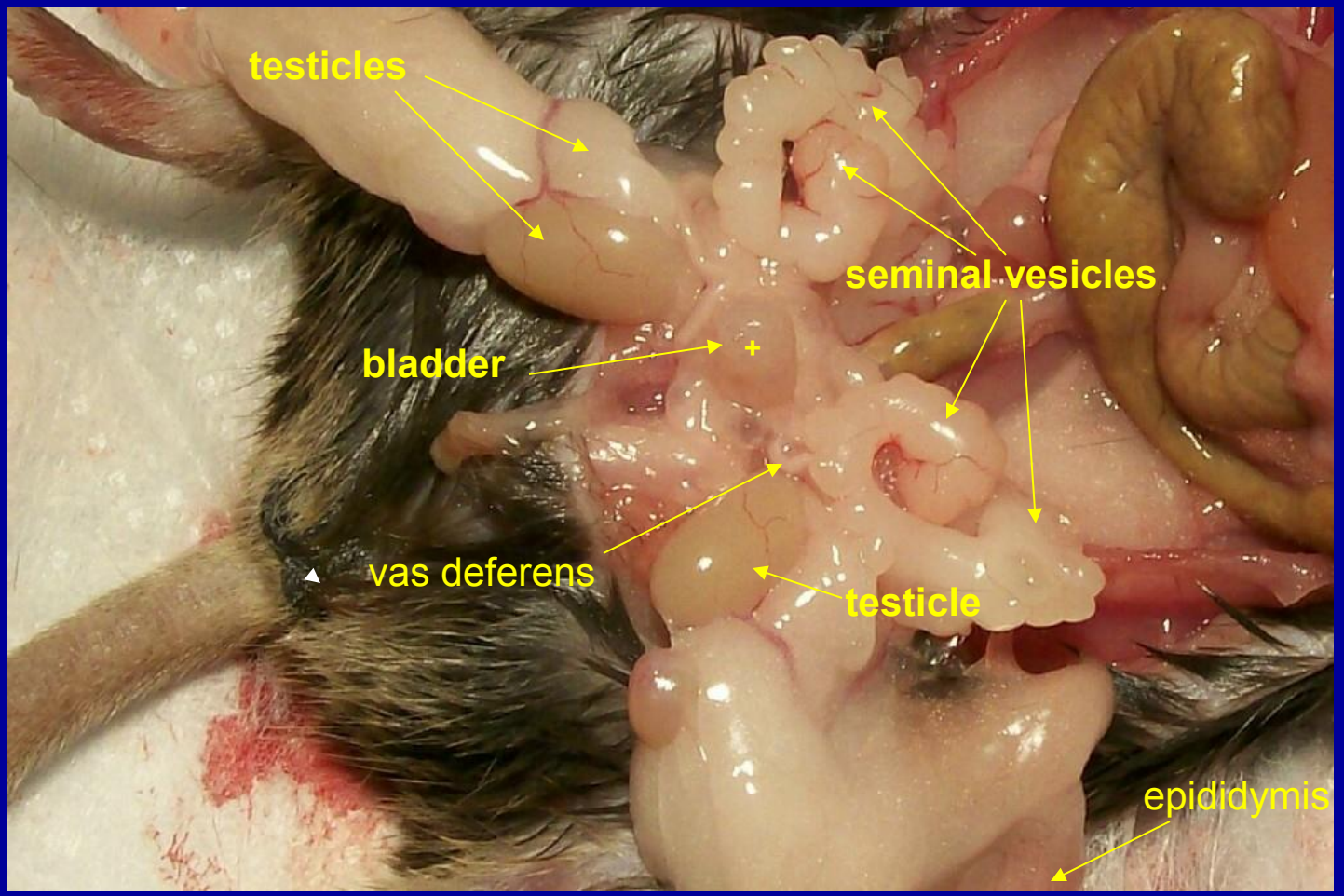
Cervix is identified below the bladder.



All organs are lifted up, and scissors passed beneath, cutting just below the cervix.

Male ureters, testicles, seminal vesicles, epididymis, vas deferens, bladder and prostate are collected together.

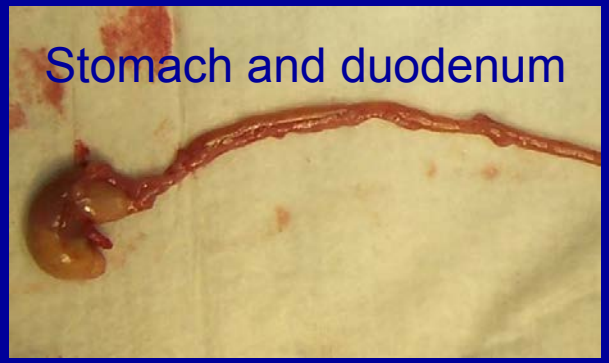
Fat pads from around testicles & epididymus are removed.



Scissors are passed behind the seminal vesicles and bladder as the entire group of organs is lifted up and cut away.

Stomach, small and large intestines and the cecum are removed as one piece. The mesenteric tissues are pulled away and the GI tract arranged in a loose "z" pattern.

The stomach and the first third of small intestine (duodenum) are removed as one. The stomach serves as a "landmark."

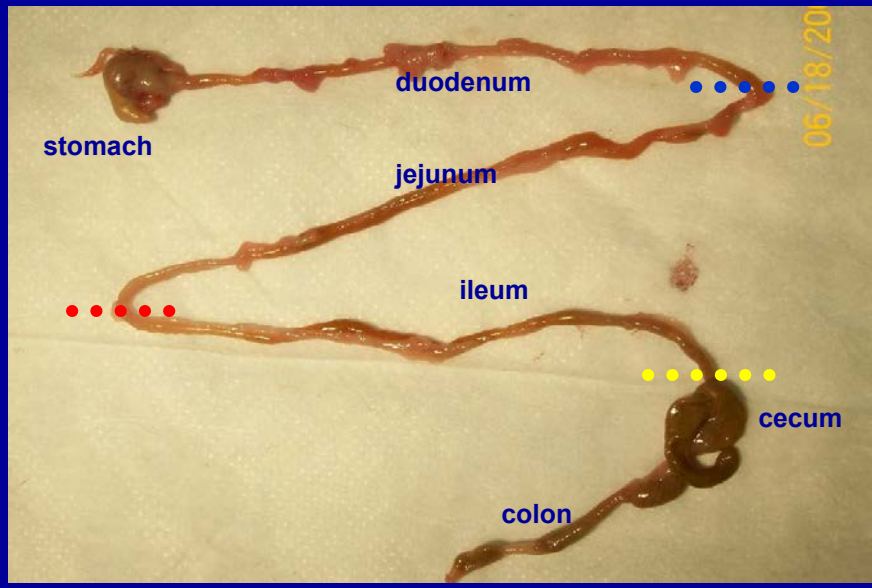


The second third (jejunum) is removed. It has no associated landmark organ.

The final third portion is the ileum, with the cecum serving as the landmark.

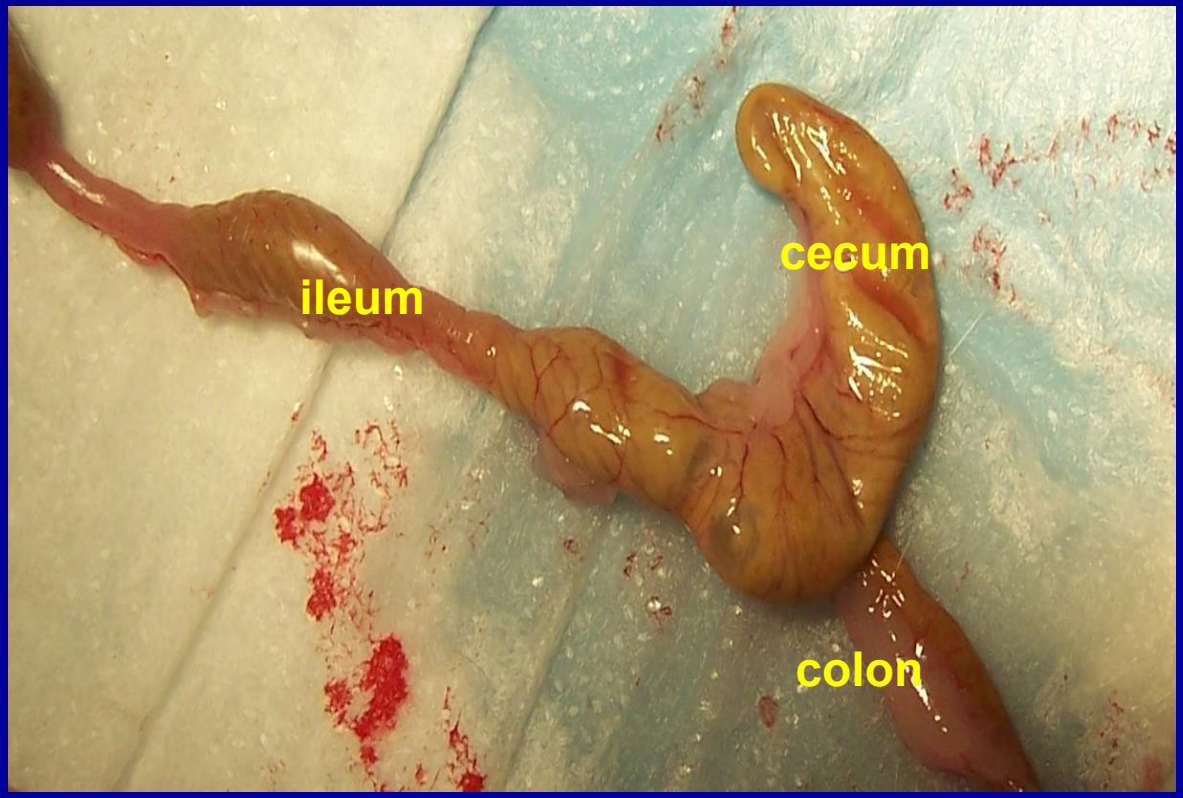
Stomach, cecum and small intestine are flushed with saline, PBS or formalin (refer to protocol).

For cassettes, all pieces with landmark organs are coiled within cassette so that the landmark (e.g., stomach, cecum) is placed in the outer most corner.



The cut end of section is at the inner most part of the coil.

Stomach, small and large intestines and the cecum are removed as one piece. The large intestine and descending colon are removed from the cecum.

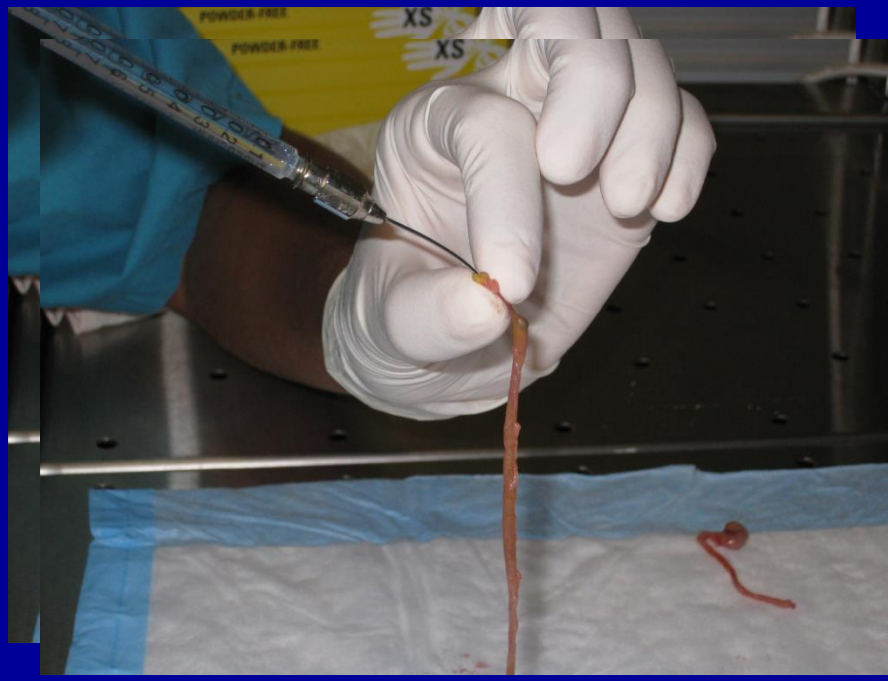


When submitting cassettes, all pieces with landmark organs are coiled within cassette so that the landmark (e.g., stomach, cecum) is located in the outer most corner.

Cut end of section is at the inner most section of the spiral.

Cassetting the Small Intestine

- The small intestine is laid out and the three segments identified and tissues trimmed per the protocol.
- The segment of interest is flushed with either saline or formaldehyde, depending on the researcher's needs.



- The tip is grasped and a 20g ball tip gavage needle is introduced and enough fluid pushed through to clear the lumen completely of content.

Cassetting the Small Intestine

- Remove excess fluid. Using the side of the needle and GENTLE pressure, push the contents through.
- If lumen is to remain open, re-inflate once content is removed.

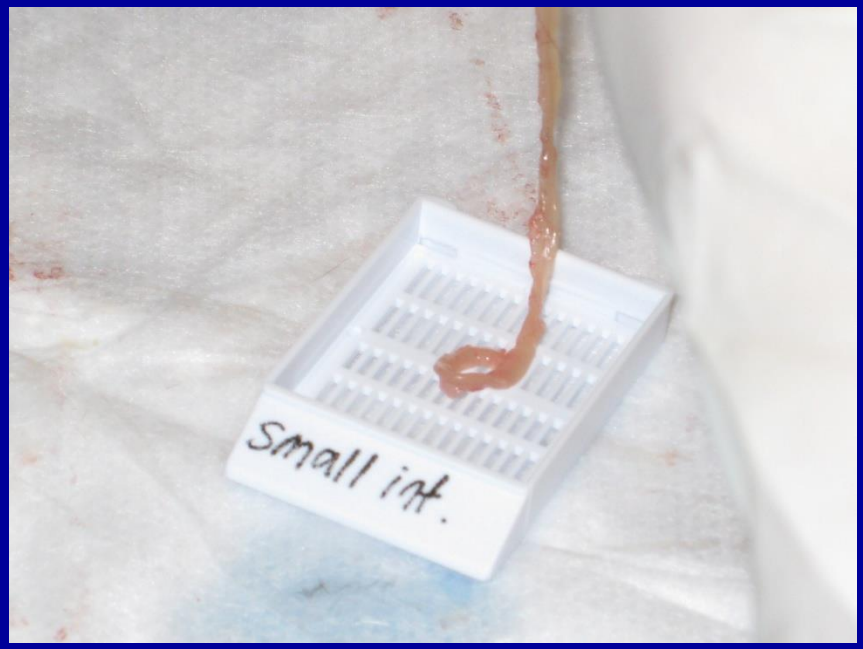
Slide #2

- TAKE CARE! Too much pressure will rip the segment.

Cassetting the Small Intestine

Slide #3

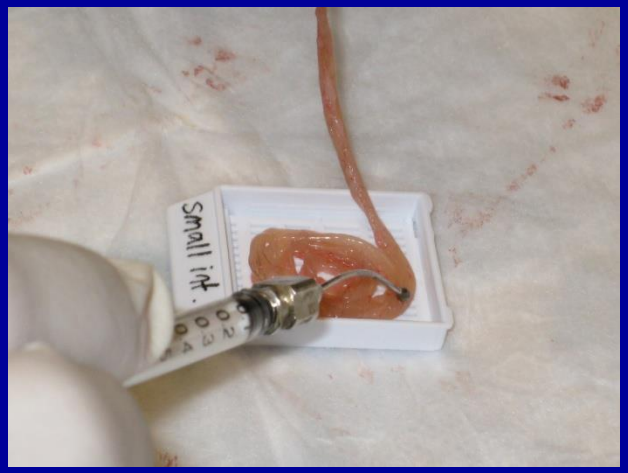
- Once contents are removed, the segment is coiled within the cassette.
- Start with the distal (farther) end in the center of the cassette. Wind the piece in a spiral configuration.

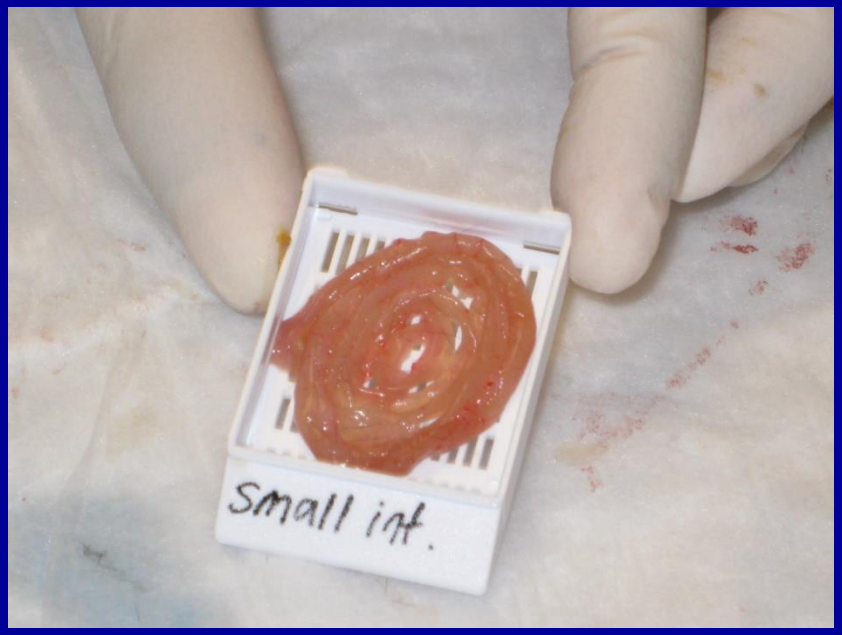


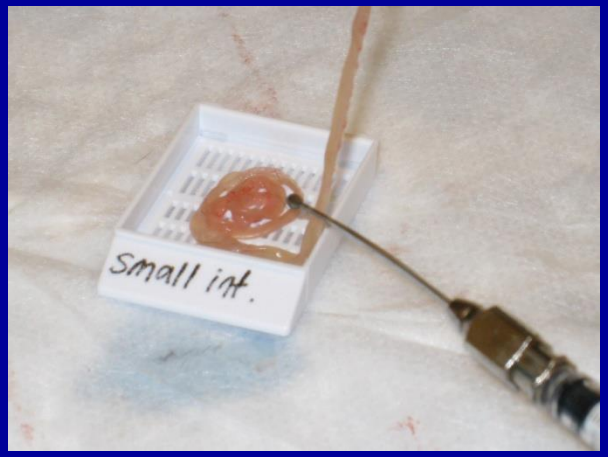
- The proximal (closest) end should end up in the corner (with or without stomach attached).

Cassetting the Small Intestine

Slide #4



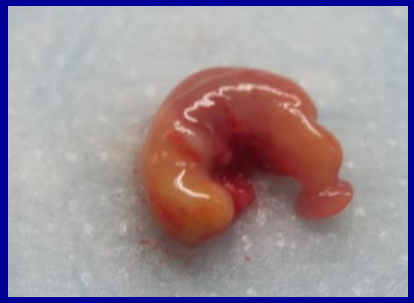


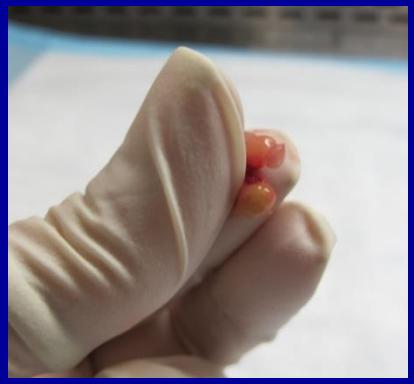


- This the jejunum. The cecum and stomach have been removed.

Cassetting the Stomach

- The stomach is grasped between the forefinger and thumb
- Scissors tips are passed through the duodenal pyloris to the esophageal pyloris

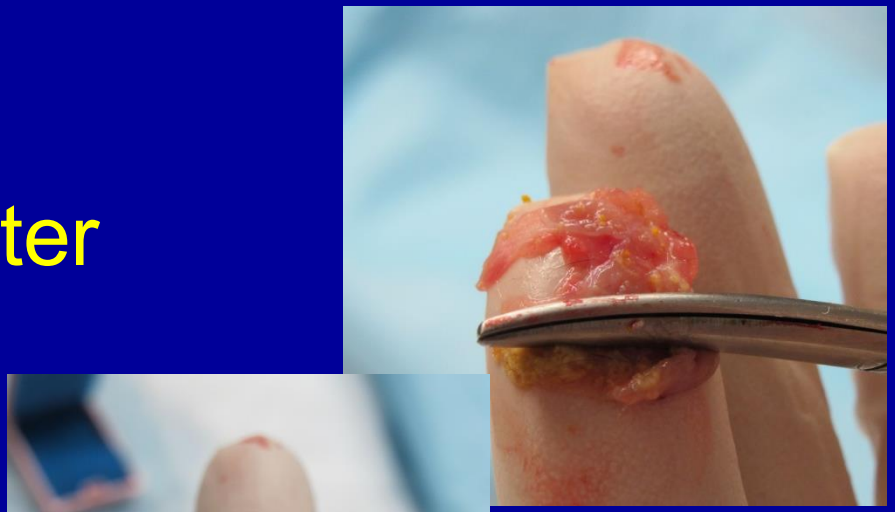


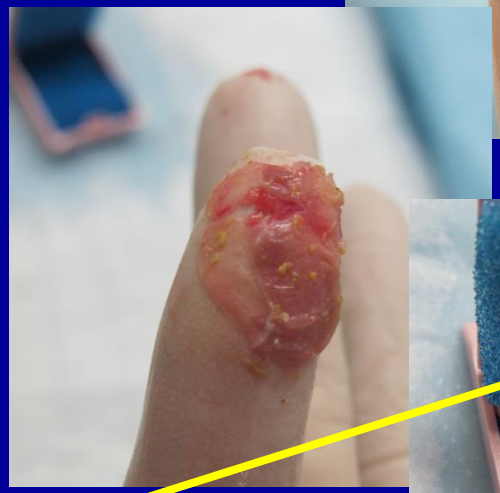


Cassetting the Stomach

Slied #2

- A single cut is then made along the greater curvature
- The tissue is then opened and content removed
- Place between two sponges to prevent curling





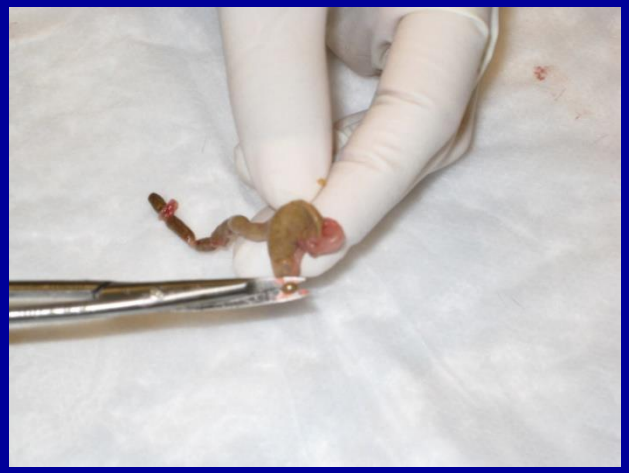
Cassetting the Large Intestine

- The technique is the same as for the small intestine. However, the large intestine is normally collected as one piece. Depending on the researcher, the cecum may be left attached to the large intestine, or attached to the ileum.

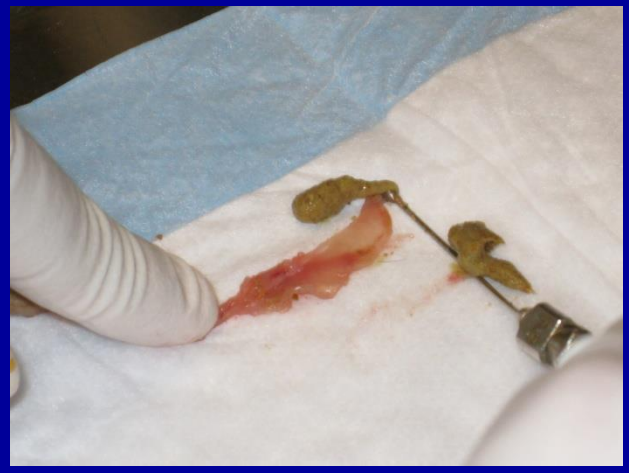
- As we have not outlined how to remove the cecal content, we will now illustrate, leaving the cecum attached to the large intestine.

Cassetting the Large Intestine

Slide #2



- First, the tip of the cecum is split, or is snipped off.
- The side of the needle is used to push the gross contents out of the lumen.
- Next, the cecum is rinsed by inserting the needle into the cut end and flushing gently with fluid until remaining content is removed. Placed between 2 sponges.



Cassetting the Large Intestine

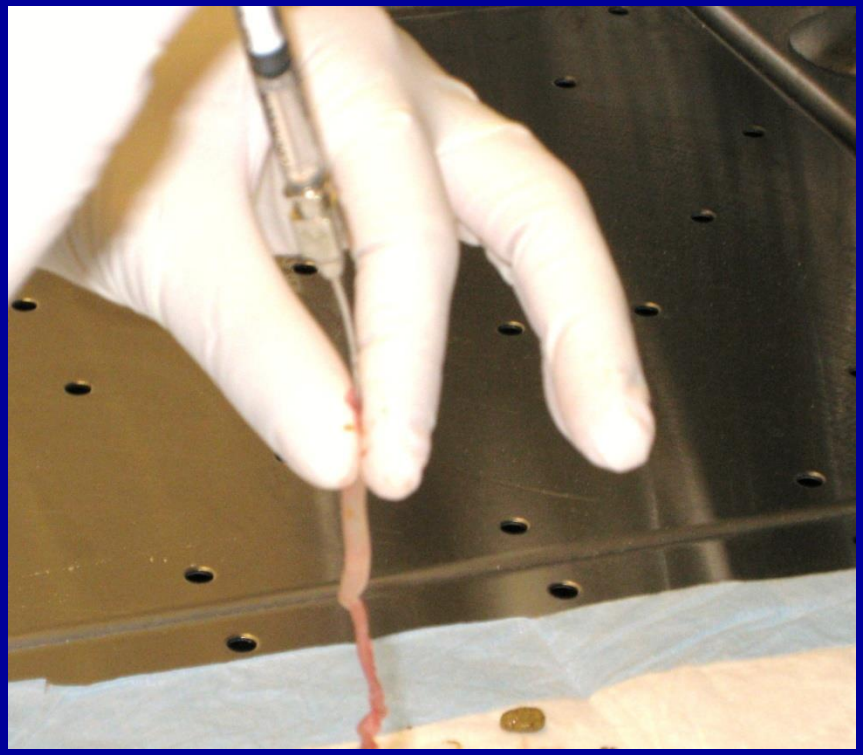
Slide #3

- Beginning at the distal end, the fecal pellets and remaining lumen content are removed.
- If contents “stick,” they are moistened with fluid and gently massaged forward until expelled.

Cassetting the Large Intestine

Slide #4

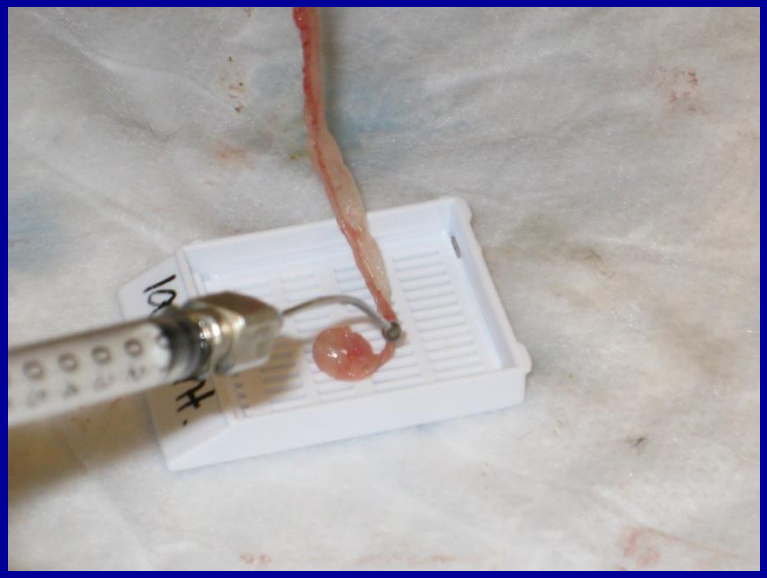
- Entering again through the cecal incision, the needle is threaded into the large intestine and the lumen is gently flushed of content, as was done with the small intestine.

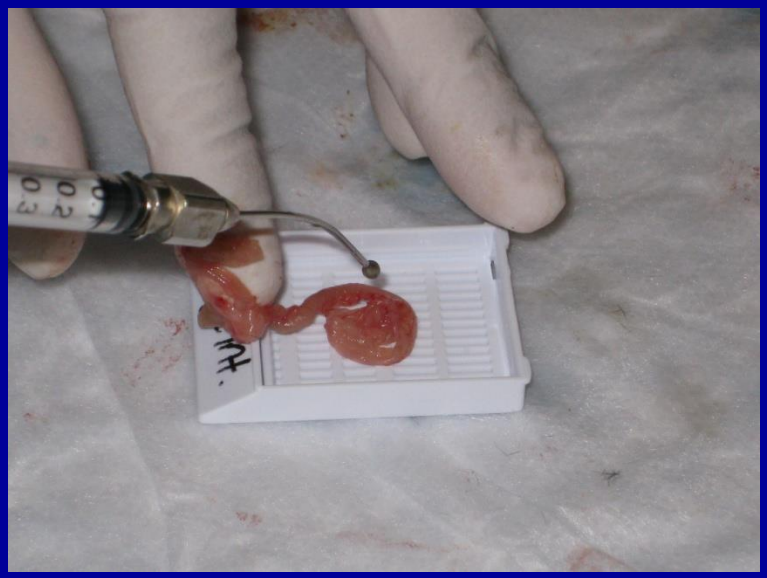


Cassetting the Large Intestine

Slide #5

- Beginning with the distal end of the large intestine, the coil is begun.
- The needle tip can be used to help arrange the spiral so that it does not overlap.

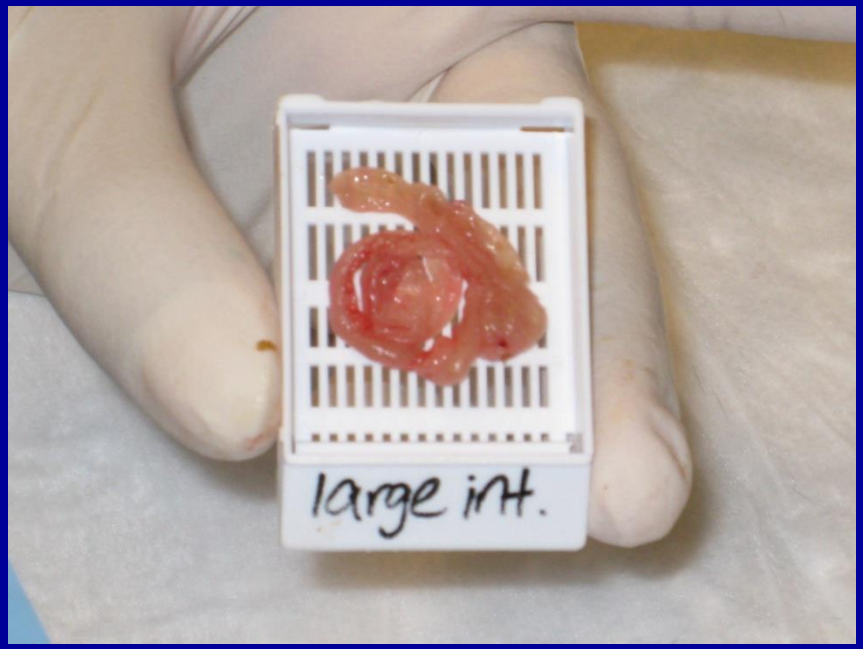




Cassetting the Large Intestine

Slide #6

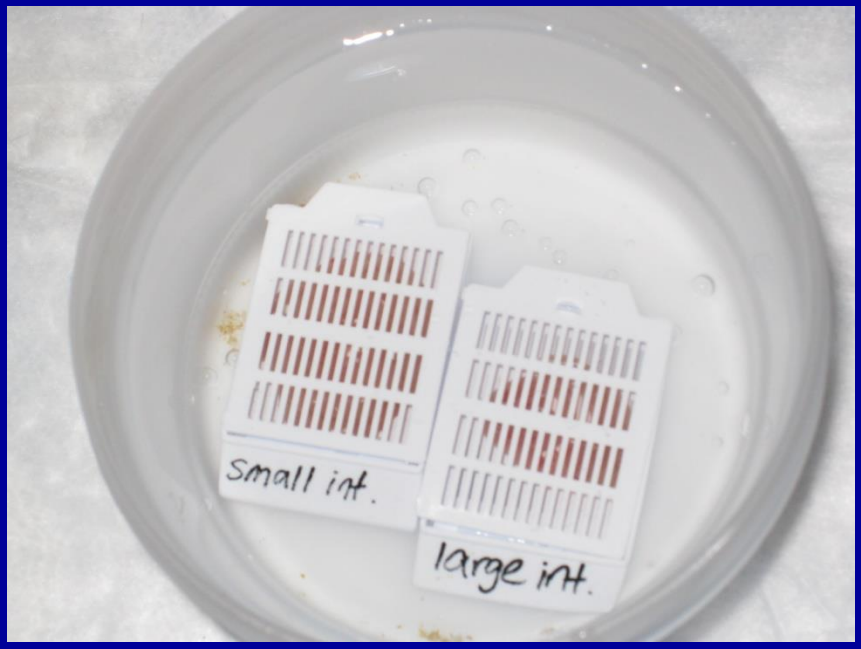
- The final coil is shown with the cecum to the upper/right side.
- NOTE: If the protocol calls for an organ (stomach, cecum, etc.) to remain attached, the coil is begun in a manner that ensures the organ is in an outer corner – regardless of if starting with proximal or distal end of the segment.
- If no organ is attached, then always begin the coil with the proximal end centered.



Final Cassettes

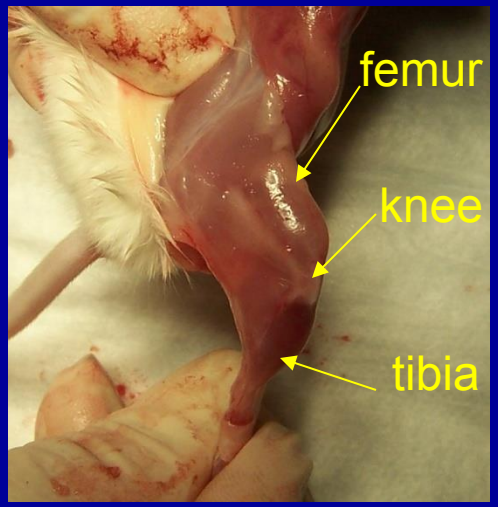
- The final cassettes are dropped in formaldehyde.

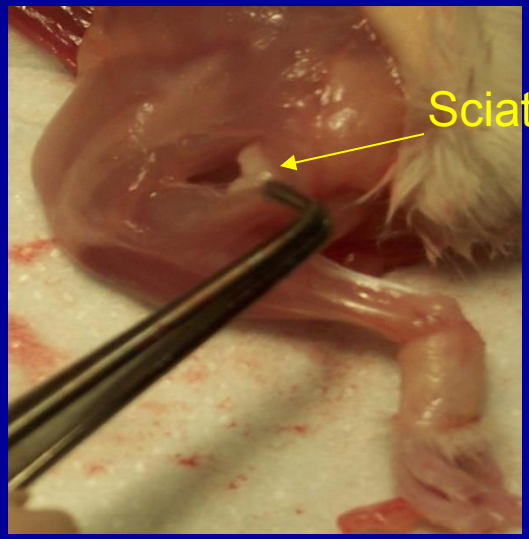
- Correct ratio of tissue to formaldehyde is 1:20.

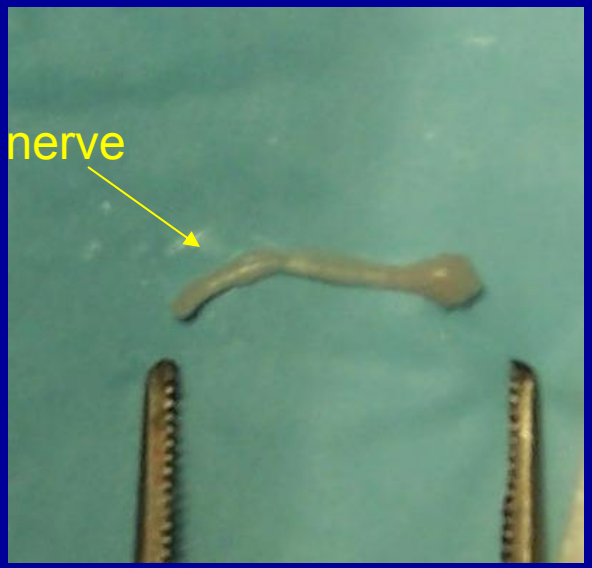


- If multiple animals are submitted, be sure to label the individual cassette with the ID number and target tissue.

+/- Isolate the sciatic nerve. It is located at the back of the leg and runs in a natural grove between two muscles. Curved forceps are placed under it and gentle blunt dissection is used to free it from surrounding tissues. Remove entire length GENTLY, as it stretches/tears easily. Cassette between 2 sponges







+/- Trim away remaining muscle. Cut through at the hip and just above the ankle. Include the femur, knee and the tibia (the fibula is not utilized). The limb is now transected/cut at the knee, leaving two lengths of bone (tibia and femur). Bone marrow is collected by threading a blunted 25 g needle into the lumen of each and flushing with solution (of choice) over a collection tube. When done correctly the bone segment will turn white.