



University Animal Care Committee

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 1 THE LABORATORY RAT

HANDLING AND RESTRAINT

- General Biology and Physiological Data
- Handling and Restraint
- Identification
- Sexing
- Sampling for Genotyping
- Blood Collection: intra-cardiac puncture
- Euthanasia
- Necropsy Explanation with pictures

The UACC would like to acknowledge the invaluable help of the Animal Resources Centre technicians in preparing this handout.

THE LABORATORY RAT

The common laboratory rat *Rattus norvegicus* is an ideal experimental animal for several reasons: abundance of literature published pertaining to them, ease of handling, high fertility rate, short gestation period, low maintenance and disease model for various human disorders and diseases.

GENERAL BIOLOGY AND PHYSIOLOGICAL DATA:

- most active at night (nocturnal)
- curious and investigative behaviour
- poor vision, acute sense of hearing and smell
- social animals, adult males may require separation if aggressive
- body temperature: 37°C
- respiratory rate: 75-115 breaths/min
- heart rate: 260-400 beats/min
- daily water consumption: 10-12 ml/100 g body weight
- daily food consumption: 10 g/100 g body weight
- oestrous cycle: 4-5 days
- duration of oestrus: 12 hours
- litter size: 6-12
- gestation: 20-22 days
- birth weight: 5 g
- weaning age: 21 days
- sexual maturity: 7 weeks
- breeding duration: 12 - 16 months
- male adult weight: 450-550 g
- female adult weight: 250- 300 g
- life span: 2.5-3.5 yrs

Score 1: Muscle wasting is advanced, fat deposits are gone and bones are very prominent.
Euthanasia is mandatory.



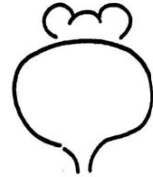
Score 2: The mouse is becoming thin and bones are prominent.
This category may be further divided subjectively as +2, 2, -2.



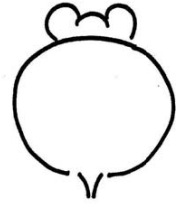
Score 3: The mouse is in optimal condition. Bones are palpable but not prominent.



Score 4: The mouse is well-fleshed, and bones are barely felt.



Score 5: The mouse is obese, and bones cannot be felt at all.



HANDLING AND RESTRAINT

Manual restraint:

- Before opening the cage observe the animals within. Nervous or young rats can escape quickly.
- Rats will not stay on top of the wire-bar lid of the cage. Always have a hand on them.
- Rats should not be held by their tail as their skin is fragile and can easily strip from the underlying tissue.
- Rats should not be scruffed by the loose skin on the back of the neck.

Three methods are commonly used:

1. "V" grip

- With your dominant hand, slide your index and middle finger along both sides of the head as far as possible and grasp the head with your knuckles on the jaw bones.
- Place your thumb and remaining fingers under both forelimbs to grasp the thorax.
- If possible, you can hold the lower body with your other hand or rest the rat on your chest for the comfort of the animal. This is especially important for larger or pregnant animals.

2. Cross leg

- With your dominant hand, position your thumb and middle finger on each elbow.
- Push on both elbows to cross the two front paws.
- With your remaining fingers support the thorax of the rat.
- If possible, you can hold the lower body with your other hand or rest the rat on your chest for the comfort of the animal. This is especially important for larger or pregnant animals.

3. Towel method

- If you have nervous or aggressive animals, you can wrap it in a towel. This method has the advantage of controlling the hind limbs which prevents potential scratching.

Restraint devices:

Several restraint devices are available in various sizes and materials (e.g., Plexiglas, plastic) and can be used when performing different techniques such as injections or blood collection. The restrainer should be small enough so that the animal cannot turn around yet allow the animal to rest comfortably and breathe normally. Observe animals to ensure that they do not overheat and never leave an animal in a restrainer unattended.



IDENTIFICATION

Rat can be identified by the following methods:

1. Cage cards
2. Temporary markings
3. Ear punching/notching
4. Ear tags
5. Tail tattoo
6. Micro-tattooing
7. Electronic identification with microchips

1. Cage Cards

- Cage cards can be used to identify individually-housed rats or a single breeding pair. They can also be used to identify groups of rats on protocols if individual identification is not necessary.
- All sections of the cage card need to be completed.

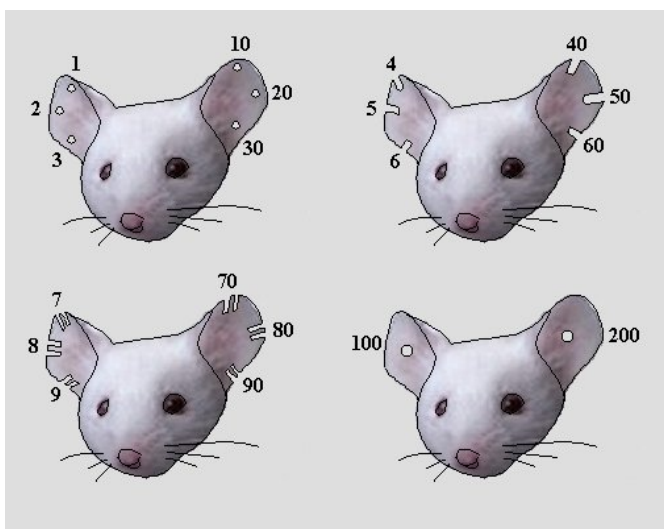
CAGE CARD		
INVESTIGATOR	DEPARTMENT	
PROTOCOL #	CONTACT	
ANIMAL ID	SEX	STRAIN
SOURCE	DATE REC'D	WT. REC'D

2. Temporary marking

- Temporary marking can be used for short term individual identification.
- Use a non-toxic, permanent marker to write numbers, bars or other distinguishable marking on the tail or the ears.
- If temporary marking is to be used for duration exceeding a week, repeat marking at least twice a week.

3. Ear punching/notching

- This method cannot be used on rodents under 2 weeks (14 days) of age.
- Restrain the animal securely and using an ear punch, punch a hole and/or notches in the ears following an identification chart.
- Whenever possible, use a simple code to limit the number of notches/punches made to the animal.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals.
- If needed, use the excised tissue as a sample for genotyping.



4. Ear tag

- Use tags of appropriate size, approximately 5 mm long.
- Rinse tags in 70% alcohol before use.
- Place the tag low on the pinna (distal $\frac{1}{3}$) so that it rests against the rat and does not bend the ear, catch on the cage or cause the rat to hold its head in a lopsided manner.
- If the tag is placed too tight it can lead to local infection or inflammation. The animal will need to be monitored for these clinical signs and the tag removed if necessary.

5. Tattooing

- Local or general anesthesia is recommended.
- Use an electric tattoo machine to write numbers on the tail.
- Ensure that needles are sterile and sharp.

6. Micro-tattooing

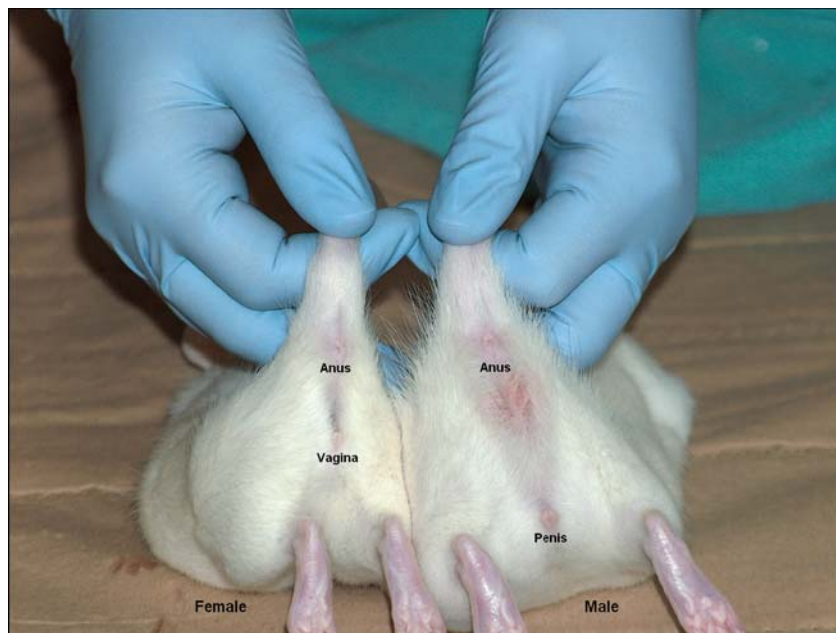
- Use a micro-tattooer to inject tattoo ink in the toe pads and/or the ears.
- Whenever possible, use a simple code to limit the number of toes tattooed.
- Have the identification chart readily available in the animal room to allow prompt identification of individuals.

7. Microchips

- Use appropriate general anaesthesia and analgesia to implant microchip.
- Do not implant microchips in animals less than 3 weeks old.
- Apply disinfectant on the skin (e.g., chlorhexidine, betadine).
- Using the implanter, inject the microchip subcutaneously in the neck area between shoulder blades.
- Have available a compatible reader to allow identification of the mice.
- Microchips can be reused after proper cleaning and sterilization (follow manufacturer's recommendation).

SEX DETERMINATION

- Sexing of mice is based upon ano-genital distance
- Males have a greater distance between the anus and urogenital opening than females.
- An opposite sex comparison is advisable initially.
- The testicles can be retracted into the abdomen; therefore, it may be easier to sex a mature male by holding its head up vertically. The genital papilla is more prominent in males than females



SAMPLING FOR GENOTYPING

Rats can be genotyped by the following methods:

1. Fecal pellet
2. Buccal epithelial cell
3. Ear punching
4. Tail snipping

1. Fecal pellet

- Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding.
- Properly identify samples to match animal identifications

2. Buccal epithelial cell

- Firmly restrain the animal by the scruff to maintain its mouth open.
- Using the swab, vigorously scrape both inner cheeks.
- Insert cotton bud into collection tube and snip off excess shaft.
- Properly identify samples to match animal identifications

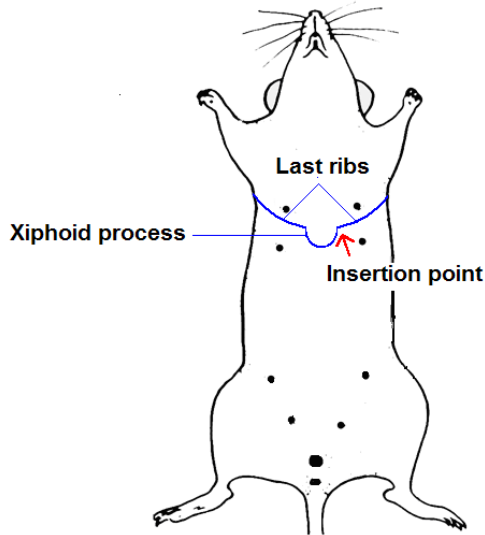
3. Ear punching

- Do not use this method in rodents under 2 weeks of age.
- Restrain the animal securely.
- Using the ear punch; punch holes and/or notches in the ears following an identification chart.
- Use the excised tissue as a sample for genotyping.

BLOOD COLLECTION

Intra-cardiac puncture

- Terminal procedure.
- This procedure must be done on anesthetized animal or one that has been just euthanized.
- Procedure:



- Place the rat in dorsal recumbency.
- Palpate the xiphoid process between the two last ribs and visualize it.
- Prepare a syringe between 1cc and 5 cc with a 20G 1 1/2 needle.
- insert the tip of the needle between the left side of the xiphoid process and the last rib.
- Once you went through the skin, gently pull back on your plunger to create a minimal negative pressure and keep it.
- Move slowly toward the heart with an angle of approximately 40-45 degree.
- Note: The heart is slightly to the left of the midline.
- When a small quantity of blood will come into the hub of the needle, stabilize your syringe and continue to pull back on the plunger slowly.

Note: If the blood flow stops, you change the angle of the needle slightly or rotate it.

EUTHANASIA

Rat can be euthanized in a variety of acceptable, effective and humane methods. Euthanasia methods can be either

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chemical or physical.

Adult rodents - Chemical methods

1. CO₂ asphyxiation

- In order to minimize stress animals should be euthanized in their home cage.
There should be a maximum of:
 - 4 adult rats weighing up to 300 g
 - 3 adult rats weighing up to 400 g
 - 2 adult rats weighing up to 500 g
 - 1 female with 1 litter (pups over 10 days old)
- Place the appropriate sized lid on the animal cage with grid removed.
- Connect the regulator hose to lid fitting.
- Do not pre-charge the chamber.
- Plug in the heater unit if necessary and available (e.g. if euthanizing many cages)
- Open the CO₂ tank valve.
- Set the regulator to the appropriate setting:
 - Standard mouse cage (7.25" x 11.5" x 5"): 2 LPM (Litres per minute)
 - Standard rat cage (12" x 9" x 6"): 5.25 LPM
 - Cages of different dimensions:

Measure the cages width, length and height and multiply them to determine the volume in cubic inches.

Then divide this by 61 to convert into liters and multiply by 20% to determine flow rate.

$$\frac{\text{height} \times \text{width} \times \text{length}}{61} \times 20\% = \text{flow rate (LPM)}$$
- Once the animals become unconscious, the flow rate can be increased to minimize the time of death. Please note that the time required for euthanasia can be several minutes.
- Maintain the CO₂ flow until the animal has stopped breathing.
- Close the valve on the tank.
- Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change in eyes.
- Following euthanasia by CO₂, physical euthanasia such as cervical dislocation is strongly recommended to ensure death.

2. Barbiturate or injectable anesthetic overdose

- Inject three times the anesthetic dose intra-venously or intra-peritoneally.

- Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change in eyes.
- A physical method of euthanasia, such as cervical dislocation, is recommended on your animals before disposal to ensure that they have been correctly euthanized.

3. Overdose of inhalant anesthetic

- Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanized.
- The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate amount of the anesthetic. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Procedures should be conducted in a chemical fume hood to prevent inhalation of the anesthetic by personnel.
- The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter through a nose cone.
- Sufficient air or O₂ must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a large container, there will be sufficient O₂ in the chamber to prevent hypoxemia.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change in eyes.
- Following euthanasia by CO₂, physical euthanasia such as cervical dislocation is strongly recommended to ensure death.

Adult Rodents - Physical methods

Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed.

Anesthesia or sedation is necessary prior to physical methods of euthanasia, unless described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).

1. Cervical dislocation

- For mice and rats under 200g, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a narrow, blunt instrument such as the dull edge of a scissor blade, acrylic ruler or cage card holder is pressed at the base of the skull.
- With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.

2. Decapitation

- Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous

manner are commercially available.

- The use of a plastic conical restraining bag is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

3. Exsanguination

- Animals may be exsanguinated to obtain blood products, but only when they are deeply anesthetized or recently euthanized by CO₂ asphyxiation.
- Collect blood from the heart. (Procedure described in blood collection section of Module 1)
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, colour change in eyes.
- A physical method of euthanasia, such as cervical dislocation or pneumothorax is recommended on your animals before disposal to ensure that they have been correctly euthanized.

4. Pneumothorax

- A pneumothorax can be performed after euthanasia by CO₂ asphyxiation or by barbiturate or injectable anesthetic overdose.
- With sharp scissors, the diaphragm is lacerated causing the lungs to collapse therefore insuring the death of the animal before disposing of it.

Neonatal Rodents

Rodents over 10 days old or older can be euthanized by the same procedures as adult rodents.

Rodents under 10 days old must be euthanized by one of the following methods:

1. CO₂ asphyxiation
2. Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended.
3. CO₂ may be used for narcosis of neonatal animals but it must be followed by another method of euthanasia (e.g. decapitation using sharp blades).
4. Keeping neonates warm during CO₂ exposure for narcosis may decrease the time to death
5. Barbiturate overdose
6. Inject 3 times the anesthetic dose IP.
7. May be followed by a physical method of euthanasia (e.g. decapitation using sharp blades).
8. Overdose of inhalant anesthetic followed by decapitation

9. Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations, therefore, alternative methods are recommended.
10. Inhalant anesthetics may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades).
11. Decapitation
12. Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.

Gestating Rodents

Gestating rodents with foetuses under 17 days old can be euthanized by the same procedures as adult rodents.




Gestating rodents with foetuses over 17 days must be euthanized by one of the following methods:

1. CO₂ asphyxiation of the mother, followed by decapitation or barbiturate overdose (IP) of the fetuses.
2. Overdose of injectable anesthetics to the mother.

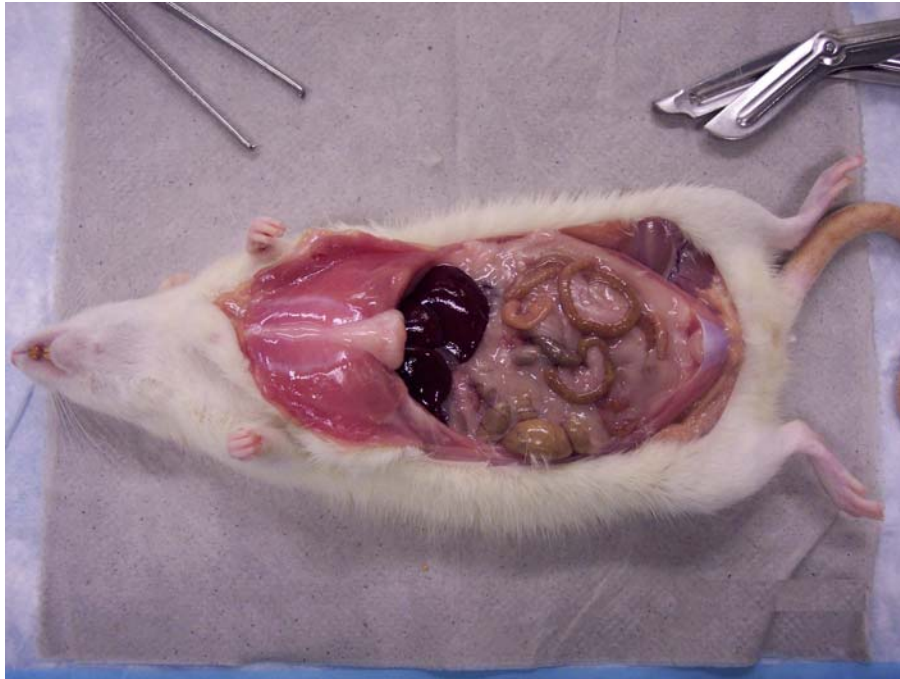
Unacceptable Euthanasia Techniques For Rodents

13. Decompression
14. Asphyxiation
15. Air embolism
16. Rapid freezing
17. Carbon monoxide
18. Methoxyflurane
19. Ether
20. Nitrogen
21. Nitrous oxide
22. Chloroform
23. Chloral hydrate
24. Poisons (strychnine, cyanide)
25. Household products and solvents (acetone, alcohol)

MOUSE EUTHANASIA

Method of Euthanasia	CO2 Asphyxiation	Barbiturate or Injectable Anesthetic Overdose	Inhalant Anesthetic Overdose	Exsanguination	Cervical Dislocation	Decapitation
 Adult mouse and gestating mouse (under 17 days gestation)	YES	YES	YES	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia
 Gestating mouse (more than 17 days gestation)	YES	YES decapitation of pups not required	YES	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia	YES After CO2 or Under General Anesthesia
Decapitation of pups required after euthanasia of mom						
 Pups under 10 days old	Only as Narcosis	YES	Only as Narcosis	NO	NO	YES

NECROPSY



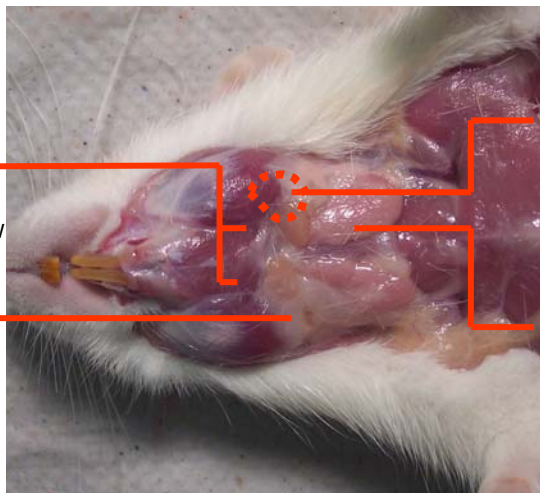
SALIVARY GLANDS

Lymph Nodes

Brown solid nodes found near the jaw line.

Parotid

This is the white, half-moon shaped tissue lying on top.



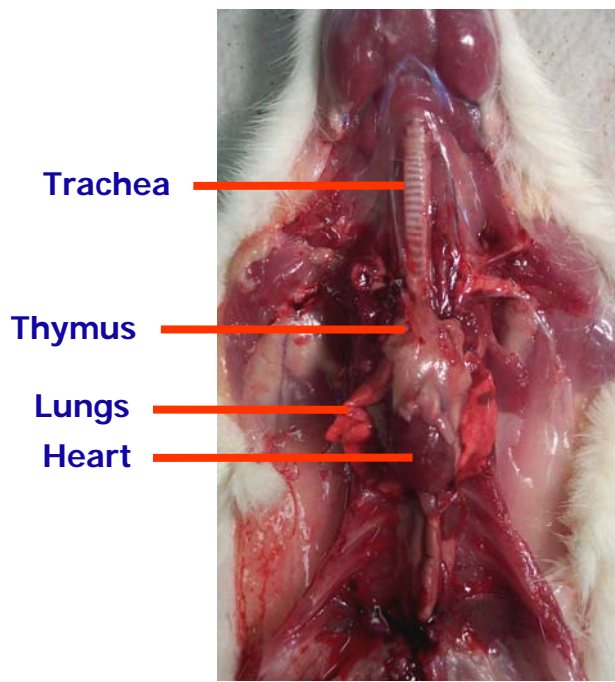
Sublingual Gland

Also known as Submaxillary gland is found attached to the corner of the submandibular gland.

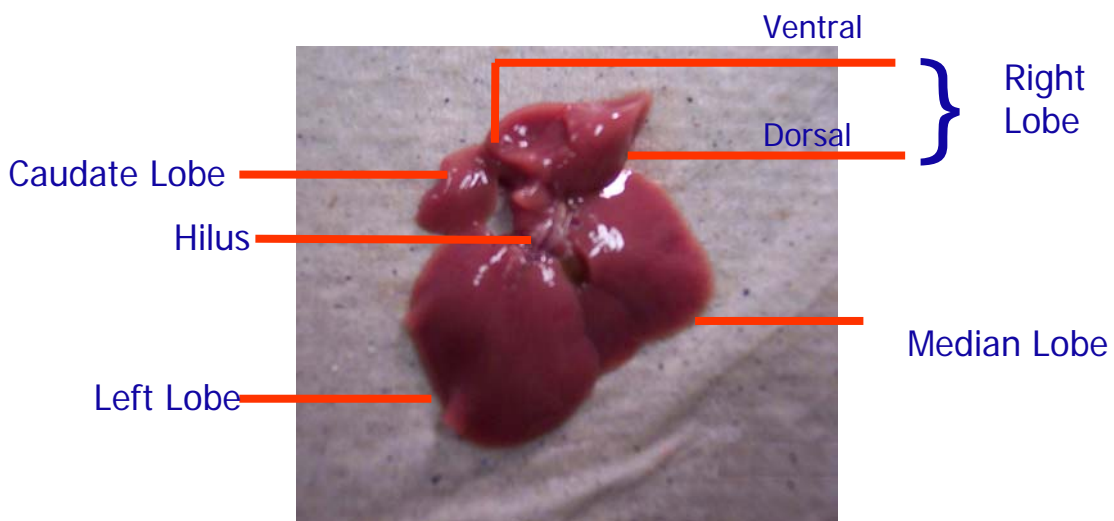
Submandibular Gland

The largest salivary gland responsible for most of the secretions.

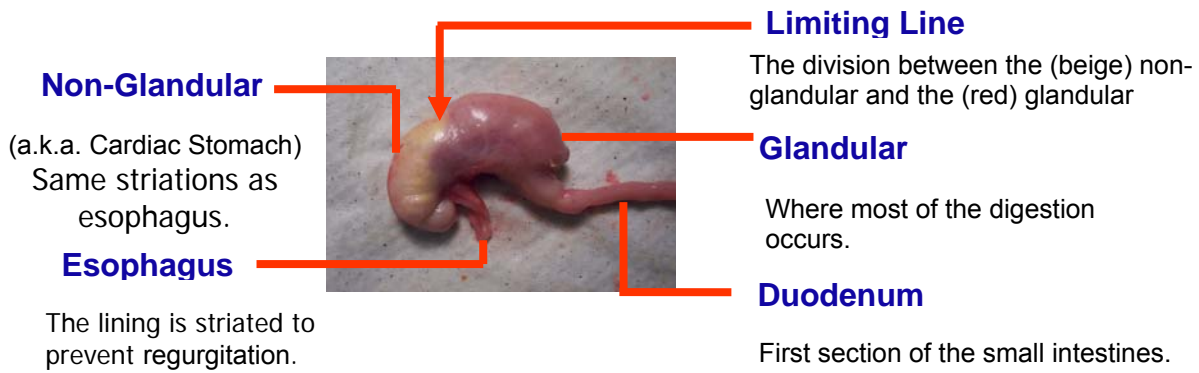
TRACHEA, THYMUS, LUNGS AND HEART



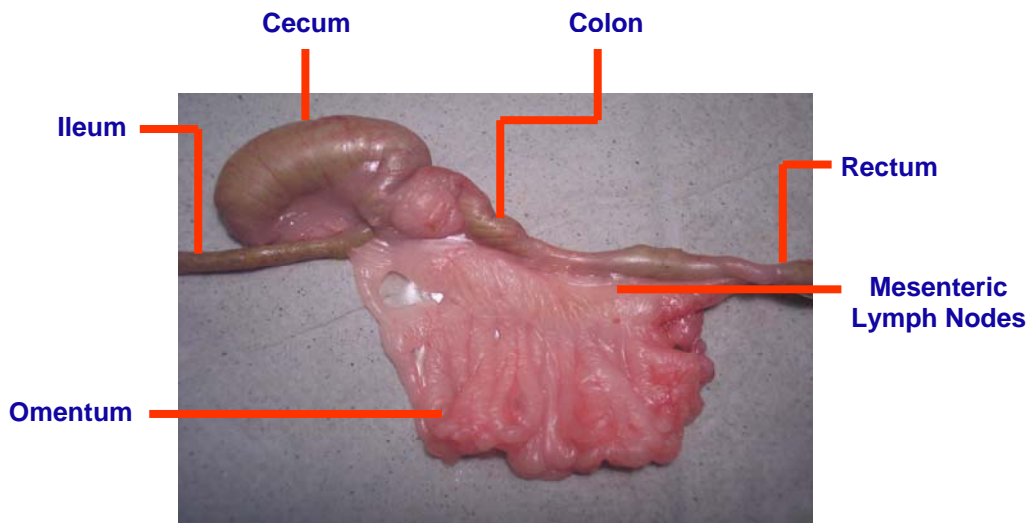
LIVER



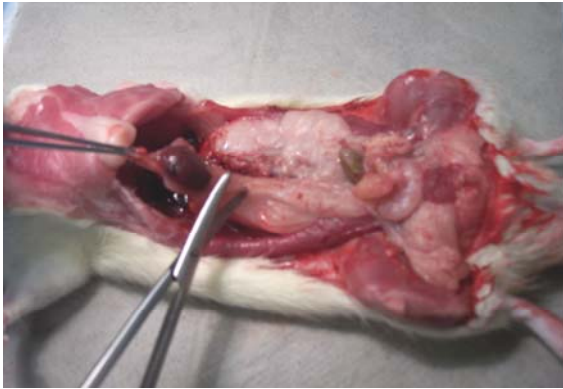
STOMACH



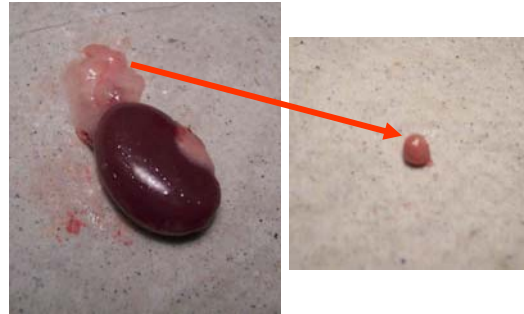
CECUM, MESENTERIC LYMPH NODES, COLON AND RECTUM.



KIDNEY



The adrenal gland can be found within the adipose tissue above the kidney, so be careful when dissecting the fat away.



It is normal to find the right kidney displaced a little more cranially than the left in both rats and mice.

THE FEMALE REPRODUCTIVE SYSTEM

Uterine Horn

You may find black dots along the horns. These are past implantation sites, where 8-12 offspring will develop for approx. 21 days.

Urinary Bladder

May be found full or empty. Will hold up to 2.5ml of urine each day in the mouse and 15ml in the rats.



Ovary

Found embedded in fat attached to the uterine horn. The oviduct, a small coiled tubule, may be found attached to the ovary.

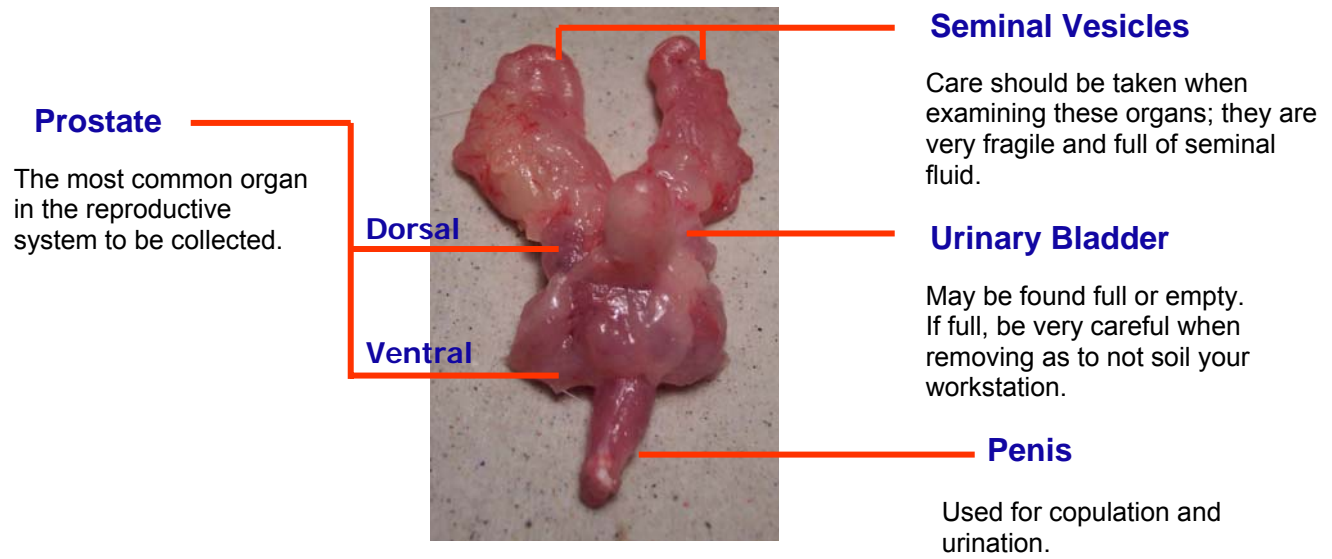
Cervix

You may find black dots along the horns. These are past implantation sites, where 8-12 offspring will have developed for approx. 21 days.

Vagina and Vulva

Used for copulation.

THE MALE REPRODUCTIVE SYSTEM



Necropsy photos courtesy of Douglas Hospital facility Animal Health Technicians