In vivo toxicological study of nanomaterials

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Introduction

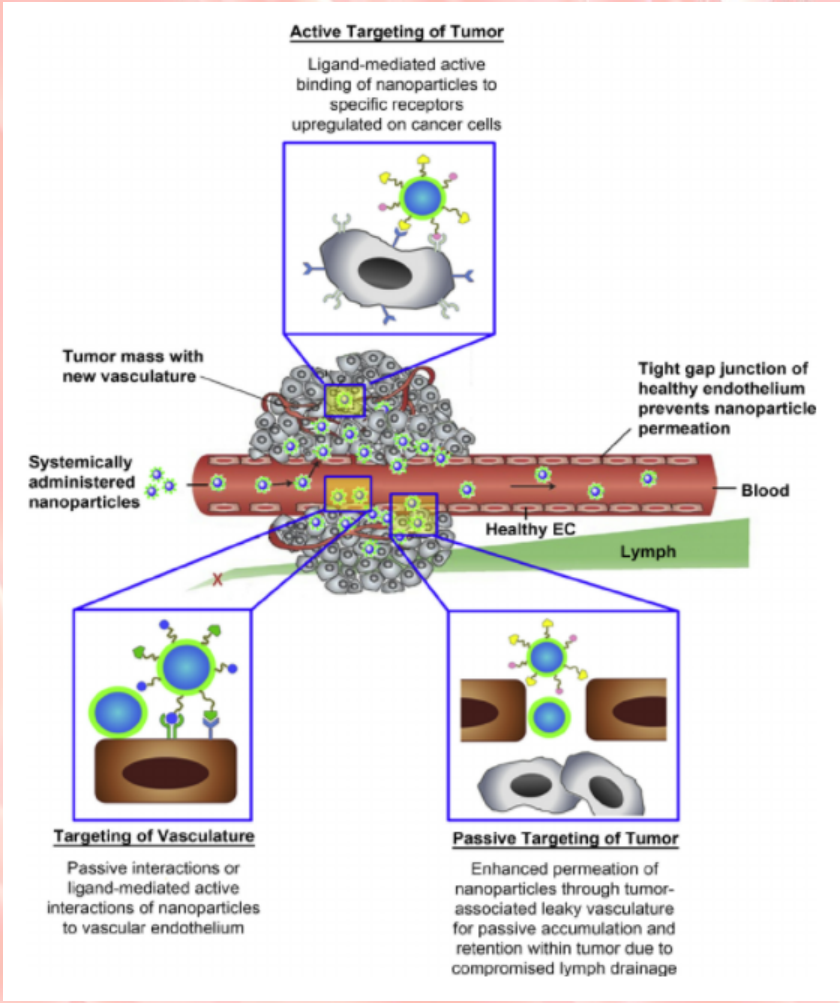
The concept of drug targeting, suggested by Paul Ehrlich almost a century ago, considered an hypothetical ‘magic bullet’ as an entity consisting of two components:

Introduction

- A conventional application of drugs is characterized by limited effectiveness, poor biodistribution, and lack of selectivity.
- These limitations and drawbacks can be overcome by controlling drug delivery. Nanomaterials are very promising as drug delivery systems (DDS). They can transport the drug to the place of action, thus, minimizing the distribution on healthy tissues and decreasing the undesirable side effects.
- Additionally they may protect the drug from rapid degradation or clearance and enhance its concentration in target tissues.
- This approach is especially important when the therapeutic and toxic concentration of the drug is very close.

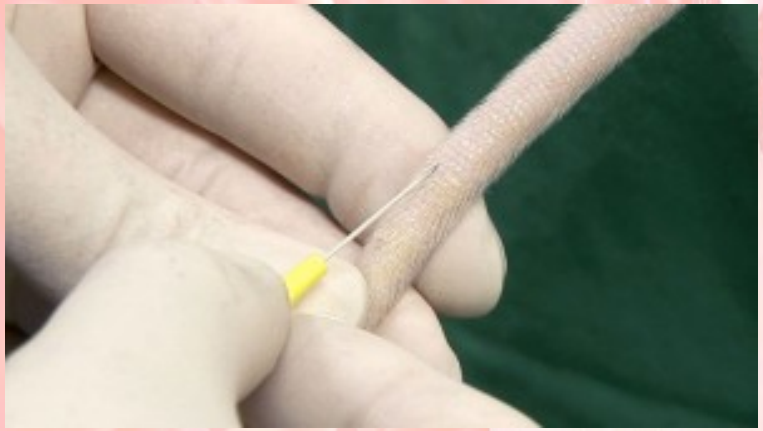
Features exploited by nanomaterials

- Enhanced permeability and retention
- Targeting due to alterations in physical characteristics
- Targeting moieties



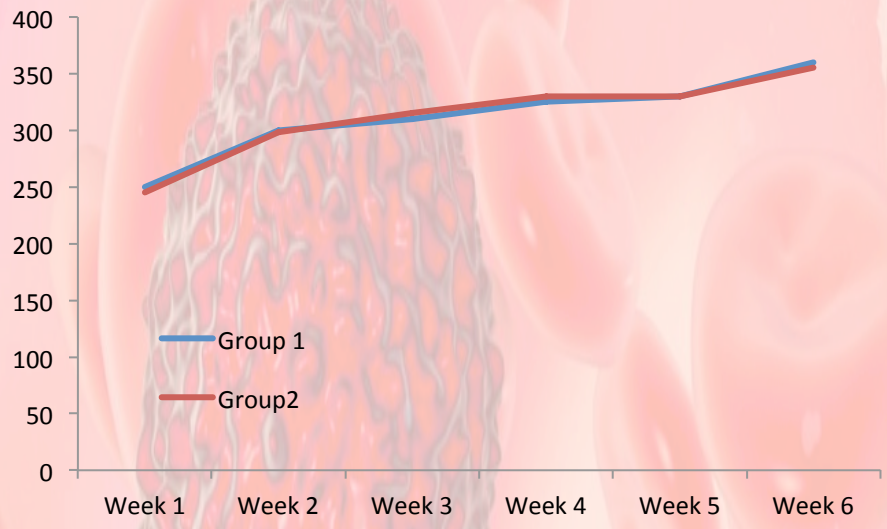
- Efficacy
- Toxicological impact

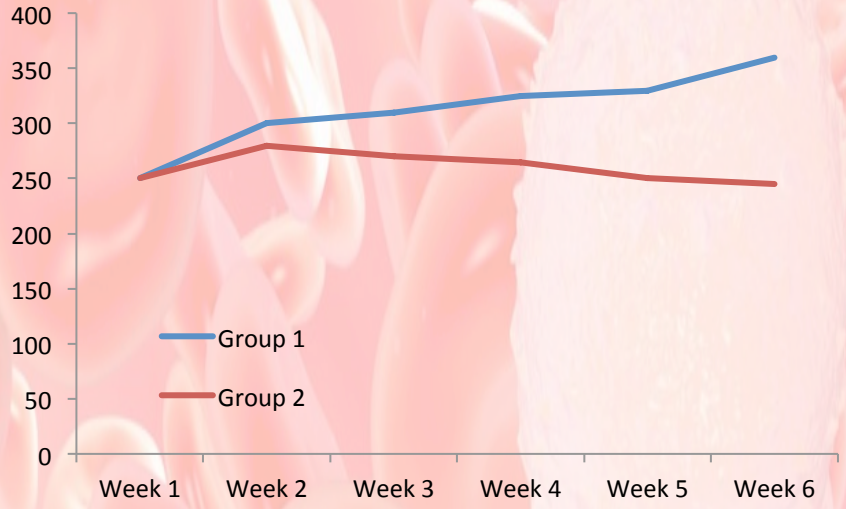
Rodents



Recommended I.V. Volumes for Rodents			
Species	Dose volume	I.V. volume (ml/Kg)	I.V. Volume (ml/25g)
Mouse	Ideal	5 bolus	0.125 bolus
	Maximum	25 slow injection	0.625 slow injection
			I.V. Volume (ml/ 250g)
Rat	Ideal	5 bolus	1.25 bolus
	Maximum	20 slow injection	5 slow injection

Body weight curves





Mouse

Small Volumes of blood required		Larger blood volumes
General anaesthesia required	General anaesthesia not required	General anaesthesia required (terminal method)
Saphenous vein	Saphenous vein	Cardiac puncture
Coccygeal vein	Coccygeal vein	Abdominal/thoracic vessel
Sublingual vein	Mandibular vein	Retro-orbital
	Blood vessel cannulation	Decapitation
	Tail snip	

Rat

Small Volumes of blood required		Larger blood volumes
General anaesthesia required	General anaesthesia not required	General anaesthesia required (terminal method)
Saphenous vein	Saphenous vein	Cardiac puncture
Coccygeal vein	Coccygeal vein/Temporary cannula	Abdominal/thoracic vessel
Sublingual vein	Jugular vein	Retro-orbital
	Blood vessel cannulation	Decapitation

Recommended blood volumes

	Repeated	Non-repeated
Mouse (for an average weight of 25 g)	Maximum <10% total blood volume (= 0.14 ml) on any single occasion and <15% total blood volume (= 0.21 ml) in 28 days	Maximum <10% total blood volume (= 0.14 ml)
	For repeat bleeds at short intervals, suggested limit <1% total blood volume (= 0.01 ml) in 24 hours and consider cannulation	Terminal sample under general anaesthesia (volume unrestricted)
Rat (for an average weight of 400 g)	Maximum <10% total blood volume (= 2.56 ml) on any single occasion and <15% total blood volume (= 3.84 ml) in 28 days	Maximum <10% total blood volume (= 2.56 ml)
	For repeat bleeds at short intervals, suggested limit <1% total blood volume (= 0.25 ml) in 24 hours and consider cannulation	Terminal sample under general anaesthesia (volume unrestricted)

Biochemical indexes of toxicological insult

Parameters	
Serum Glutamic Oxaloacetic Transaminase (SGOT/AST)	Acute injury of liver cells
Serum Glutamic Pyruvic Transaminase (SGPT/ ALT)	
Alkaline Phosphatase (ALP/SAP)	Chronic liver dysfunction
Bilirubin total (tBIL),	Liver function
Bilirubin direct (dBIL)	
Creatinine (Cr)	Markers of renal functions
Urea (Ur)	
Albumin (Alb)	Defective hepatic synthesis or increased loss from kidneys

Complete blood count

Parameter		Causes related to substance administration
PCV, RBC, Hgb	↑	Dehydration
	↓	Anaemia
WBC	↑	Infection, necrosis, severe inflammation
	↓	Anaphylaxis, toxicity
Neutrophils	↑	Chronic stress, necrosis, inflammation
	↓	Infection, toxicity, bone marrow suppression
Lymphocytes	↑	Chronic stress, necrosis, inflammation
	↓	Infection, toxicity, bone marrow suppression
Platelets	↑	Haemorrhage, acute or chronic inflammation
	↓	Toxicity, bone marrow suppression, anaphylaxis

General vs local anaesthesia

Safety in pregnancy

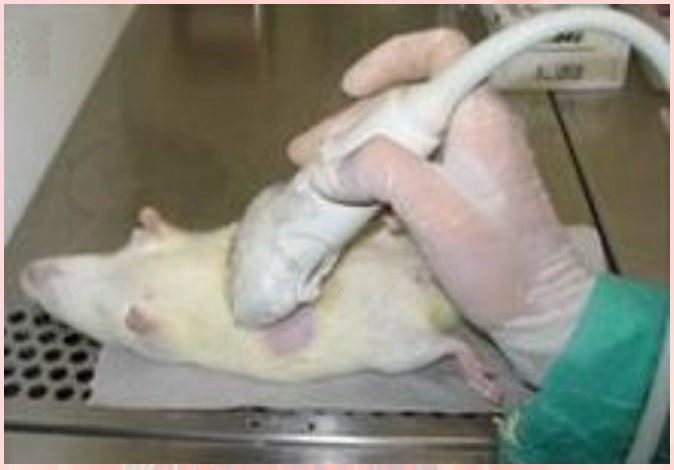
- Studies have shown that some nanomaterials may cross the placenta and cause deleterious effects on fetal growth, while others don't. The size and/or surface charge have been incriminated for these differences.

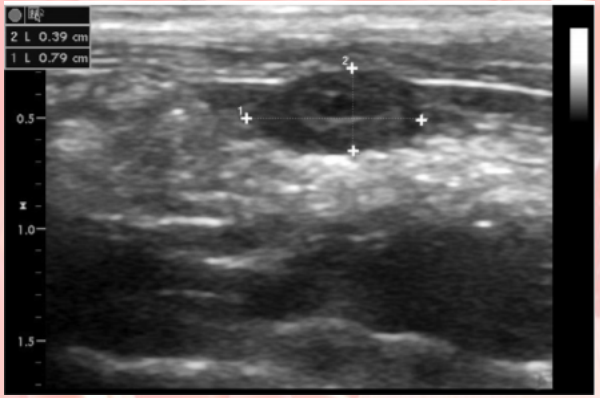
- In both humans and rats the placenta has a discoid shape and belongs to the haemochorial type, while changes, during pregnancy, in blood count and biochemical parameters are closely analogous.

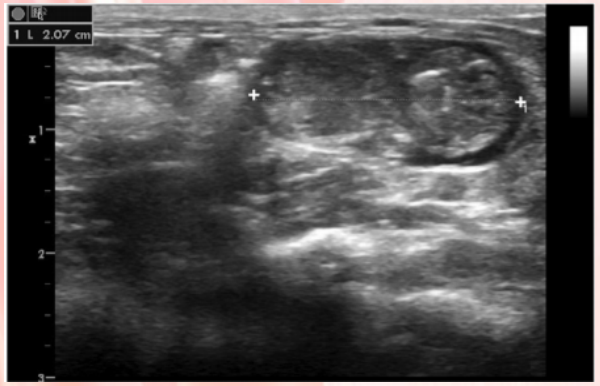
Ultrasonography

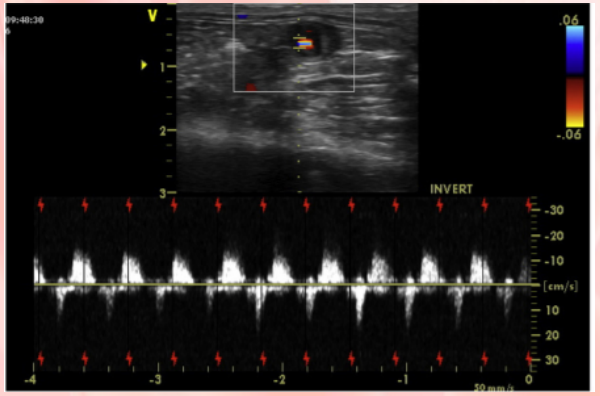
- In humans ultrasonographic volumetric parameters are used to evaluate normal development of fetuses during pregnancy.

- While heart rate has been employed to monitor fetal hypoxaemia and complications related to growth restriction due to placental insufficiency.



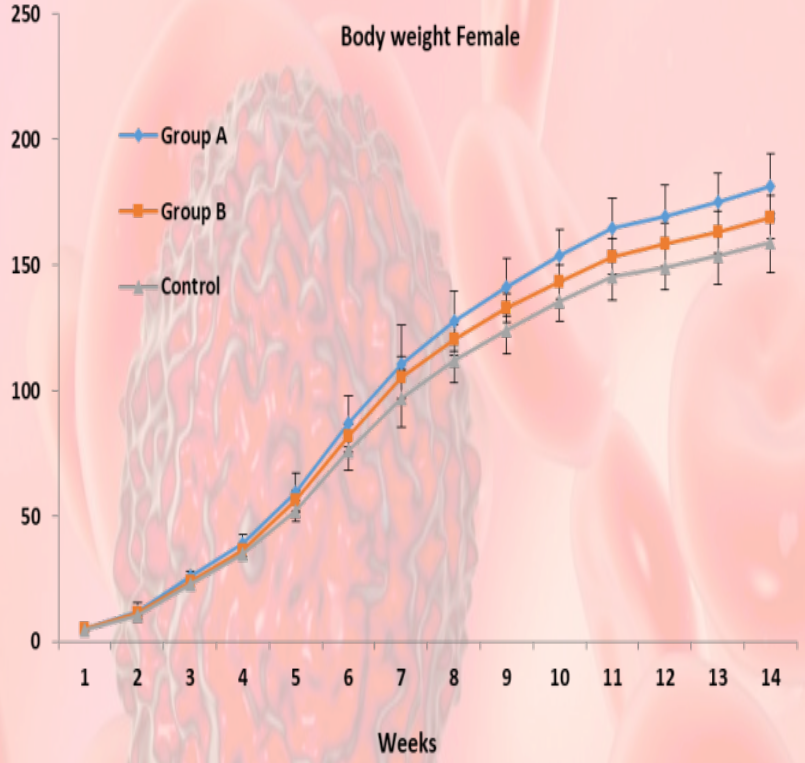


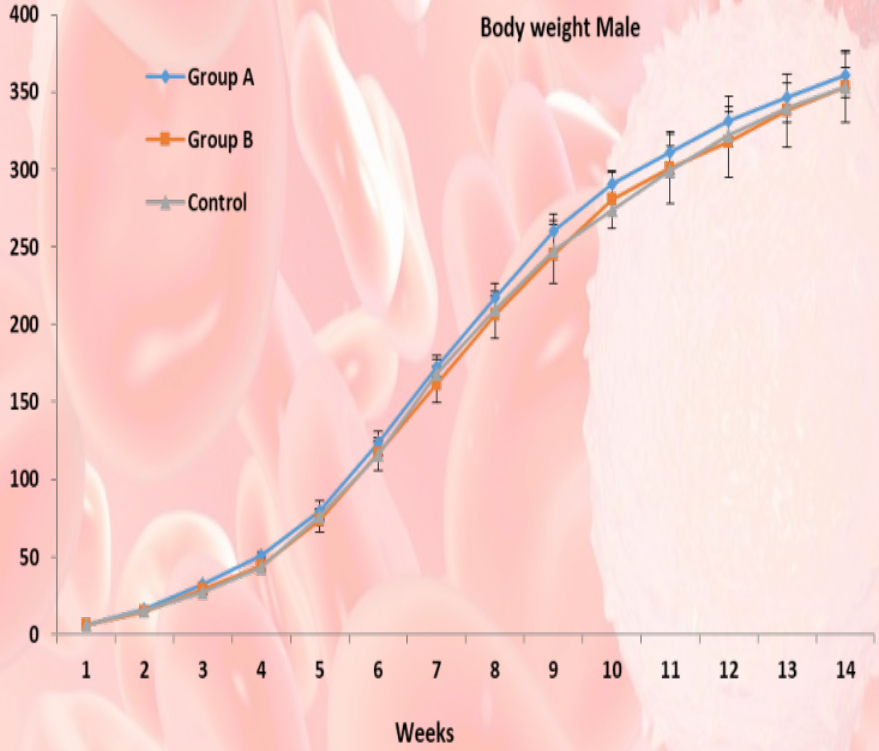




- Width of placenta
- Length and width of embryonic sac
- Foetus length
- Heart rate

Growth weight curves of offspring





Ante mortem examination

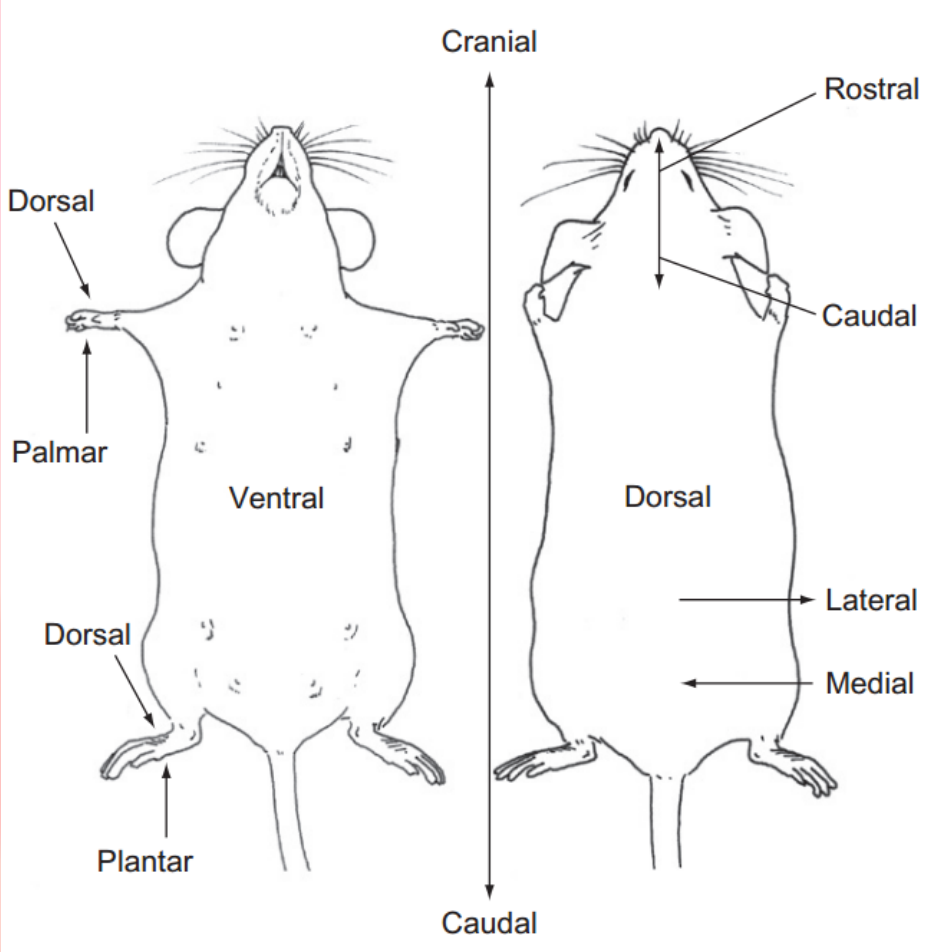
- Behavior of the animal and response to external stimuli
- Fur and skin and record any lesions
- Weigh (Doses of anaesthesia, ratio of organ to body weight)

Inspect all orifices

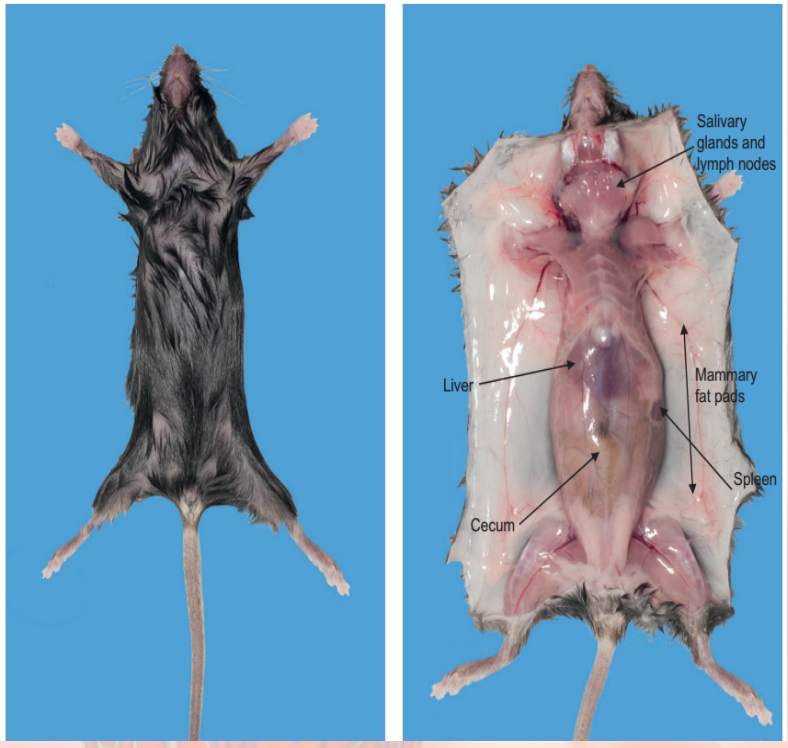
- Macroscopic lesions
  - Evaluation and recording
    - Size
    - Weight
    - Photograph
- Harvest and manage appropriatelly the biological samples

- Necropsies should be conducted as soon as possible after euthanasia
- 15 min after death
- Never freeze
- In need refrigerate at 0 to 4 ^{o}C (loss of RNA after 30-40 min)

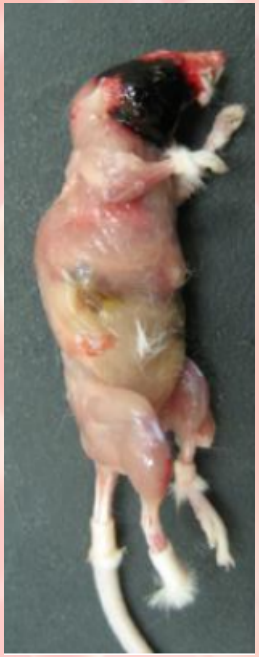
Describing gross lesions



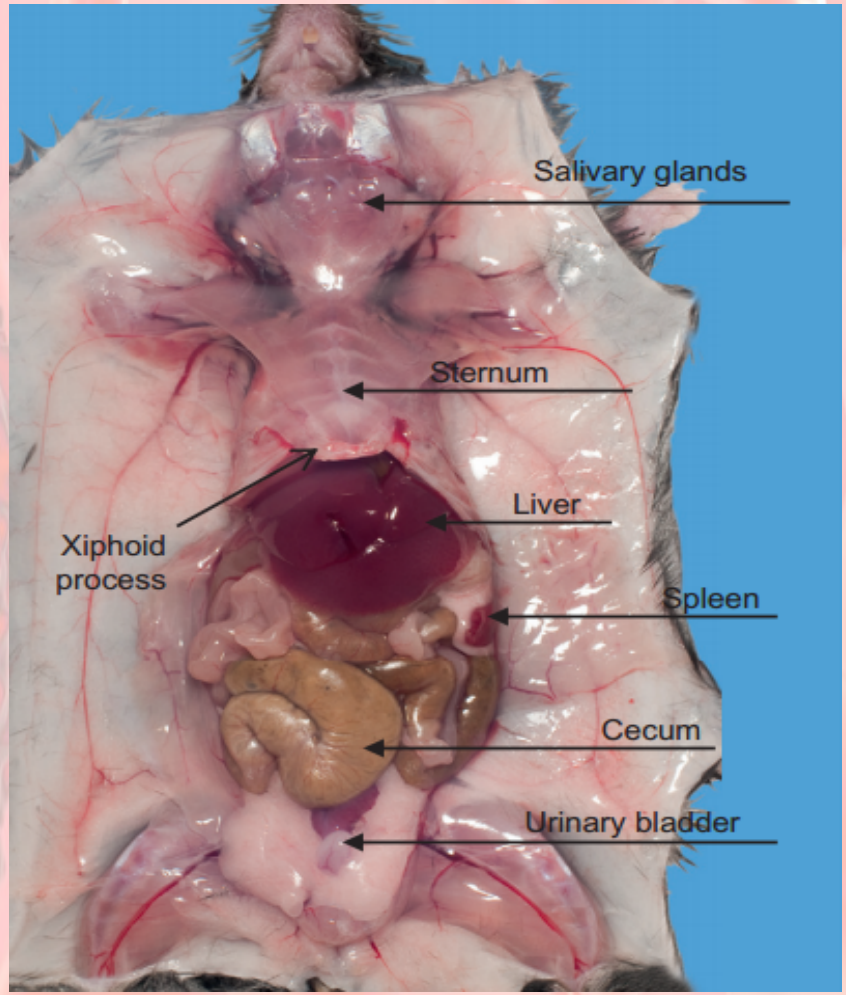
- Location:
  - Organ
  - Sub-location
- Distribution:
  - Focal
  - Multifocal
  - Multifocal to coalescing
  - Diffuse
- Color
- Size:
  - Measure 3 dimensions if possible
- Consistency:
  - Soft
  - Firm
  - Hard



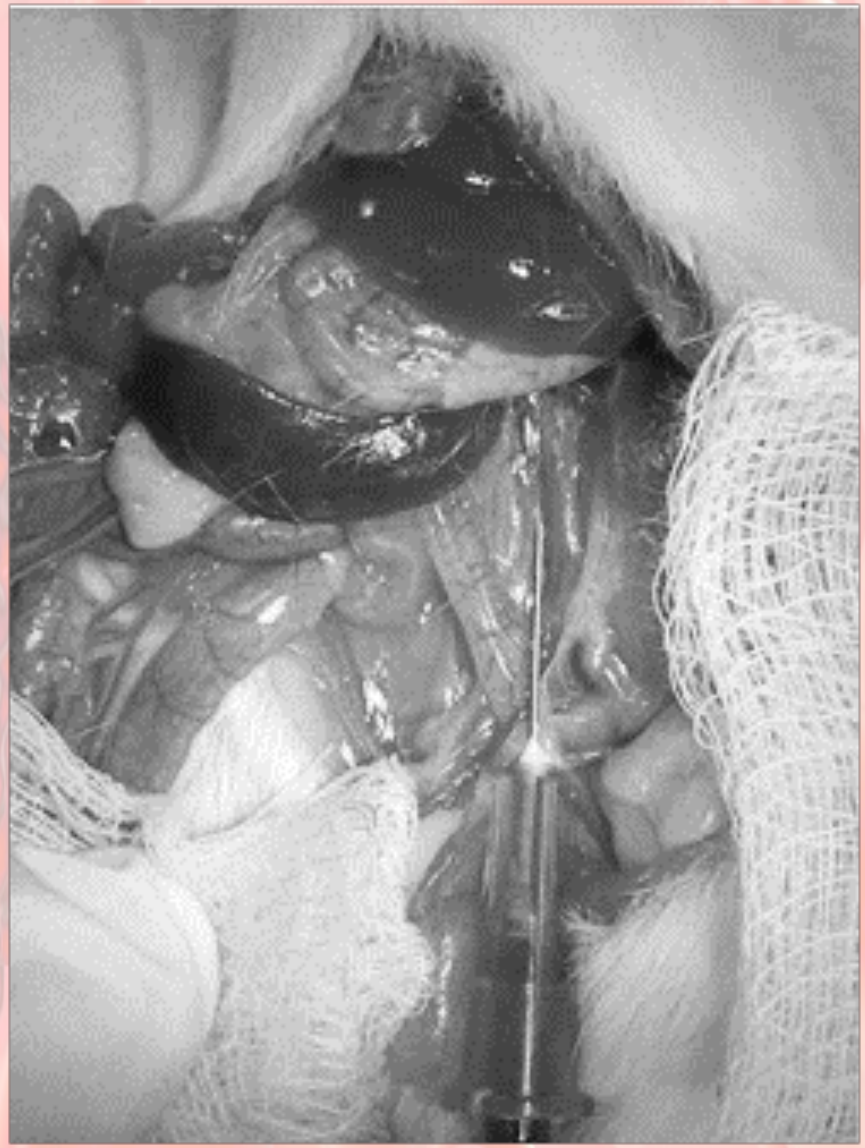
- SC Haemorrhages
- SC Abscesses
- SC Tumors



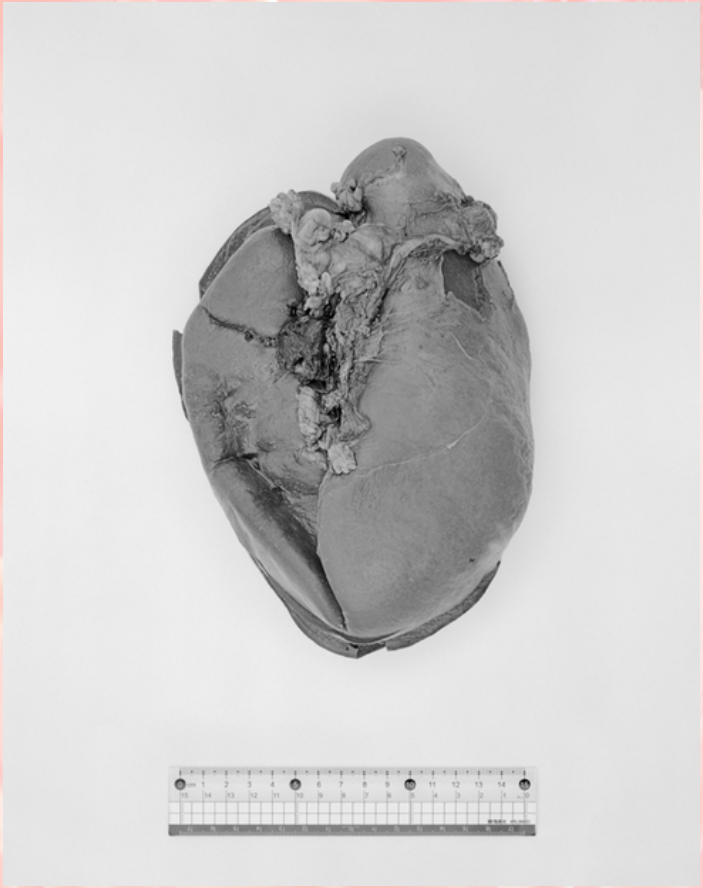
Abdominal cavity



Abdominal cavity



Examine, weigh, measure and fixate



List of organs for weighing

Rat

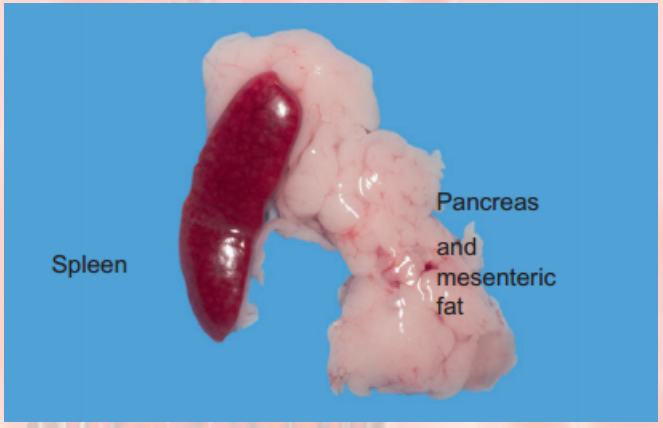
- Liver
- Kidney
- Heart
- Adrenal glands
- Brain
- Testes
- Prostate
- Epididymes
- Spleen
- Thymus
- Thyroid/parathyroid
- Pituitary gland

Mice

- Liver
- Kidney
- Heart
- Adrenal glands
- Brain
- Testes
- Spleen
- Thymus
- Thyroid/parathyroid
- Pituitary gland

Sellers, R., et al., Toxicologic Pathology 35, 751–755 (2007)

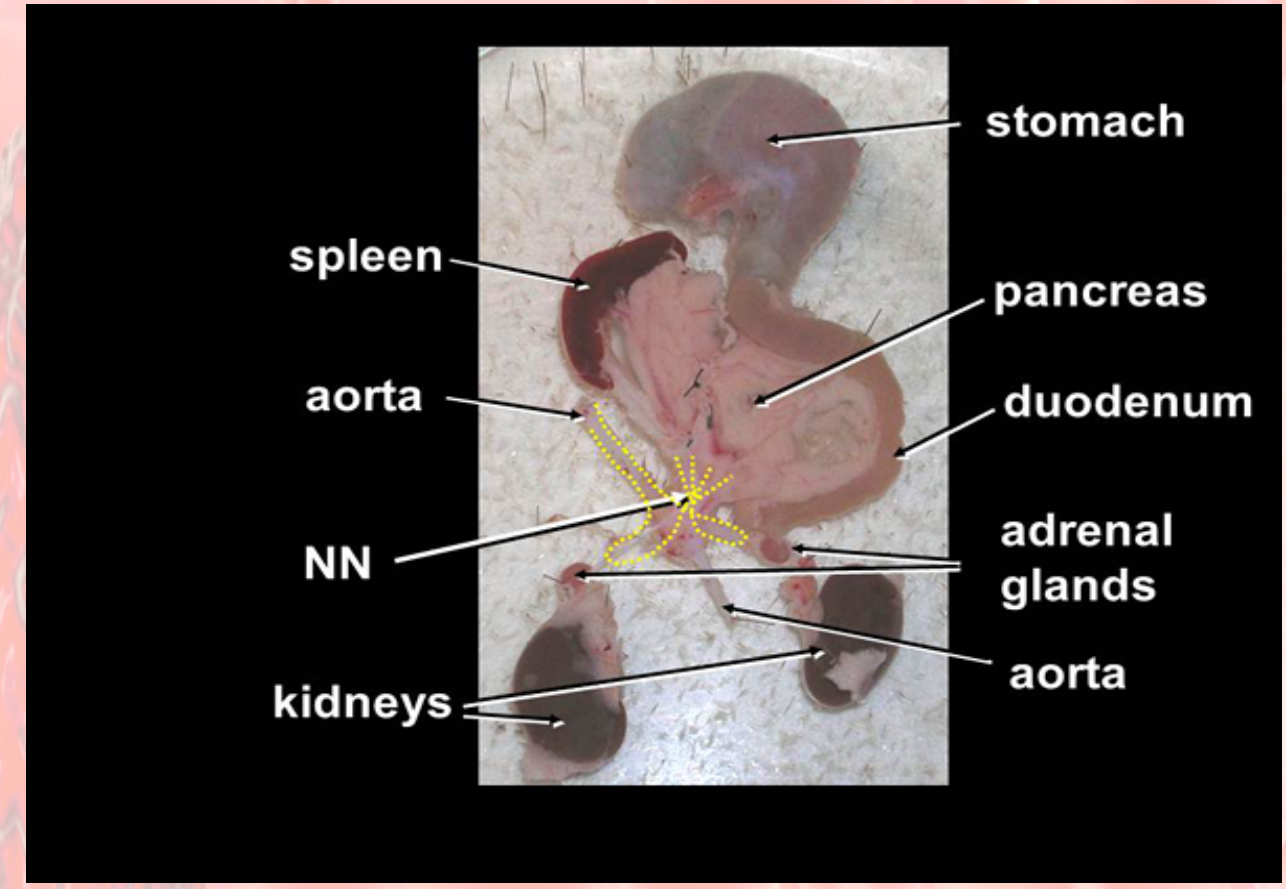
Abdominal cavity



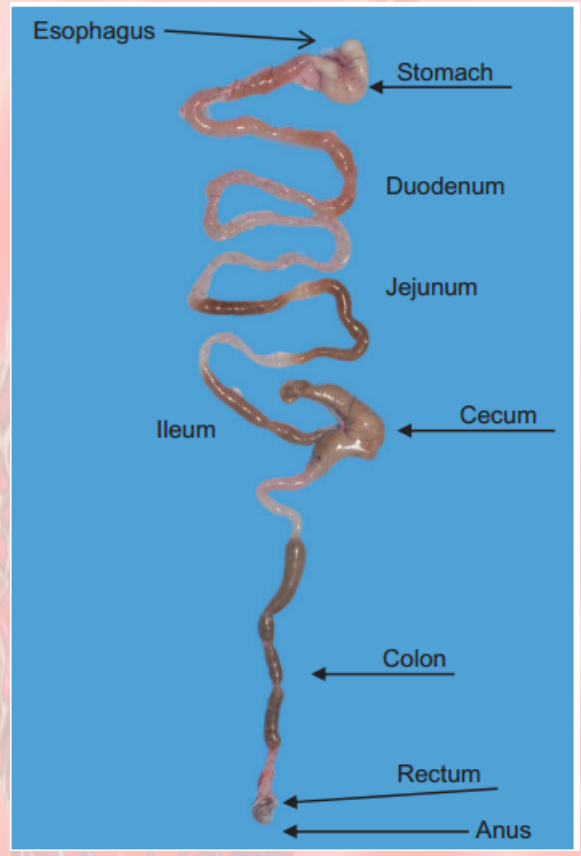
Spleen and pancreas removed en block

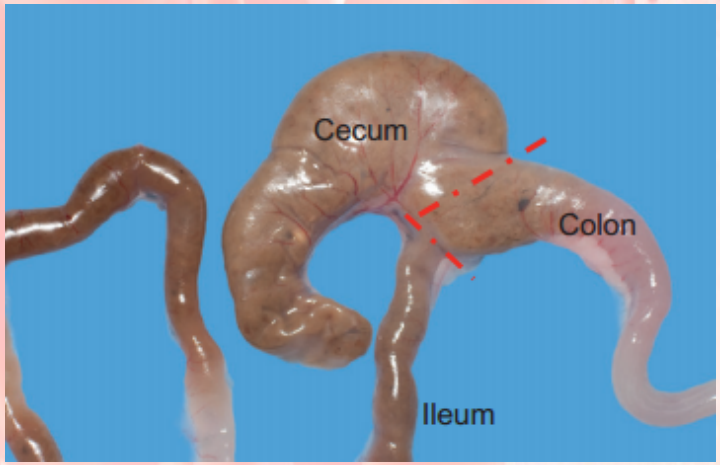
The pancreas is located under and attached to the spleen. It is diffuse and embedded in variable amounts of mesenteric fat

Rat pancreas



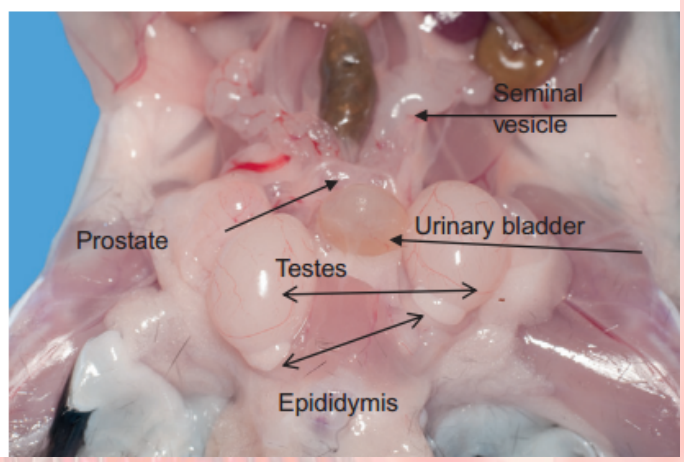
Abdominal cavity

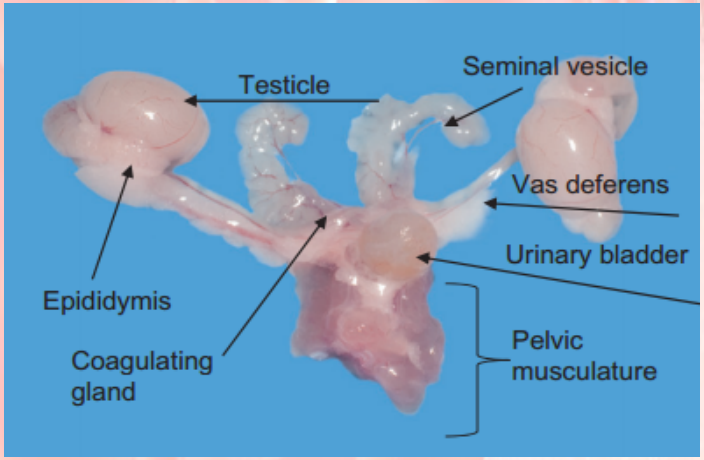


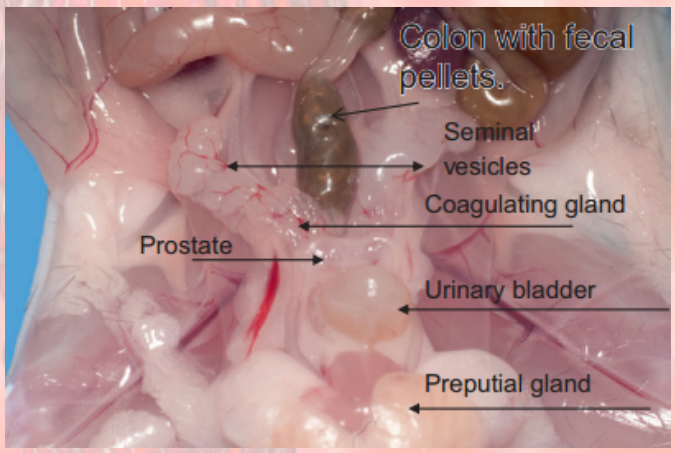


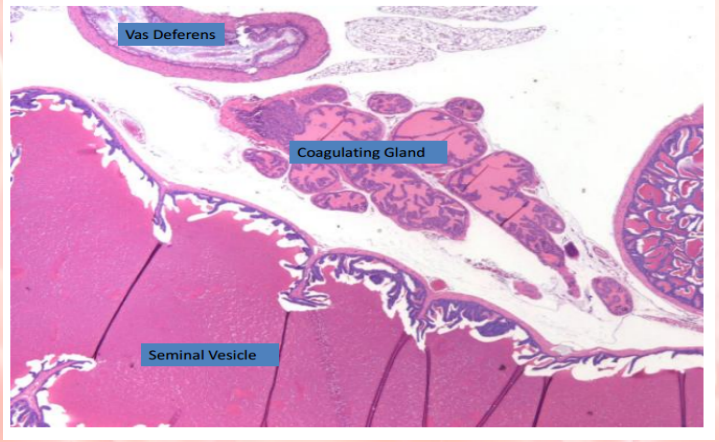
Dashed lines indicate approximate incision lines to separate the structures

Abdominal cavity

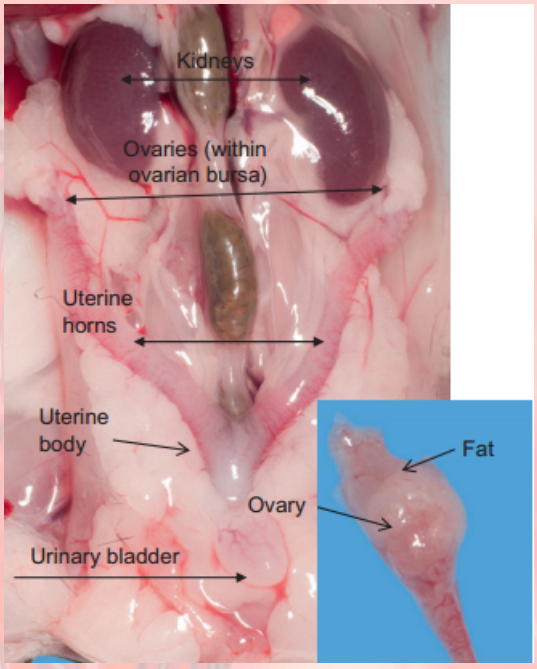


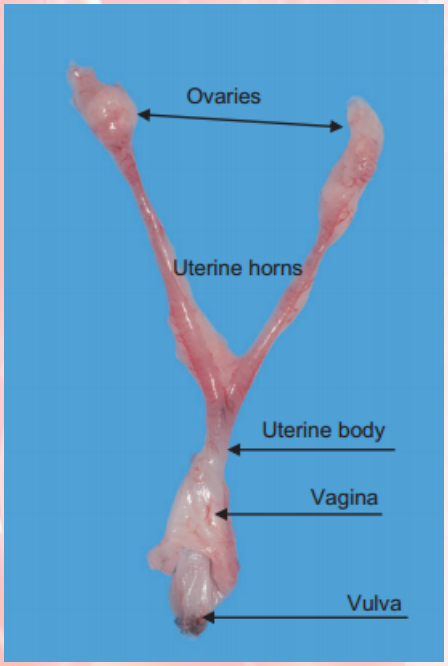




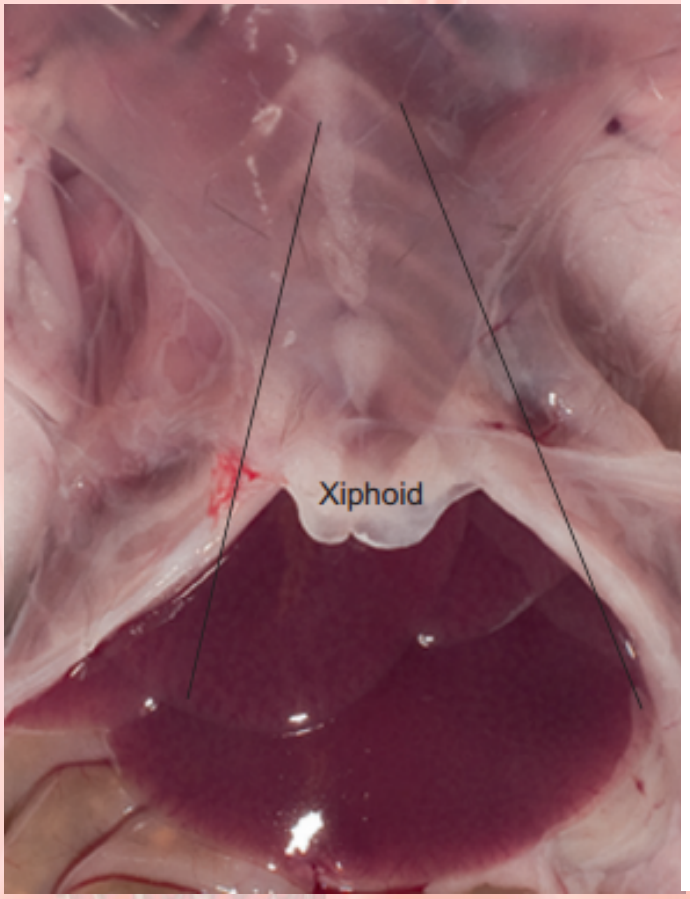


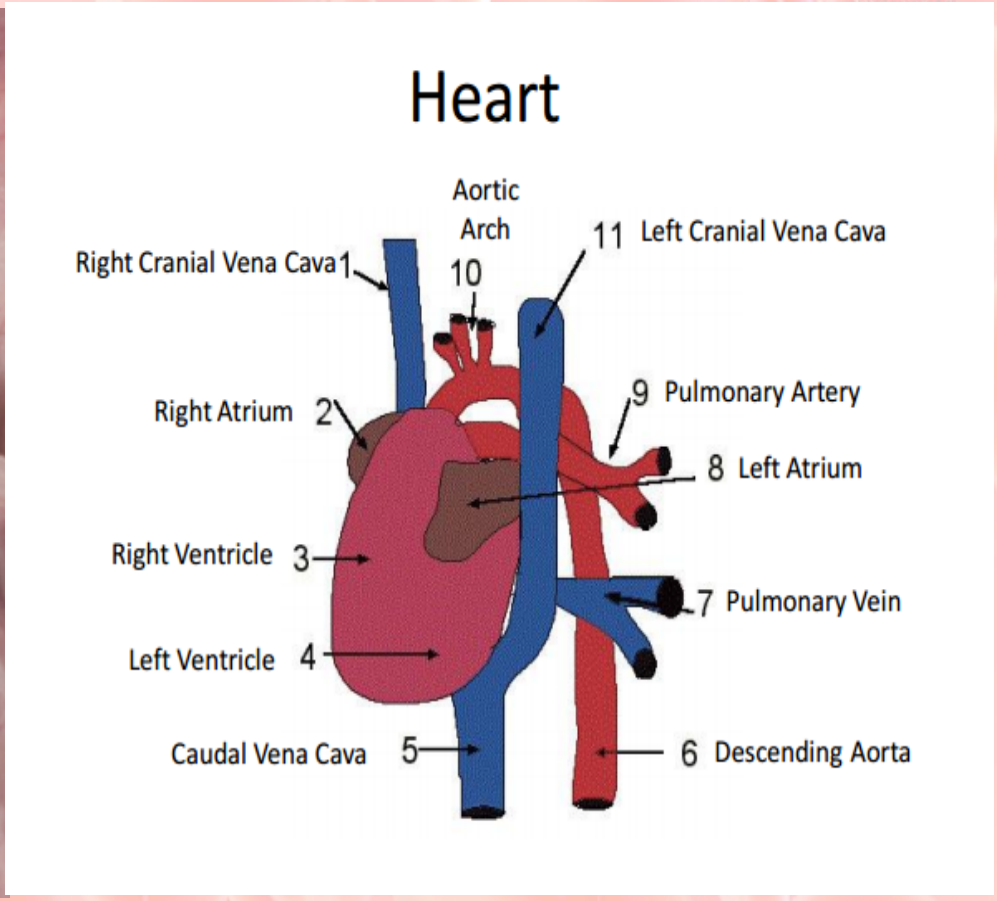
Abdominal cavity



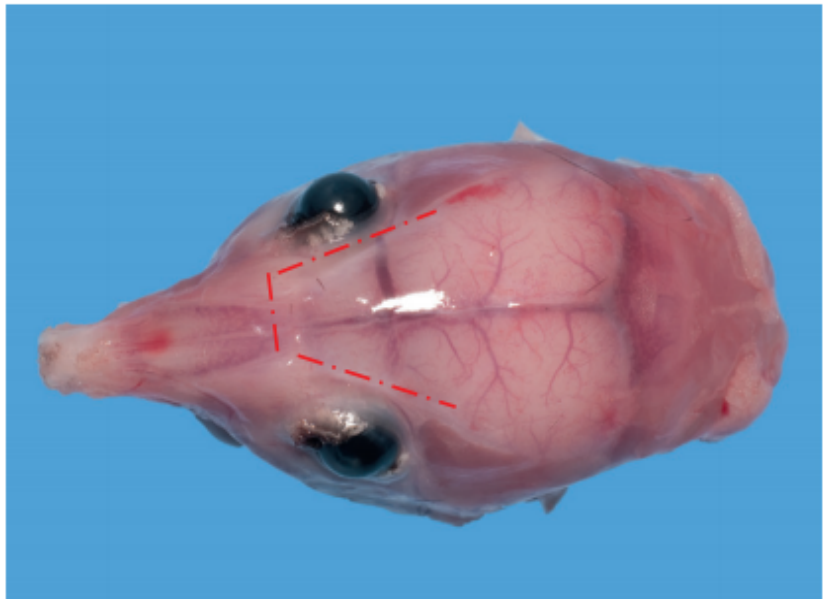


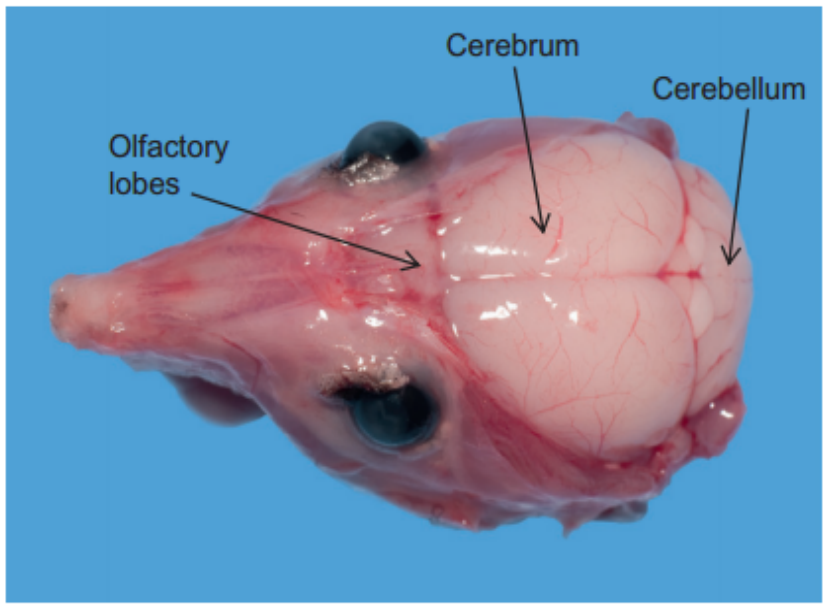
Thoracic cavity

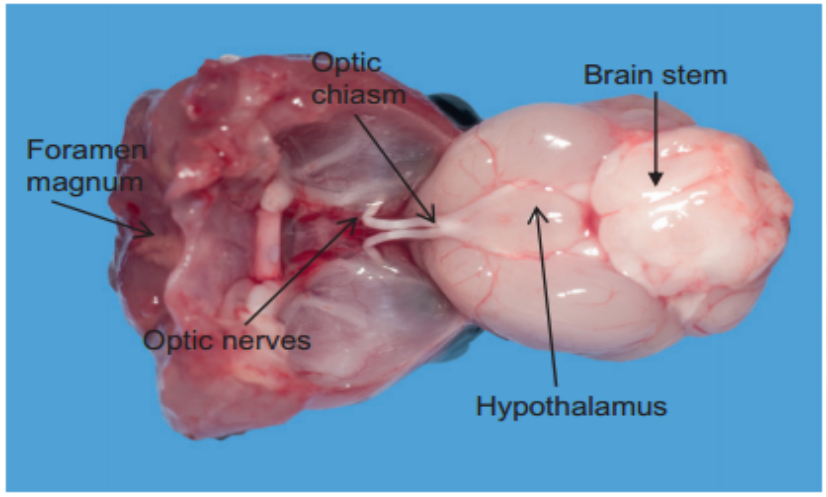




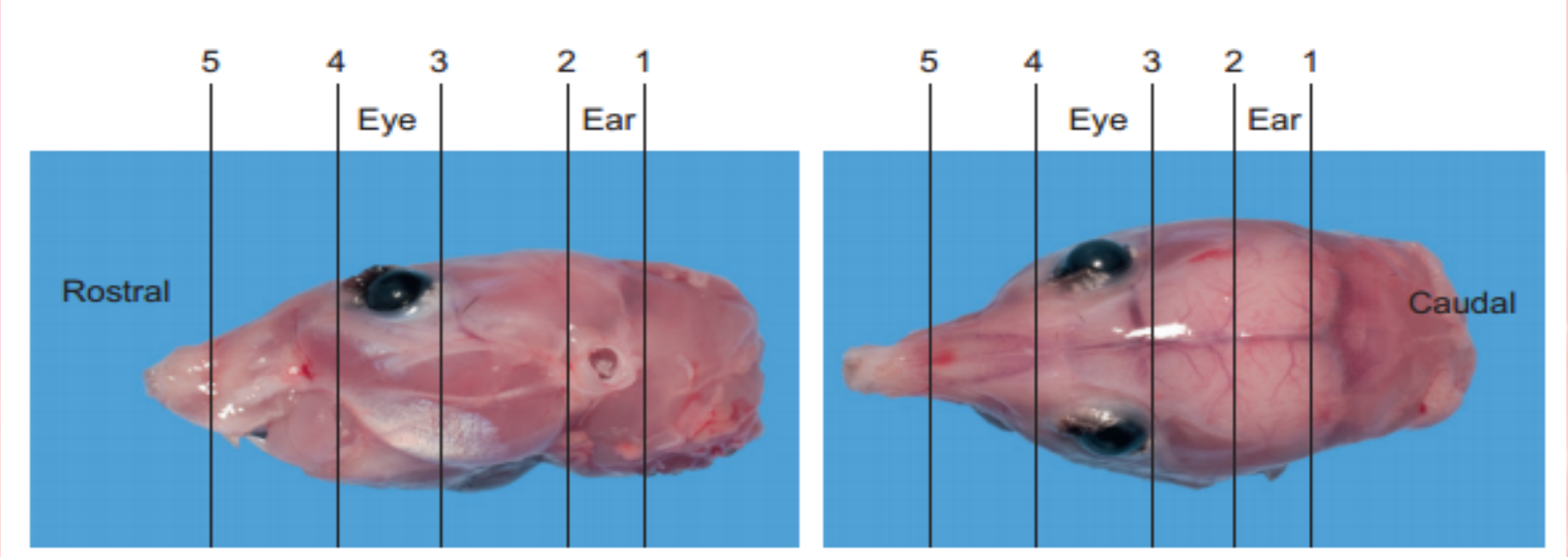
Cranial cavity

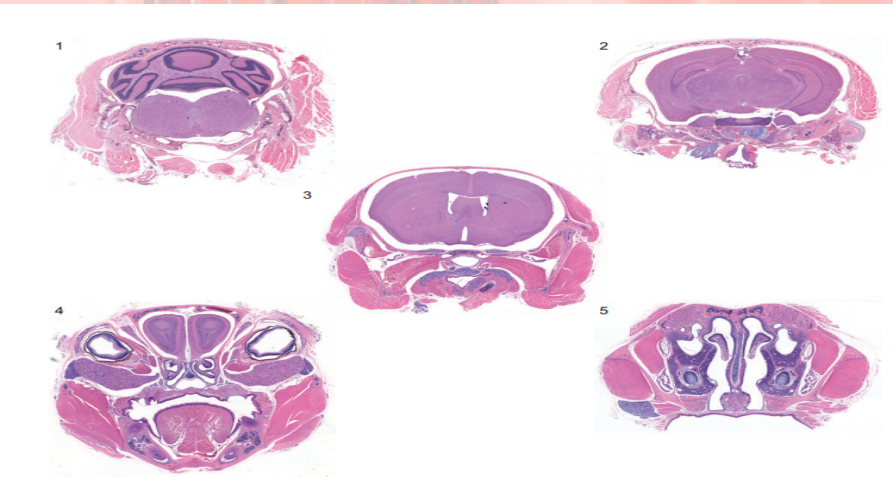






Cranial cavity





Once the skin is removed, the entire head can be placed en block directly into the decalcification (decal) solution

Thank you for your attention