Soft Tissue Anomalies

Introduction and Survey Results

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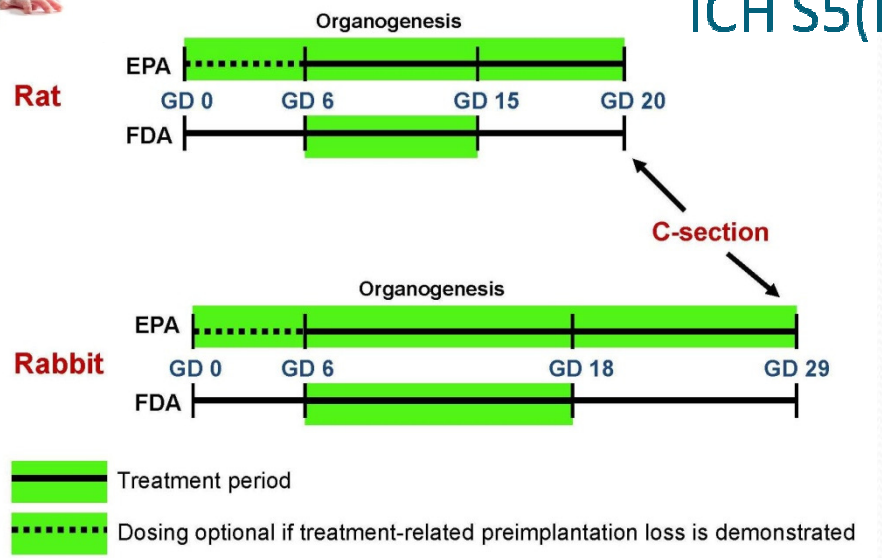
Prenatal Developmental Toxicity Study

OPPTS 870.3700; OECD 414

Embryo-Fetal Developmental Toxicity Study

ICH S5(R2)



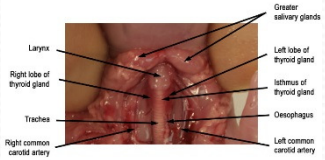
- **Exposure** of dams during major period of fetal organogenesis or during entire duration of gestation
- **Laparohysterectomy** conducted immediately prior to expected day of parturition
- **Maternal evaluation**:
  - Clinical observations
  - Body weight, food consumption, and/or water consumption
  - Necropsy findings
    - Macroscopic pathology
    - Ovarian corpora lutea counts
    - Non-reproductive organ weights are optional
  - Evaluation of gravid uterus
    - Gravid uterine weight
    - Implantation status
      - Counts (live, dead, early and late resorptions, empty implantation sites)
      - Placement in uterine horns
    - Examination of placental and amniotic fluid
- **Fetal evaluation**:
  - Fetal sex
  - External examination
  - Visceral (soft tissue) examination
  - Skeletal examination

Images from www.irdg.com and Google Images

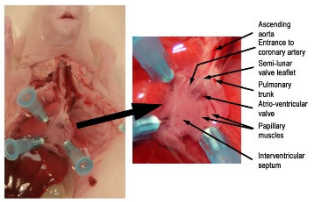
Typical Methods for Soft Tissue Evaluation

- Selection of fetuses for soft tissue evaluation (per guidelines)
  - 50% of litter for rodents
  - 100% of litter for rabbits
- Wilson's technique (serial section)
  - Fetuses are preserved and decalcified in Bouin's solution
  - Head
  - Cervical, thoracic, and abdominal organs
    - May alternatively be dissected post-fixation
- Staples technique (fresh dissection)
  - Cervical, thoracic, and abdominal organs examined in situ
    - Staples dissection of heart
  - Head
    - Removed, preserved/decalcified in Bouin's solution, then serial sectioned
    - Optional single coronal section of rabbit heads to visualize internal brain morphology

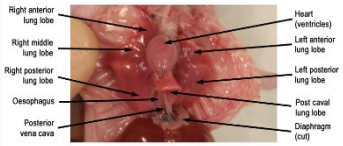
Soft Tissue Evaluation



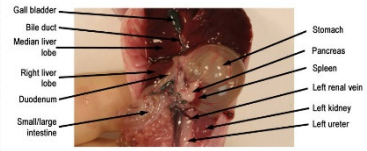
Ventral cervical region - main structures



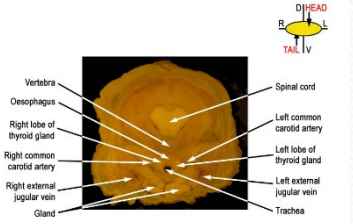
Heart examination - features of left ventricle



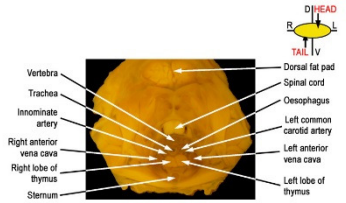
Thoracic cavity - lobulation of lungs



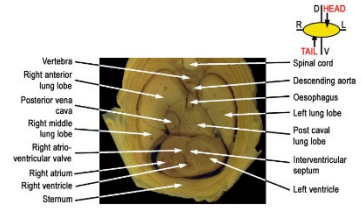
Abdominal viscera



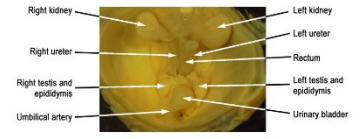
Section 2
Viewed from head aspect



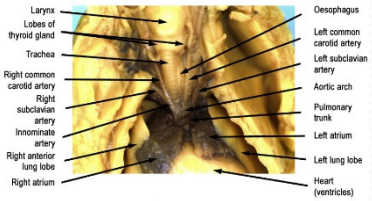
Section 7
Viewed from head aspect



Section 13
Viewed from head aspect

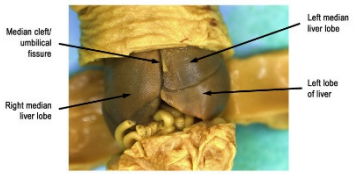


Male body wall section
viewed from ventral aspect



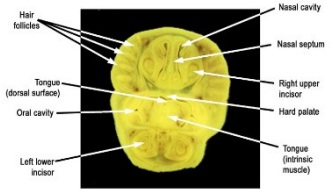
Throacic cavity -
detail of cardiovascular system

Heart - first section

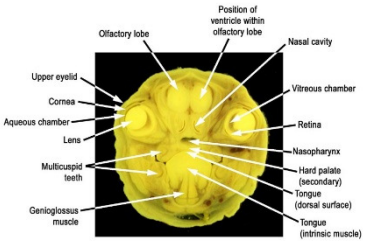


Liver - diaphragmatic aspect

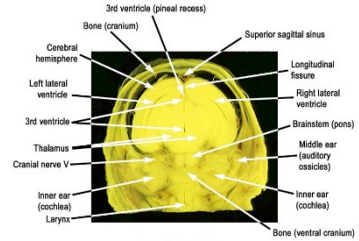
Abdominal contents - digestive tract



Section 2 Occipital view



Section 7 Occipital view



Section 10 Occipital view

Images from www.irdg.co.uk

History: 4th Workshop Soft Tissue Survey (2002)

- Index of agreement (IA) = (M-V)/(M+V+U)x100
- Visceral findings: low agreement observations discussed
  - Low agreement for M or V (equal numbers)
  - More than 1/3 (>7) respondents answered U or N
- Observations categorized as:

1. Number (e.g., absent, supernumerary, doubled)
2. Shape (e.g., misshapen, defect, cyst, etc.)
3. Location (e.g., malpositioned, transposed, etc.)
4. Size (e.g., size descriptor, small/enlarged, narrow/dilated, elongated/distended/short)
5. Color (e.g., discoloration as consequence of functional impairment)

History: 4th Workshop Soft Tissue Survey (2002)

Conclusions for visceral observations

- There was high agreement for M or V determination for only a few observations
- Low agreement observations should remain in the Gray Zone, with the decision to classify as M or V dependent on:

1. Severity
2. Historical control data
3. Occurrence in isolation
4. Difficulty in determining relationship with an abnormal process

- A new category of “Not Malformation” (Unclassified) was agreed upon for visceral functional changes

8th Workshop Soft Tissue Survey

- 280 individual soft tissue findings
  - Version 2 terminology
  - Also included species other than rodent and rabbit (primate)
- Coded responses:
  - Malformation, Variation, Unknown, or Grey Zone
  - For Gray Zone findings: Grade, Severity, Persistence
- 18 respondents
- Agreement indices calculated:
  - IA = (M-V)/(M+V+U+G)x100
  - **IA = (M-V)/(M+V+G)x100**

8th Workshop Soft Tissue Survey

- Agreement indices on M or V classification
  - +75-100: 68
  - +50-74: 53
  - +0-49: 94
  - -0-49: 51
  - -50-74: 13
  - -75-100: 1
- Good agreement on M or V classification (IA $\geq$ 75%)
  - Malformations: 68 (+100%: 31)
  - Variations: 1 (-100%: 1)
- Poor agreement on M or V classification (IA $\leq$ 25%)
  - Malformations: 55
  - Variations: 19

$$IA = (M-V)/(M+V+G)x100$$

8th Workshop Soft Tissue Survey

- Low agreement for M or V classification:
  - Equal no. of respondents classifying finding as M or V: 5
  - More than 1/3 respondents (>6) classifying finding as Unknown: 7

8th Workshop Soft Tissue Survey

- Findings with Low agreement for M or V classification:
  - Equal no. of respondents classifying finding as M or V: 5
- Esophagus – dilated
- Gallbladder – bilobed
- Intestine – cyst
- Thorax – fluid-filled
- Intestinal wall - thick

8th Workshop Soft Tissue Survey

- Findings with Low agreement for M or V classification (cont.):
  - More than 1/3 respondents (>6) classified finding as Unknown: 7
- Common carotid trunk – present
- Inner ear - discolored
- Inner/middle ear – discolored
- Gum (periodontium) – discolored
- Aqueous chamber/humor – fluid-filled
- Inner ear – red material
- Inner/middle ear – red material

8th Workshop Soft Tissue Survey

- Proposal from 4th Workshop: classification as “Not Malformation” (Unclassified)
  - Findings that are likely consequences of functional disorder
  - More severe than a variation due to health impact on offspring
- “Unclassified” note in current survey findings (mostly variations)
  - +75-100: 0/68
  - +50-74: 0/53
  - +0-49: 4/94
  - -0-49: 37/51
  - -50-74: 8/13
  - -75-100: 0/1

8th Workshop Soft Tissue Survey

- "Not Malformation" (Unclassified) observations
  - Discolored
  - Red material
  - Altered texture
  - Consistency
  - Fluid-filled
  - Blood-filled (pericardium)

8th Workshop Soft Tissue Survey

- For Gray Zone anomalies, Grade was most often selected as the additional information needed
  - +75-100: (60, 0, 0)/69
  - +50-74: (146, 29, 1)/159
  - +0-49: (431, 170, 3)/547
  - -0-49: (217, 14, 0)/349
  - -50-74: (25, 1, 1)/64
  - -75-100: (0, 0, 0)/0

Sum scores: (Grade, Size, Persistence)/Gray Zone