

**ENDOCRINE DISRUPTION:
A GUIDANCE DOCUMENT
FOR HISTOLOGIC
EVALUATION OF ENDOCRINE
AND REPRODUCTIVE TESTS**

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Endocrine Disruption: Guidelines for Histopathologic Evaluation

General Introduction

These guidelines have been prepared to provide the pathologist reading slides from endocrine and reproductive studies (e.g. the updated TG407) with sufficient information to be able to identify the often subtle histopathologic changes that are associated with endocrine disruption in target tissues. The guidelines have been structured to provide the following information on potential target tissues for endocrine disrupting chemicals:

1. Normal structure and function
2. Normal morphological variation of structure
3. Morphological patterns associated with hormone disruption
4. Recommended terminology and severity grading
5. Critical aspects of histopathologic evaluation
6. References

Normal Structure and Function

When evaluating an endocrine tissue for evidence of hormone imbalance it is essential to be familiar with the normal structure and function of the tissue. When dealing with a functional unit such as the male and female reproductive systems it is also necessary to consider the various parts (testis, epididymis, prostate and seminal vesicles for male and ovary, uterus, and vagina for female) as integrated units because there is considerable functional and endocrinologic interdependence of the tissues on one another. Section 1 of each tissue will provide a brief review of the normal physiology and morphology of the tissue that is deemed relevant for identifying changes in hormonal balance.

Normal Morphological Variation of Structure

The results of hormone imbalance (endocrine disruption) can produce subtle histopathologic changes that can be challenging for the pathologist to recognize. Although it is important to identify these subtle indicators of endocrine disruption, it is also important that the pathologist does not mistakenly over diagnose changes that constitute normal morphological or physiological variation of structure within the tissues being examined. The second section provides examples of common variations that may be mistaken for drug induced changes.

Morphological Patterns of Hormone Disruption

There are many different ways of disrupting hormonal balance including altered steroidogenesis, altered androgen/estrogen metabolism, hormone receptor agonism/antagonism, increased clearance of hormones and administration of estrogenic/androgenic xenobiotics. In many cases, the pattern of changes in the various hormone dependant tissues provides an indication of the underlying cause of the hormone disruption. To aid interpretation of these underlying causes, Section 3 provides a guide to some of the most common characteristic patterns that can be recognized.

Recommended Terminology and Severity Grading

To try and ensure consistency of identification and interpretation of changes associated with endocrine disruption, it is critical that consistent terminology, consistent grading and consistent thresholds of recording are used by those charged with the task of examining TG407 studies. Section 4 provides guidance on recommended terminology and grading of findings

Critical Aspects of Histopathologic Evaluation

This guidance document has been prepared to aid the pathologist in detecting changes that are characteristic of endocrine disrupting chemicals. The endocrine system is, by its nature, a dynamic self regulating system that functions within a range of “normal variability”. This normal physiologic range is reflected by a normal range of morphological features and it is important that the pathologist does not over interpret these normal variations in structure, especially since their evaluation of “normality” within the study is based on only 5 control animals/sex. The final section 5 provides guidance on the critical aspects of the histopathologic evaluation that will help the pathologist to identify those changes that are outside the “normal” expected range of variation. This attempts to provide practical guidance on things to be aware of so that you do not under or over diagnose changes.

References

Finally, a reference list is provided for each organ system that provides additional reading material when additional detail is needed.

Part 1: Guidelines for Dissection and Trimming of Endocrine tissues

A comprehensive set of Guides for Organ Sampling and Trimming in Rats and Mice has been devised and published online and in a series of manuscripts in Experimental Toxicologic Pathology by the Registry Nomenclature Information System (RENI). The guides can be readily accessed online from the following website: <http://www.item.fraunhofer.de/reni/trimming/trimm.php?lan=en>

Close adherence to these guides is recommended to obtain consistent and appropriate sampling of the various endocrine tissues. Critical aspects of sampling and sectioning are briefly summarized below.

Male Reproductive System

Critical aspects of tissue dissection and handling of the testes and epididymides have been reviewed by (Foley, 2001). Additional guidance on sampling, fixation, and sectioning is also provided by (Lanning *et al.*, 2002; Creasy, 2003)

Testes

It is essential that the testes are fixed in an appropriate fixative such as Modified Davidsons or Bouins Fixative. They should NOT be fixed in formalin since this results in cellular shrinkage and precludes detection of the types of subtle morphological changes that are likely to occur with endocrine disruption. Modified Davidsons fixative (Latendresse *et al.*, 2002) is preferred to Davidsons fixative since PAS stain does not stain the spermatid acrosome (required for detailed staging of the spermatogenic cycle) when conventional Davidsons fluid is used. Bouins fixation results in differential tubular shrinkage, with tubules in the center of the testis showing greater shrinkage than more peripherally located tubules. The shrunken central tubules are often surrounded by interstitial proteinaceous fluid. This is an artifact of fixation and should not be mistaken as a real finding.

A transverse or longitudinal section of the testis may be used, or one of each may be employed. It is important to include the rete testis in the section since this can provide evidence of estrogen induced fluid disturbances in the rete and efferents ducts.

It is important to avoid trauma and squeezing of the testis during the necropsy dissection since this will result in sloughing of the germ cells into the tubular lumen, which may be mistaken for a test article related change.

Epididymides

The epididymis can be preserved in the same fixative as the testis or in formalin. Formalin provides slightly better preservation of cellular detail. It is important to ensure

that the epididymis does not dry out between dissection and fixation, to prevent drying artifact, particularly of the corpus.

A longitudinal section that incorporates the initial segment, head, body and tail of the epididymis is essential for a comprehensive evaluation of changes. Findings are often localized in specific regions of the epididymis. In addition, the location of sloughed testicular germ cells within the length of the epididymis provides a useful indication of the timing (after the start of dosing) the disturbance in spermatogenesis occurred in the testis.

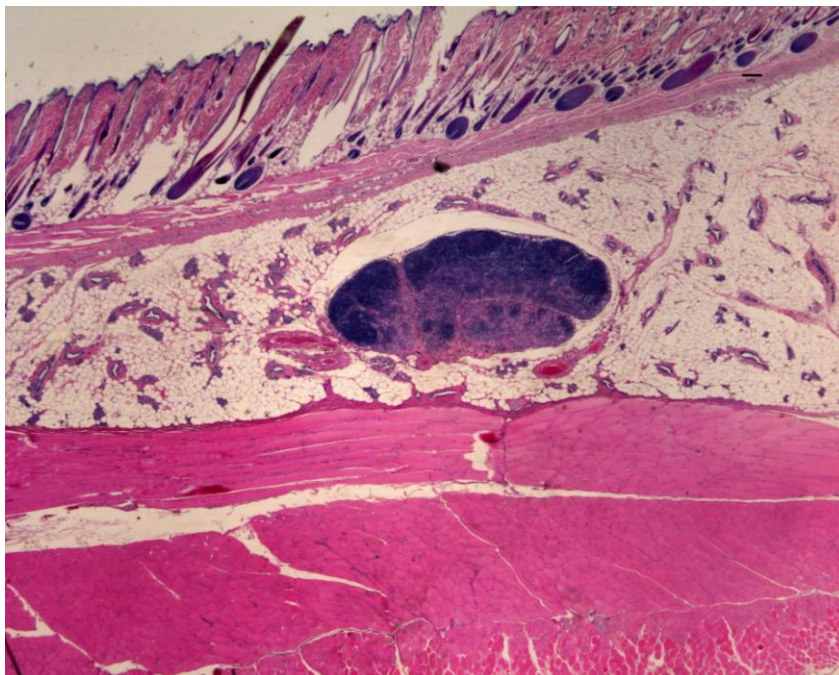
Prostate and Seminal Vesicles

Prostate and seminal vesicles are best fixed in formalin.

The amount of secretory content of the accessory sex organs is very androgen dependant and represents a large proportion of the weight of the glands. Therefore it is critical that the organ weight of these tissues includes all the secretory content. This can be best achieved by dissecting out the accessory sex organs attached to the urinary bladder as a unit. The bladder should then be removed from the accessory sex gland unit over a weigh boat to ensure that any fluid that leaks is caught and weighed with the gland. Since the organ weight is generally more sensitive than the histopathological appearance of the organ, this procedure is critical.

Mammary gland

Sampling of the inguinal mammary gland is recommended.



A good section of mammary gland is necessary to ensure a general overview of the gland rather than a regional sample that may only include the terminal end buds. The best way of ensuring this for males and females is to use the lateral iliac lymph node as a landmark for sampling. In females, both the nipple and the lymph node may be used as landmarks.

Female Reproductive System

All female reproductive tract tissues are best fixed in formalin. Because the oestrous cycle-associated morphological changes observed in the reproductive tract do not occur uniformly along its length, sampling of tissues from specific areas of the vagina, uterus and ovaries is essential if an accurate histological evaluation of the system is to be performed and meaningful comparisons between animals made. Additional guidance on preparation of the uterus and vagina for sampling, fixation, and sectioning is also provided by the OECD Report of the Initial Work Towards the Validation of the Rodent Uterotrophic Assay - Phase 1 (No. 65: <http://www.oecd.org/document>).

Vagina

A transverse section should be taken from the mid-vagina, avoiding the posterior (caudal) one-third of the organ as this is covered by a permanently keratinised stratified squamous epithelium. Care should be taken to avoid incorporating vulval or vaginal skin in this section. Weighing of the vagina is not required by the TG 407.

Uterus

The ovaries and vagina should be separated from the uterus, which is then weighed prior to fixation. After fixation, a transverse section should be taken midway along the length of each uterine horn. A longitudinal horizontal section should also be obtained from the uterine cervix/body and posterior (caudal half) of the attached horns.

Ovary

At necropsy, the ovaries should be separated from the oviducts and weighed before fixation. Once fixed, the ovaries should be halved longitudinally and a section obtained from the middle of the organ. Suboptimal sampling (for example, from the periphery of the ovary) will result in sections that fail to include a representative selection of follicular and luteal structures.

Pituitary

The small size of the pituitary, and its location within the sphenoid bone of the skull can make it difficult to remove without damage. Fixation in-situ is recommended, followed by careful dissection and post fixation weighing. This procedure also minimizes the possibility of the tissue drying out prior to fixation.

Sectioning of the pituitary should aim to include the maximum area of the pars distalis while still including the pars nervosa and pars intermedia. A consistent sampling is necessary, since different types of secreting cells are located in different areas within the pars distalis (see example in the RENI dissection guide for lactotroph distribution).

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