5. MORPHOLOGICAL PATTERNS OF ENDOCRINE DISRUPTION

Introduction

5.1 Endocrine disrupting substances can affect the hypothalamic-pituitary-ovarian (HPO) axis in several ways, producing alterations in the female reproductive tract that may be detected histologically. The updated TG 407 is concerned with detecting substances with oestrogenic, antioestrogenic, androgenic and antiandrogenic activity. Potent endocrine disruptors typically cause overt histological changes in the vagina, uterus and ovary that are relatively straightforward to identify. Ethinyl oestradiol (a potent oestrogenic compound), for example, induces ovarian atrophy in association with hyperplastic and hypertrophic changes in the uterus and vagina.

5.2 Detection of substances with weak endocrine activity is more problematic. Compounds such as genistein and nonylphenol do not usually cause profound histopathological changes in individual reproductive tract organs; they may, however, disrupt the synchrony of the normal oestrous cycle-associated morphological alterations in the reproductive tract. Thus, whilst individual tissues may be histologically normal, they fail to correlate in terms of oestrous cycle stage when collectively evaluated as a unit. The challenge of identifying asynchronous morphological changes in the female reproductive tract when assessing TG 407 studies is discussed further below.

Overview of morphological patterns of endocrine disruption

5.3 A useful classification of the morphological alterations that may be observed in the female rodent reproductive tract following xenobiotic administration has been described by Yuan (1998). This scheme comprises three types of morphological response, based on the combined histological appearance of the vagina, uterus and ovary and is summarised below:

- Type I atrophic vagina, uterus and ovary
- Type II atrophic ovary with hyperplastic/hypertrophic uterus and vagina
- Type III hyperplastic/hypertrophic ovary, uterus and vagina

5.4 Using this classification scheme, Table 5.1 provides an overview of the morphological alterations likely to be encountered in the female reproductive tract following administration of (anti)oestrogenic or androgenic substances. The expected histopathological changes and underlying mechanisms of endocrine disruption associated with each type of response are summarised. Although compounds with (anti)oestrogenic or androgenic activity typically induce type I and II responses, the type III response is included here for reference. Figures 5.1 to 5.8 illustrate examples of the morphological alterations that may be observed. This part of the guidance document concludes with a summary of recommended terminology for the recording of these changes.

5.5 Note that overt histopathological changes associated with short-term antiandrogen administration are typically limited to the male reproductive tract (Kunimatsu et al, 2004), although ovarian interstitial stromal cell hyperplasia and/or hypertrophy has been described in rats following subacute oral dosing with flutamide, a potent androgen antagonist (Andrews et al, 2001).
Table 5.1 – Overview of the morphological responses associated with endocrine disruption (modified after Yuan, 1998).

<table>
<thead>
<tr>
<th>MORPHOLOGICAL PATTERN</th>
<th>EXPECTED MORPHOLOGICAL ALTERATIONS</th>
<th>EXAMPLE SUBSTANCE</th>
<th>MECHANISM OF ENDOCRINE DISRUPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>VAGINA</strong></td>
<td><strong>UTERUS</strong></td>
<td><strong>OVARY</strong></td>
</tr>
<tr>
<td></td>
<td>Thin, atrophic epithelium</td>
<td>Thin, atrophic endometrial and glandular epithelium consisting of low columnar cells</td>
<td>Atrophy with inactive interstitial glands; glandular cells are small and spindle-shaped</td>
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<td></td>
<td>comprising 2-3 cell layers</td>
<td>(Figure 5.1)</td>
<td>(Figure 5.3)</td>
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<td></td>
<td>(Figure 5.1)</td>
<td>Sparse endometrial glands and atrophic myometrium</td>
<td>Reduction in numbers of follicles and corpora lutea (may not be observed in short-term studies)</td>
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<td></td>
<td></td>
<td></td>
<td>Anovulatory follicular cysts may be present (Section 4.5)</td>
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<tr>
<td><strong>Type I</strong></td>
<td></td>
<td></td>
<td><strong>CGS 18320B</strong></td>
</tr>
<tr>
<td>(Atrophic vagina, uterus and ovary)</td>
<td></td>
<td></td>
<td>(non-steroidal aromatase inhibitor)</td>
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<td></td>
<td>Note</td>
<td></td>
<td>Endogenous production of ovarian oestrogen is thus reduced, initiating widespread atrophic reproductive tract changes</td>
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<tr>
<td></td>
<td>Atrophic changes are more readily detected in the vagina compared with the uterus and ovary</td>
<td></td>
<td>Reduced endogenous oestrogen levels may impair positive oestrogenic feedback on the HPO axis, disrupting the preovulatory LH surge and triggering the formation of anovulatory follicular cysts</td>
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</tbody>
</table>

- Atrophic changes are more readily detected in the vagina compared with the uterus and ovary  
- Ovarian atrophy may be particularly difficult to detect in short-term studies. Early (primordial to tertiary) follicular stages develop independently of hormonal stimulation and are thus frequently observed in the atrophic ovaries of non-cycling rats from subacute studies. Similarly, corpora lutea formed during previous cycles may also be present  
- Therefore, in short-term studies the presence of normal follicular and luteal structures does not necessarily imply unperturbed reproductive function
Table 5.1 (cont.) – Overview of the morphological responses associated with endocrine disruption (modified after Yuan, 1998).

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAGINA</td>
<td>UTERUS</td>
<td>OVARY</td>
</tr>
<tr>
<td><strong>Type II</strong> (Hyperplastic/hypertrophic vagina and uterus with atrophic ovary)**</td>
<td>Hyperplasia and/or hypertrophy of epithelium</td>
<td>Hyperplasia and/or hypertrophy of luminal and glandular epithelium</td>
<td>Atrophy with inactive interstitial glands</td>
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<tr>
<td></td>
<td>Keratinisation of superficial epithelium – may be incomplete with retention of nuclei in cells of stratum corneum</td>
<td>Polymorphonuclear cell infiltration of endometrium</td>
<td>Reduction in numbers of follicles and corpora lutea (may not be apparent in short-term studies)</td>
</tr>
<tr>
<td></td>
<td>Areas of epithelial hypertrophy with mucification may be noted. Superficial epithelial cells are hypertrophied and contain large mucin-filled vacuoles (Figures 5.2a to 5.2c)</td>
<td>Foci of squamous metaplasia present in luminal and glandular epithelium</td>
<td>Anovulatory follicular cysts +/- intrafollicular granulosa cell hyperplasia may be observed (Section 4.5) (tamoxifen/raloxifene)</td>
</tr>
<tr>
<td></td>
<td>Epithelial mucification typically predominates over keratinisation following androgen administration</td>
<td>Cystic endometrial glands may be observed</td>
<td>Intersitial stromal cell hyperplasia and/or hypertrophy may be noted (Figures 5.7 and 5.8) (tamoxifen/raloxifene &amp; flutamide)</td>
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<td></td>
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<td></td>
<td><strong>Tamoxifen/raloxifene</strong> (selective oestrogen receptor modulators)</td>
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<td><strong>Methyl testosterone</strong> (androgen agonist)</td>
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**Ethynyl oestradiol** (oestrogen agonist)
Table 5.1 (cont.) – Overview of the morphological responses associated with endocrine disruption (modified after Yuan, 1998).

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Type III</strong></td>
<td><strong>VAGINA</strong></td>
<td><strong>UTERUS</strong></td>
<td><strong>OVARY</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Hyperplasia and/or hypertrophy of epithelium</strong></td>
<td><strong>Hyperplasia and/or hypertrophy of luminal and glandular epithelium</strong></td>
<td><strong>Enlarged with increased numbers of corpora lutea and tertiary follicles. These changes are less pronounced with prolactin analogues</strong></td>
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<td></td>
<td><strong>Keratinisation of superficial epithelium – may be incomplete with retention of nuclei in cells of stratum corneum</strong></td>
<td><strong>Increased numbers of luteal cysts (luteinised anovulatory follicles) may be observed (Section 4.4)</strong></td>
<td><strong>Gonadotrophic/luteotrophic effect</strong></td>
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<tr>
<td></td>
<td><strong>Areas of epithelial hypertrophy with mucification may be noted. Superficial epithelial cells are hypertrophied and contain large mucin-filled vacuoles (Figures 5.2a to 5.2c)</strong></td>
<td></td>
<td><strong>Substances with gonadotrophic activity trigger continuous follicle maturation and corpora lutea formation</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Proliferative changes associated with prolactin analogues are typically limited to mild epithelial hypertrophy with mucification</strong></td>
<td></td>
<td><strong>The increased ovarian activity results in elevated oestrogen and progesterone levels that initiate proliferative changes in the uterus and vagina</strong></td>
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<td></td>
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<td><strong>Analogues of LH, LHRH, FSH and prolactin</strong></td>
<td><strong>Prolactin analogues may be luteotrophic in effect, prolonging the lifespan of corpora lutea. This results in persistent progesterone secretion and the development of a pseudopregnancy-like state</strong></td>
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<td><strong>Dopamine-depleting compounds (e.g. reserpine) impair the release of dopamine (a prolactin-inhibiting factor) from the hypothalamus. By increasing endogenous prolactin secretion, such substances can have an indirect luteotrophic effect</strong></td>
</tr>
</tbody>
</table>
Selected examples of morphological alterations associated with endocrine disruption

A. Vagina

Figure 5.1 – Vagina: atrophy (rat, H&E). Detection of chemically induced atrophy in the vagina is straightforward. Note the marked attenuation of the vaginal epithelium which comprises 2-3 layers of small flattened cells. Generalised atrophy of the female reproductive tract occurs normally in aged, reproductively senescent rats, but should not be encountered as a background finding in the young, sexually mature animals specified by the TG 407.

Figure 5.2a – Vagina: hypertrophy with mucification (rat, H&E). Superficial epithelial cells are hypertrophied with large, intracytoplasmic mucin-filled vacuoles (V). Only the two most basal cell layers of the stratum germinativum (SGerm) are unaffected. Mucification of the vaginal epithelium is a potential alteration associated with progestagenic substances following priming by oestrogen. It is also the expected morphological response of the vagina to administration of large doses of androgens. Luteotrophic compounds (e.g., prolactin analogues) typically cause mild epithelial hypertrophy with mucification.

Figure 5.2b – Vagina: hypertrophy with mucification (rat, H&E). Very large, intracytoplasmic mucin-filled vacuoles (V) are present within hypertrophied superficial stratum germinativum cells.
Figure 5.2c – Vagina: hypertrophy with mucification in a postpartum rat (H&E). Compare this with the section of normal vagina in prooestrus shown below. The exaggerated hypertrophy and mucification (H) of the superficial stratum germinativum (SGerm) are clearly visible.

Figure 5.2d – Vagina: prooestrus (rat, H&E). The presence of a superficial stratum mucification and underlying eosinophilic stratum corneum allows differentiation of normal prooestrus from vaginal hypertrophy with mucification. Note the small size of the mucin-filled intracytoplasmic vacuoles associated with the stratum mucification (arrowheads).
B. Uterus

Figure 5.3 – Uterus: atrophy of luminal epithelium (rat, H&E). A low columnar epithelium lines the uterine lumen and endometrial glands (not shown) are reduced in number.

Figures 5.4a and 5.4b – Uterus: squamous metaplasia (rat, H&E). Figure 5.4a shows squamous metaplasia (SM) in the luminal epithelium of the endocervix – a spontaneous background finding in a control animal.

Figure 5.4b illustrates hyperplasia/hypertrophy of the luminal epithelium (HE) together with a focus of squamous metaplasia (SM) in a rat treated with the synthetic oestrogen diethylstilbestrol.
Figures 5.5 and 5.6 – Ovary: atrophy (rat, H&E). Figure 5.5 (above) shows an obvious reduction in the number of follicles and corpora lutea; this characterises ovarian atrophy as observed in long-term studies and aged female rats. In subacute studies, this alteration may not be observed, reducing the sensitivity of the ovary as an endpoint for the detection of (anti)oestrogenic effects. Changes in the ovarian stroma and interstitial glands, however, may be induced by the short-term administration of endocrine disrupting substances (Figures 5.7 and 5.8). Figure 5.6 (below) shows a normal ovary from a young, sexually mature control animal for comparison.
Reproductive tract asynchrony

5.6 As previously noted, weak endocrine disruptors are difficult to detect as they fail to disrupt the HPO axis of mature female rats and produce overt histopathological changes in the vagina, uterus and ovary. Such substances may, however, be associated with asynchrony of the oestrous cycle-associated morphological changes that normally occur in the reproductive tract.

5.7 Observation of potential reproductive tract asynchrony in short-term studies is a difficult and ambitious enterprise, even for an experienced toxicological histopathologist. A good working knowledge of the normal histological appearance of the reproductive tract at each stage of the oestrous cycle is essential. Careful evaluation of the female control rats (limited to 5 animals in TG 407 studies) should be performed in order to gain an appreciation of the range of physiological and background spontaneous changes that are normal for the strain and age of rat utilised.
5.8 For asynchrony to be confidently diagnosed, a profound lack of correlation in the histological appearance of the reproductive tract tissues should be observed. As previously discussed, reproductive tract staging is initially based on the appearance of the vagina; the presence of markedly incompatible morphological alterations in the uterus and ovary is consistent with asynchrony.

5.9 Given the highly dynamic nature of the female reproductive system and small group sizes utilised in TG 407 studies, great care should be taken by the study pathologist to avoid erroneously interpreting minor normal variations in morphology as evidence of reproductive tract asynchrony.
SUMMARY OF RECOMMENDED TERMINOLOGY

VAGINA

- Epithelial atrophy
  - attenuated vaginal epithelium comprising 2-3 cell layers

- Epithelial hyperplasia/hypertrophy
  - thickened vaginal epithelium comprising increased number of cell layers and/or enlarged cells; may occur with:
    - keratinisation: superficial epithelial cells are variably keratinised
    - mucification: superficial epithelial cells are variably vacuolated
    - keratinisation and mucification: combination of changes is present

UTERUS

- Atrophy
  - attenuated low columnar luminal and glandular epithelium
  - sparse endometrial glands and atrophic myometrium

- Epithelial hyperplasia/hypertrophy
  - thickened luminal and/or glandular epithelium comprising increased number of cell layers and/or enlarged cells

- Endometrial inflammatory cell infiltration
  - polymorphonuclear inflammatory cell infiltration of endometrial stroma and/or glands

- Cystic endometrial glands
  - dilation/cystic change in endometrial glands

- Squamous metaplasia
  - transformation of luminal/glandular columnar epithelium into a stratified squamous epithelium

OVARY

- Atrophy
  - inactive interstitial glands +/- reduced numbers of follicles/corpora lutea
  - may occur with:
    - anovulatory follicular cysts
    - interstitial stromal cell hyperplasia and/or hypertrophy

- Increased numbers of follicles/corpora lutea

- Luteal cysts
  - luteinised anovulatory follicles
  - large cystic cavity surrounded by luteinised and non-luteinised granulosa cells

FEMALE REPRODUCTIVE TRACT

- Asynchrony
  - marked: lack of correlation in the histological appearance of the uterus and/or ovary relative to the vagina
6. REFERENCES AND BIBLIOGRAPHY


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