

# Draft NTP Technical Report TR 587 Tetrabromobisphenol A (TBBPA) Pathology Review

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## Outline

- NTP original transverse versus residual longitudinal uterine sections (rat)
- Uterine Malignant Mixed Müllerian Tumor (rat)
- Atypical endometrial hyperplasia (rat)
- Renal tubule cytoplasmic alteration (male mice)

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# Original Transverse Versus Residual Longitudinal Uterine Reviews

- Original transverse tissue review
  - Transverse section through each uterine horn
  - Cervix/vagina not processed, unless gross lesions
- Residual longitudinal tissue review
  - Remaining formalin fixed cervix, vagina, and uterine remnants were sectioned longitudinally
- Rationale for residual longitudinal tissue review
  - Unable to determine the primary location for adenocarcinomas in the cervix and vagina
  - Needed to review all the cervixes for hyperplasia/fibrosis



O = Ovaries

H<sub>ds</sub> = Uterine horn, distal segment

H<sub>ts</sub> = Uterine horn, transverse section

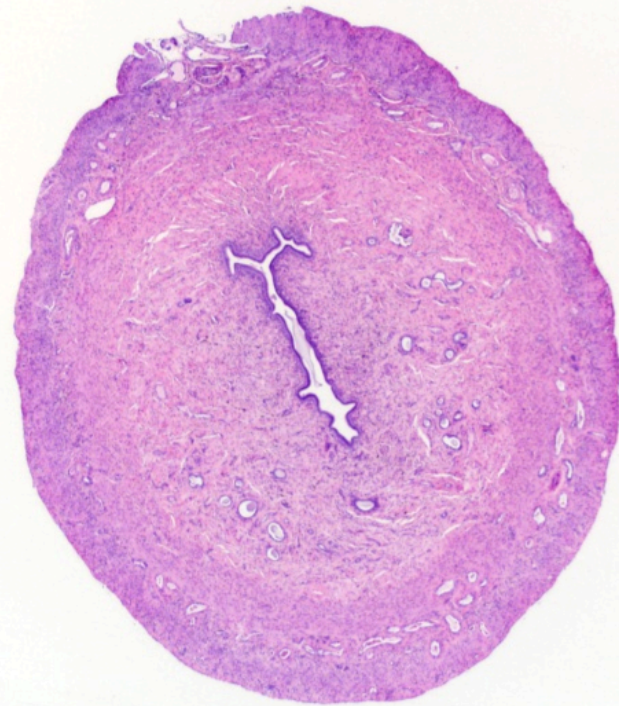
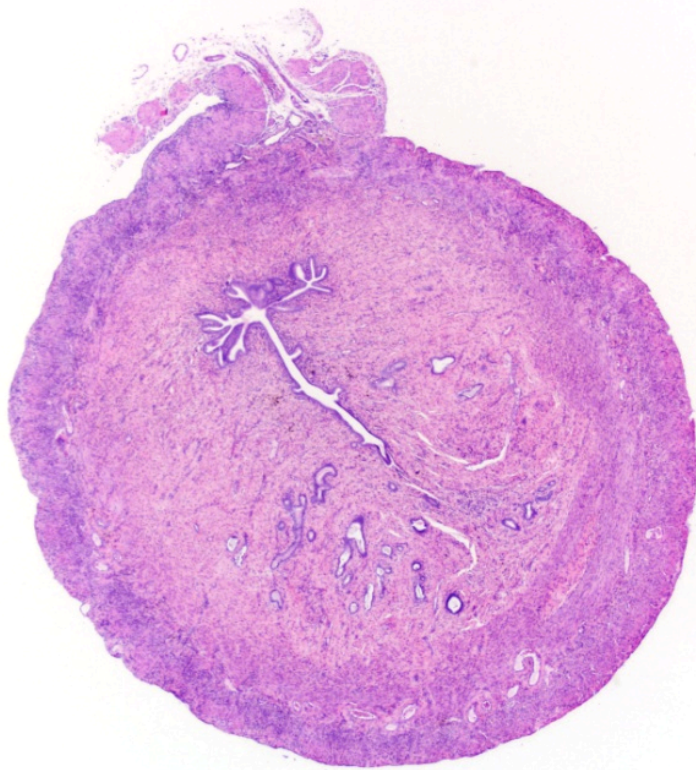
H<sub>ps</sub> = Uterine horn, proximal segment

B = Uterine body

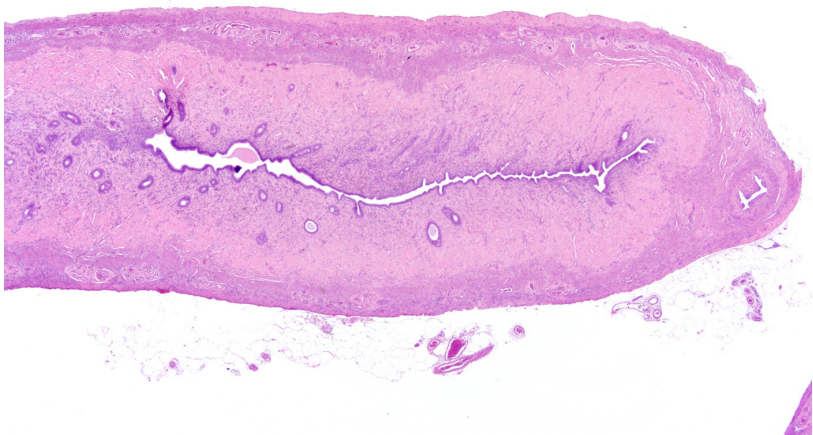
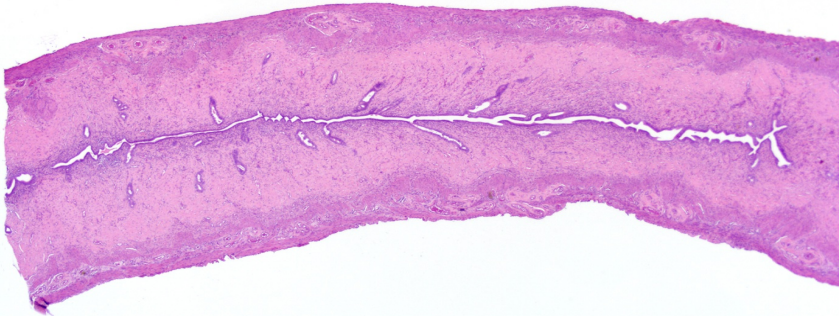
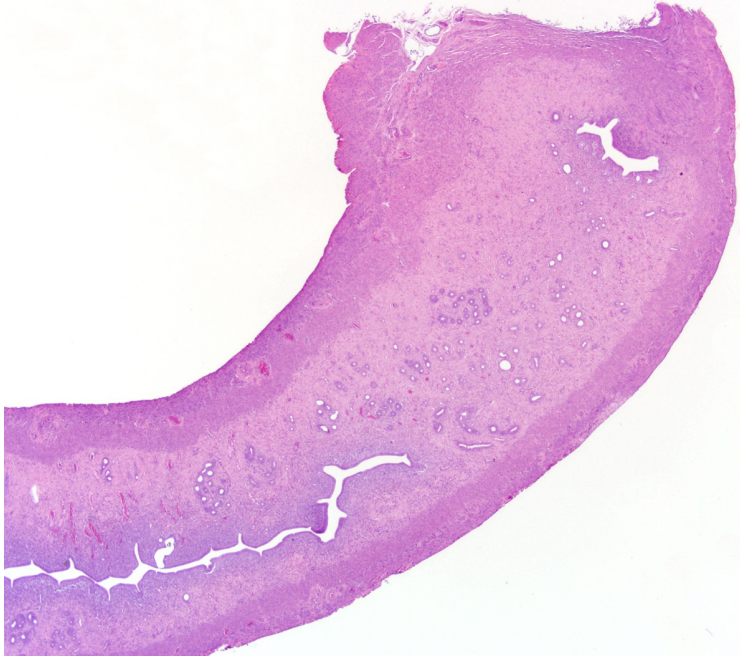
C = Cervix

V = Vagina

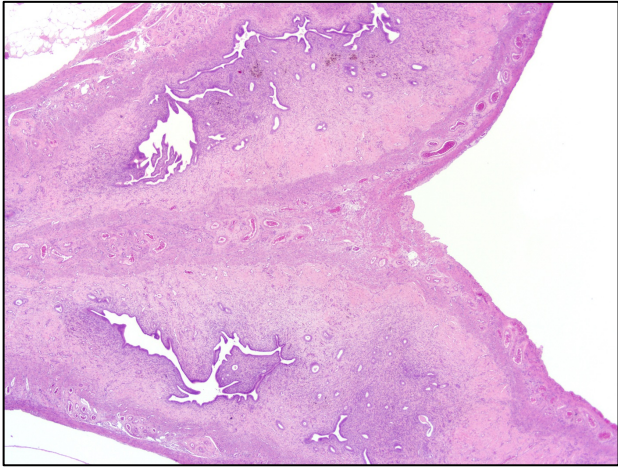
## Original Review: Transverse Section From Each Uterine Horn



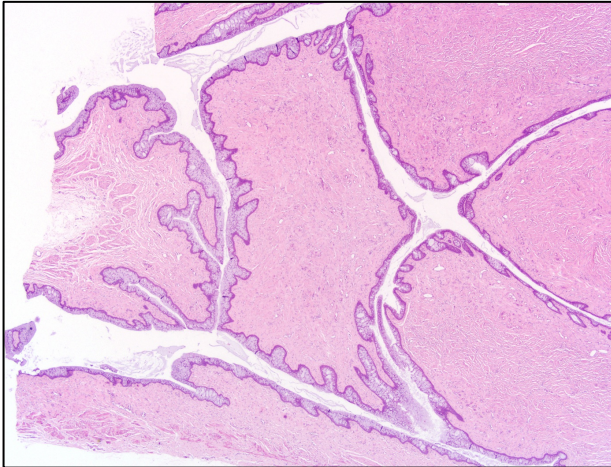
# Longitudinal Sections of Left and Right Uterine Horns



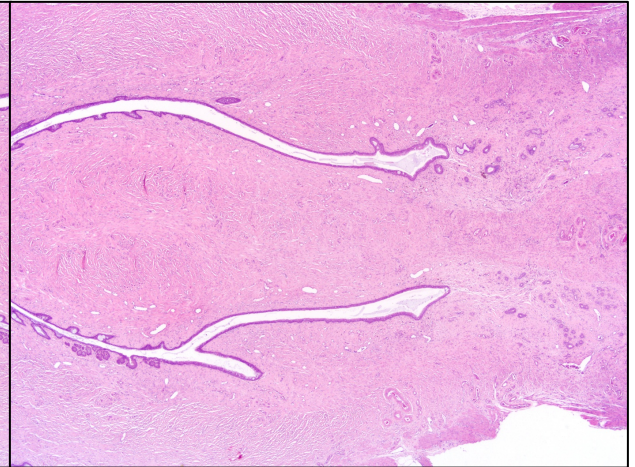
# Additional Sections of Longitudinal Tissue



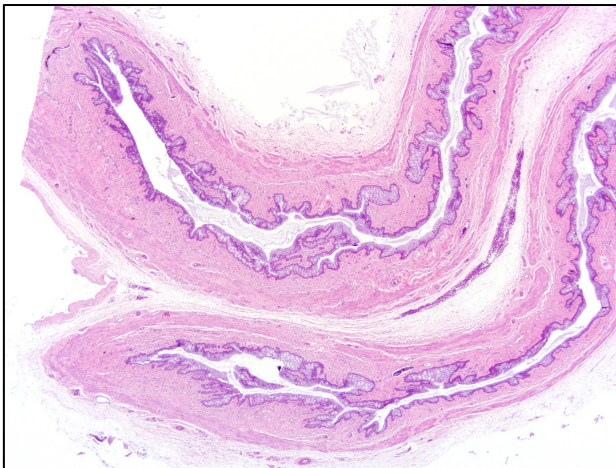
Uterus Body



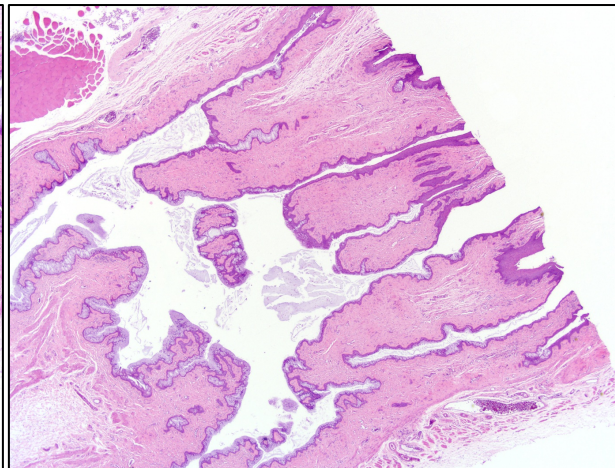
Distal Cervix



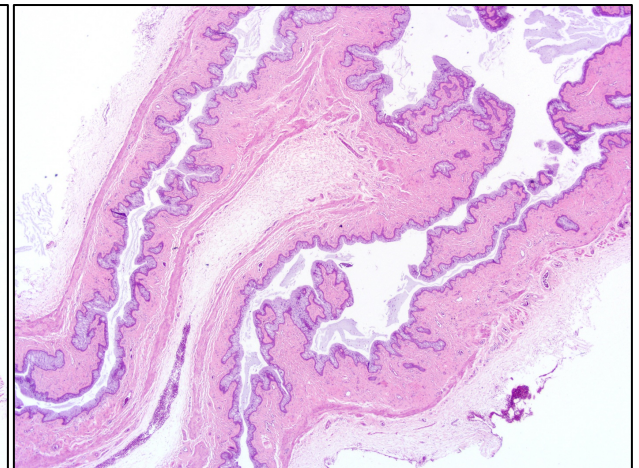
Proximal Cervix



Distal Vagina

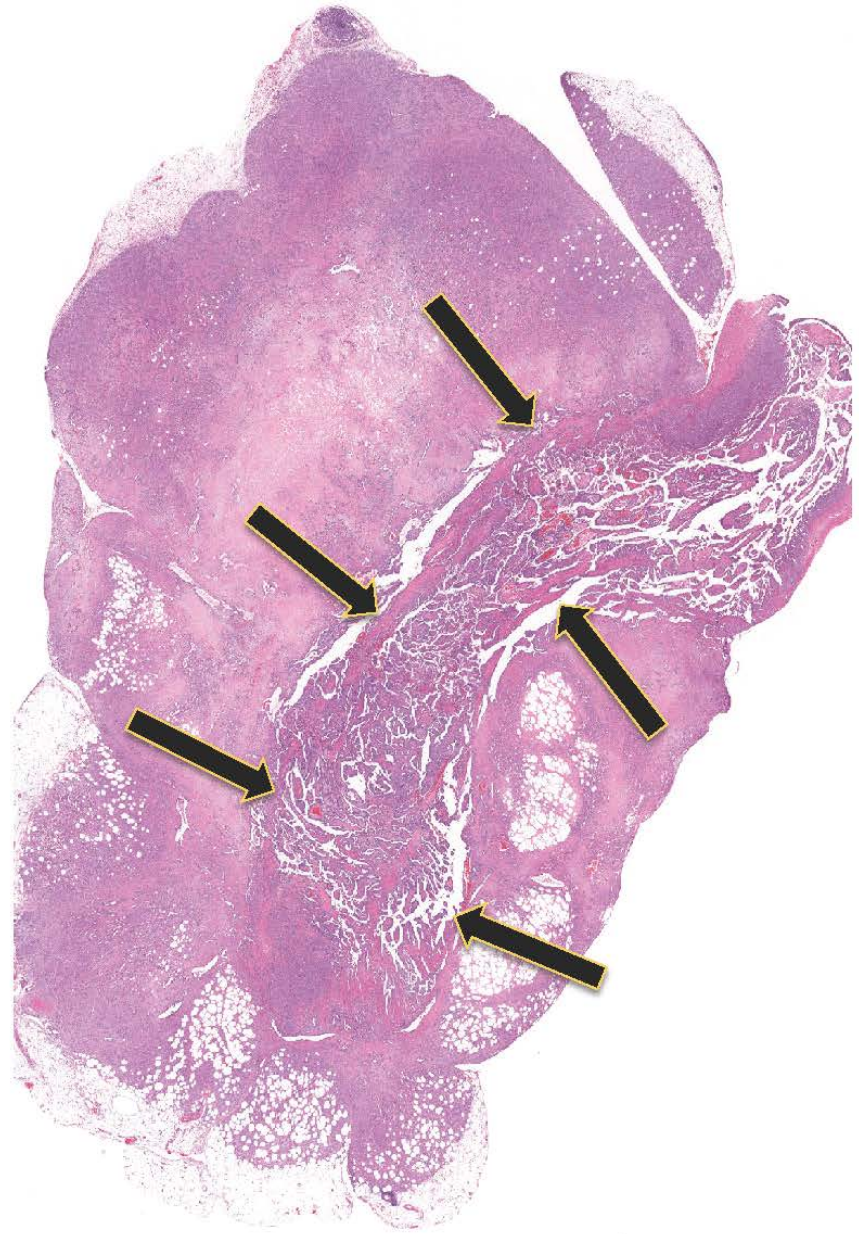
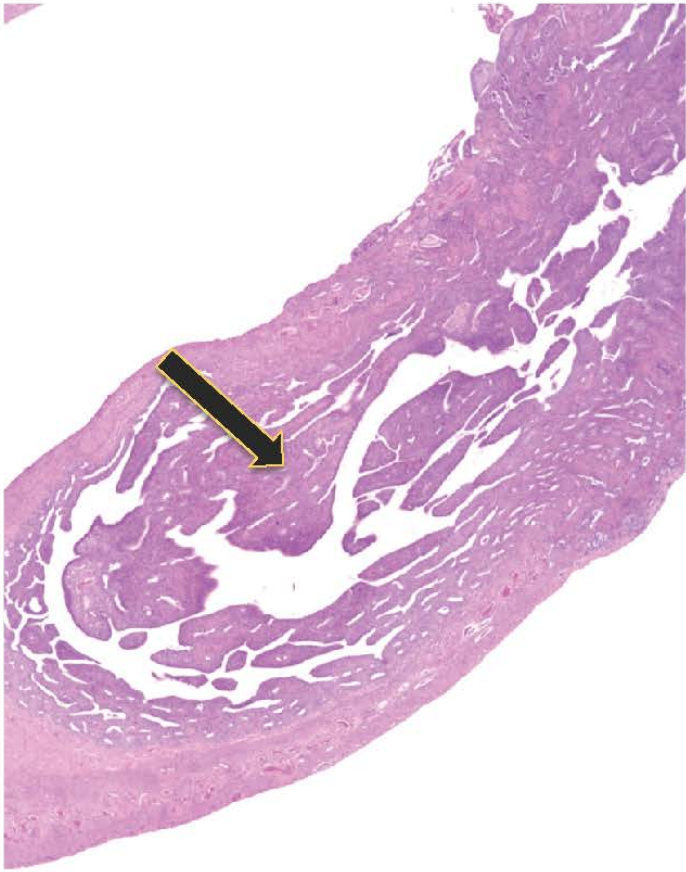


Proximal Vagina



Vagina Meets Cervix





## ORIGINAL TRANSVERSE REVIEW

Adenoma	0**	0	3	4
Adenocarcinoma (multiples included)	3*	3	8	9
Malignant Mixed Müllerian Tumor (MMMT)	0	4	0	2
Adenoma, Adenocarcinoma, or MMMT	3**	7	11*	13**

## COMBINED ORIGINAL TRANSVERSE AND RESIDUAL LONGITUDINAL REVIEWS

Adenoma	3	2	4	6
Adenocarcinoma (multiples included)	4**	10	15**	16**
Malignant Mixed Müllerian Tumor (MMMT)	0	4	0	2
Adenoma, Adenocarcinoma, or MMMT	6**	11	16**	19**

\* Positive trend test or significantly different ( $p \leq .05$ ) from the control group by Poly 3 test

\*\* Positive trend test or significantly different ( $p \leq .001$ ) from the control group by Poly 3 test

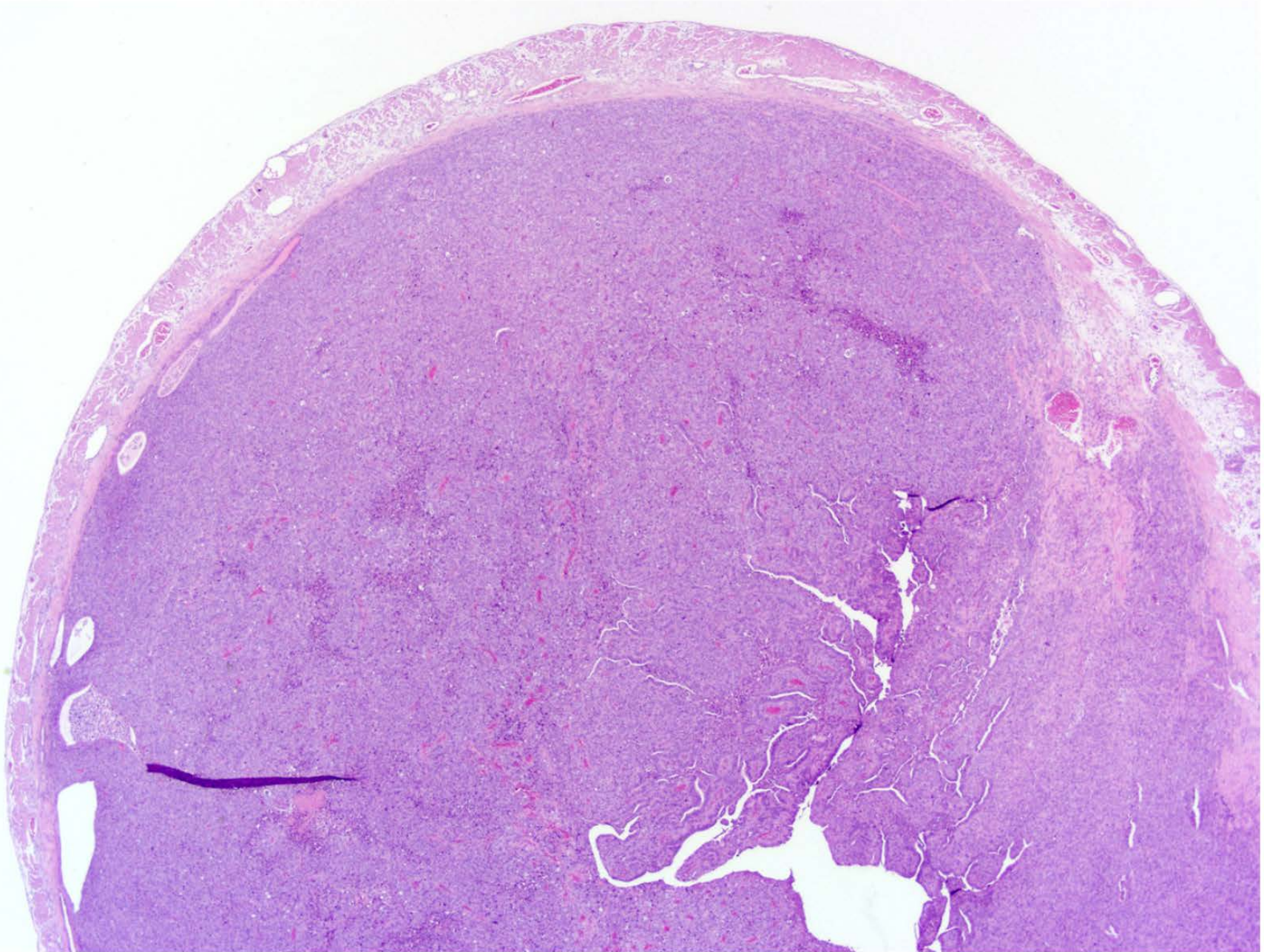
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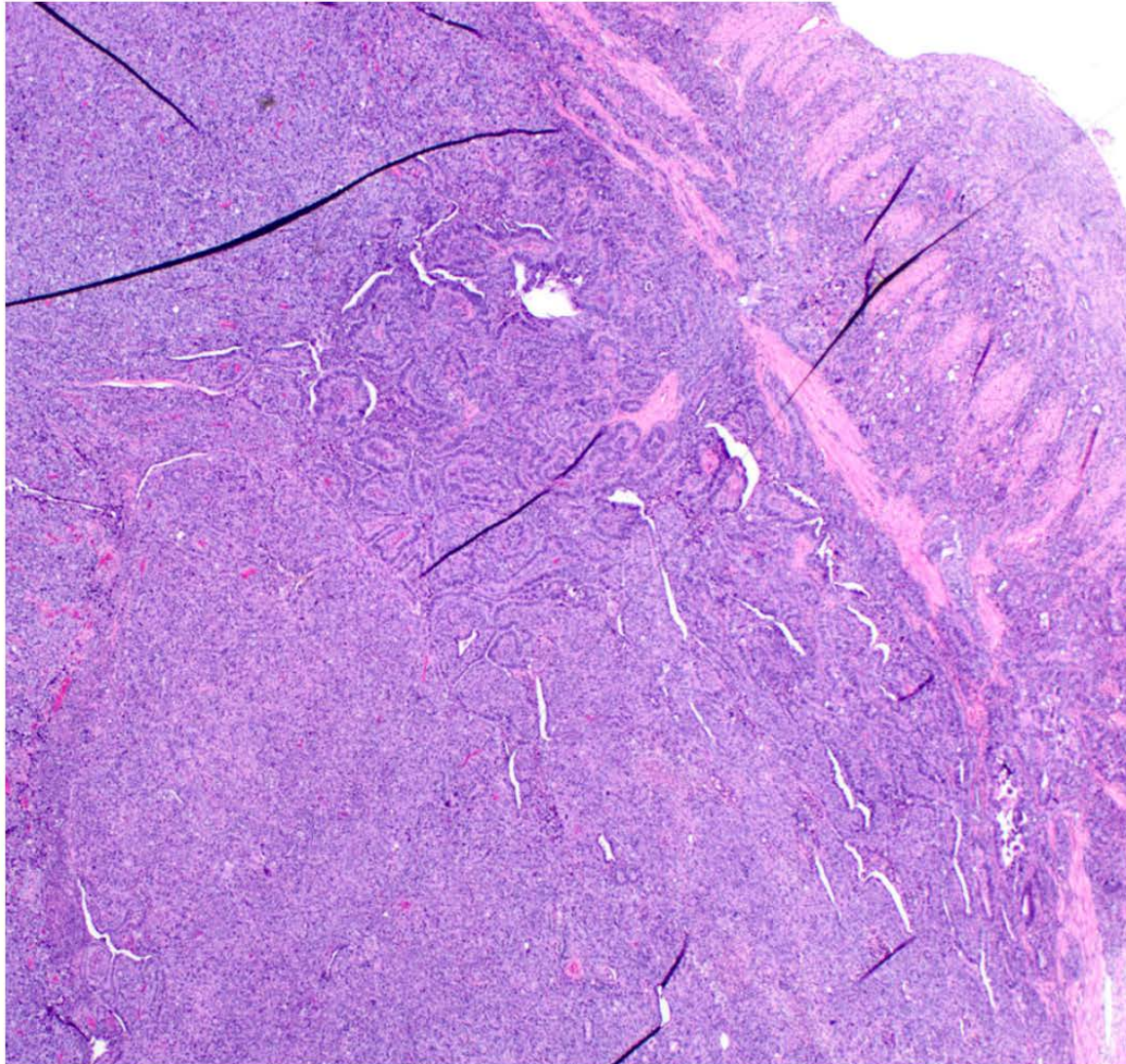
## Malignant Mixed Müllerian Tumors (MMMTs)

- Subset of tumors had an unusual solid growth pattern with areas of glandular formation
- Solid areas had histological features of both malignant epithelial and mesenchymal cells
- Were identified in the original review due to their large sizes
- 4/6 had metastases (liver, pancreas, spleen, lung, mesentery, stomach, urinary bladder)
- In all cases the metastatic tumor was the epithelial component

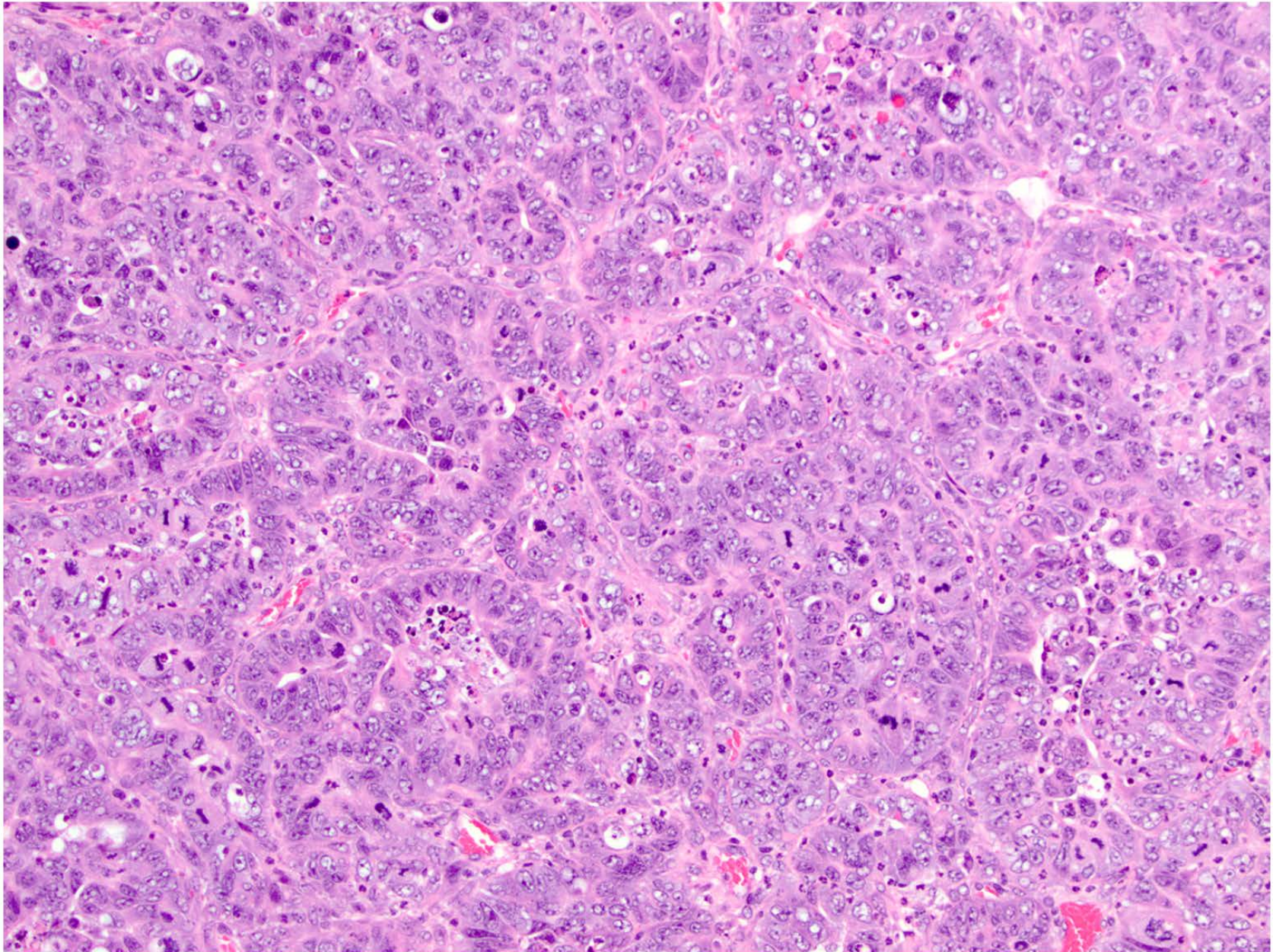
## Low Magnification of an MMMT



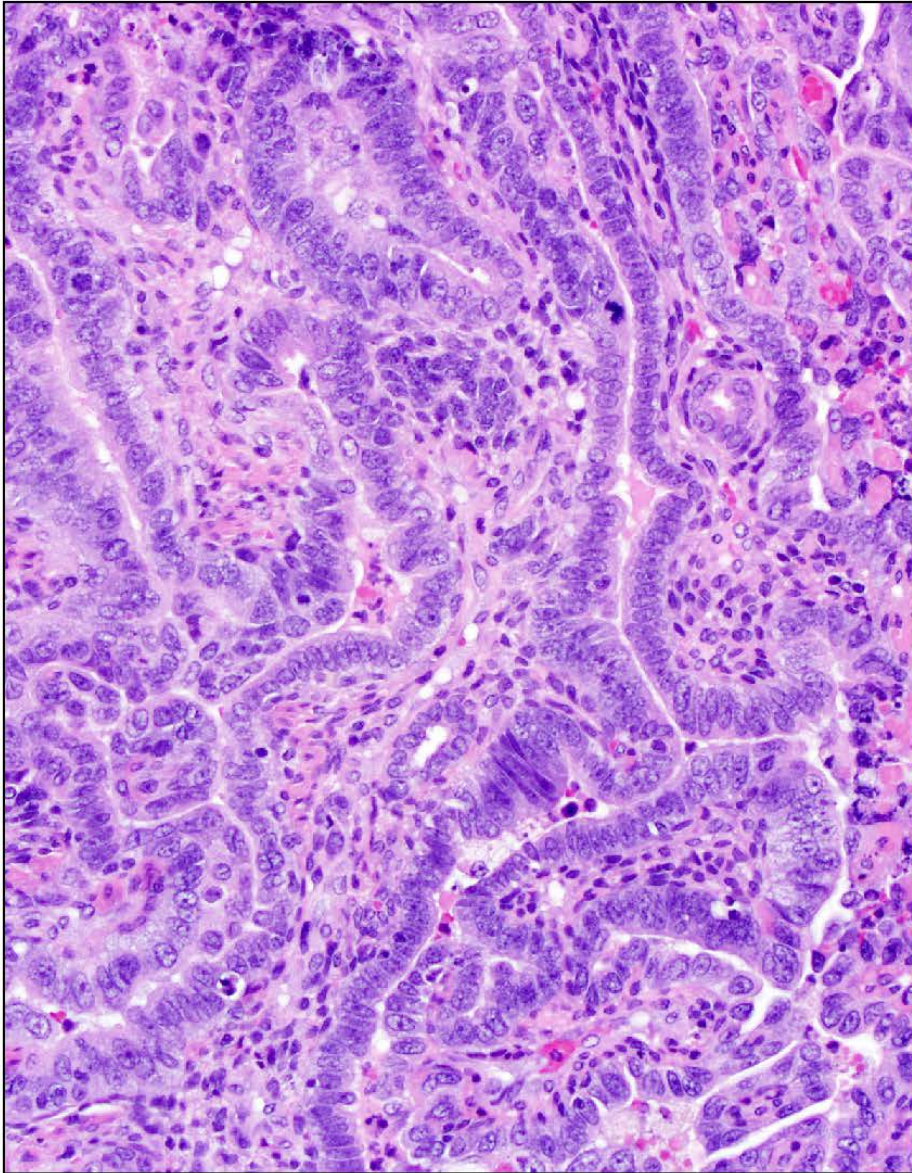
# Epithelial Component of an MMMT



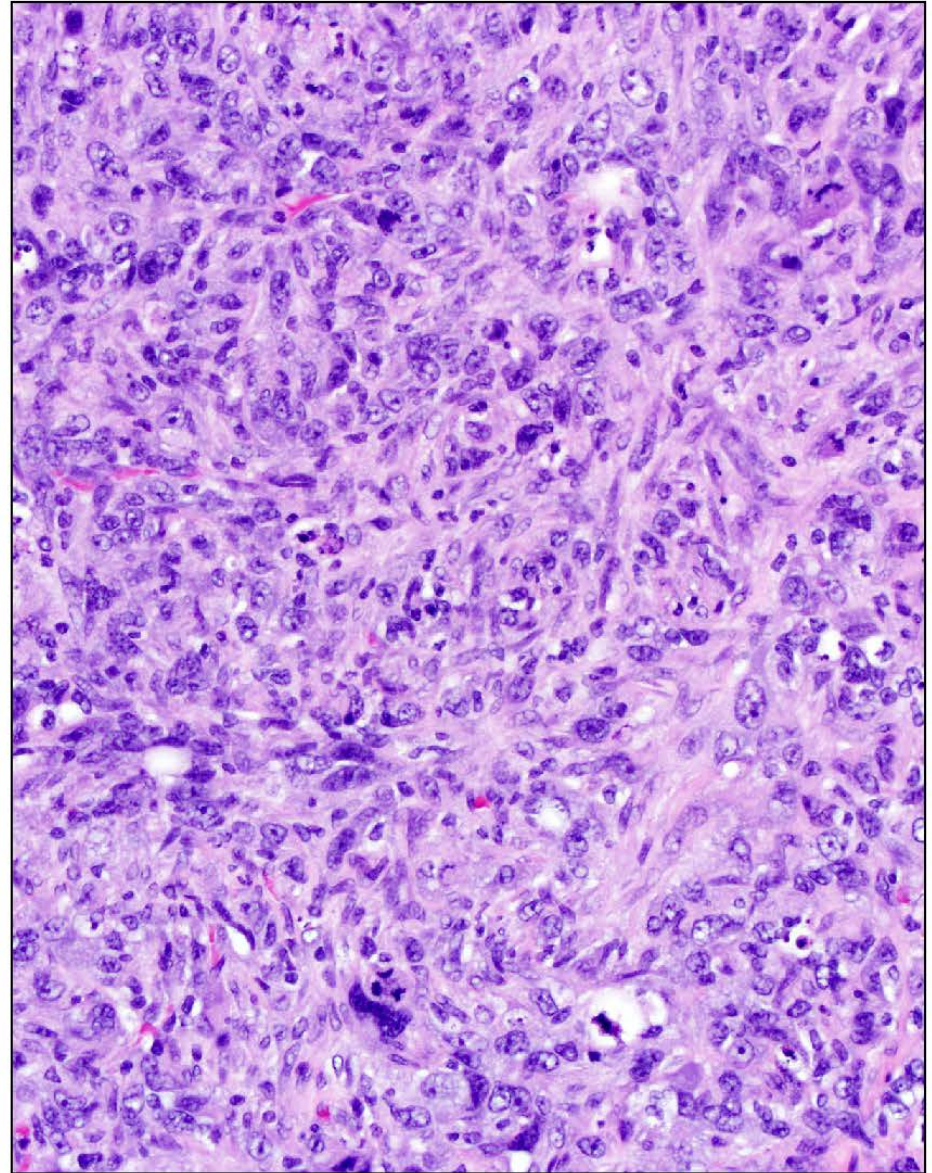
## Area of MMTT with Glandular Formation



Adenocarcinoma



MMMT



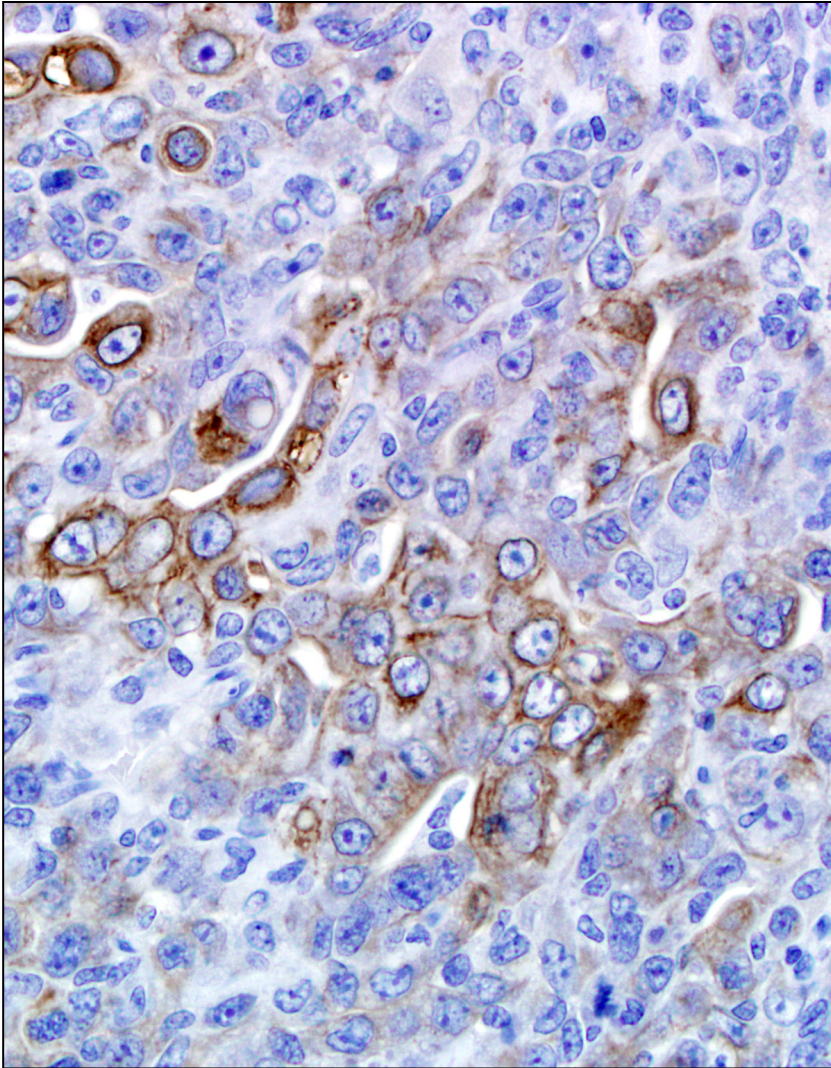


# Malignant Mixed Müllerian Tumors (MMMTs)

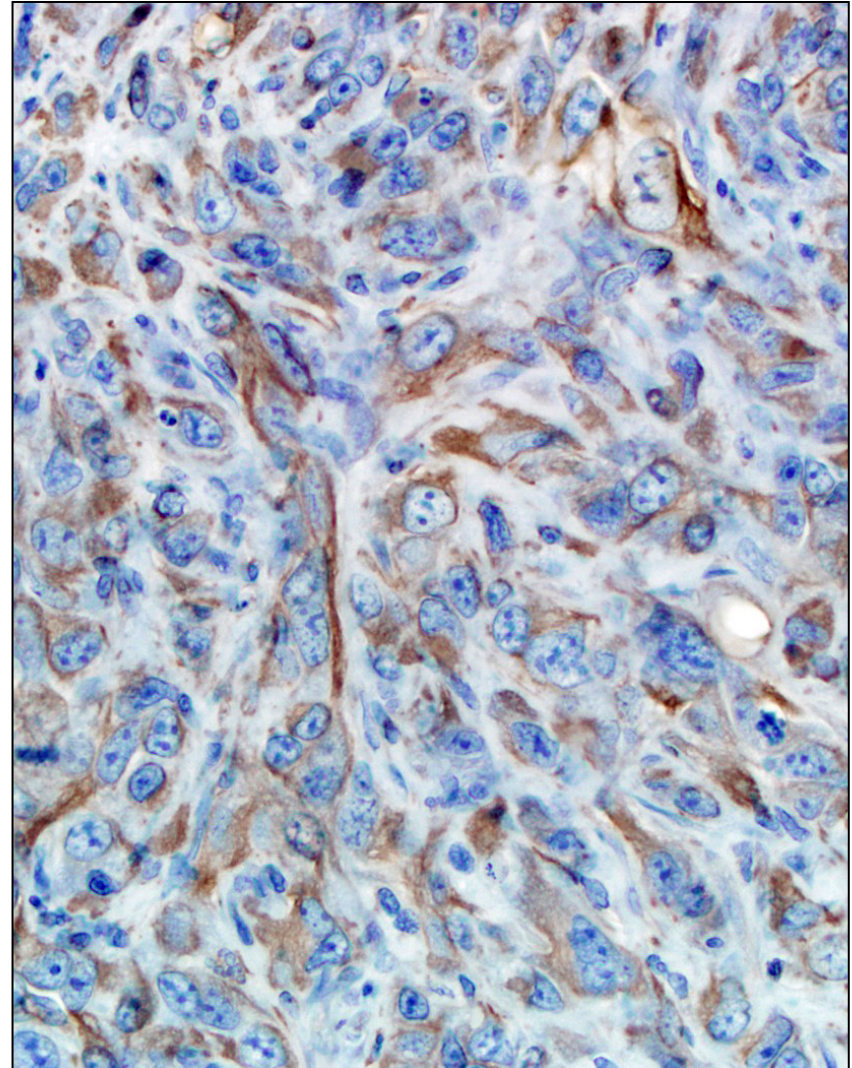
- Question was if these tumors contained both neoplastic epithelial and mesenchymal cells
- IHC stains were used to evaluate the MMMTs and the uterine adenocarcinomas
  - MMMTs
    - Cytokeratin and vimentin positive neoplastic cells
  - Adenocarcinomas
    - Only cytokeratin positive neoplastic cells

# MMMT

Cytokeratin



Vimentin



## MMMTs in Humans

- Account for ~5% of all malignant tumors derived from the body of the uterus in women, also called carcinosarcomas or metaplastic carcinomas
- Also diagnosed in the cervix, Fallopian tubes, ovaries, vagina or peritoneum but with a much lower frequency than in the uterus
- Worse prognosis than adenocarcinoma: more aggressive, more likely to metastasize
- For a stage 1 tumor (confined to the uterus) the 5 year survival rate is 50%
- Predisposing factors are similar to those of adenocarcinomas and include obesity, exogenous estrogen therapies, nulliparity, tamoxifen therapy, aromatase therapy, pelvic irradiation

# Histogenesis of MMMTs in Humans

- Most are monoclonal, derived from a single stem cell
- The carcinomatous component is the “driving force”
- Theories of histogenesis (not mutually exclusive):
  - Combination theory: Both components are derived from a single stem cell that undergoes divergent differentiation early in the evolution of the tumor.
  - Conversion theory: The sarcomatous element derives from the carcinoma during the evolution of the tumor.
- Evidence is clinical, histopathological, immunohistochemical, ultrastructural, tissue culture, molecular data
- W.G. McCluggage. Malignant biphasic uterine tumors: carcinosarcomas or metaplastic carcinomas? 2002. *J Clin Pathol.* 55:321-325

## MMMTs in Animals

- Very rare spontaneous neoplasm in animals
  - Not reported in any previous NTP studies with F344 rats
  - Published case reports for
    - 5/600 LEWIS (LEW/HAN) rats
      - *Toxicol Pathol* (1990)18:417–422
    - 2/1152 Wistar (CrI:[Wi]BR) rats
      - *Berl Munch Tierarztl Wochenschr* (1990) 103:431
    - 1/4060 Wistar (Hsd/Cpb:WU Spf) rats
      - *Vet Pathol* (2010) 47(6):1105-1110
    - 1 Wistar Hannover GALAS [BrIHan:WIST@Jcl (GALAS)] rat
      - *J Toxicol Pathol* (2011) 24:63-67
- Never reported as treatment-related in animals
- Combined with adenomas and adenocarcinomas because the epithelial component is considered the “driving force”

# Outline

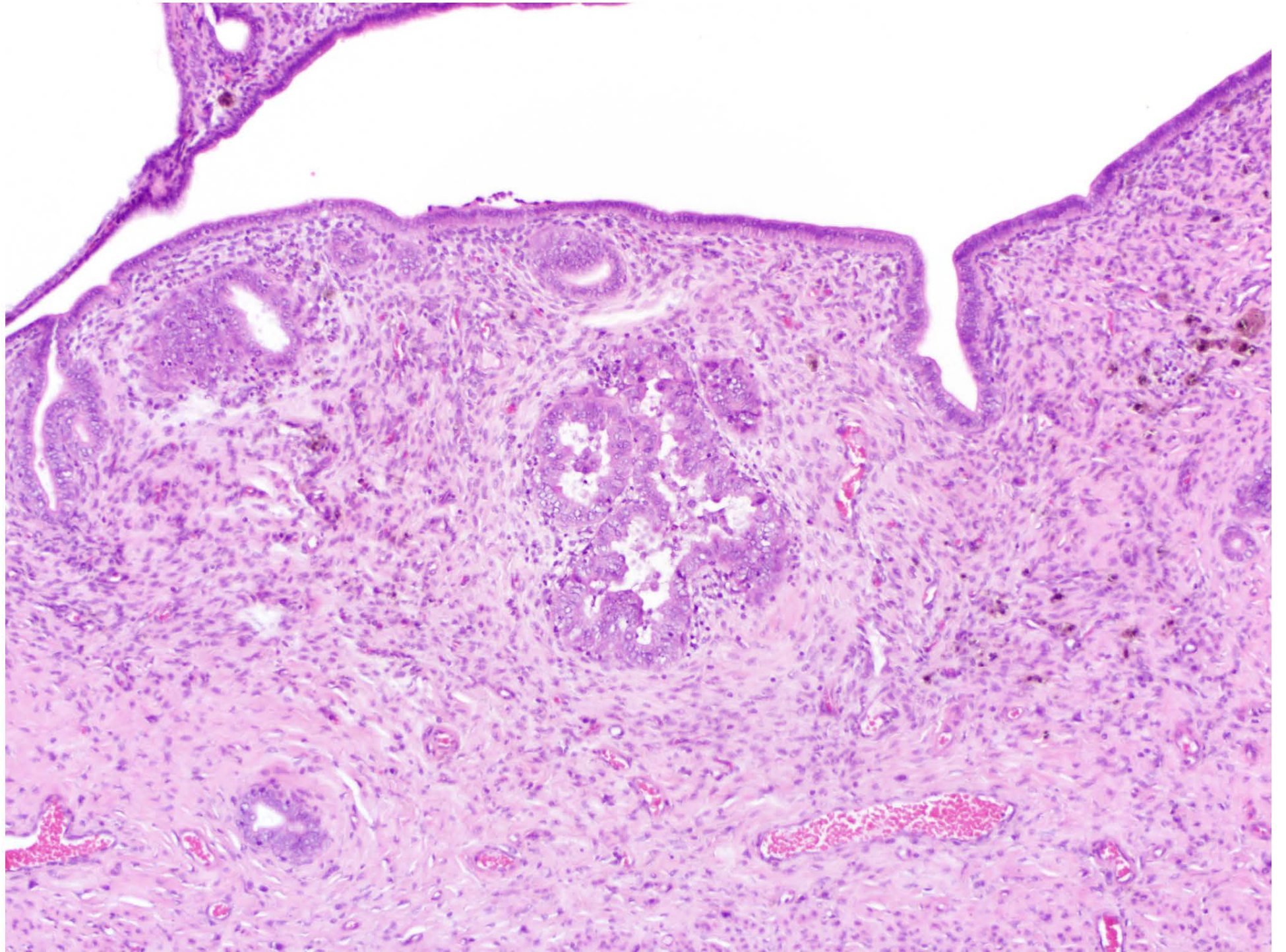
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# Atypical Endometrial Hyperplasia

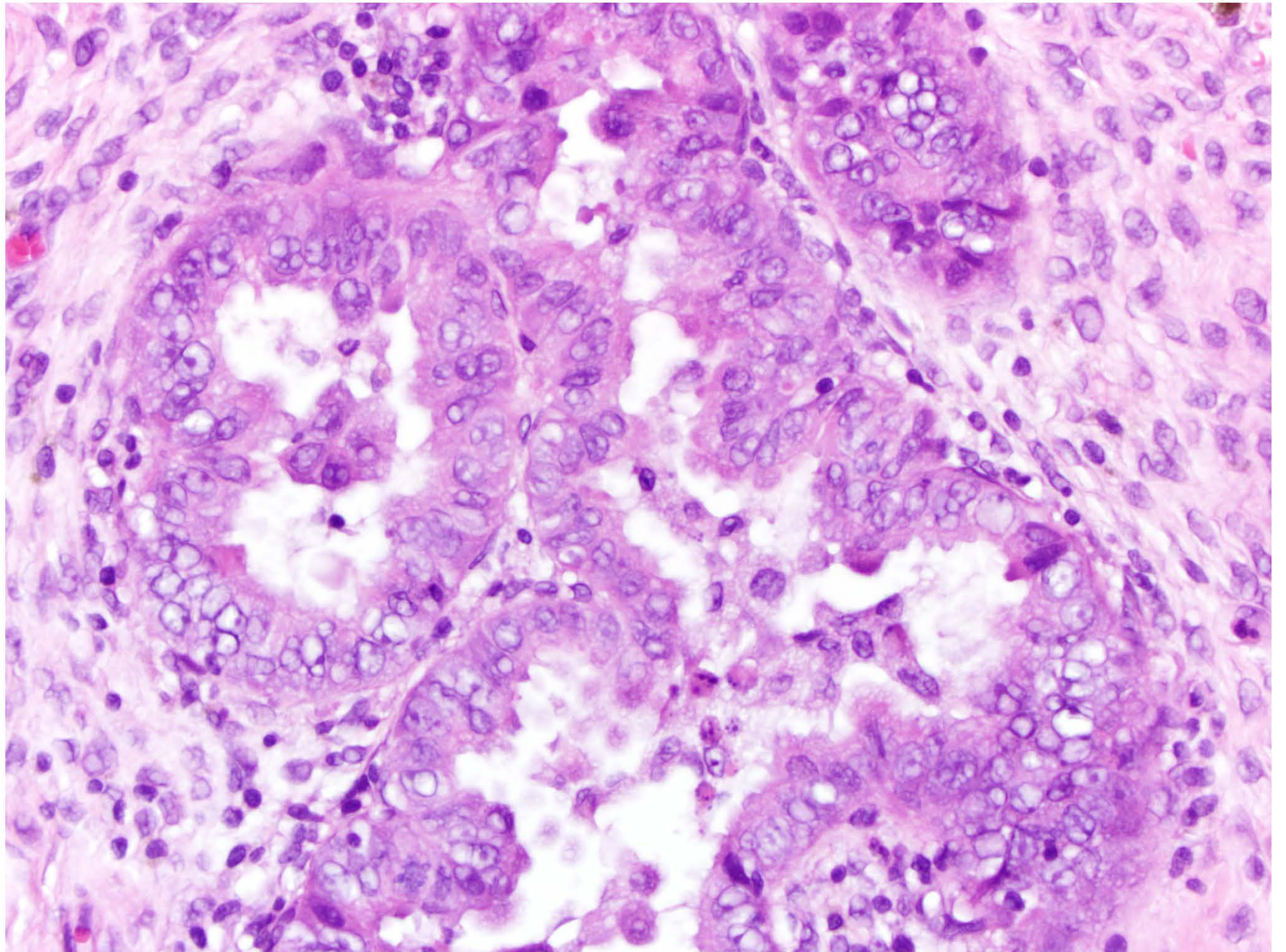
	0mg/kg	250mg/kg	500mg/kg	1000mg/kg
Endometrium, hyperplasia, atypical	2* (2)	13** (1.3)	11** (2)	13** (2.8)

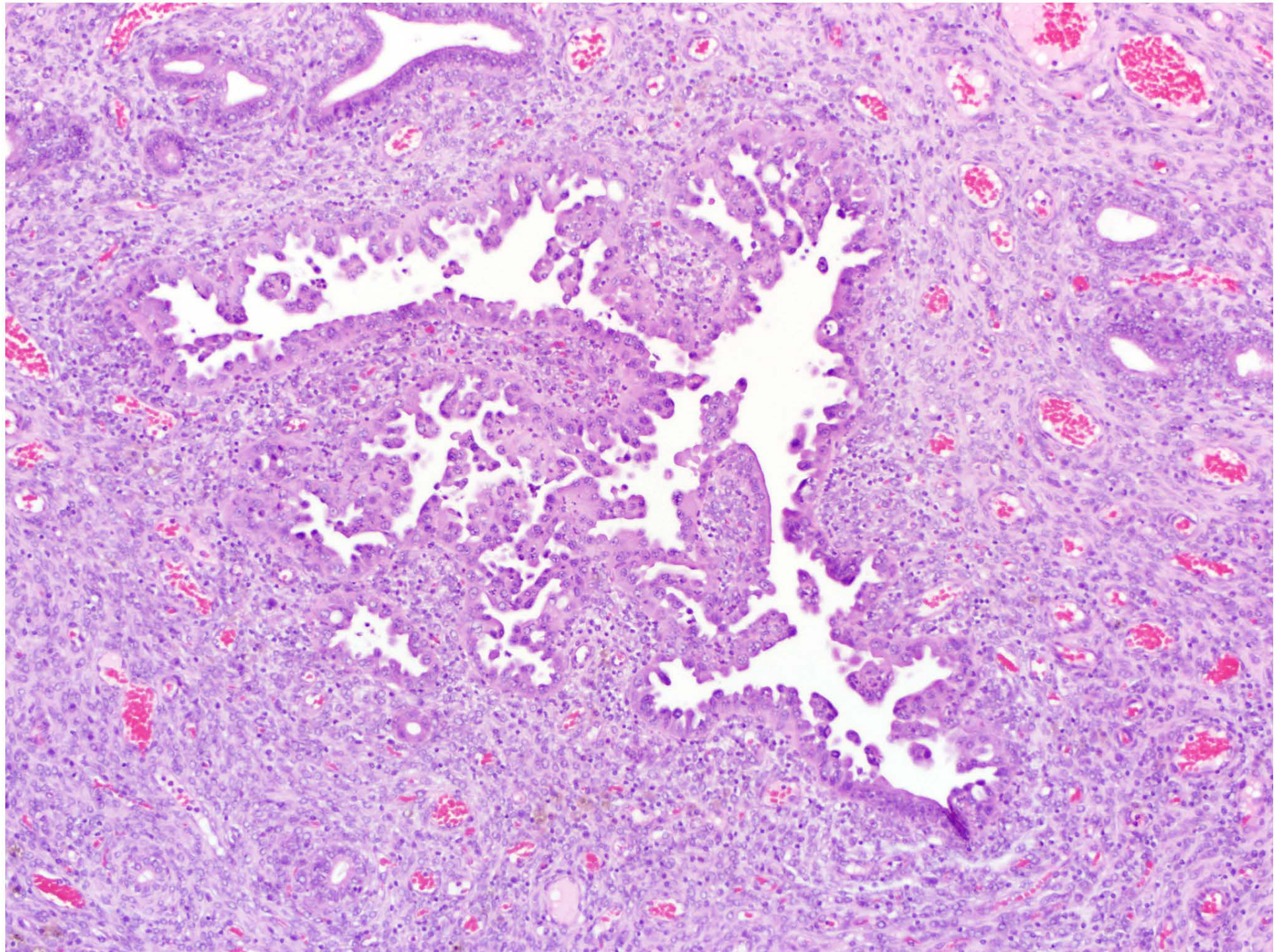
Mean severity grades, 1-4, in parentheses

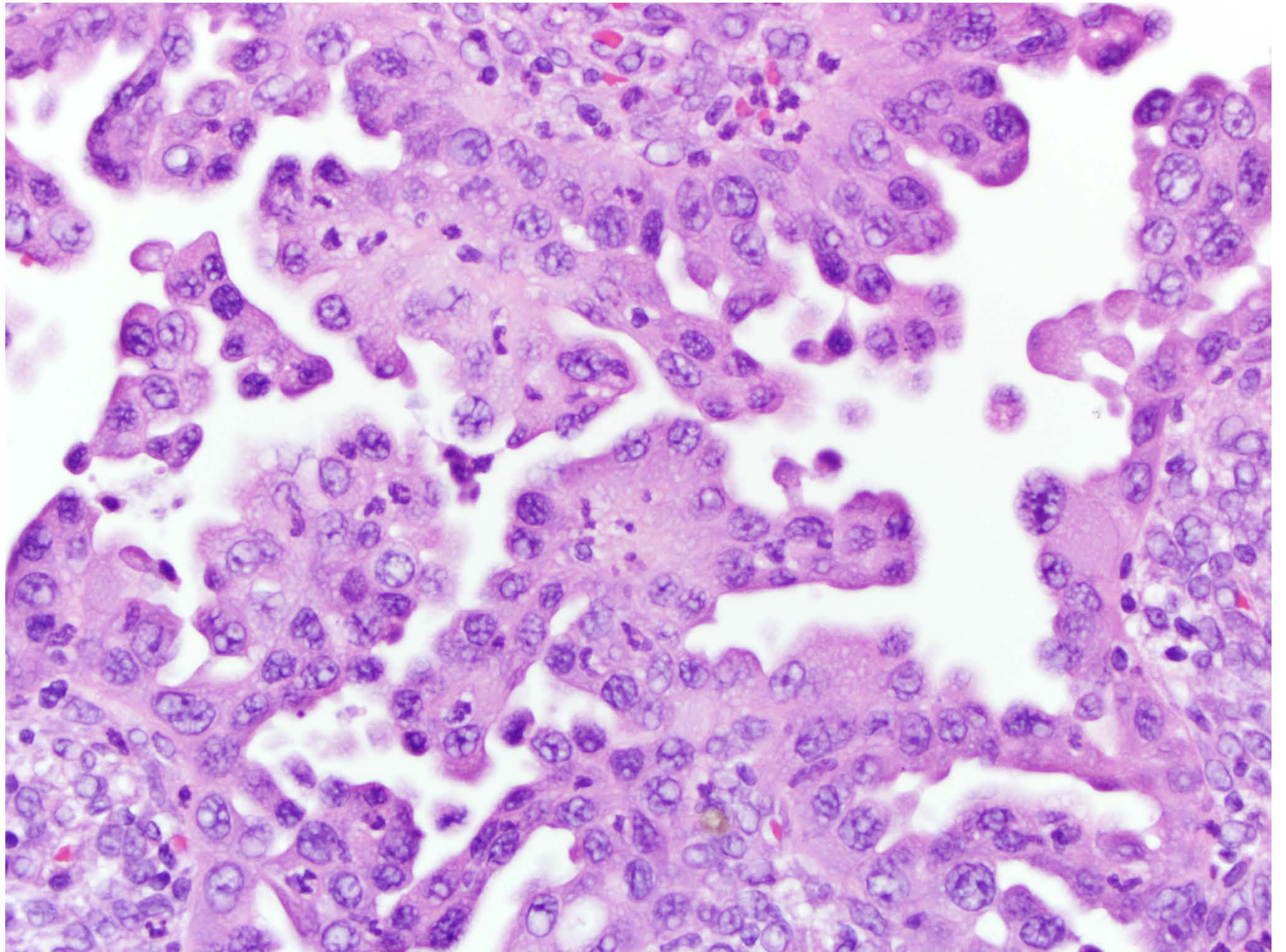
These lesions were not present in the original transverse review; were mostly small and focal and therefore not likely to be detected in a single section.











# Atypical Endometrial Hyperplasia: Humans

- Considered preneoplastic
- A significant risk factor for the development or co-existence of endometrial cancer
- 22% of patients with AH eventually develop cancer
- Most result from high levels of estrogens with insufficient levels of progesterone-like hormones
- Predisposing factors include obesity, polycystic ovary syndrome, estrogen producing tumors (granulosa cell tumor), some estrogen replacement therapies.

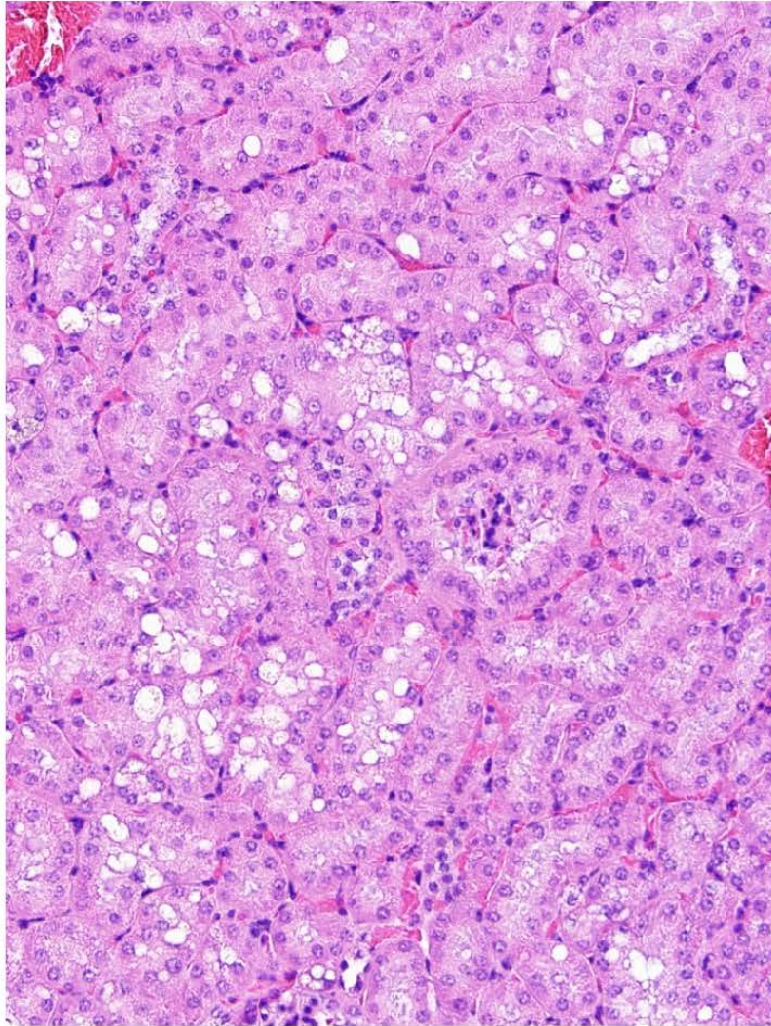
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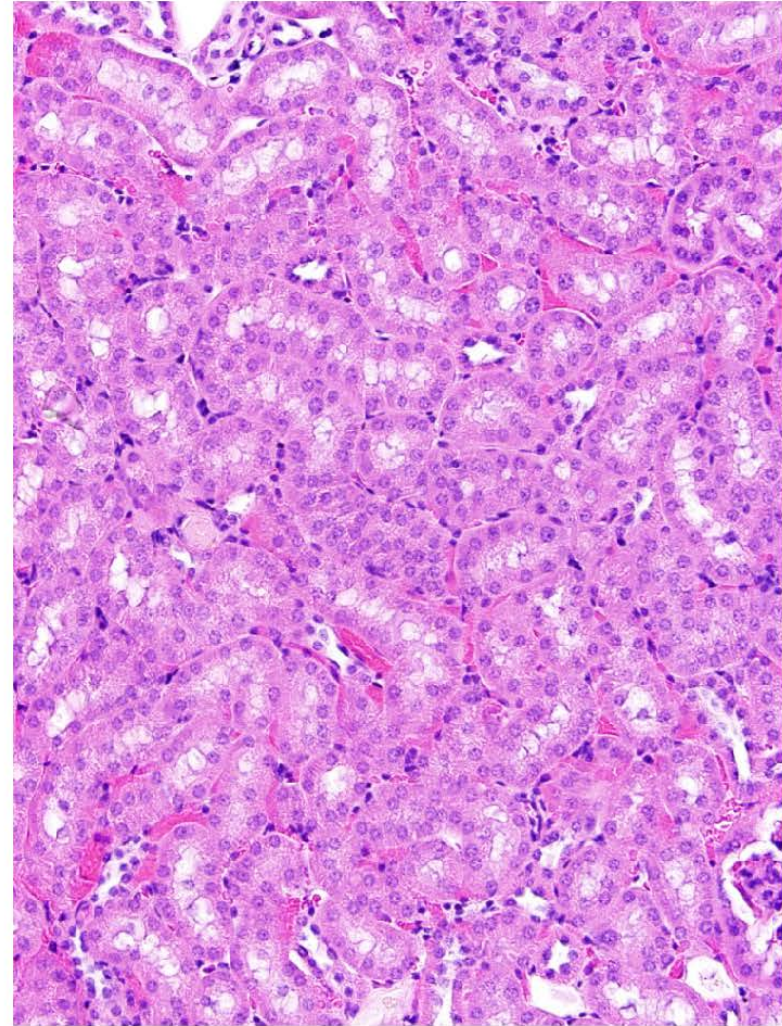
## Renal Tubule Cytoplasmic Alteration

- Decreased cytoplasmic vacuolization of renal proximal tubule epithelial cells when compared to controls
- Male mice only
  - Subchronic study: 0, 0, 0, 0, 10<sup>\*\*</sup>(1.0), 10<sup>\*\*</sup>(2.0)
  - Chronic study: 0<sup>\*\*</sup>, 20<sup>\*\*</sup>(1.9), 47<sup>\*\*</sup>(2.4), 46<sup>\*\*</sup>(2.6)
- Normally there are autophagic vacuoles in the outer cortex of male B6C3F1 mice
- Present due to loss and regeneration of cortical tubule cells
- Using orchietomy, has been shown to be dependent on endogenous testosterone

# Renal Tubule Cytoplasmic Alteration



Normal Control



Loss of cytoplasmic vacuoles

# Summary

- Residual longitudinal study
  - Revealed additional adenomas and adenocarcinomas
  - Supported the original call of “clear evidence”
  - Atypical hyperplasias were identified that were not present in the original slides
- MMMTs
  - First report of MMMTs in an NTP study
  - Rare tumors and considered more aggressive than adenocarcinomas
  - Found in the original transverse sections due to their large size
  - Combined with adenomas and adenocarcinomas because the current histogenesis theory and epithelial metastases indicate that the epithelial component is the driving force



# Summary

- Atypical hyperplasia
  - Treatment related in rats
  - Rare and potentially preneoplastic
  - Not found in the original transverse sections due to small lesion size
- Renal tubule cytoplasmic alteration
  - Treatment-related in male mice
  - Found in subchronic and chronic studies
  - May be associated with altered hormonal status