Testicular Germ Cell Neoplasia: Molecular Pathways & Challenging Morphologic Aspects

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MEDICINE

Overview

- Epidemiology & Risk Factors
- Genetics & Novel Markers
- Gender & Age Factors in Gonadal GCT
- IGCNU
- Diagnostically “Problematic” Germ Cell Tumors (GCT)

Testicular Germ Cell Neoplasms

Epidemiology & Risk Factors

- **Germ Cell Tumors (GCT) are most common malignancy in men aged 15-44y.**
- **500,000 cases Worldwide/8,250 cases in the US (370 death).**
- **Incidence increases after puberty, peaks in third decade.**
- **Modal age of NS-GCT is a decade earlier than S-GCT.**
- **Geographical variation: Life time risk is 0.4-0.7% in US, 1% in Nordic region and least in Asia and Caribbean.**

Testicular GC Neoplasms

Epidemiology & Risk Factors

- **Steep increase in incidence in US and Northern Europe.**
- **Decreased incidence in men born during WWII**
  - risk determined early in life?
- **Risk of invasive GCT parallels prevalence of IGCNU**
  - Cryptorchid testis
  - Contralateral testis in GCT pts
- **Depletion of IGCNU pool with age.**

Testicular GC Neoplasms

Epidemiology & Risk Factors

- **Abnormal In-utero conditions affect primordial GC?**
  - **Male genitalia abnormalities : Cryptorchidism, Maldescent, Hypospadia**
  - **Gonadal dysgenesis: 45,X/46,XY (10-50% risk)**
  - **Low birth weight**
  - **Older maternal age**

Abnormal diff. of primordial GC ⇨ ***IGCNU*** ⇨ ***Invasive GCT***

Testicular Neoplasms

Epidemiology & Risk Factors

- **Familial Risk: 2% of GCT have familial origin. 8xRR in siblings**
- **Male infertility: Shared causality with GCT**
- **Adulthood exposures/factors:**
  - Physical activity
  - Socioeconomics
  - Immunosupression
- **YST in infants and Spermatocytic Seminoma in older pts: lack association with IGCNU ⇨ origin from differentiated spermatogonia**

Testis
Cryptorchidism

- Incidence: 1-2%.
- IGCNU: 0.4% in cryptorchid testis biopsied at pexy.
- Currently Bx at time of orchiopexy recommended only if karyotypic abnormalities /malformation present.
- 3-5% life time risk (4-7 times RR) for malignancy in affected Testis.
- 2-3 RR in contralateral Testis
- 80 % of GCT are seminomas

Testis
Cryptorchidism

- Orhiopexy even before 2 year of age is not protective from subsequent GCT.
- Comparable stage of GCT at time of presentation in orchiopexy Vs no intervention pts.
- Animal models support link of increased risk of IGCNU in crytorchid to hormonal environment during pregnancy rather than abdominal location (endocrine disrupters)
- Mother smoking: risk for bilateral cryptorchidism?
- Micro: atrophic tubules, thick BM, leydig cell hyperplasia, microlithiasis, IGCNU.
- Microletiasis alone not a risk for GCT.

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- **Genetics & Novel Markers**
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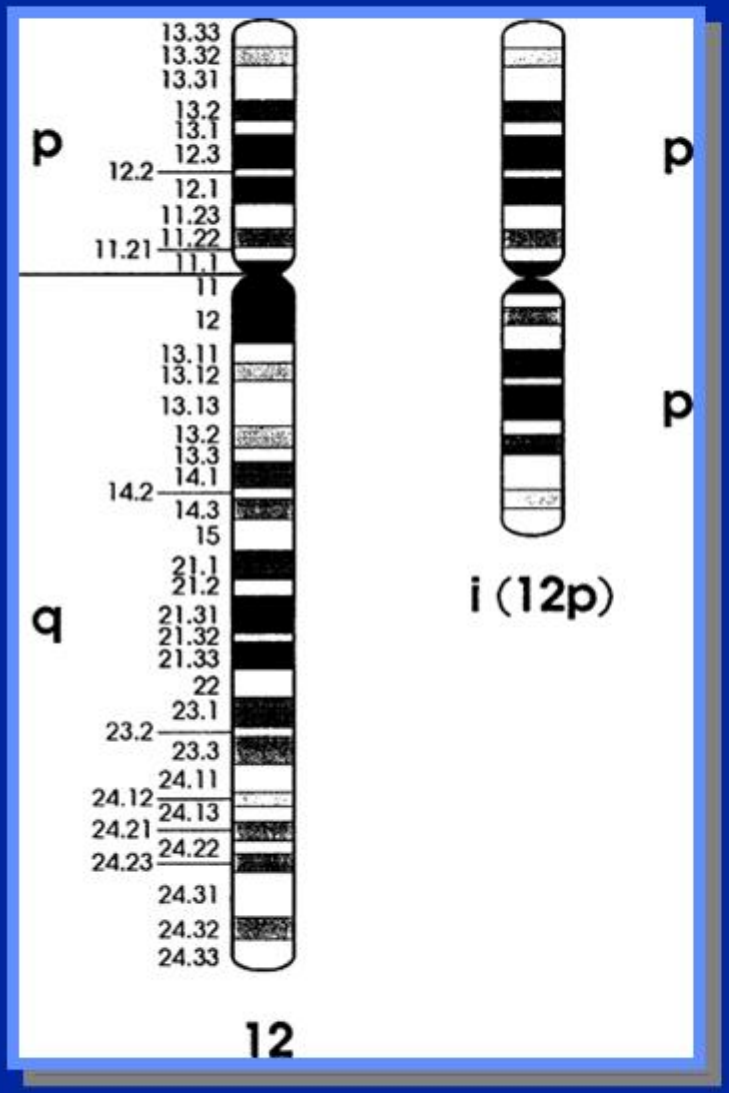
Testicular Neoplasms

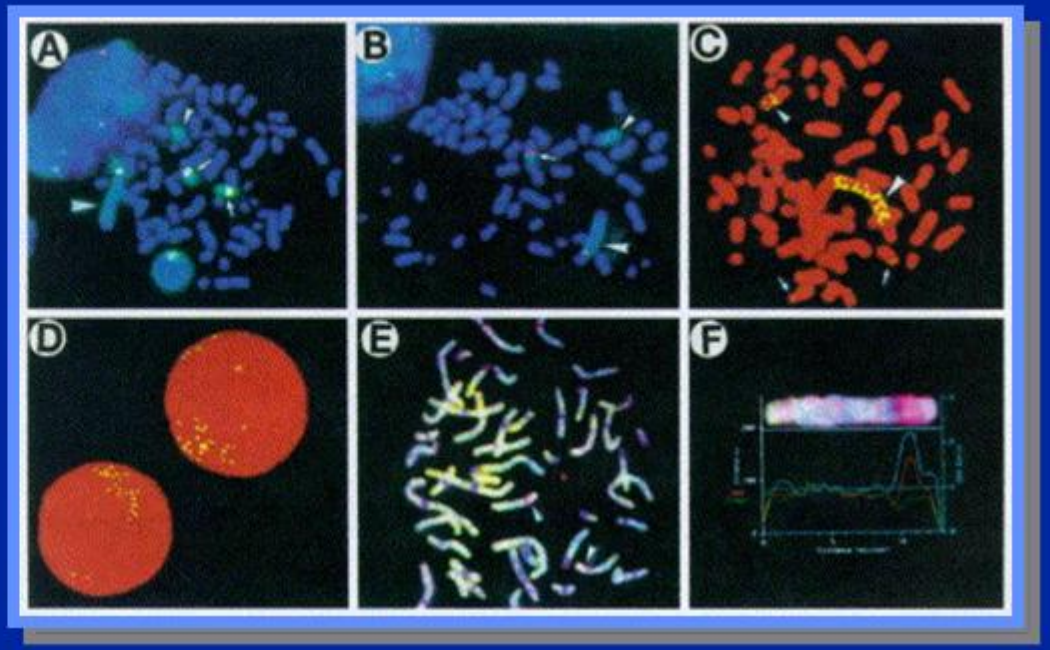
Genetics

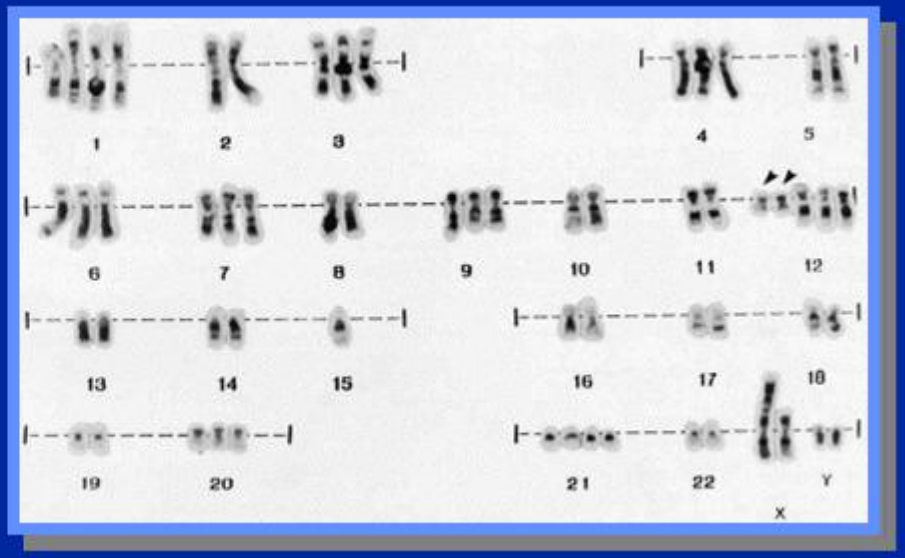
Over-representation of Chromosome 12p Region:

- **Consistently present structural aberration in GCT**
- **i (12p): present in up to 80% of cases including extratesticular tumors**
- **i (12p): uniparental origin (disomey)**
- **Other abnormalities include additional copies of parts of 12p chromosome**
- **Amplification of 12p11.2-p12.1 region: apoptosis resistance genes such as DAD-R**
- **i (12p) is rare in IGCNU**
- **i (12p) is rare in Infantile GCT (YST: -6q; Teratoma: 2N, no karyotipic change)**

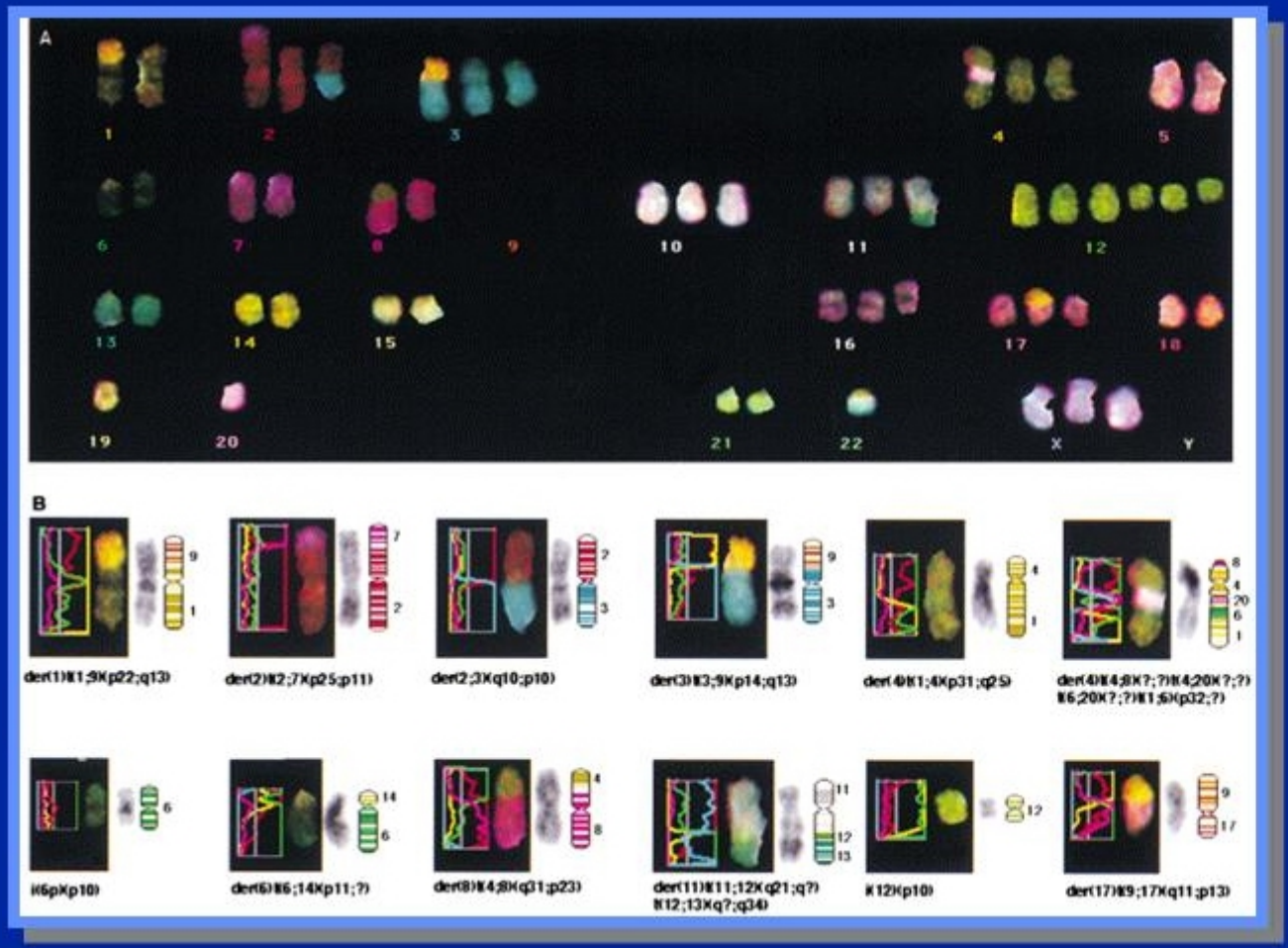
- **FISH or CGH analysis for ip12 can be helpful in DDX of GCT vs Somatic Ca. In extratesticular location.**

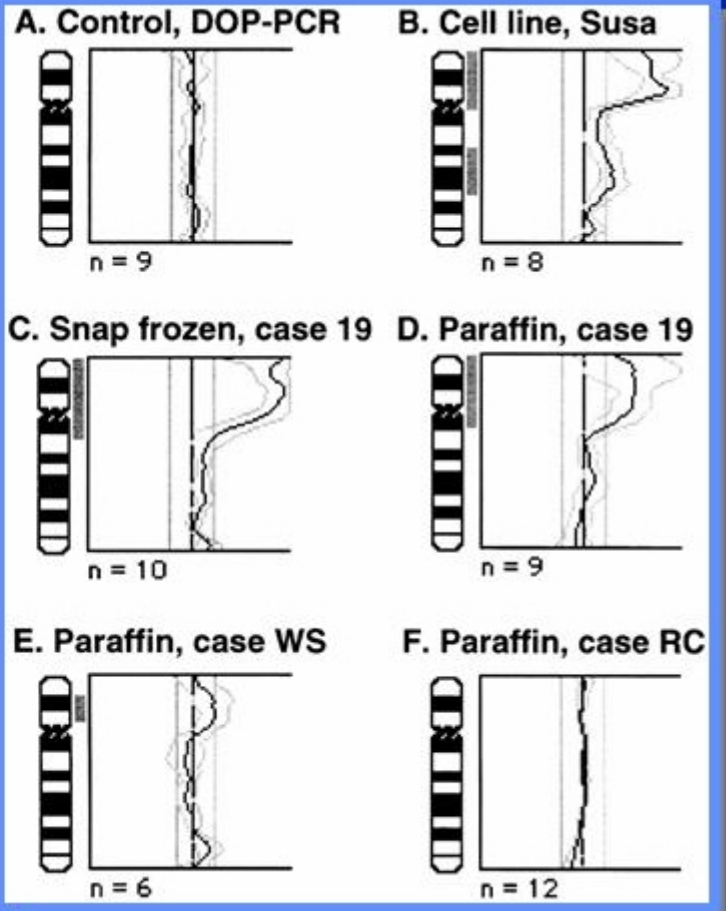






Sandberg AA. et al. J Urology 1996


A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
B


A. Control, DOP-PCR
n = 9
B. Cell line, Susa
n = 8
C. Snap frozen, case 19
n = 10
D. Paraffin, case 19
n = 9
E. Paraffin, case WS
n = 6
F. Paraffin, case RC
n = 12

Testicular GC Neoplasms

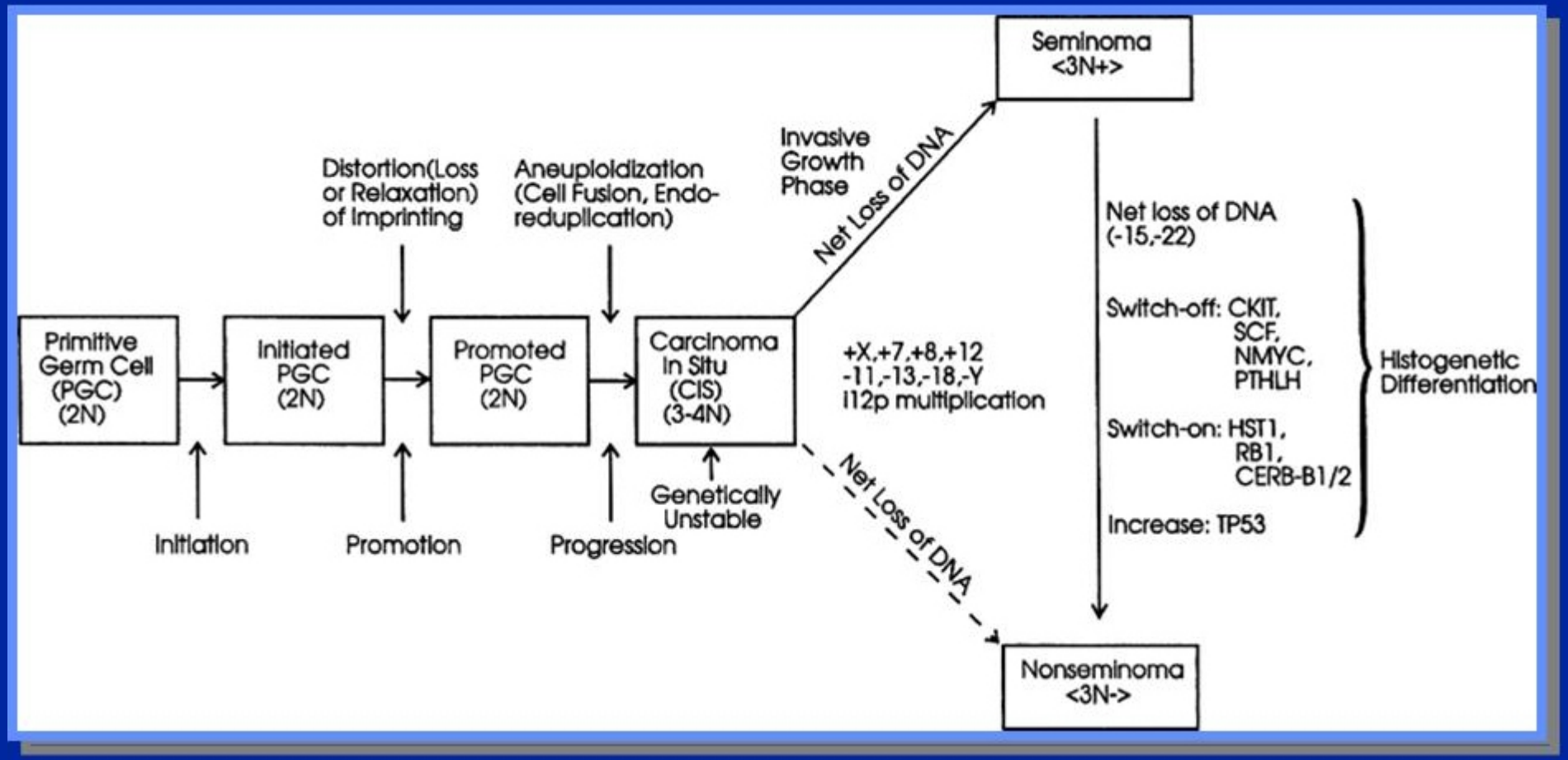
Genetics

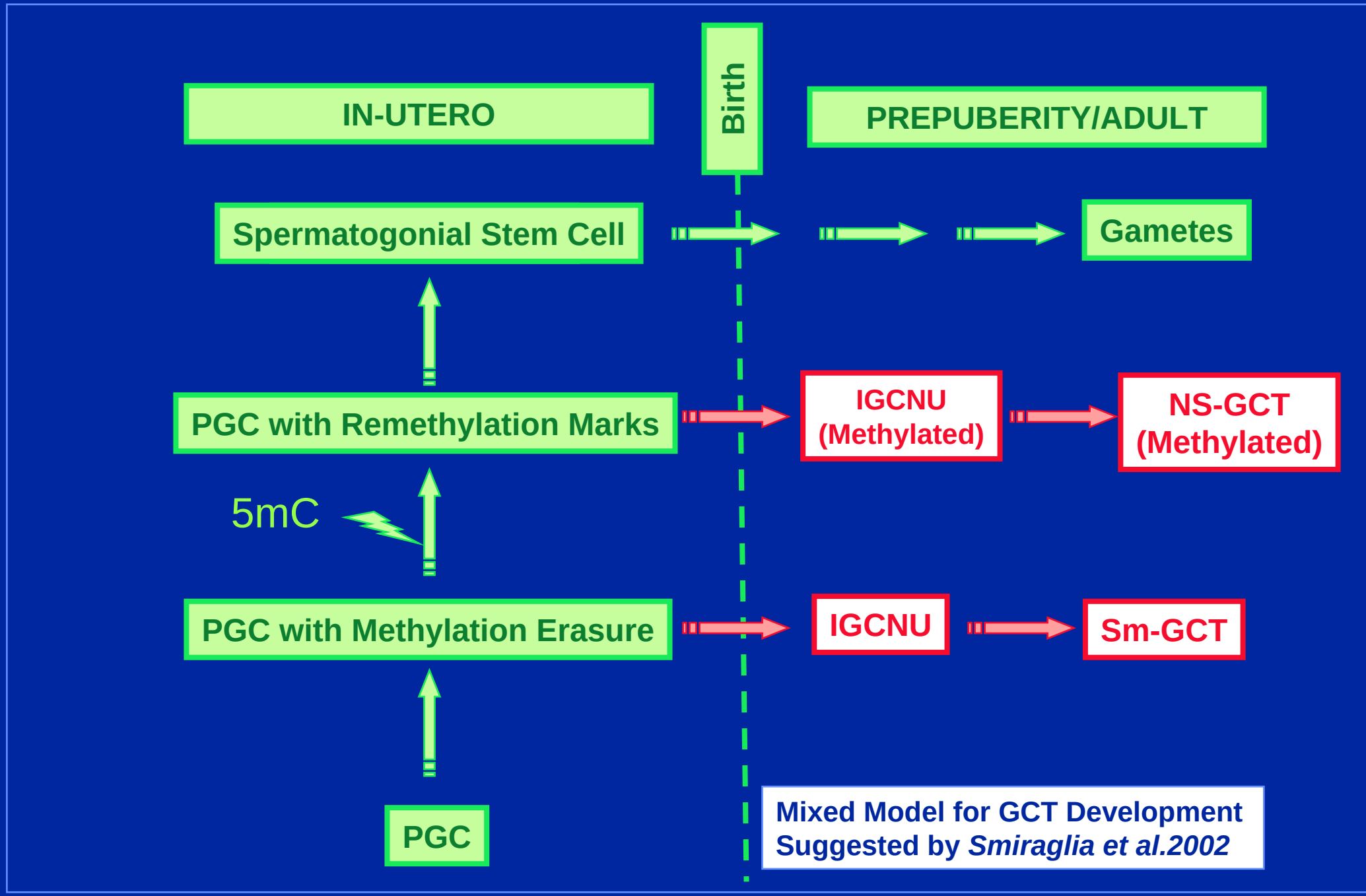
Therapy Predictive Biomarkers

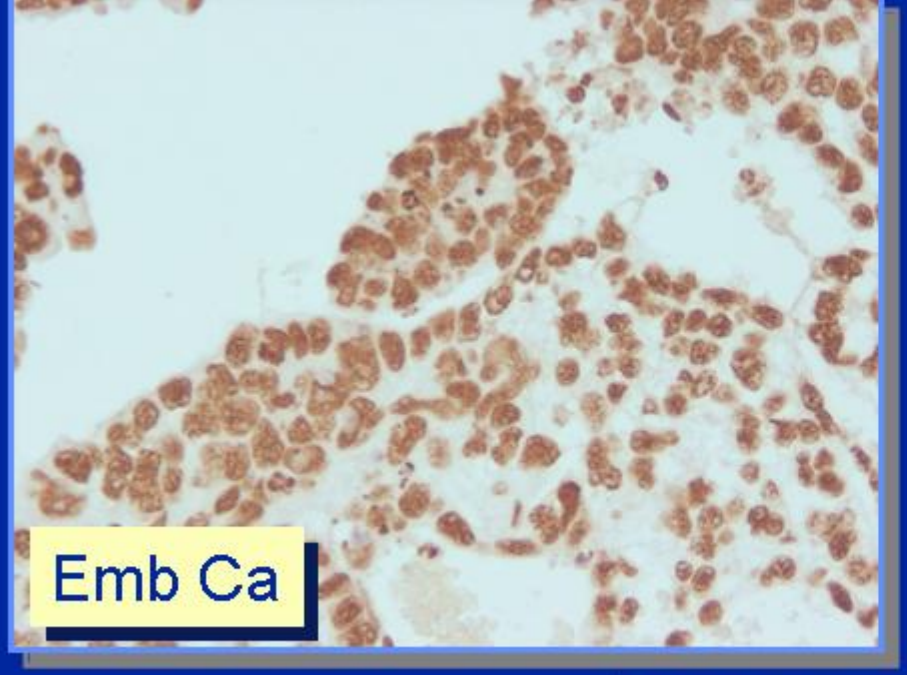
- **DNA damage detection/Apoptosis initiation programs:**
  - WT p53 overexpression is associated with chemosensitivity
  - Disturbance in MMR is found in cisplatin refractory seminoma

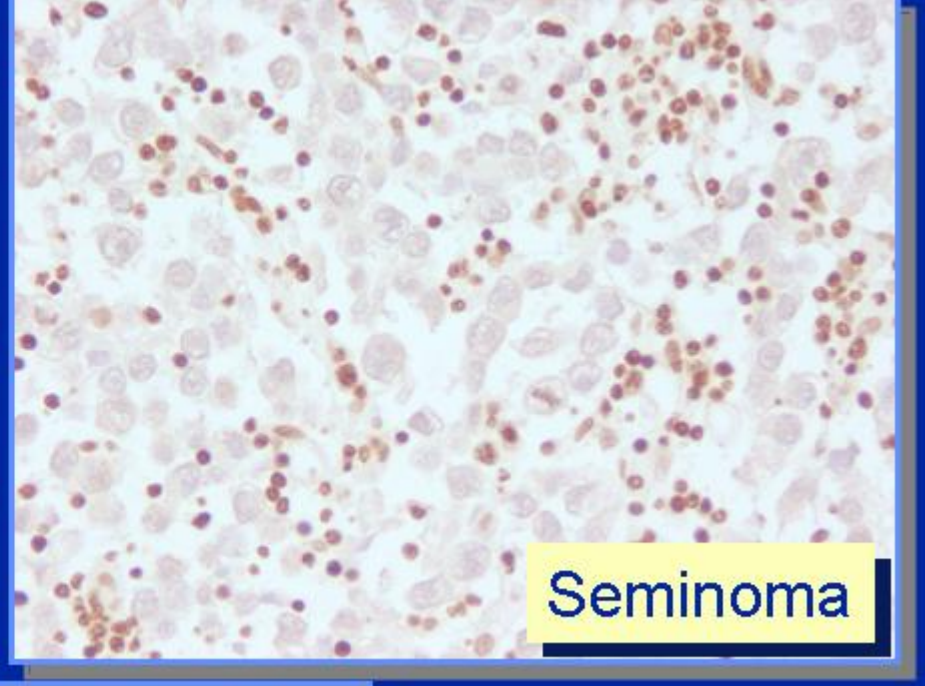
Epigenetics:

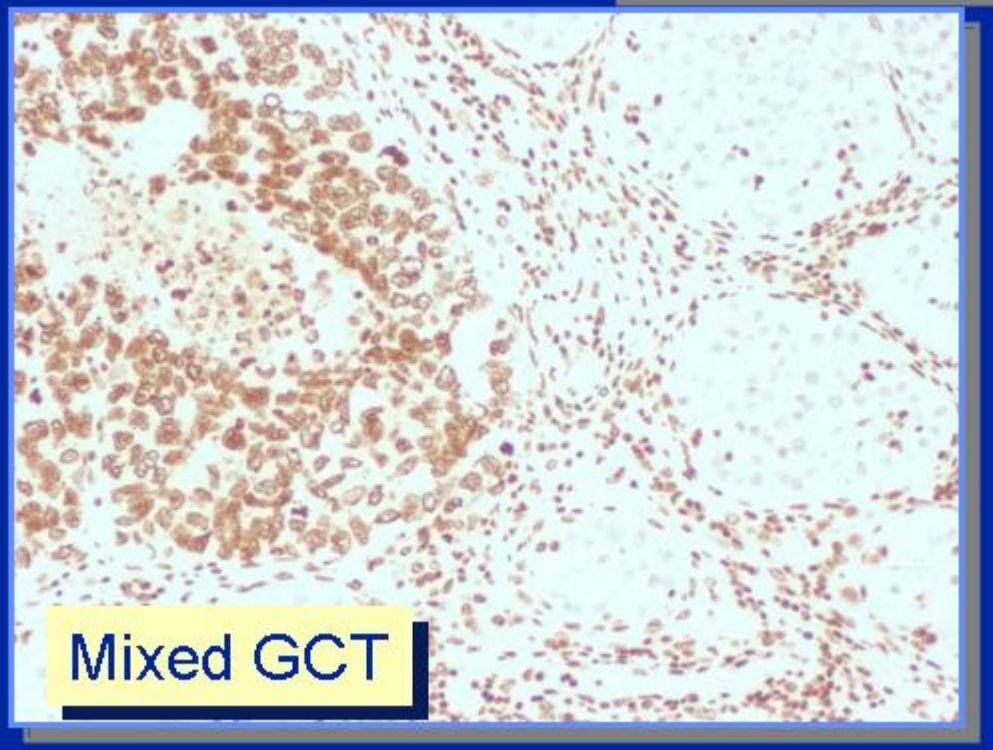
- **Genomic Imprinting:**
  - In Utero: Erasure of genetic imprinting in Primordial Germ Cell
  - Global Methylation differences between S-GCT and NS-GCT may offer clues to early stages of histogenesis of GCTx

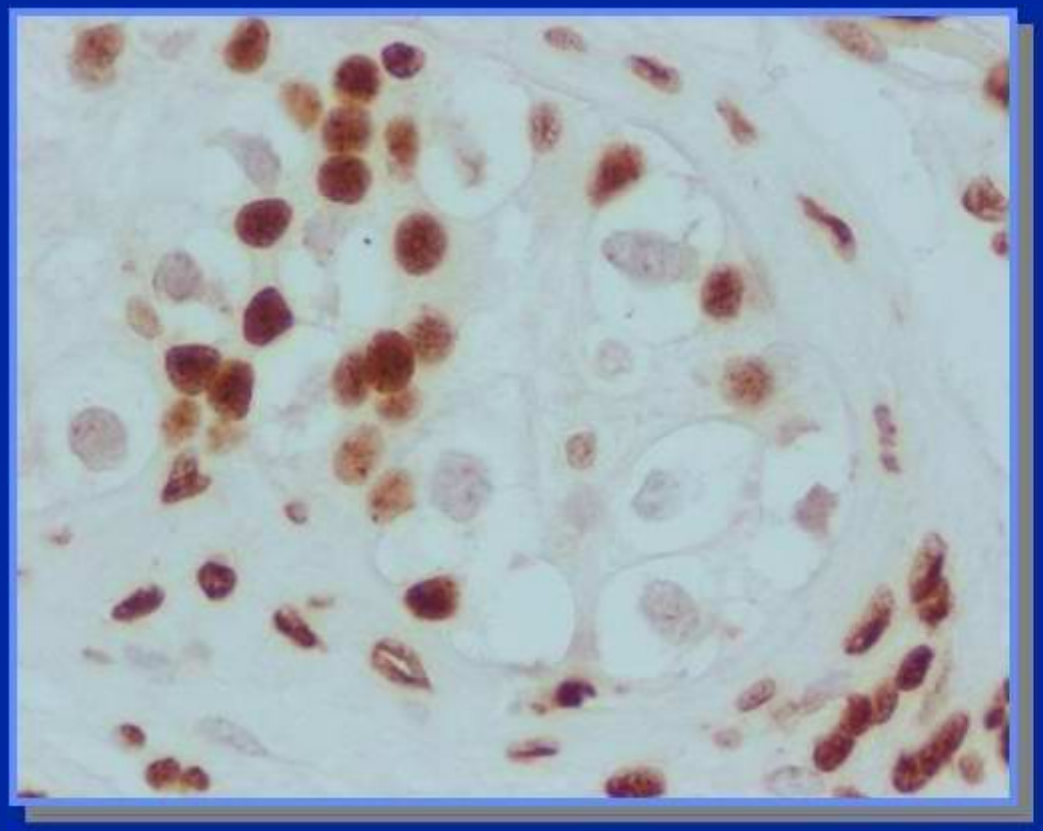

Seminoma
<3N+>
Distortion(Loss
or Relaxation)
of Imprinting
Aneuploidization
(Cell Fusion, Endo-
reduplication)
Invasive
Growth
Phase
Net Loss of DNA
Net loss of DNA
(-15,-22)
Switch-off: CKIT,
SCF,
NMYC,
PTHLH
Switch-on: HST1,
RB1,
CERB-B1/2
Increase: TP53
Histogenetic
Differentiation
Primitive
Germ Cell
(PGC)
(2N)
Initiated
PGC
(2N)
Promoted
PGC
(2N)
Carcinoma
In Situ
(CIS)
(3-4N)
+X,+7,+8,+12
-11,-13,-18,-Y
i12p multiplication
Genetically
Unstable
Net Loss of DNA
Initiation
Promotion
Progression
Nonseminoma
<3N->
Sandberg AA. et al. J Urology 1996


IN-UTERO
Birth
PREPUBERITY/ADULT
Spermatogonial Stem Cell
Gametes
PGC with Remethylation Marks
IGCNU
(Methylated)
NS-GCT
(Methylated)
5mC
PGC with Methylation Erasure
IGCNU
Sm-GCT
PGC
Mixed Model for GCT Development
Suggested by *Smiraglia et al.2002*

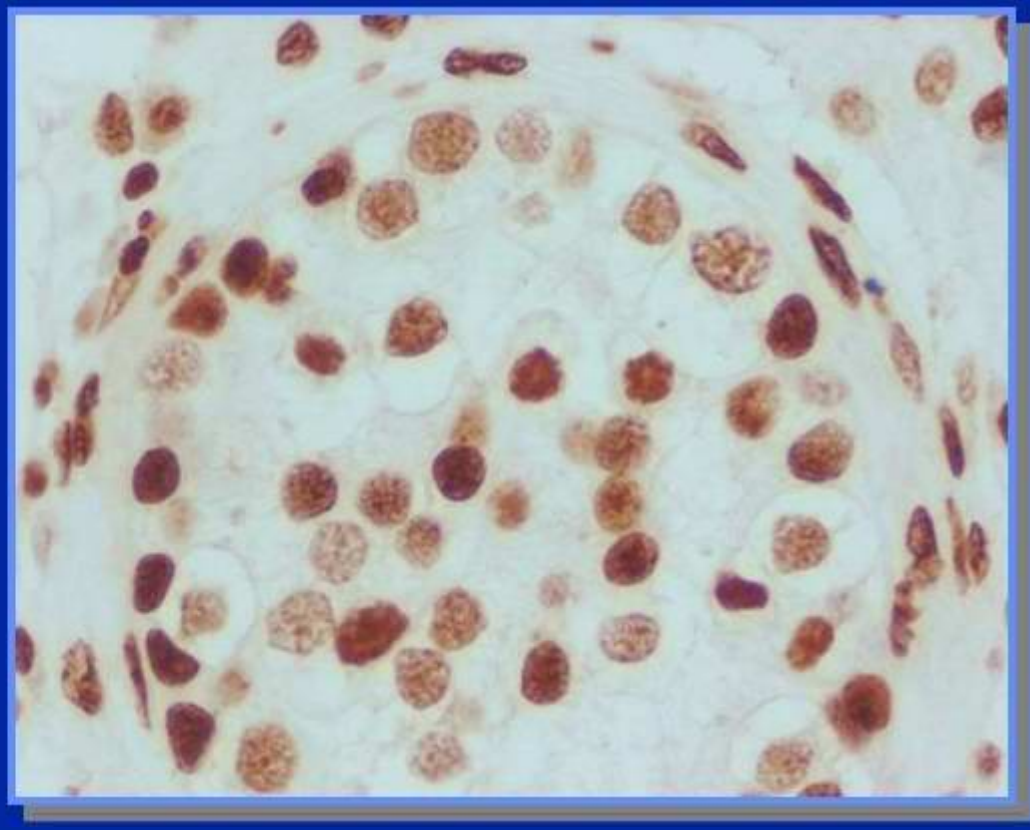

Emb Ca


Seminoma

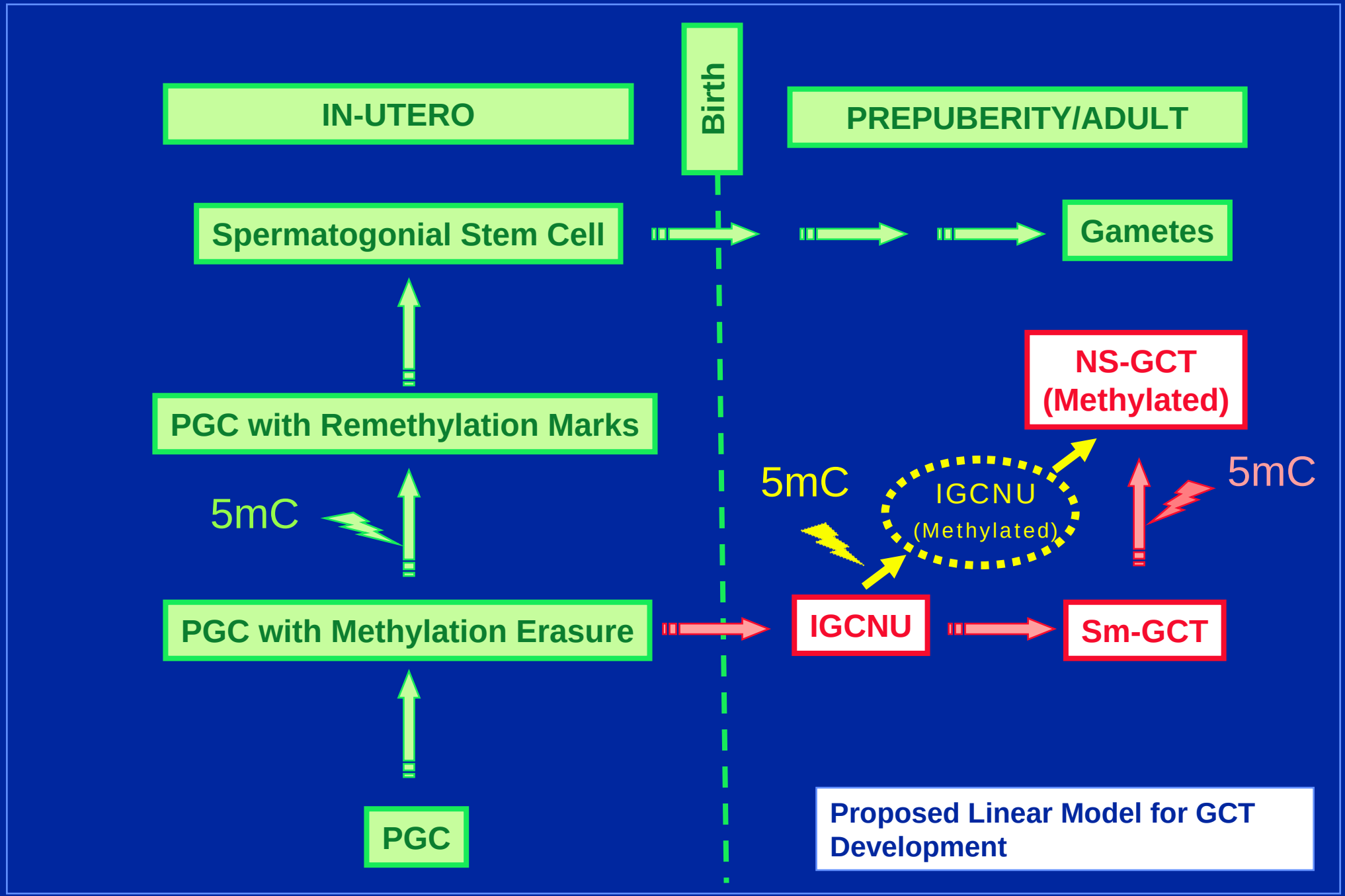

Mixed GCT



IGCNU



Normal


IN-UTERO
Birth
PREPUBERITY/ADULT
Spermatogonial Stem Cell
Gametes
NS-GCT
(Methylated)
PGC with Remethylation Marks
5mC
5mC
IGCNU
(Methylated)
5mC
PGC with Methylation Erasure
IGCNU
Sm-GCT
PGC
Proposed Linear Model for GCT Development

Testicular Germ Cell Neoplasms

Novel Diagnostic Markers

C-kit (CD117):

- Membranous positivity in IGCNU, Seminoma and Embryonal Carcinoma.
- Tyrosine Kinase (TK) receptor.
- Mutations in exons 17 and 11 ? Target of therapy for TKI in refractory seminoma

OCT4 (POU5F1; OCT3)

- Nuclear transcription factor expressed in pleuripotent embryonic and stem cells
- Nuclear staining.
- Positive in IGCNU, seminoma, embryonal carcinoma and germ cell component of gonadoblastoma.
- Greater sensitivity (100%) than c-kit and PLAP including extratesticular seminomas.
- Greatly specific: only 3/3439 TMA “somatic” carcinoma cases were found to be positive (Clear cell RCC, NSCLC)

Testicular Germ Cell Neoplasms

Novel Markers

Podoplanin

- MAB: D2 -40 and M2 A
- Transmembrane mucoprotein.
- Membranous staining.
- Excellent sensitivity for IGCNU and seminoma (diffuse staining in metastatic/extratesticular seminoma)
- Positive in non seminomatous GCT (lower sensitivity)
- Also labels lymphatic endothelium, vascular neoplasms, epithelioid mesothelioma.

Activator protein - 2γ (Ap-2γ)

- Nuclear transcription factor involved in embryonic morphogenesis
- Functionally related to c-kit and PLAP expression.
- Nuclear staining.
- Strong sensitivity for IGCNU, seminoma
- Positive in non seminomatous GCT (lower sensitivity)
- Also expressed in somatic neoplasms: melanoma , breast and ovarian carcinoma.

MARKER	IGCNU	CLASSIC SEMINOMA	SPERMATOCYTIC SEMINOMA	EMBRYONAL CARCINOMA	YST
C-kit (CD117)	+	+	+/-	+/-	+/-
OCT3/4	+	+	-	+	-
PLAP	+	+	+/-	+	+
AE1/AE3	-	-	-	+	+
CD30	-	-	-	+	+/-
AFP	-	-	-	+/-	+

Modified from Ulbright TM. Mod pathol 2005

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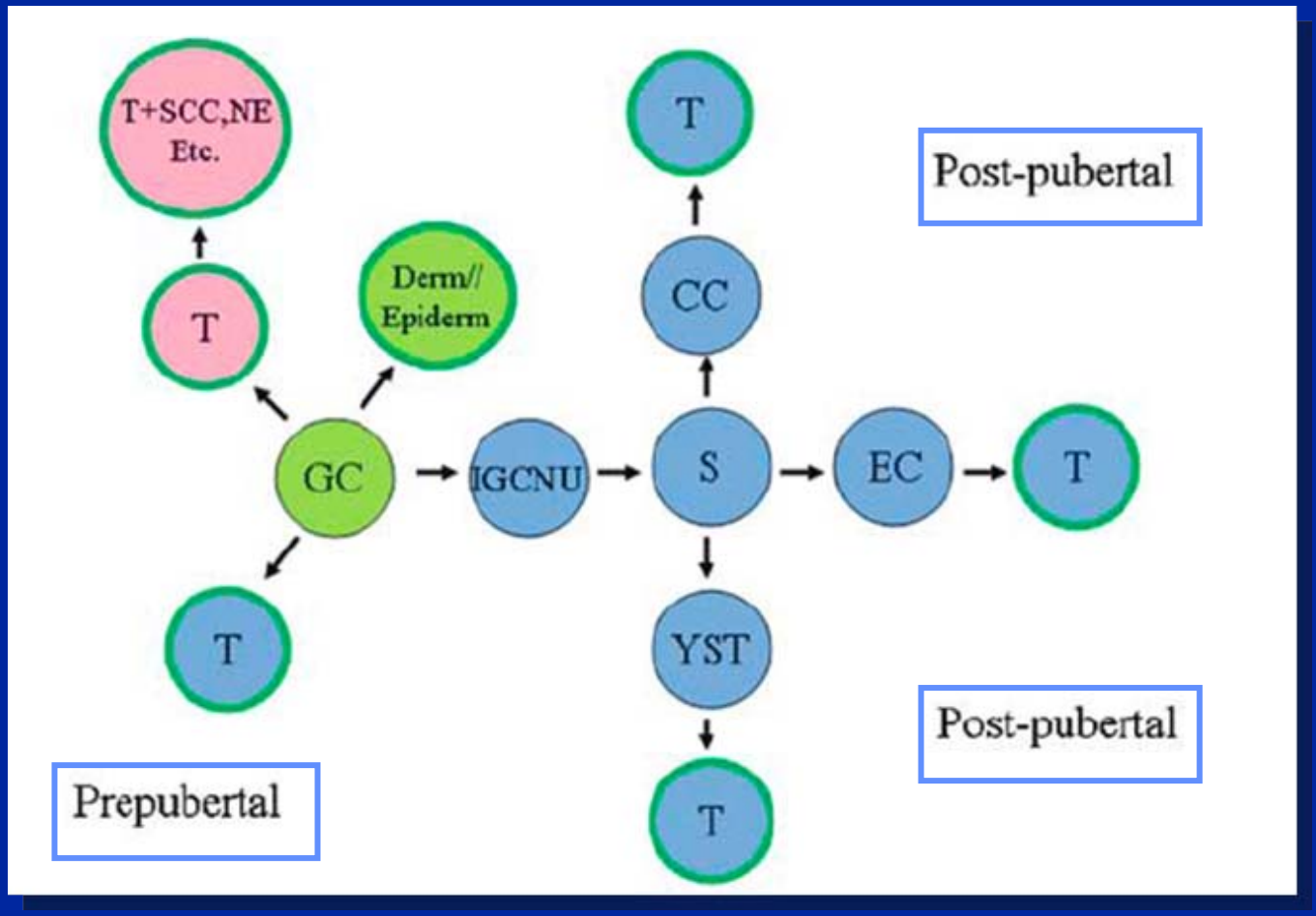
Gonadal Germ Cell Neoplasms

Tesicular Vs Ovarian



Ulbright TM. Mod pathol 2005

Gonadal Germ Cell Neoplasms

Testicular Vs Ovarian

- **Incidence/Histologic type:**
  - GCT account for 98% of testicular but only **1% of all ovarian tumors**.
  - Majority of male GCT are seminomas and mixed Sem & NS-GCT
  - Majority of ovarian GCT are mature **teratomas/dermoid cyst** (very rare in male)
  - Mixed GCT: 1/3 of testicular but only 1% of ovarian GCT

- **Pure teratomas** form 95% of ovarian but only 5% of testicular GCT but are a common component (50%) of mixed testicular GCT
- **Spontaneous regression** is limited to testicular GCT.
- **Spermatocytic seminoma** lacks an ovarian counterpart.

Prepubertal Testicular GCT

Pure YST:

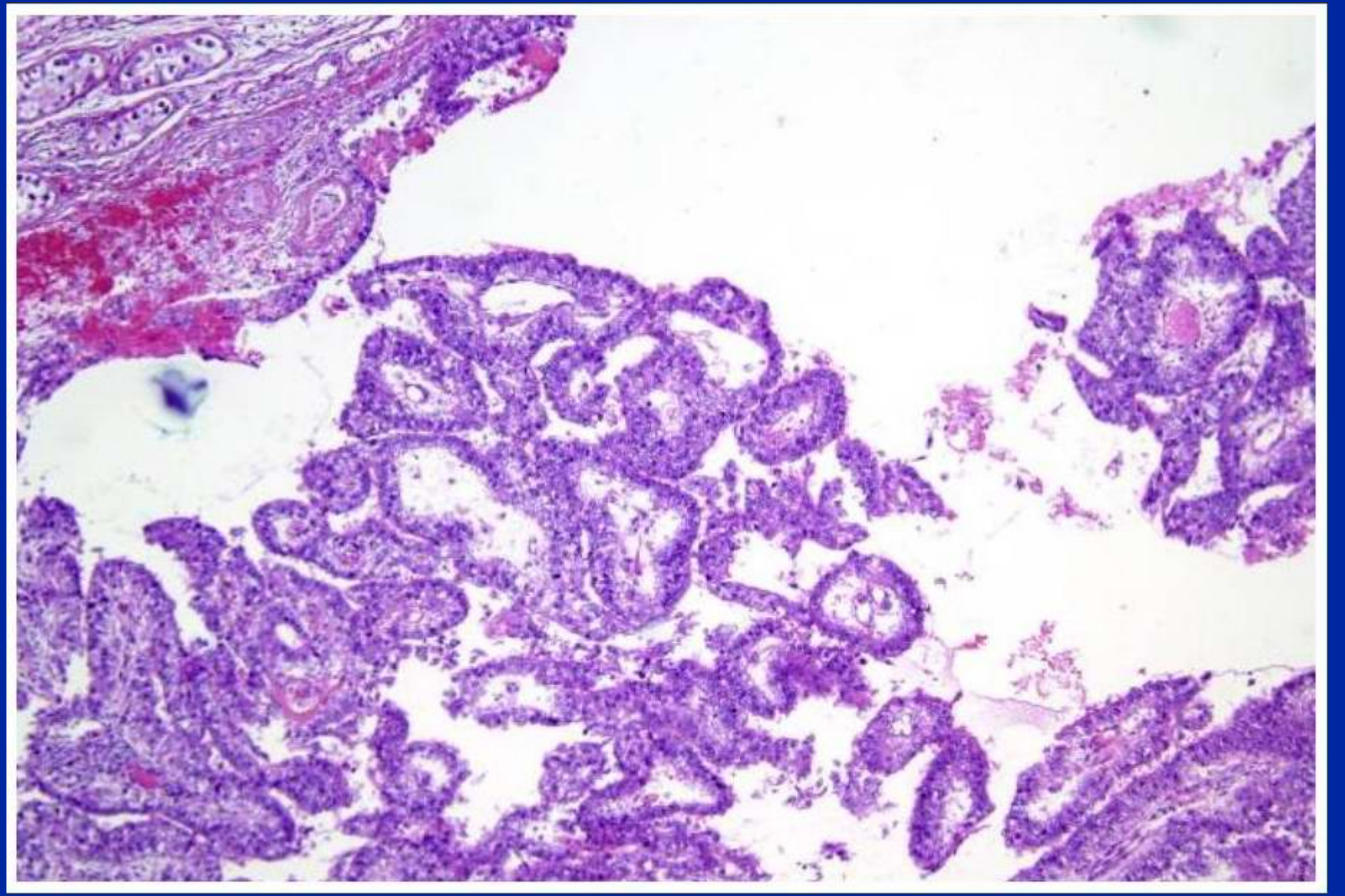
- Most common GCT in children.
- Median age 16 months of age
- 20% present with metastasis (lung and RPLN)
- Age not a factor in prognosis.
- PGX: Stage , AFP levels and Vascular invasion

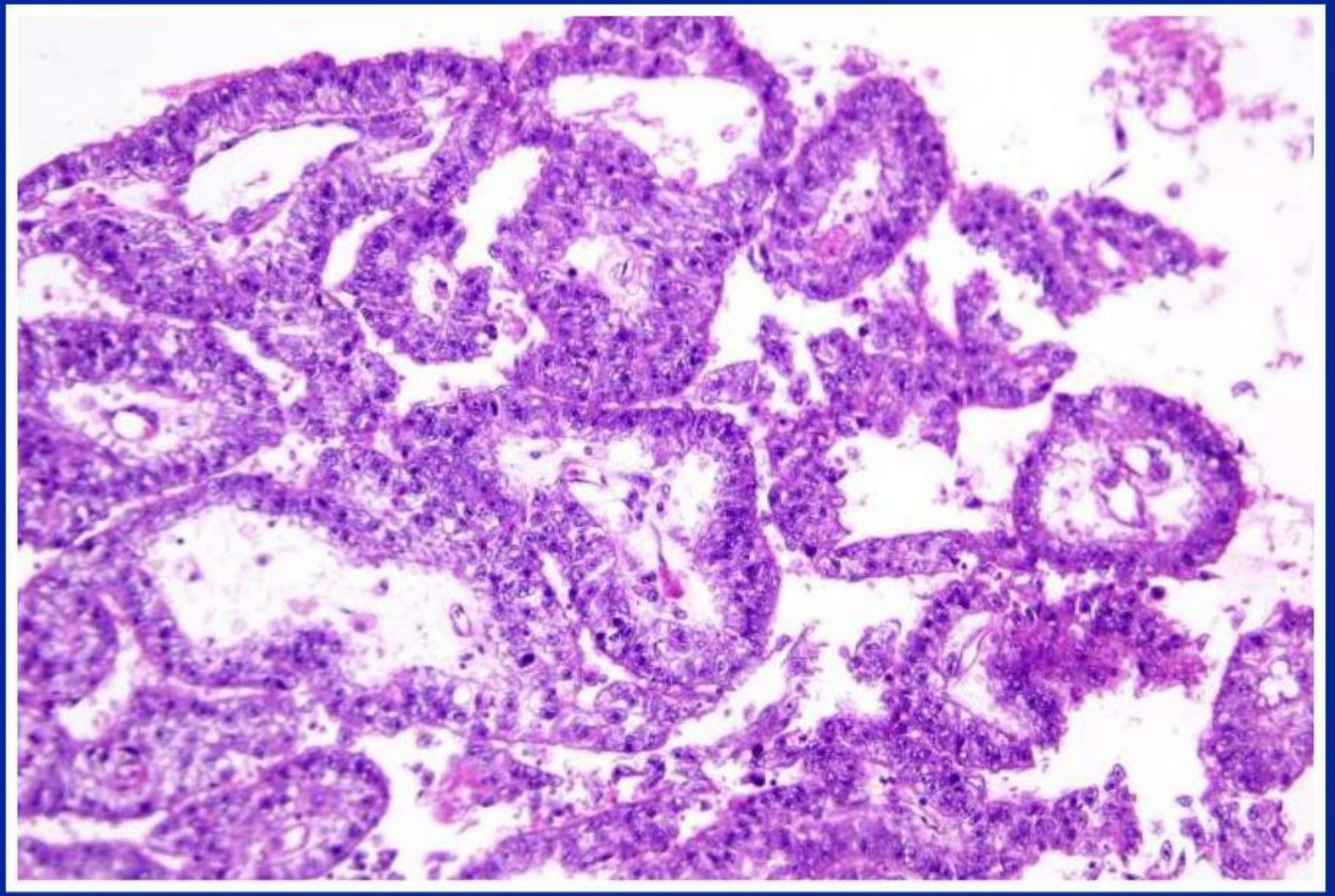
Pure Teratoma

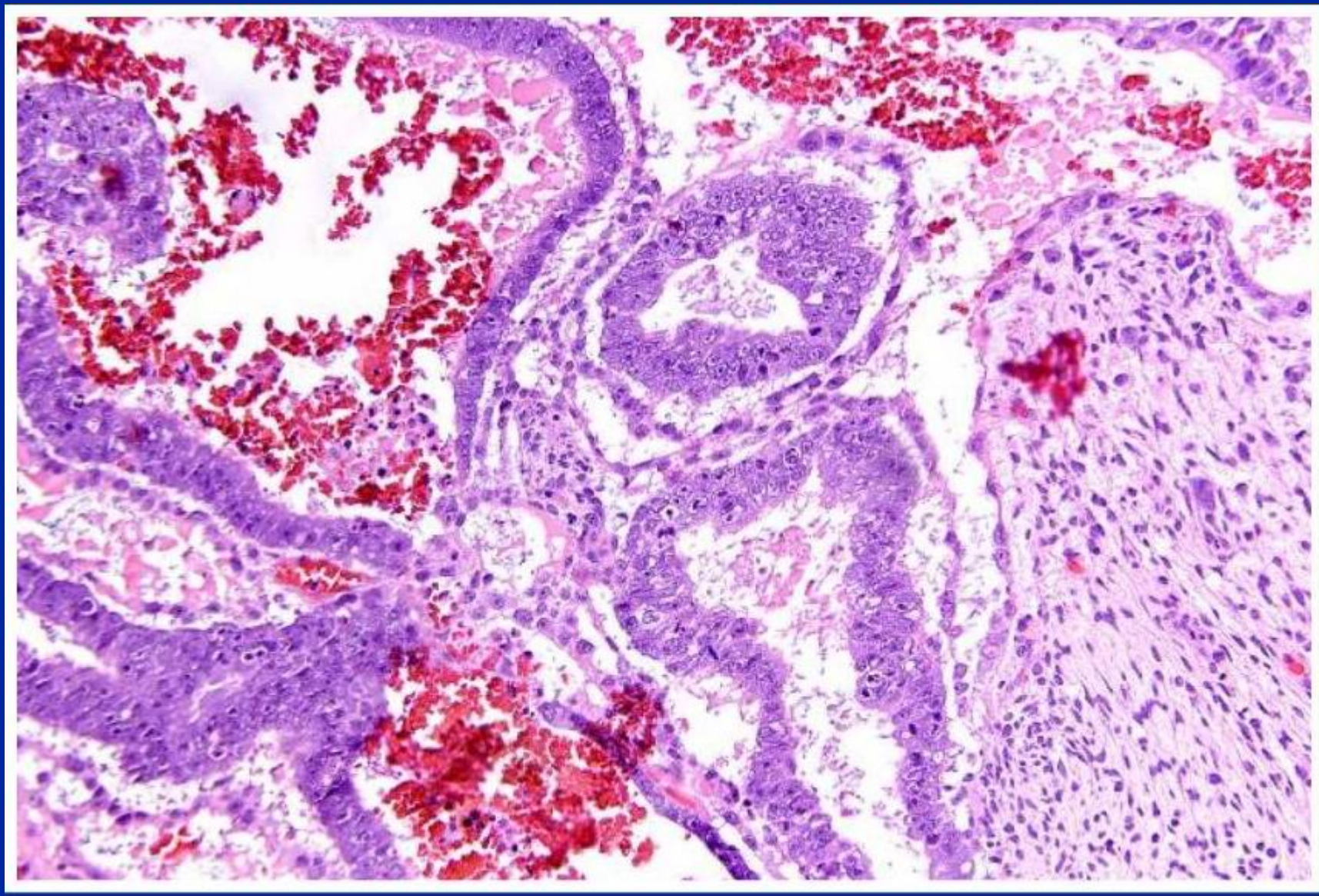
- Prepubertal testicular teratomas are benign
- Thought to originate from benign GC (diploid)
- 36% of GCT in children
- 2/3 occur in 1-2 year old (some peri-natal)

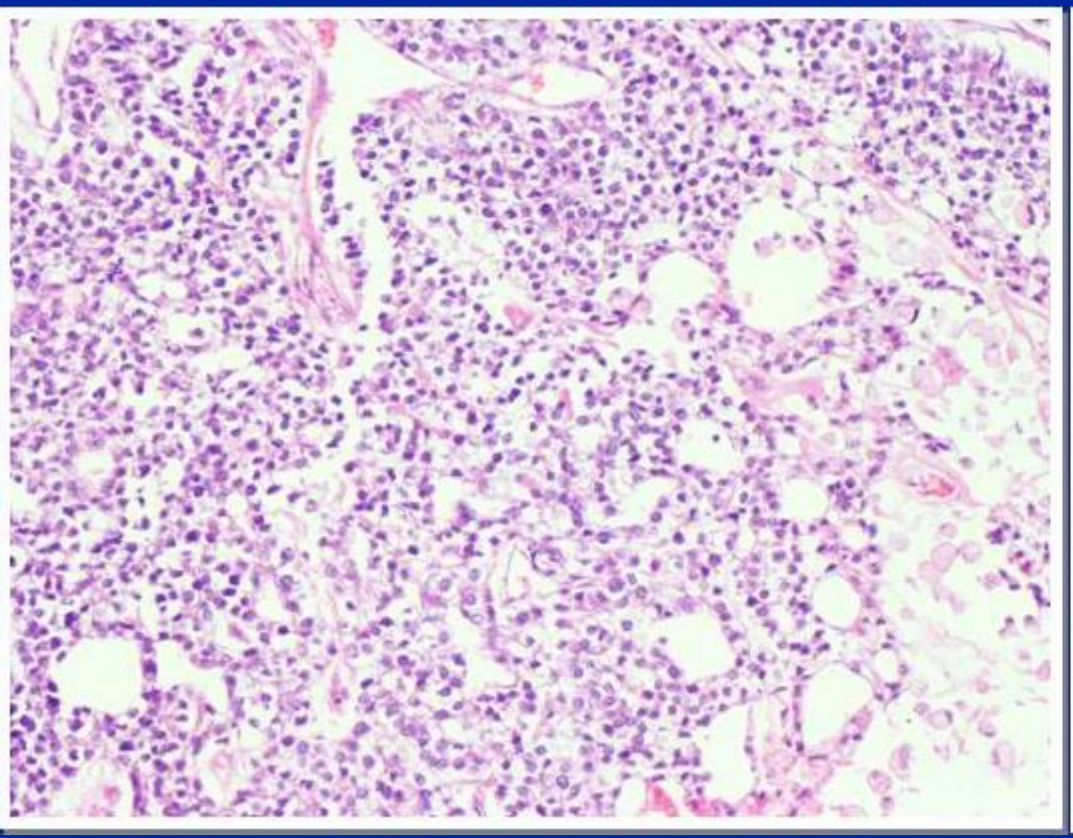
Other Types

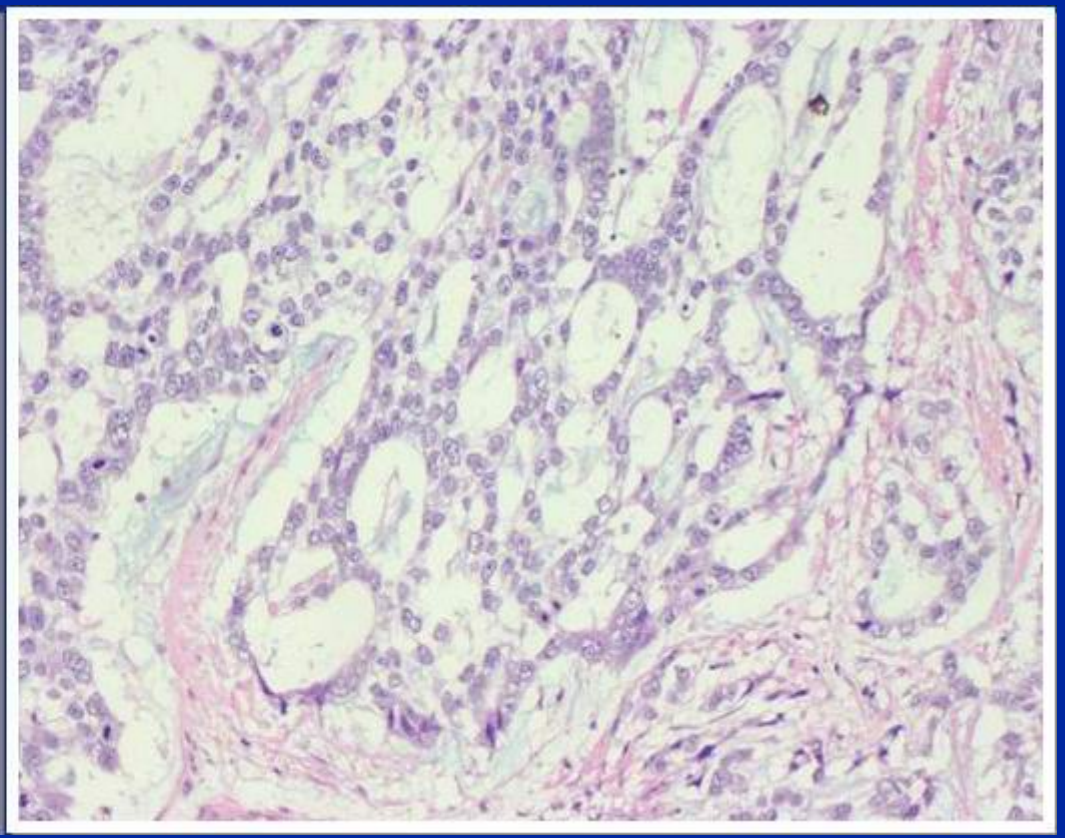
- Mixed GCT Rare in prepuberatal pts.
- Pure Choricarcinoma has poor PGX (12% survival)

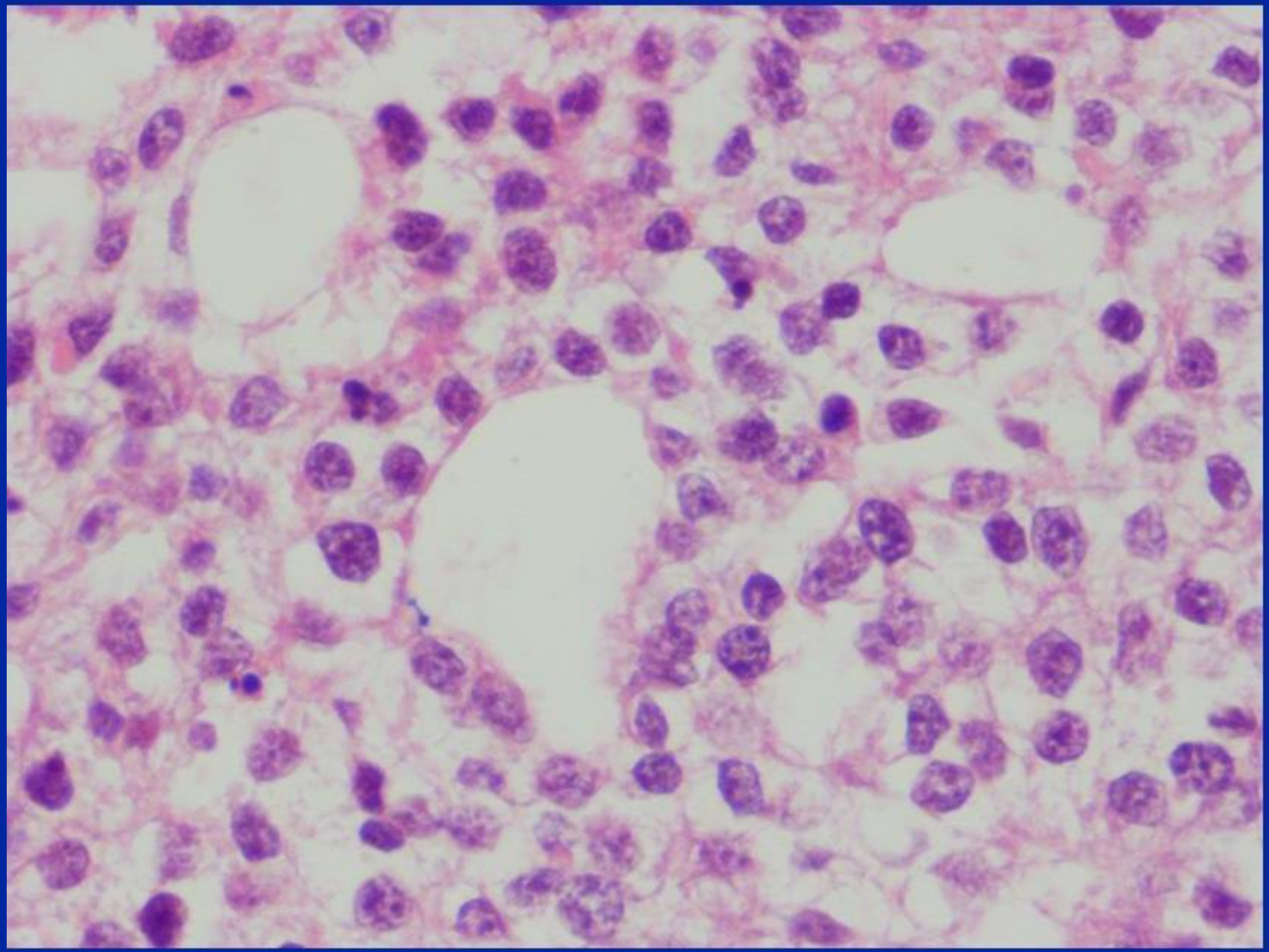












Adult Testicular Teratoma

- **All postpubertal testicular teratomas in adult males are malignant.**
- **No prognostic difference between mature or immature elements.**
- **Both elements originate from other NS- GCT.**
- **Preteratomatous malignant transformation: Clonal relation to NS-GCT elements.**
- **Metastasis may differ in histologic type from their testicular primary.**

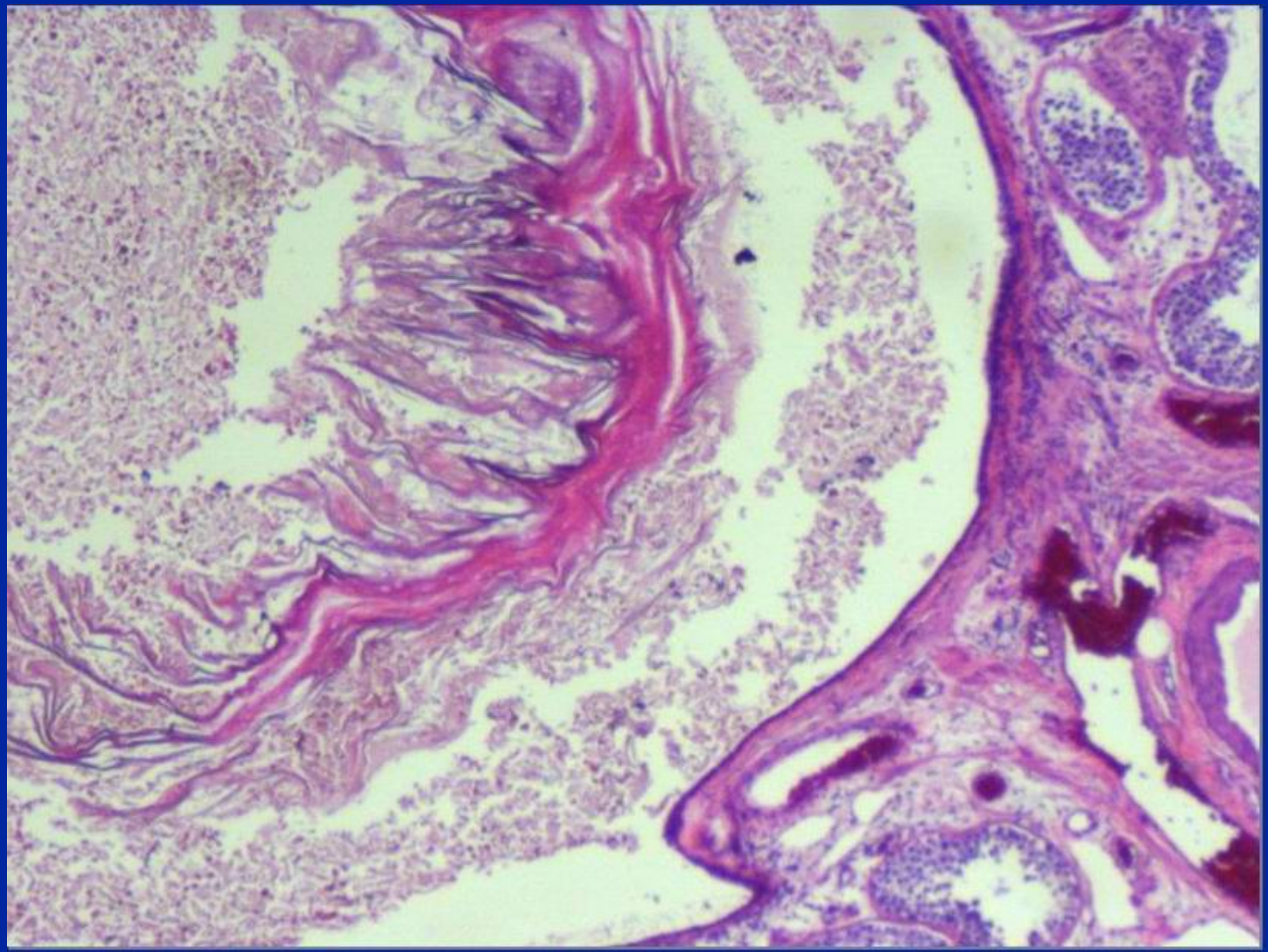
- **ONLY In post Rx RPLND, characterizing mature /immature elements is required.**

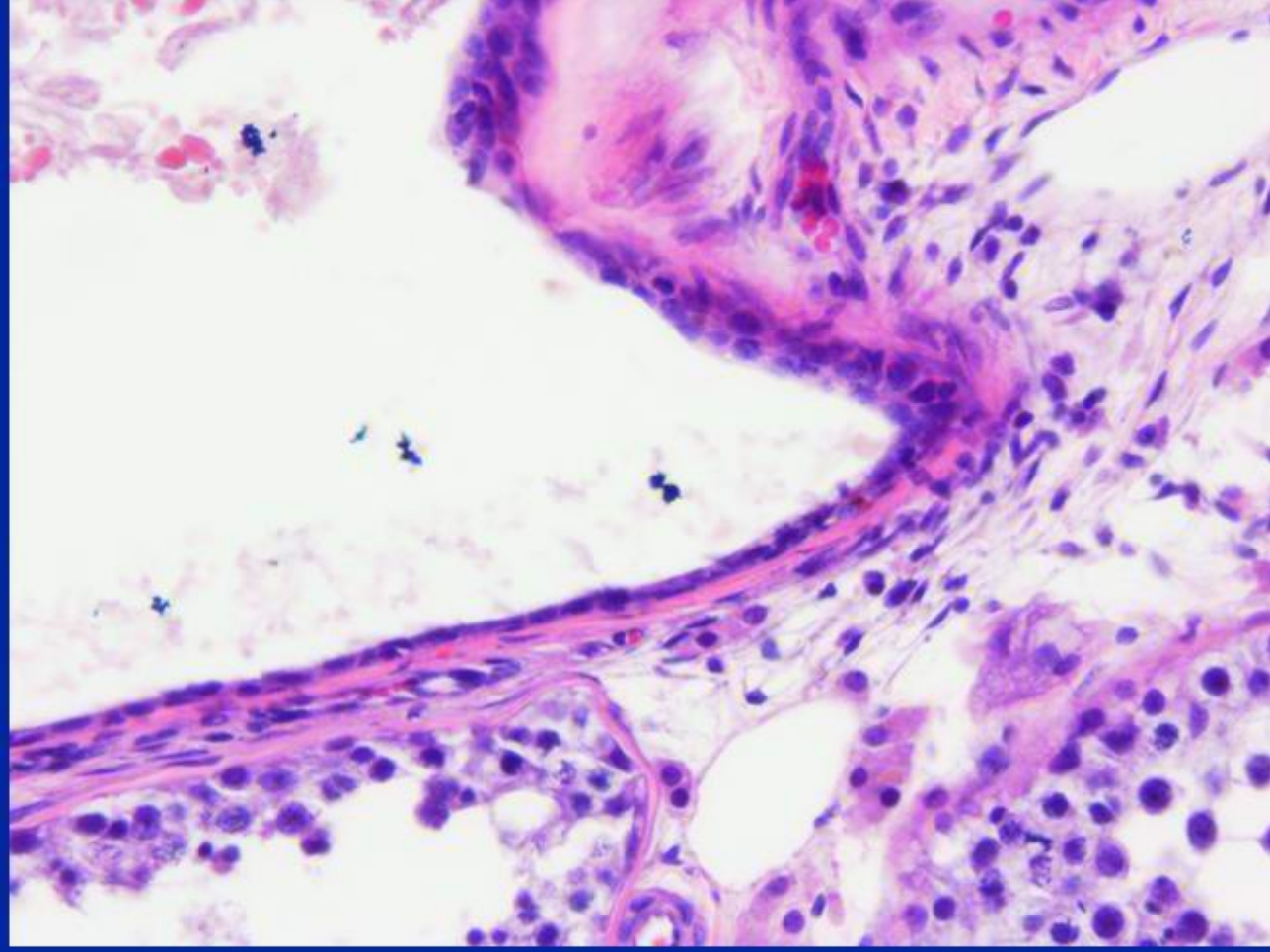
Adult Testicular Teratoma

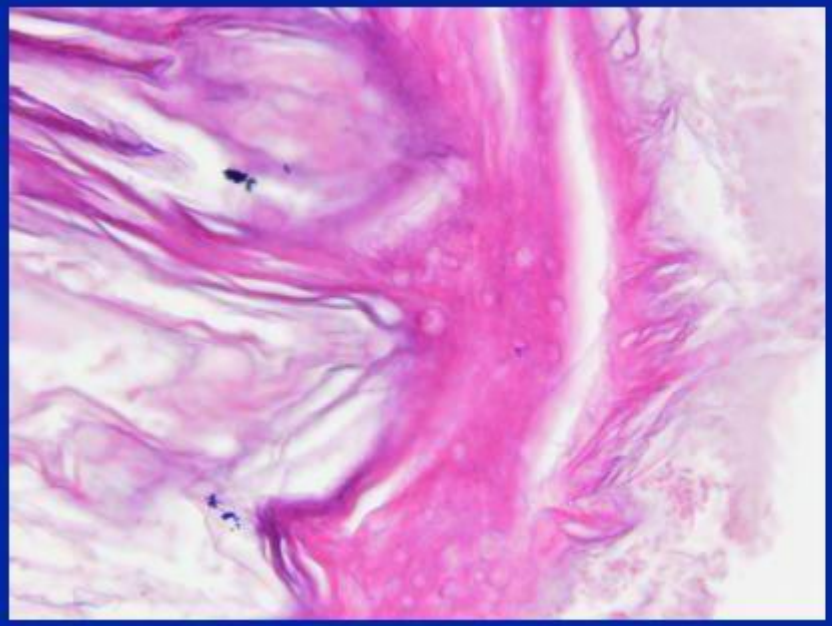
Teratoma with Somatic-Type Malignancy/Teratoma with Malignant Transformation

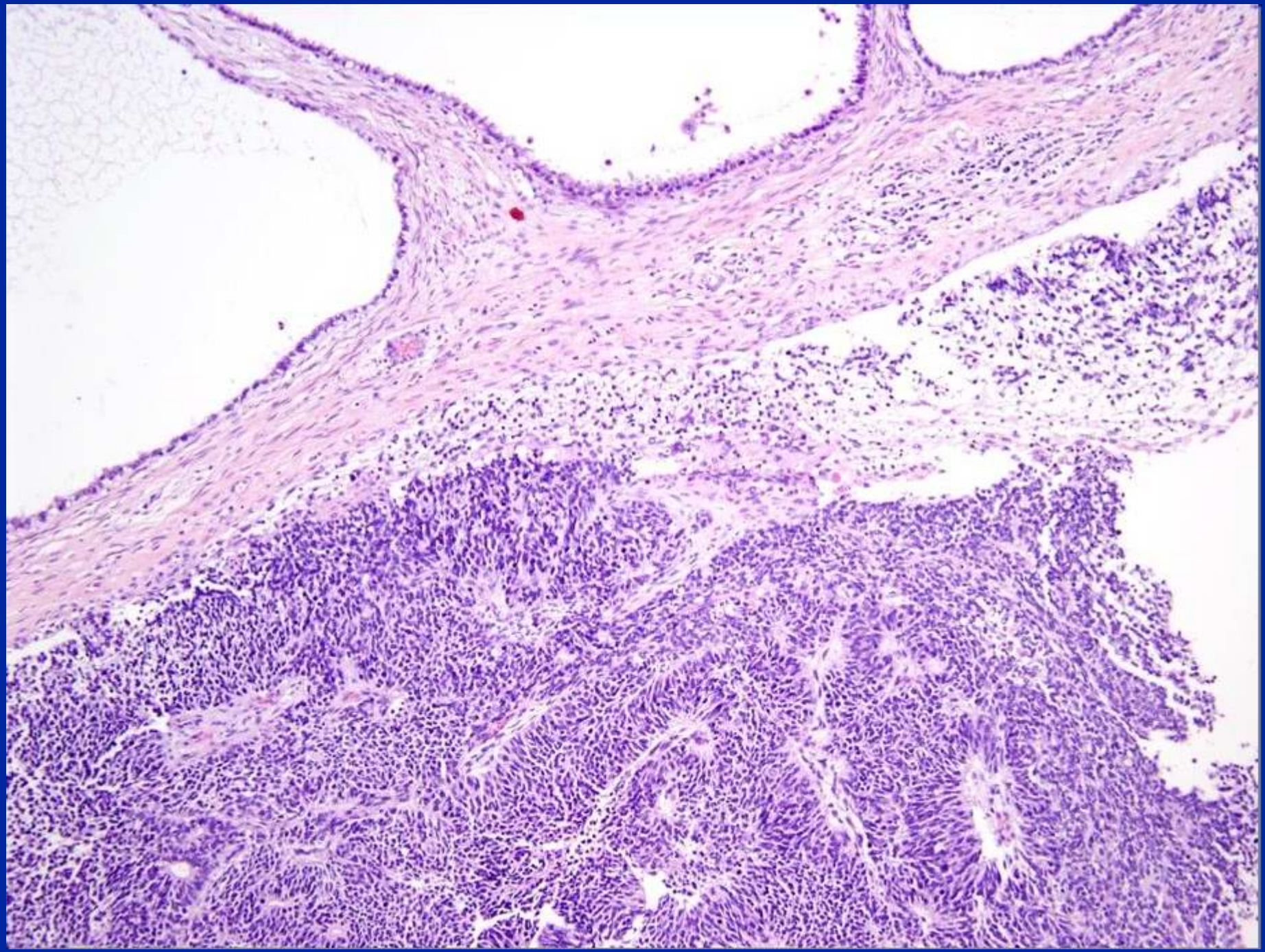
- Can occur in testicular or metastatic site.
- Expansile/overgrowth (4x field rule)
- 1/2 are undifferentiated sarcoma
- Rhabdosarcoma, leiomyosarcoma and others.
- PNET
- WT (very rarely)
- Adenocarcinoma, SCCa.

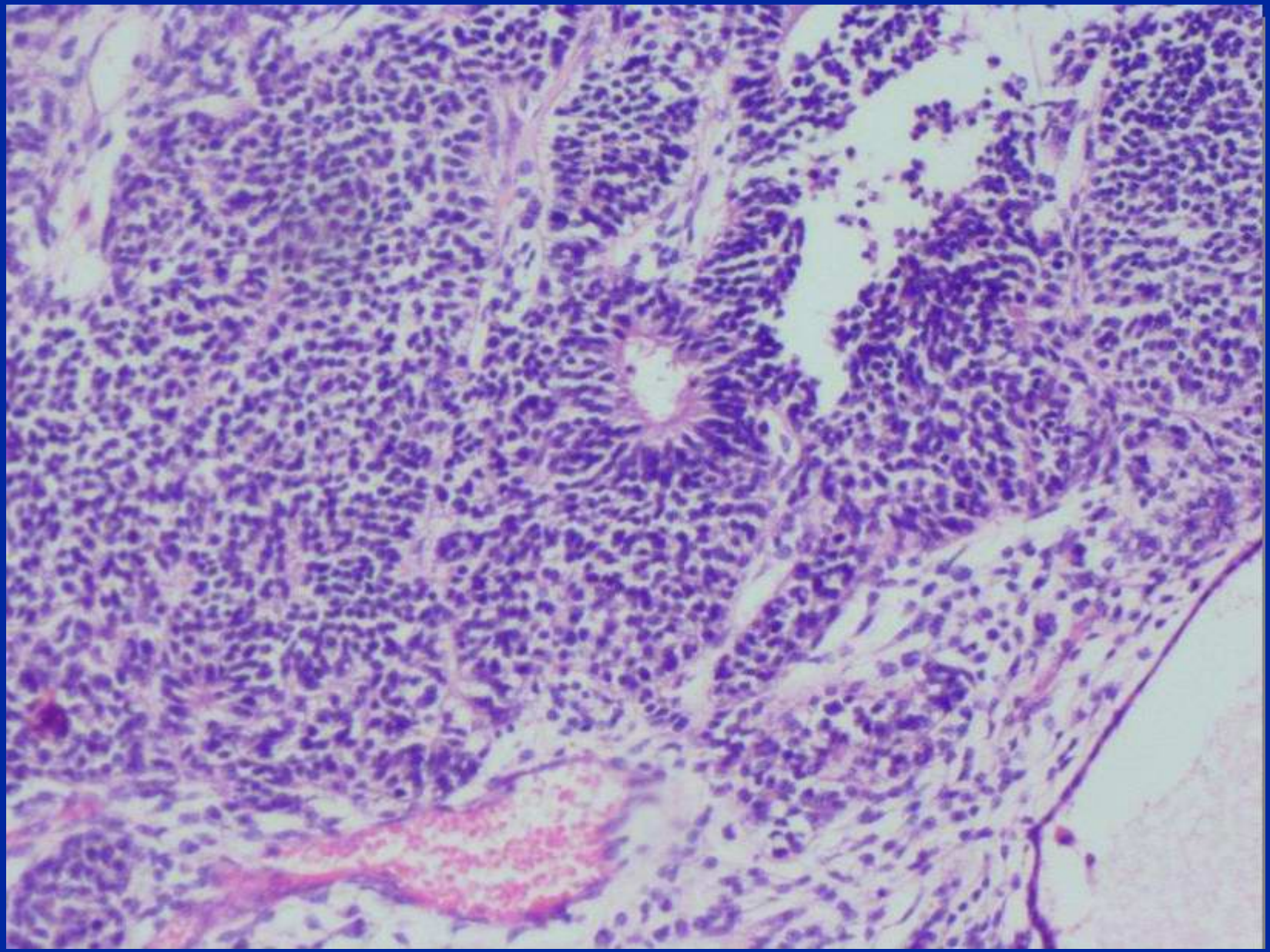
- Some share ip12 with GCT origin others show DZ specific genetic change (11;22 etc…)
- PGX: poor in metastatic site, ? not affected in testicular primary
- Rx: Do not respond to GCT Rx. Surgery and corresponding type specific Rx

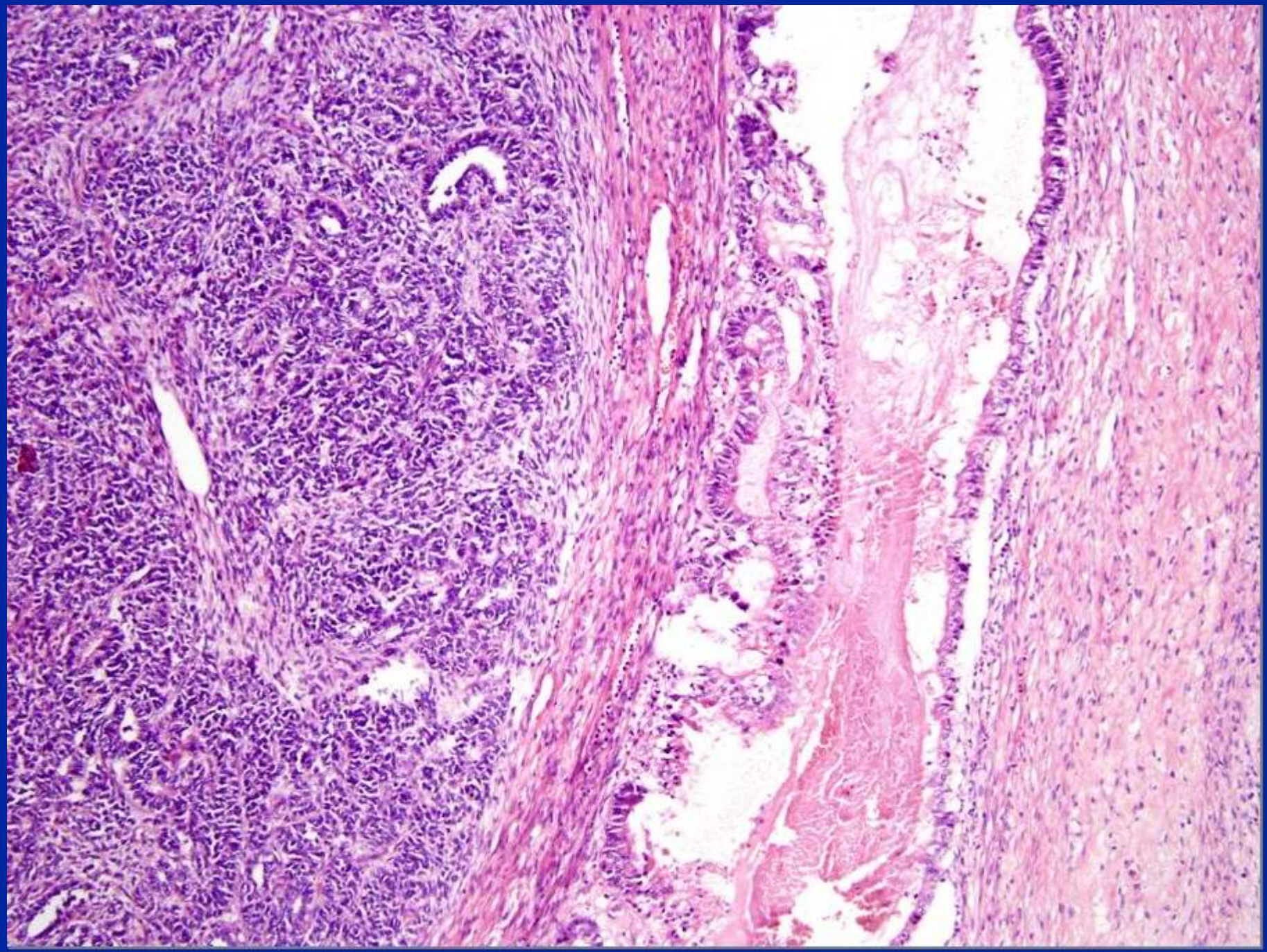


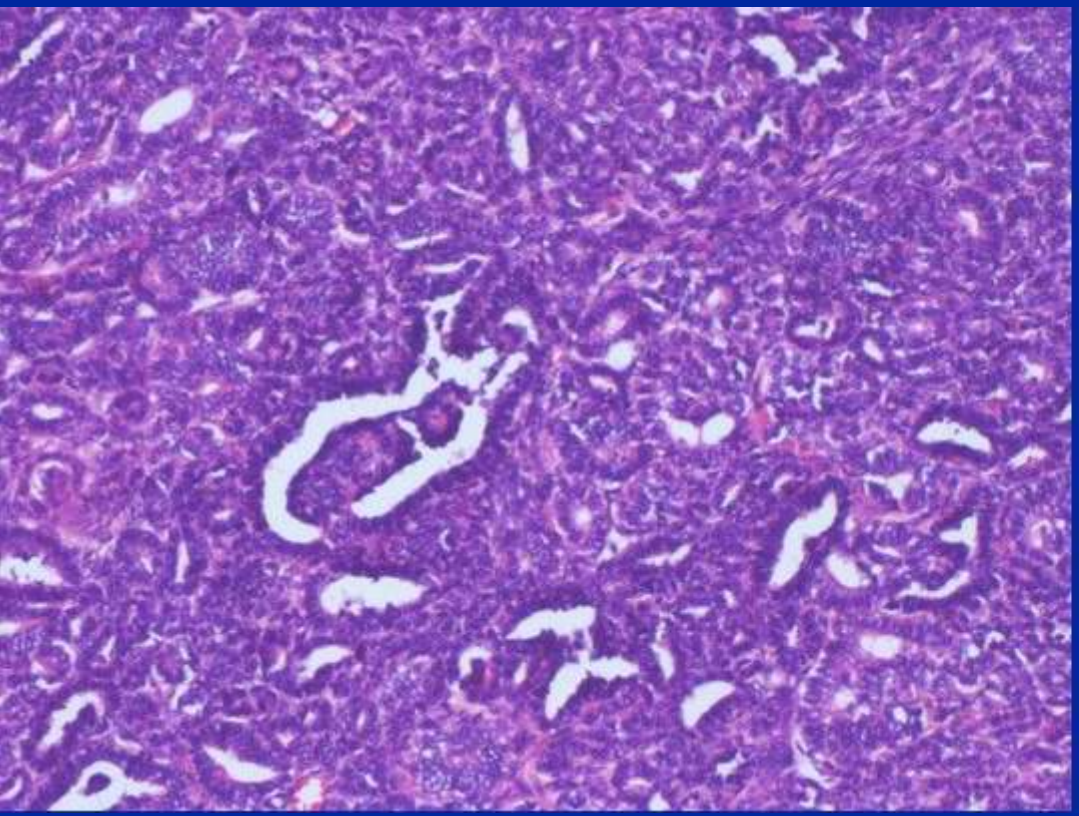


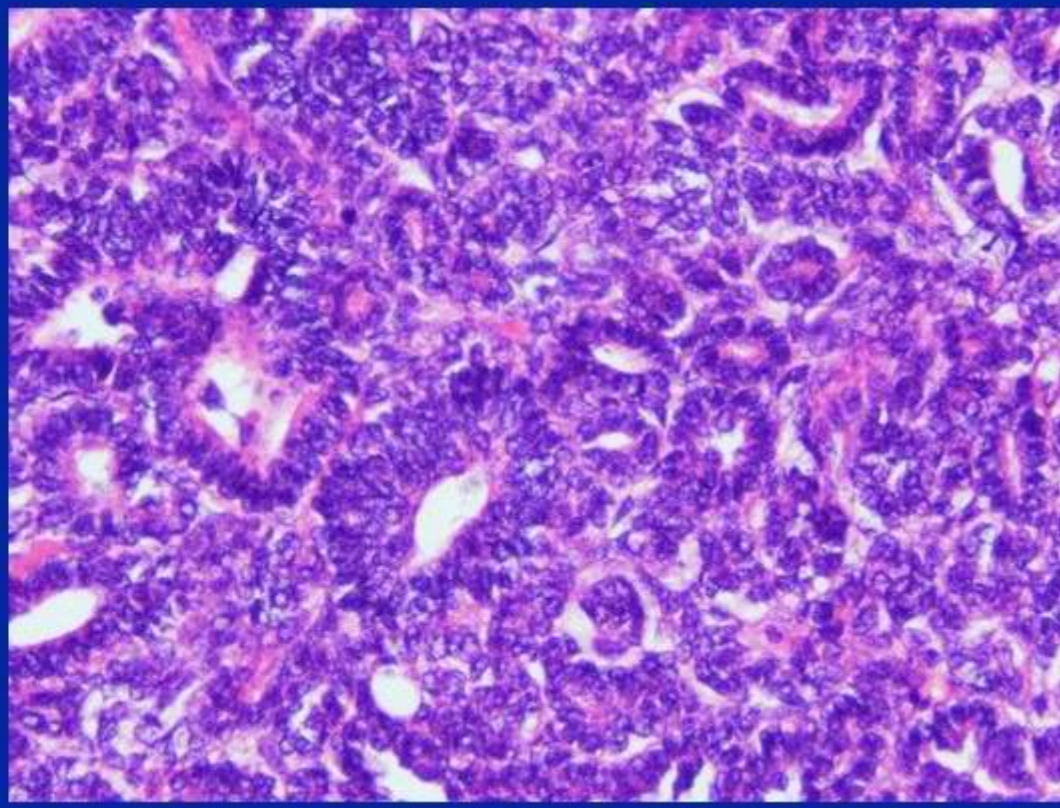


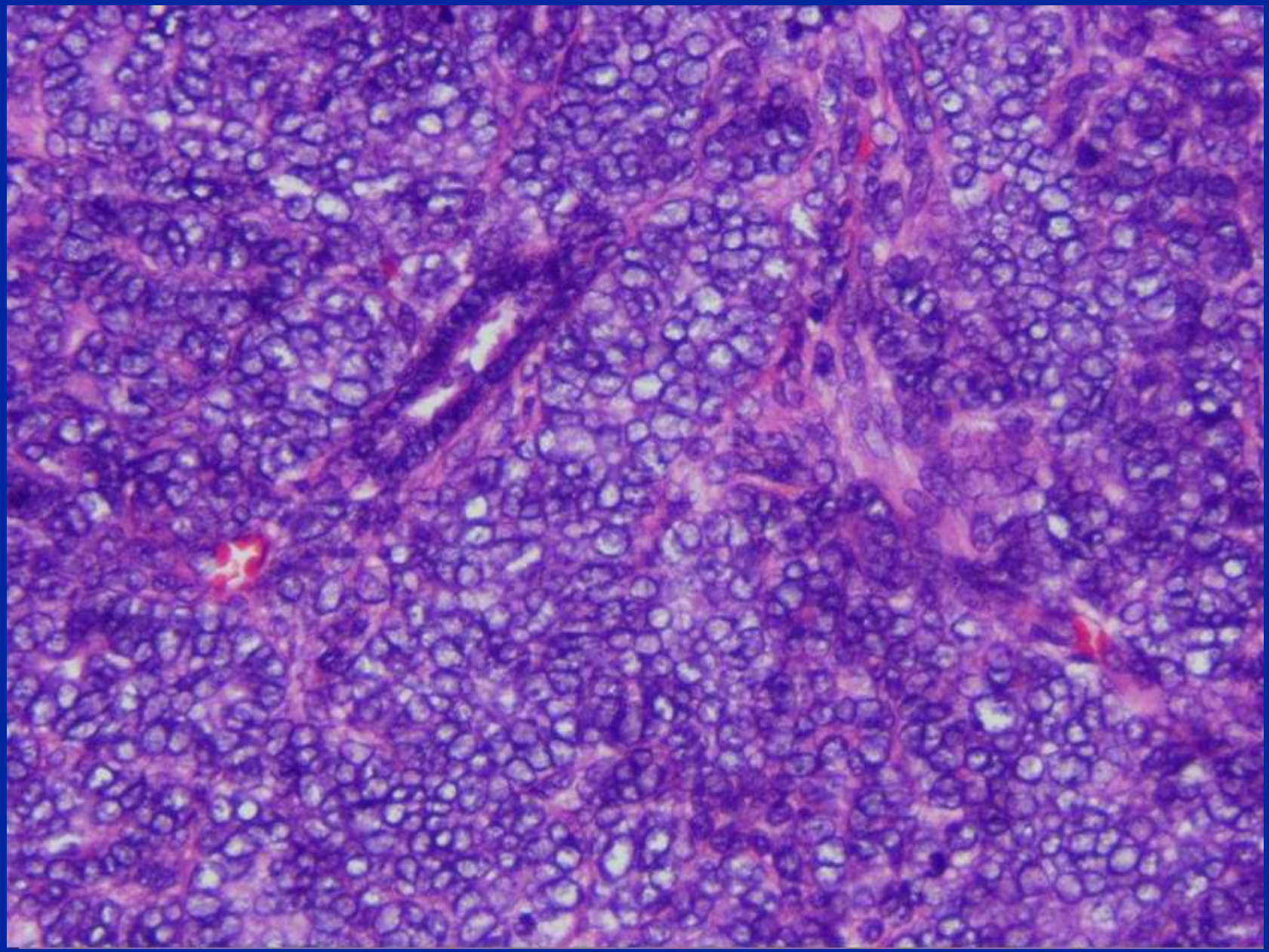


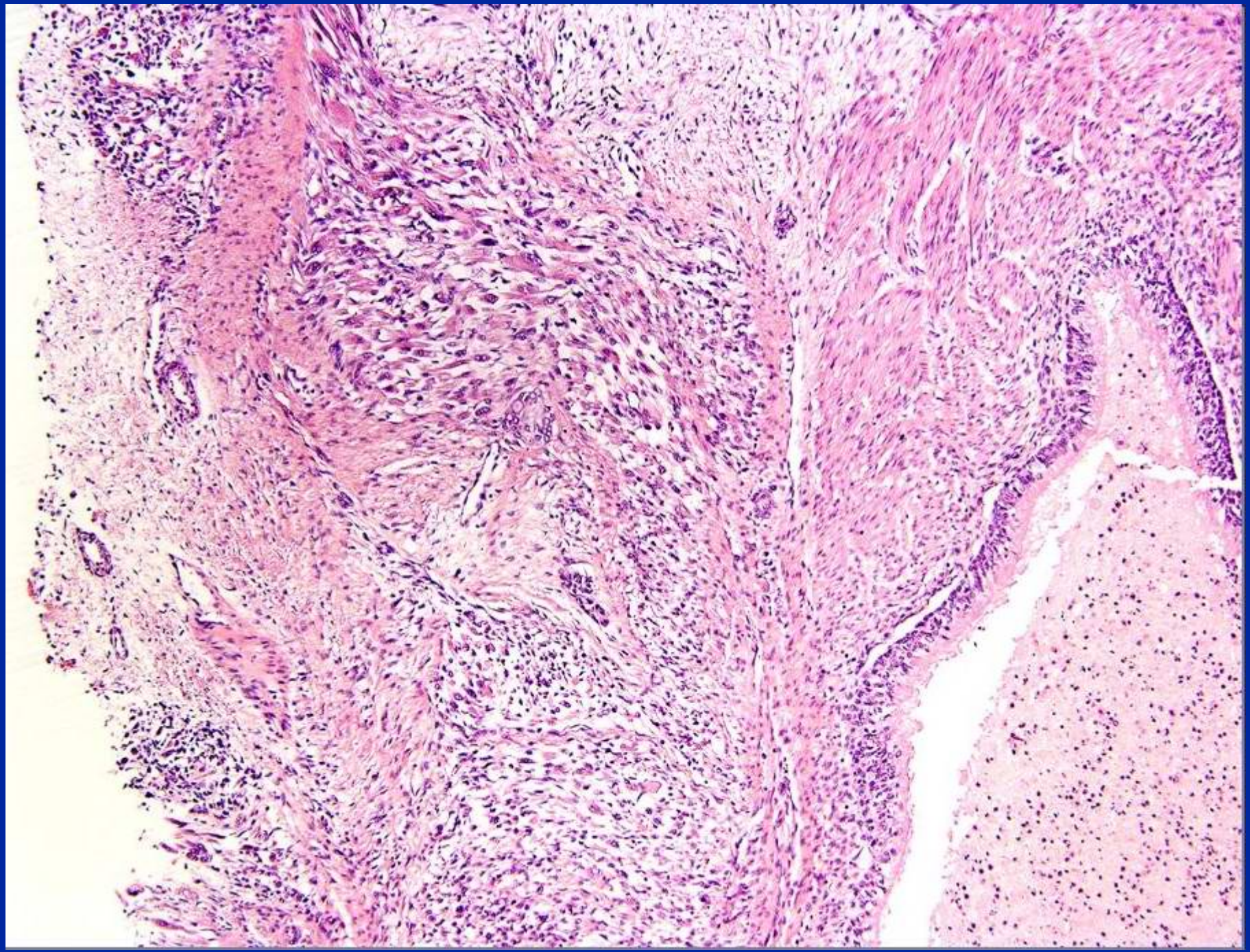


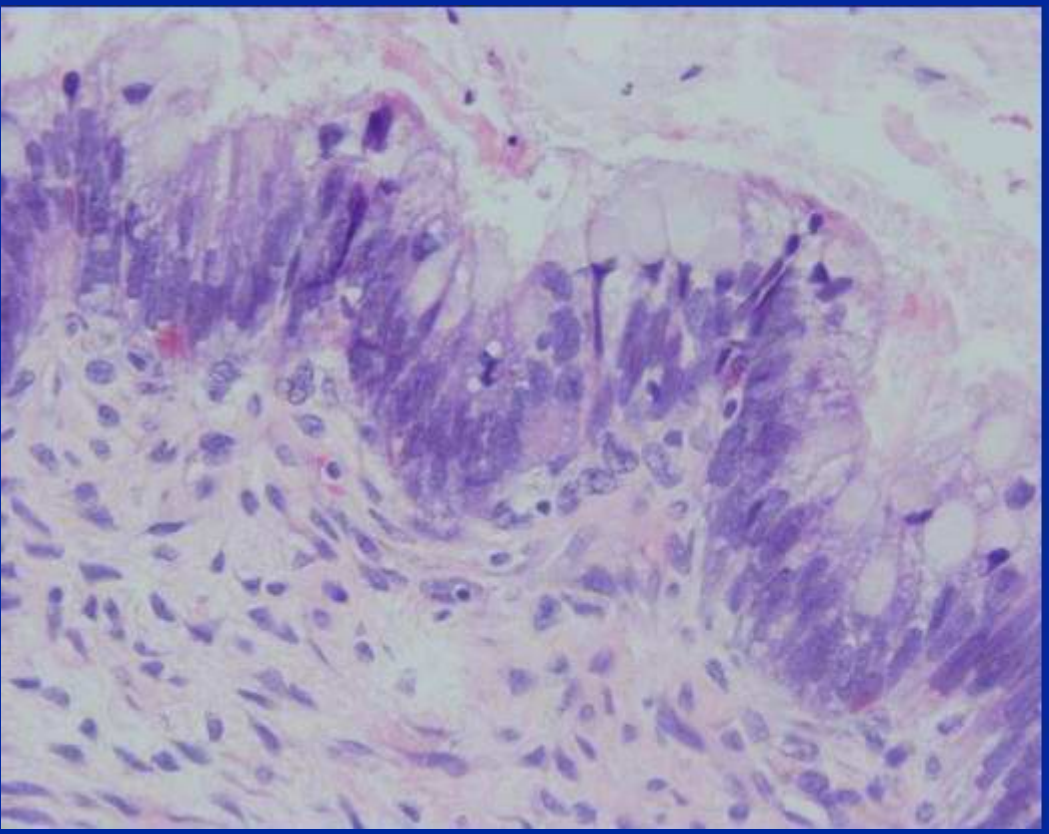


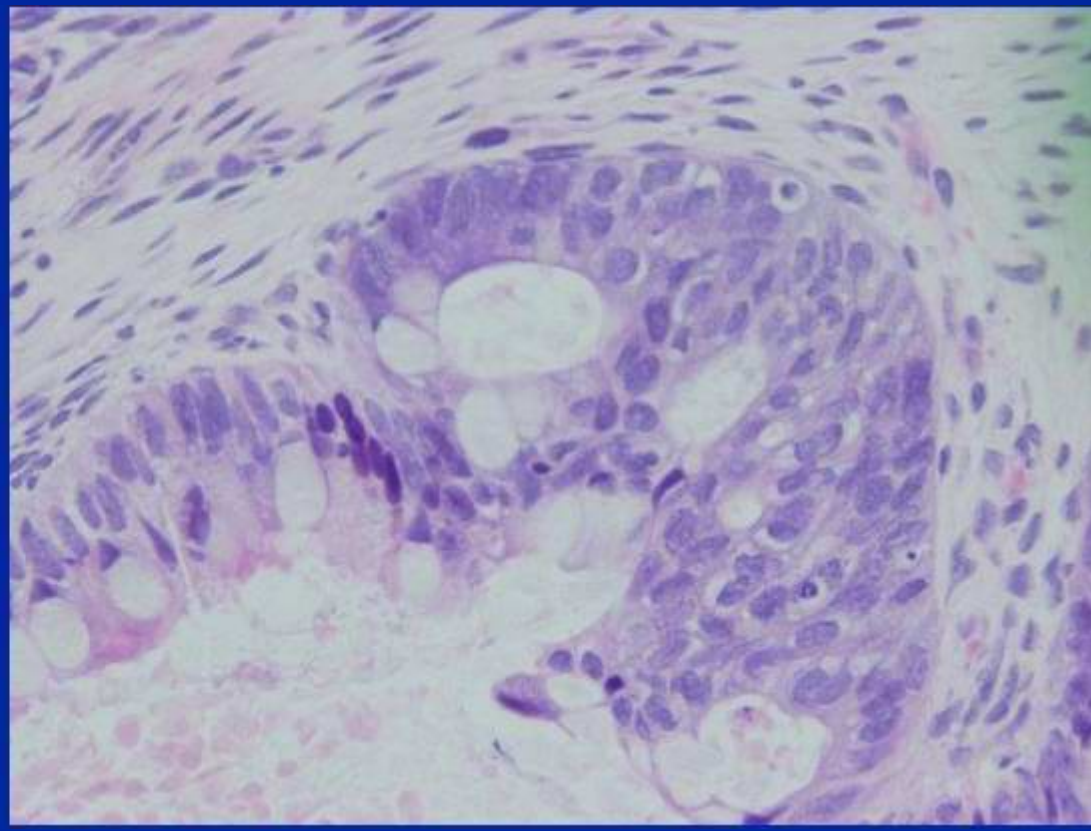


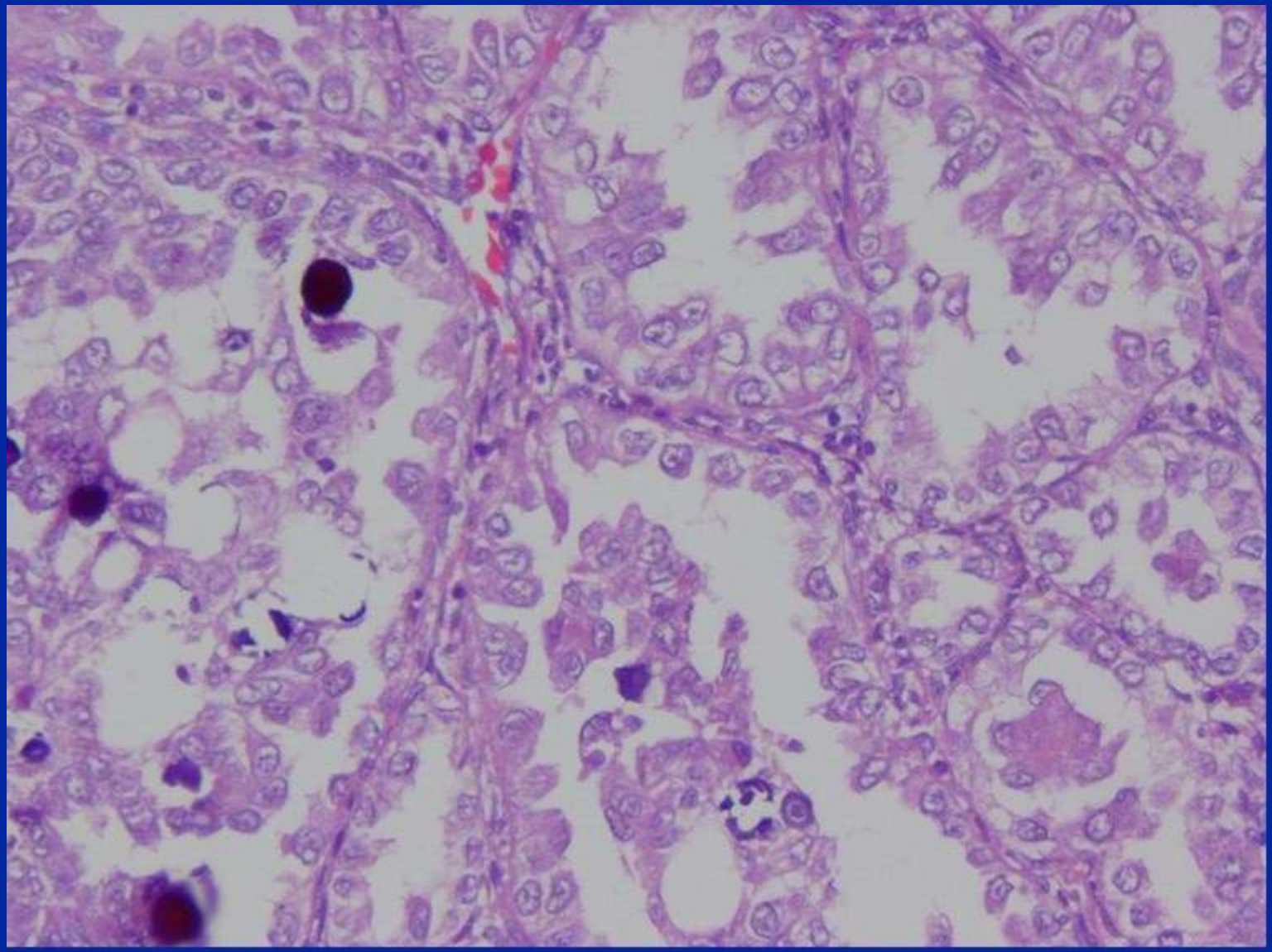












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- Gender & Age Factors in Gonadal GCT
- **IGCNU**
- Diagnostically “Problematic” Germ Cell Tumors (GCT)

Intratubular Germ Cell Neoplasm Unclassified
IGCNU

- **Incidence:**
  - 1% incidence in testicular biopsies taken as part of Infertility W/U
  - Cryptorchid testis (0.4-2%)
  - up to 25% of testis in Gonadal Dysgenesis pts
  - IGCNU present in association with the majority (80%) of invasive testicular GCT cases of adults
  - GCT pts: 5% IGCNU in contralateral testicular biopsy

- **Significance:**
  IGCNU risk of progression to invasive GCT: 50% in 5 years (infertilty and contralateral testis data) ⇨
  ???Management: XRT, organ preserving resection, surveillance

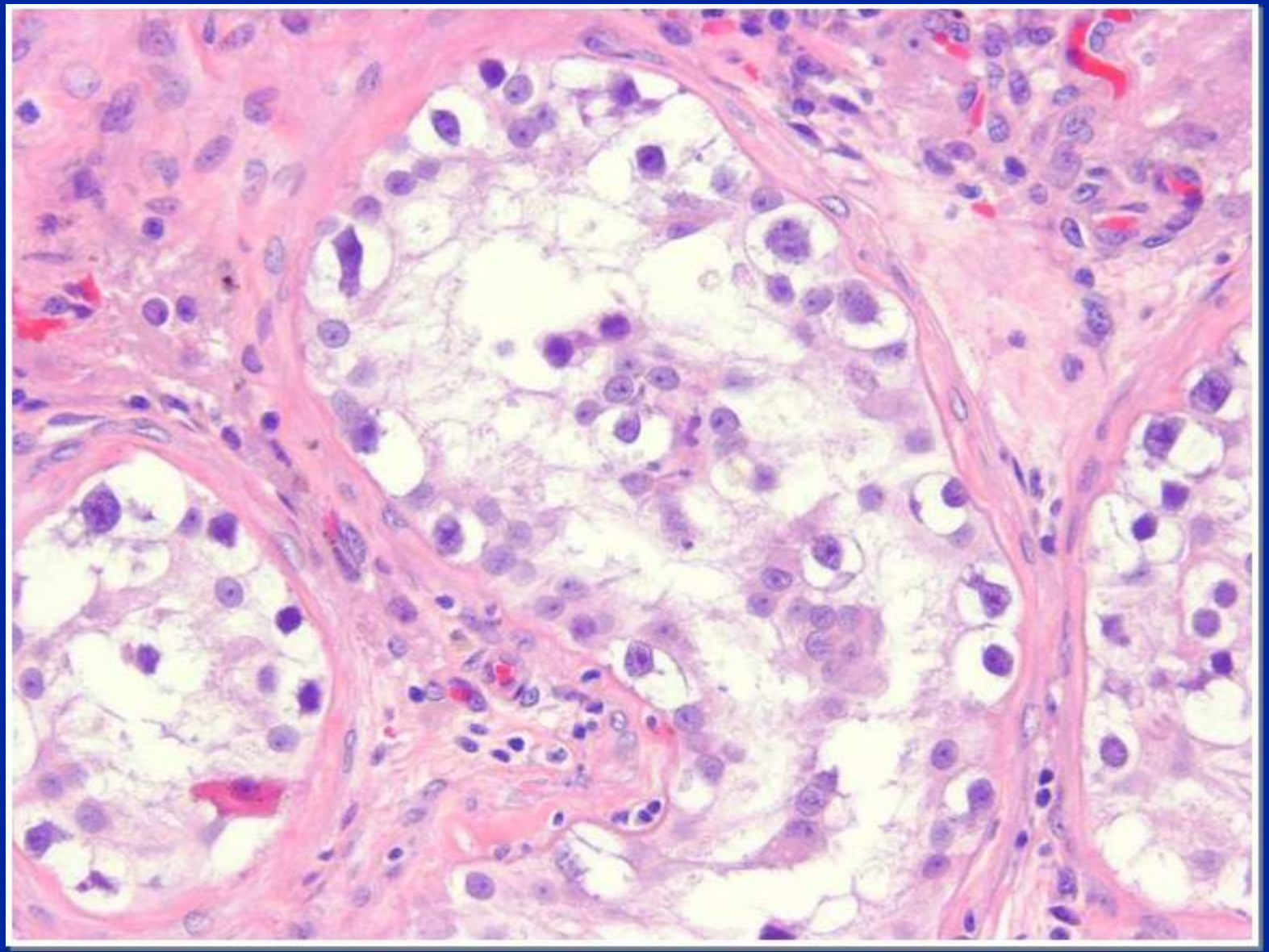
Intratubular Germ Cell Neoplasm Unclassified
IGCNU

- **DDX:**
  - Intratubular seminoma
  - Intratubular spermatocytic seminoma
  - Spermatogenic arrest
  - Prepubertal spermatogonia (giant spermatogonia)

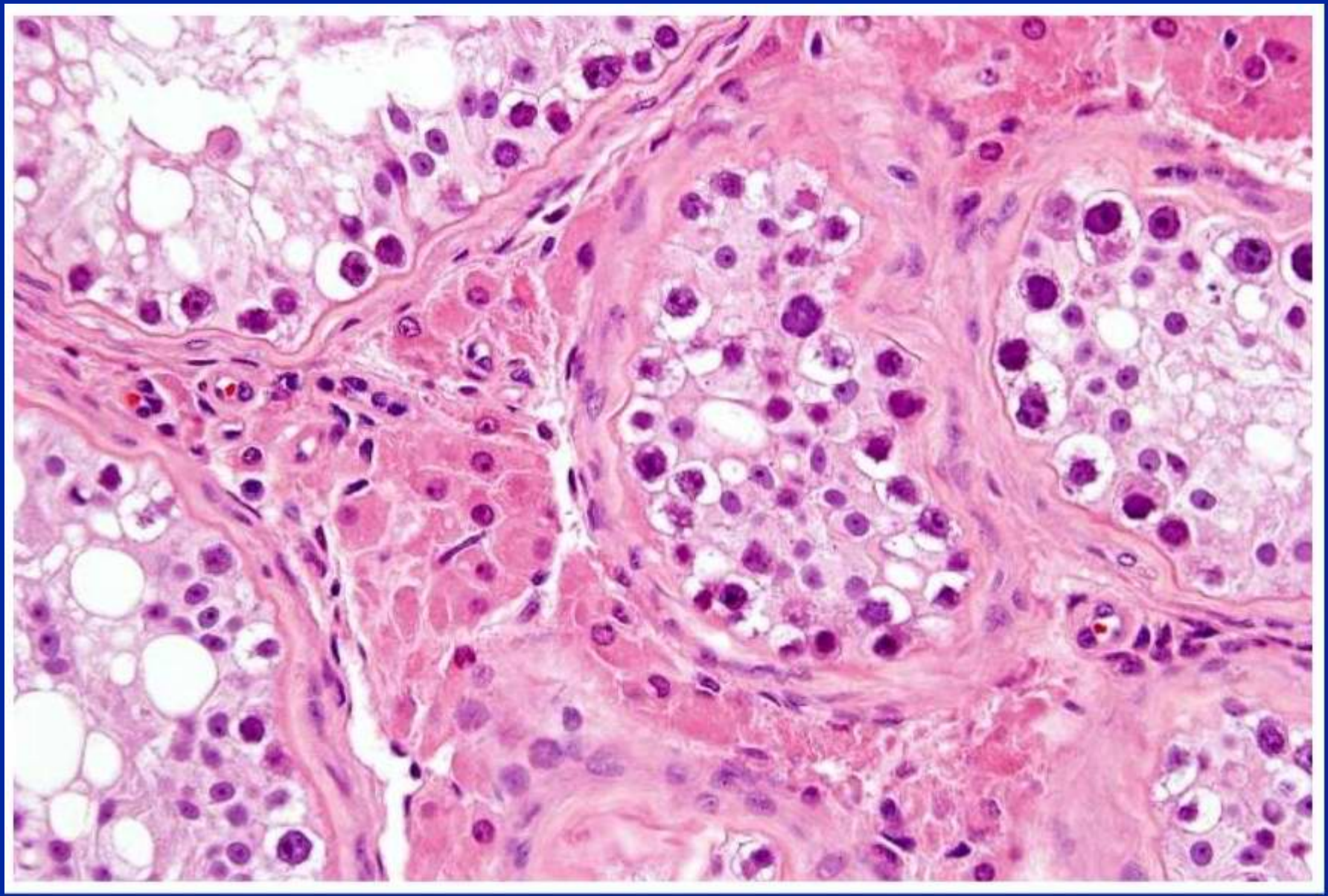
- **IHC :**
  - PLAP(+), C-kit (+), OCT3/4 (+)

- **IGCNU can extend via pagetoid spread to rete testis and epididymis**
- **Microinvasion**

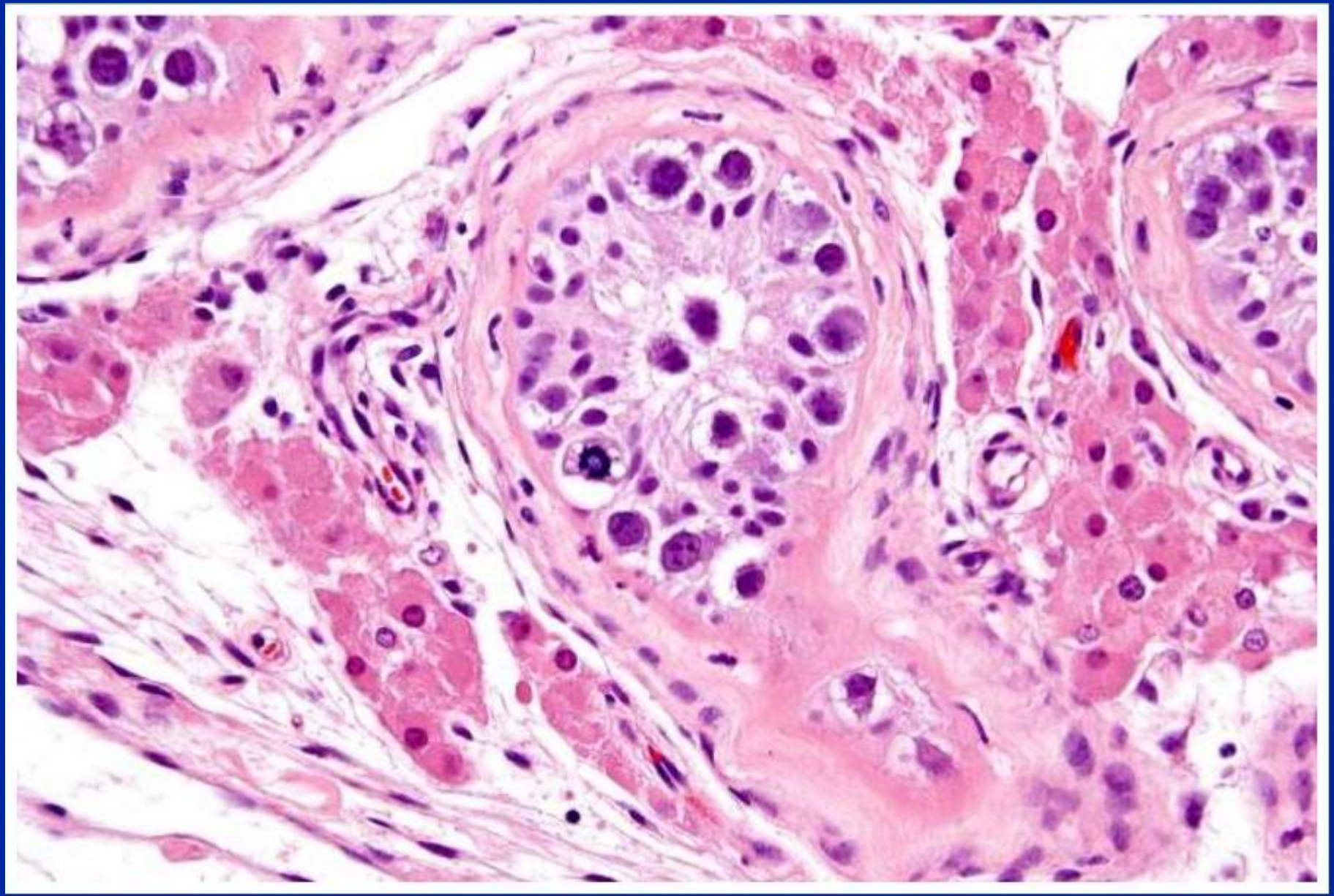
IGCNU

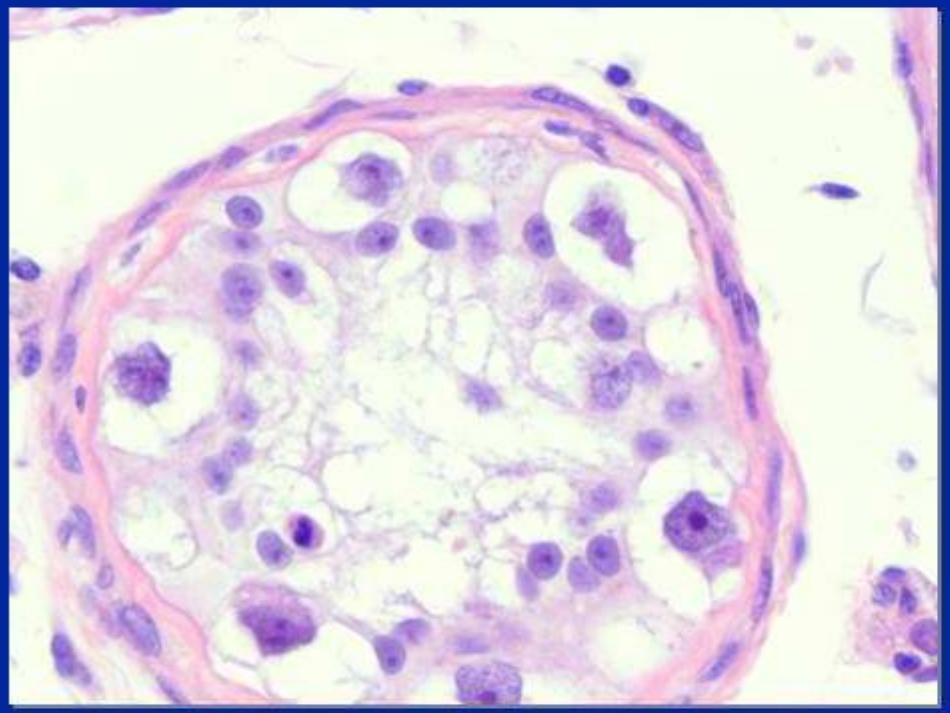


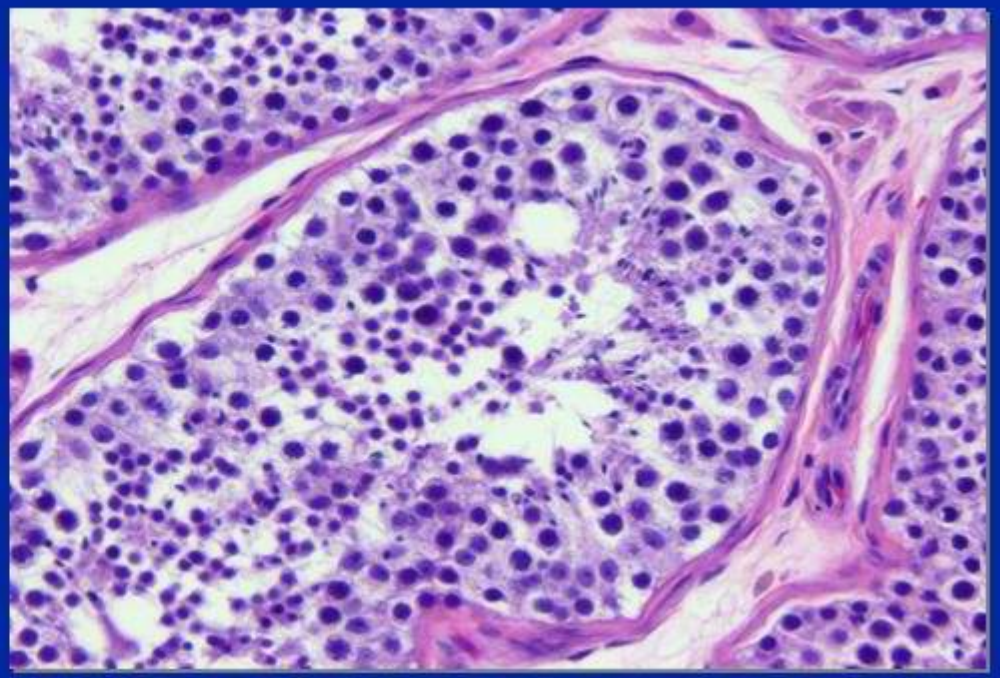
IGCNU

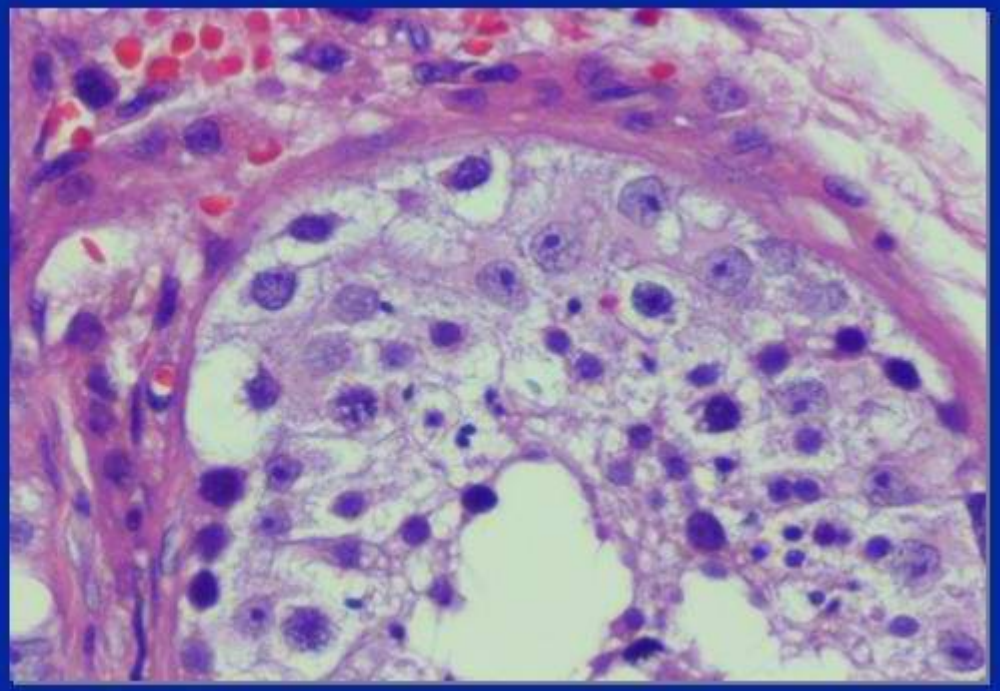


IGCNU

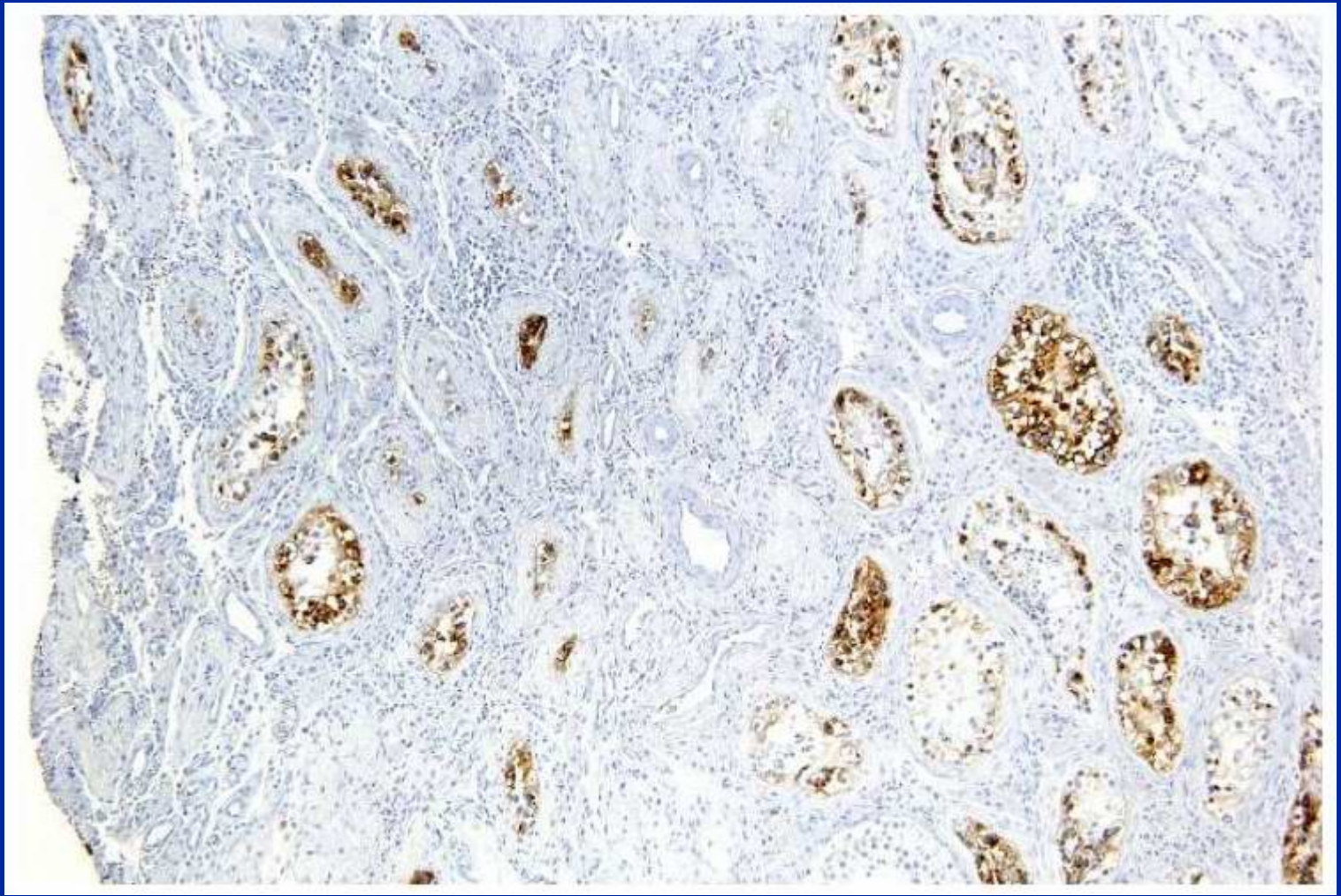


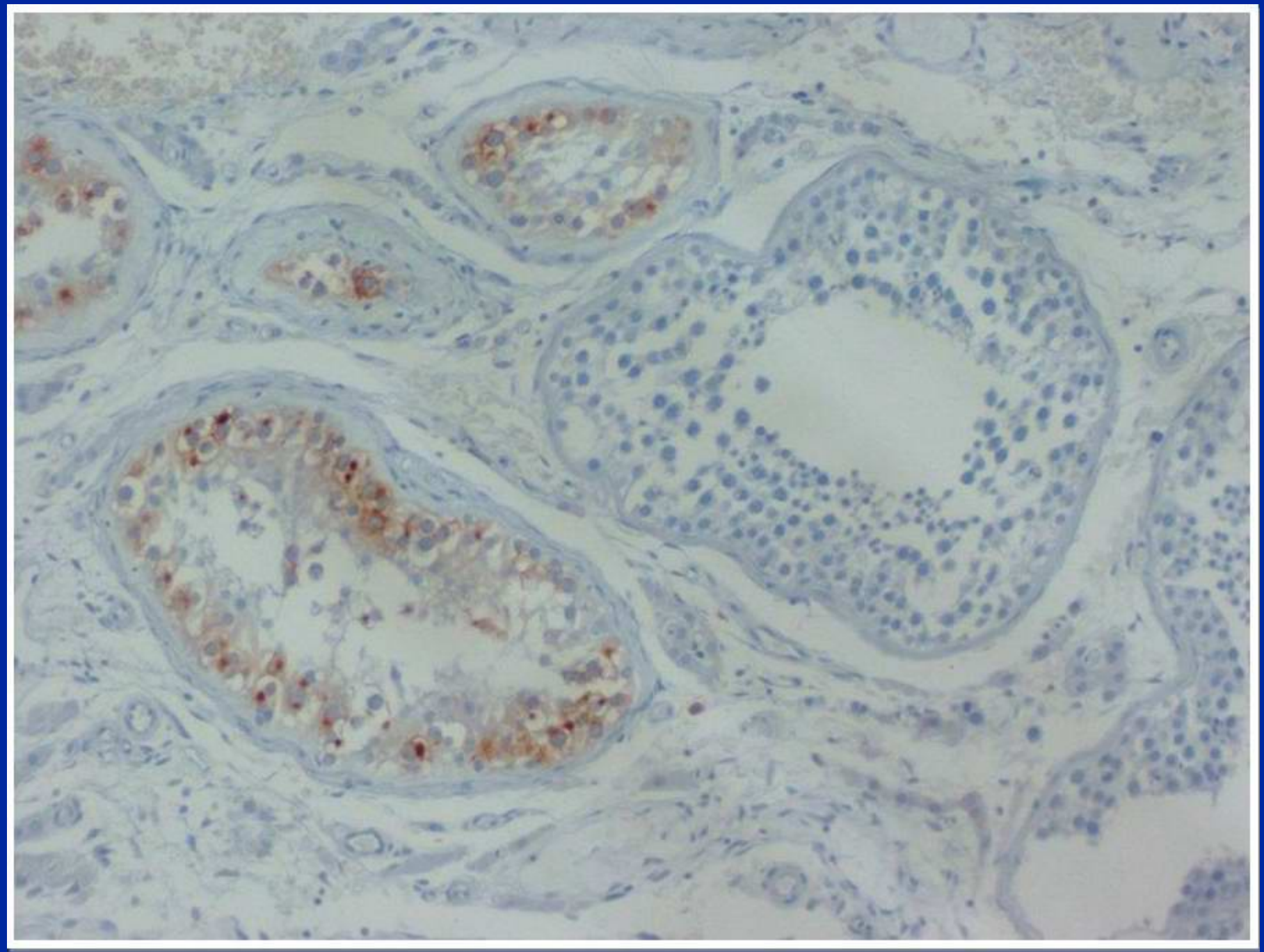


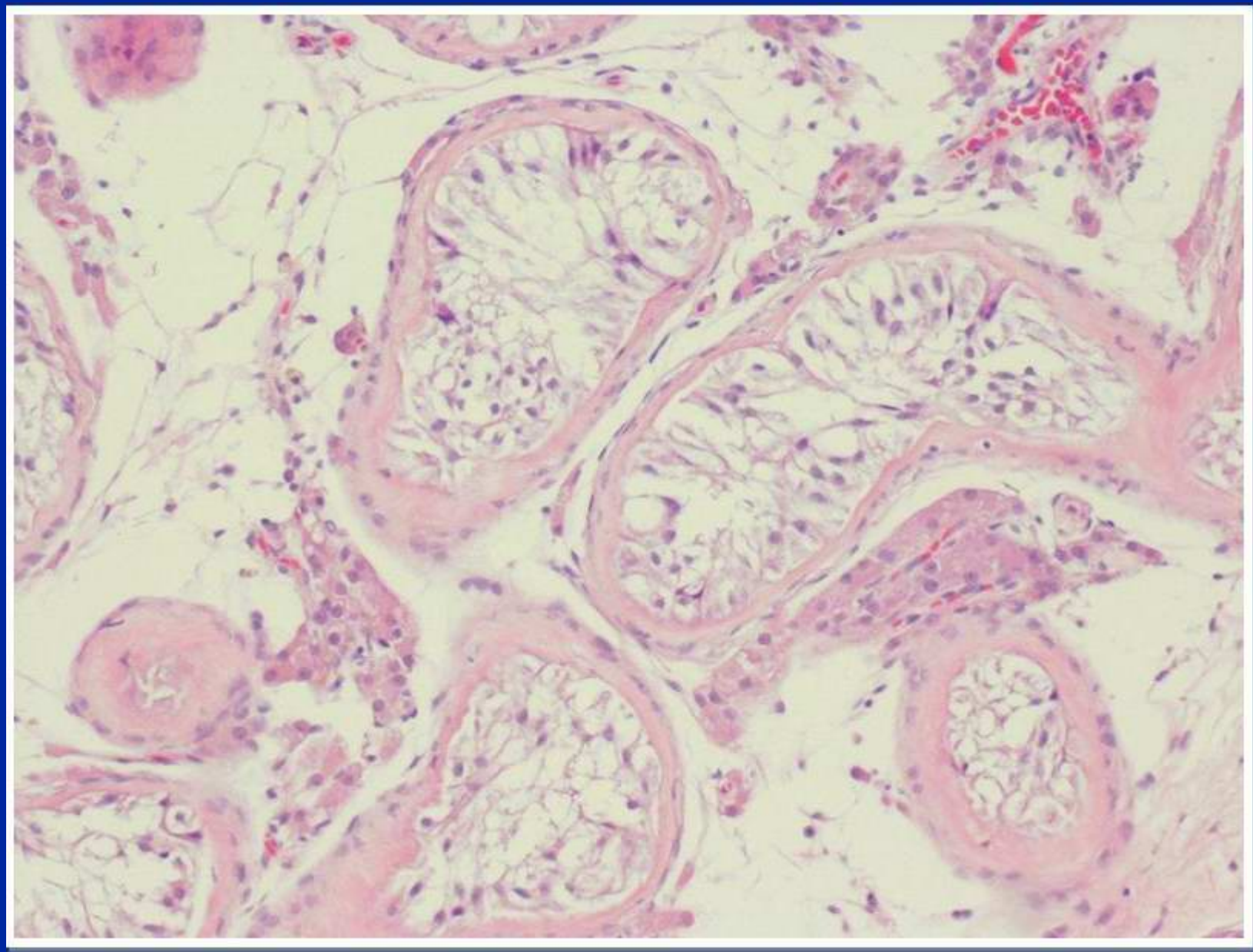


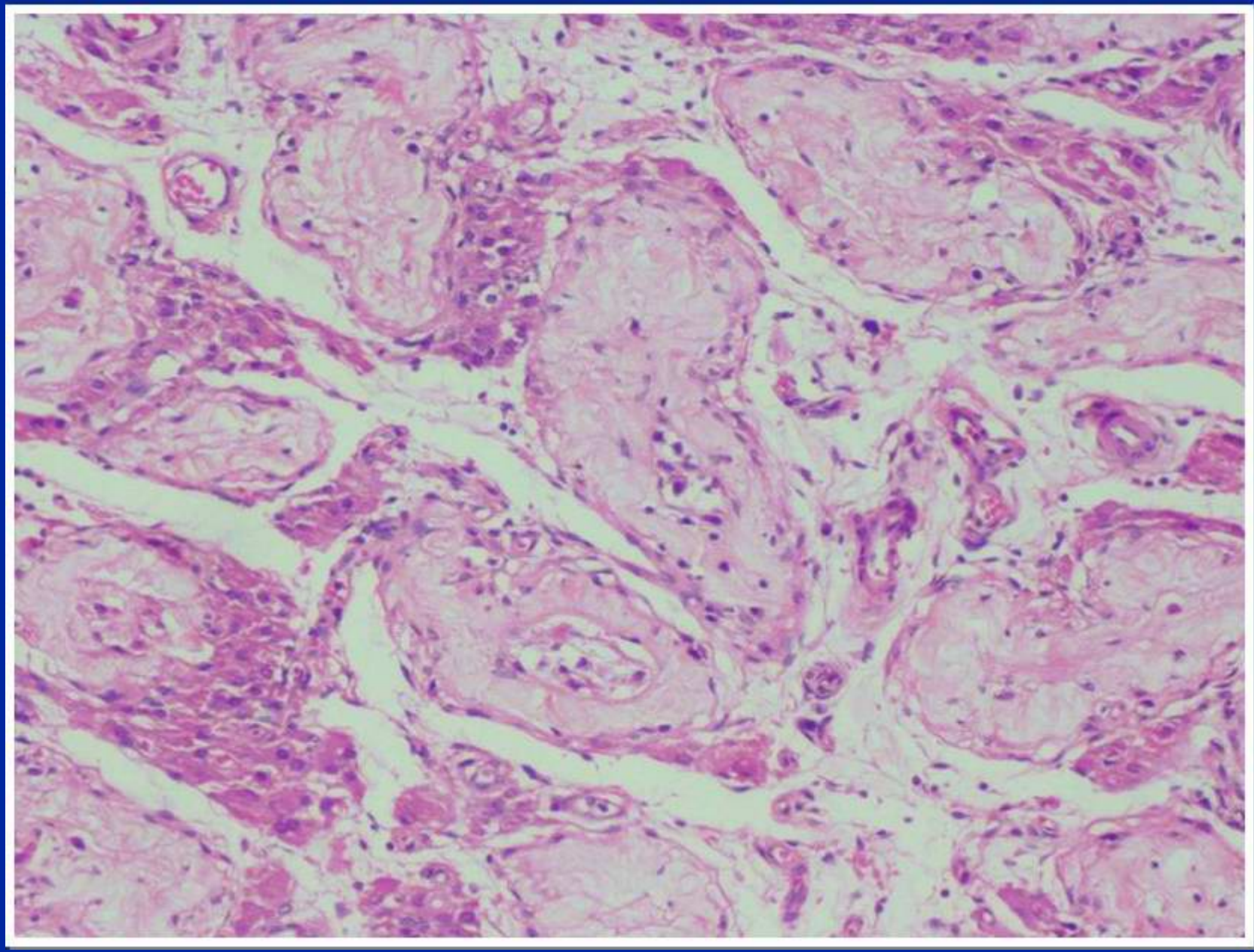


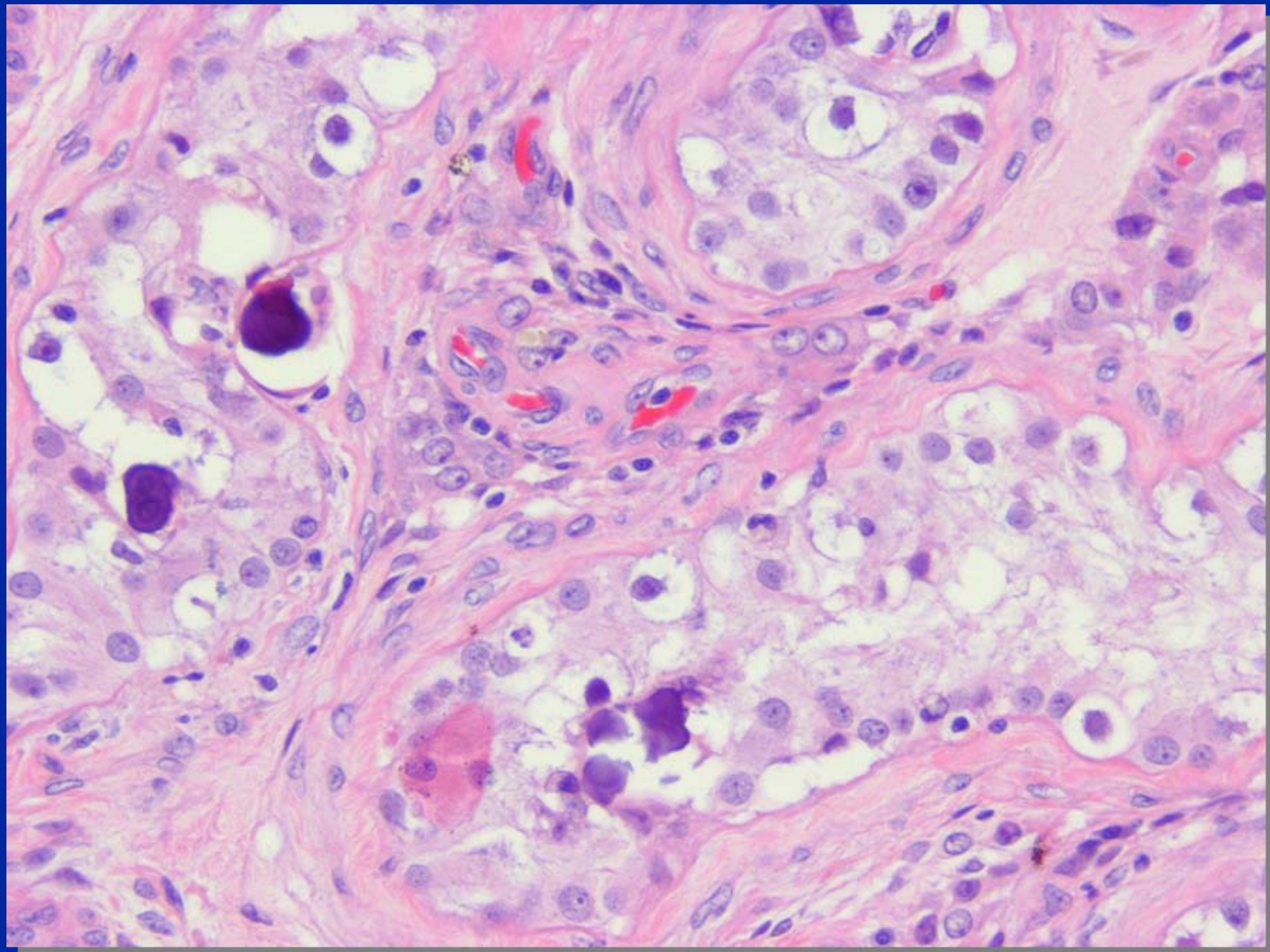
PLAP



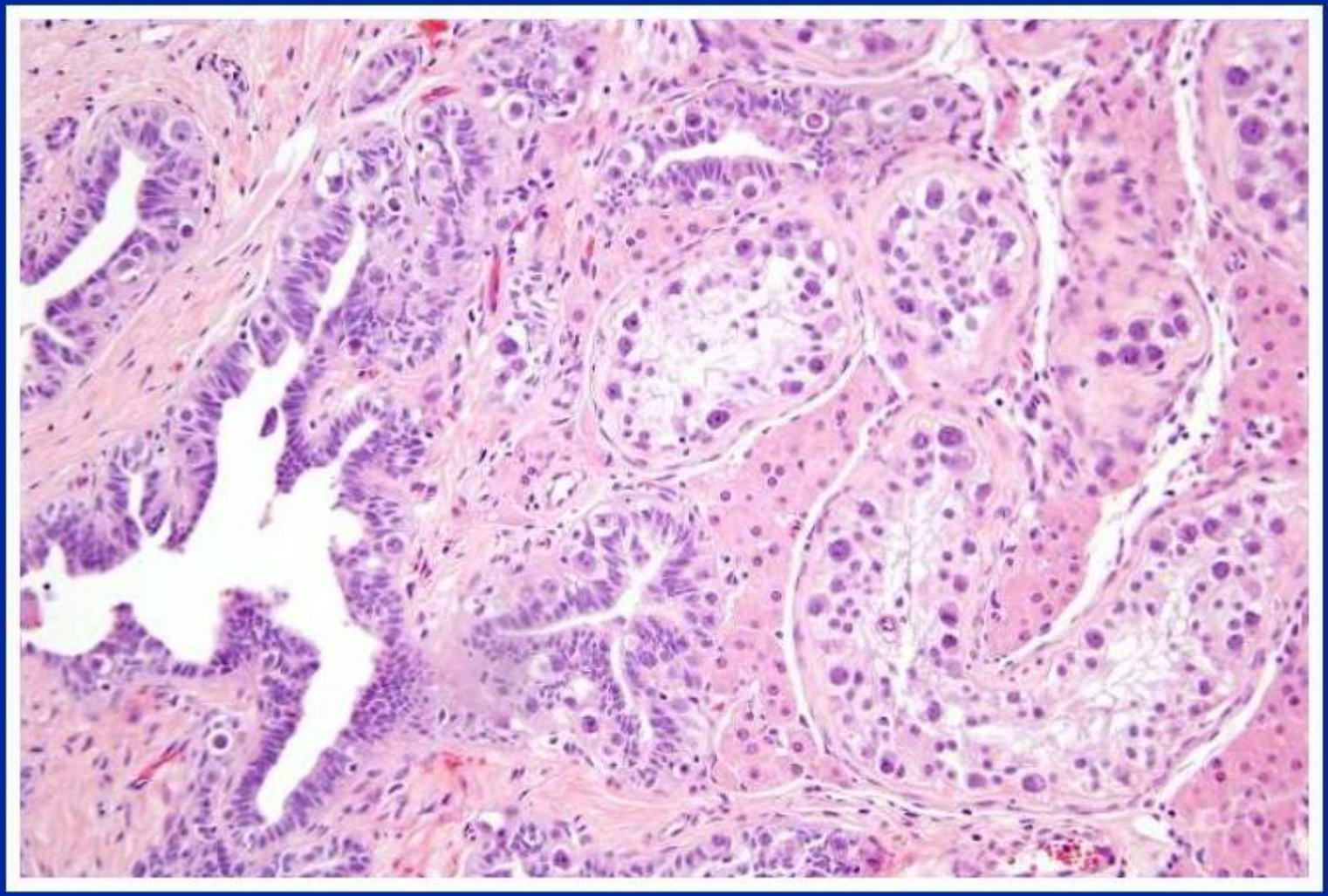




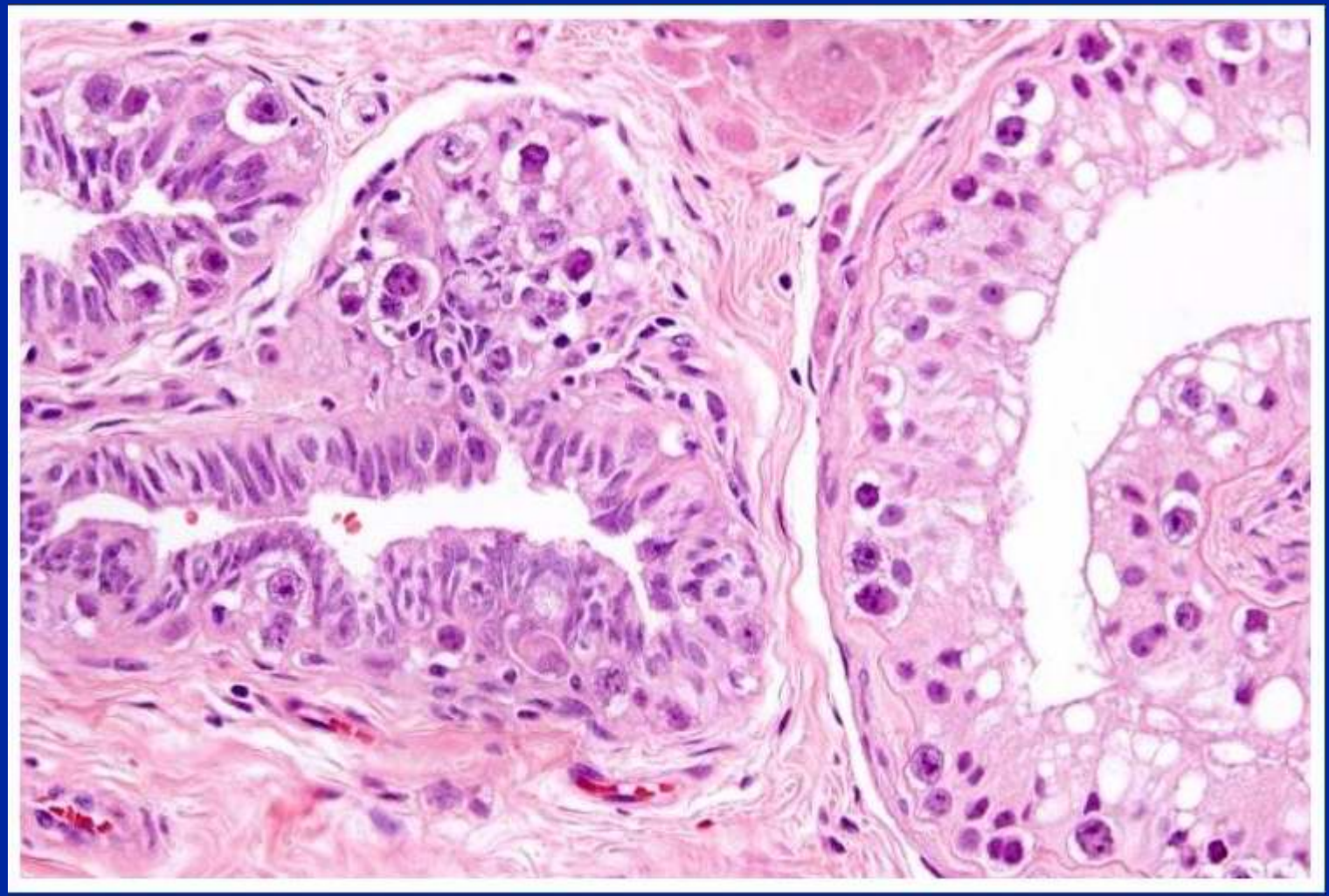




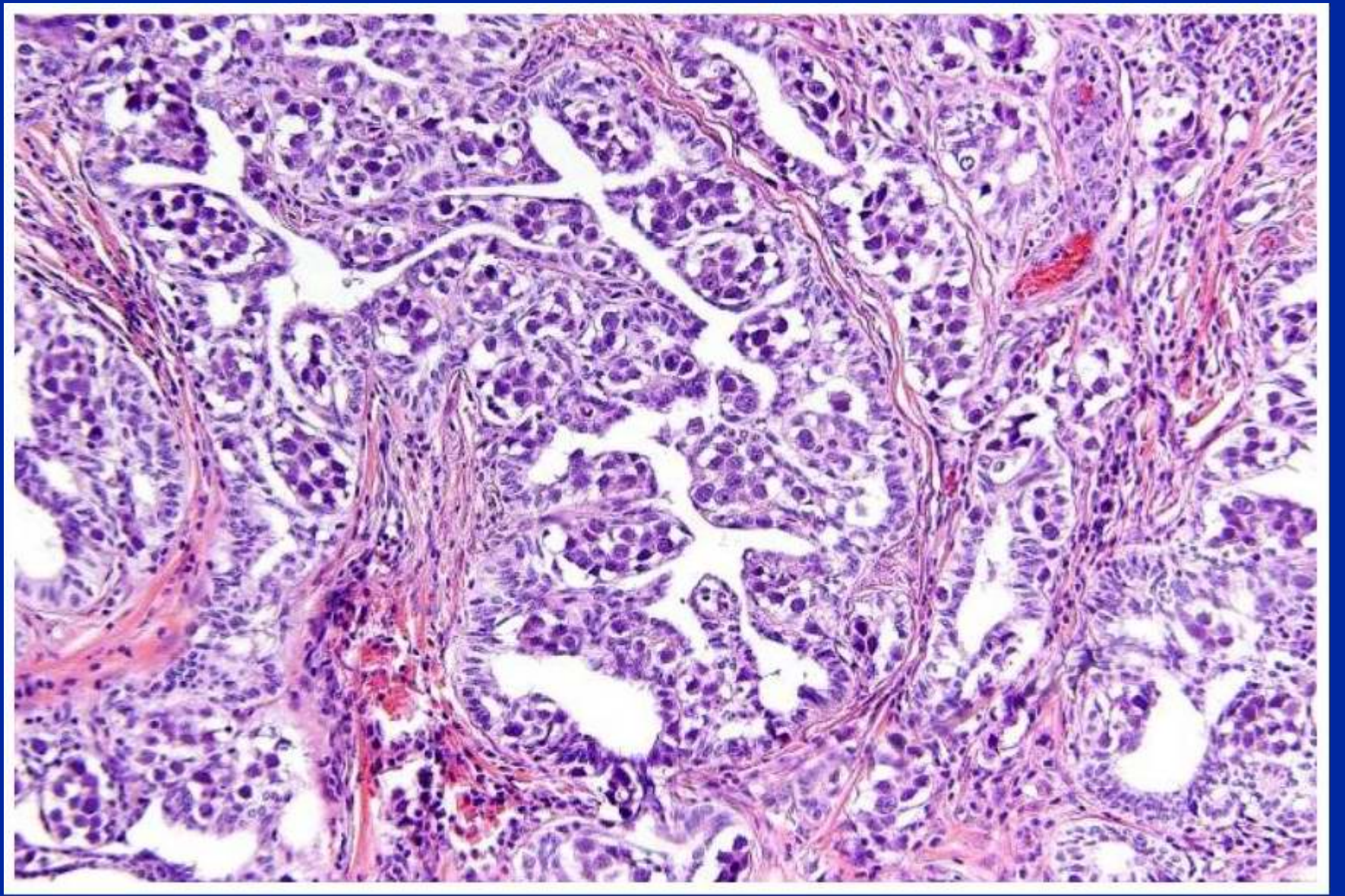
IGCNU in Rete

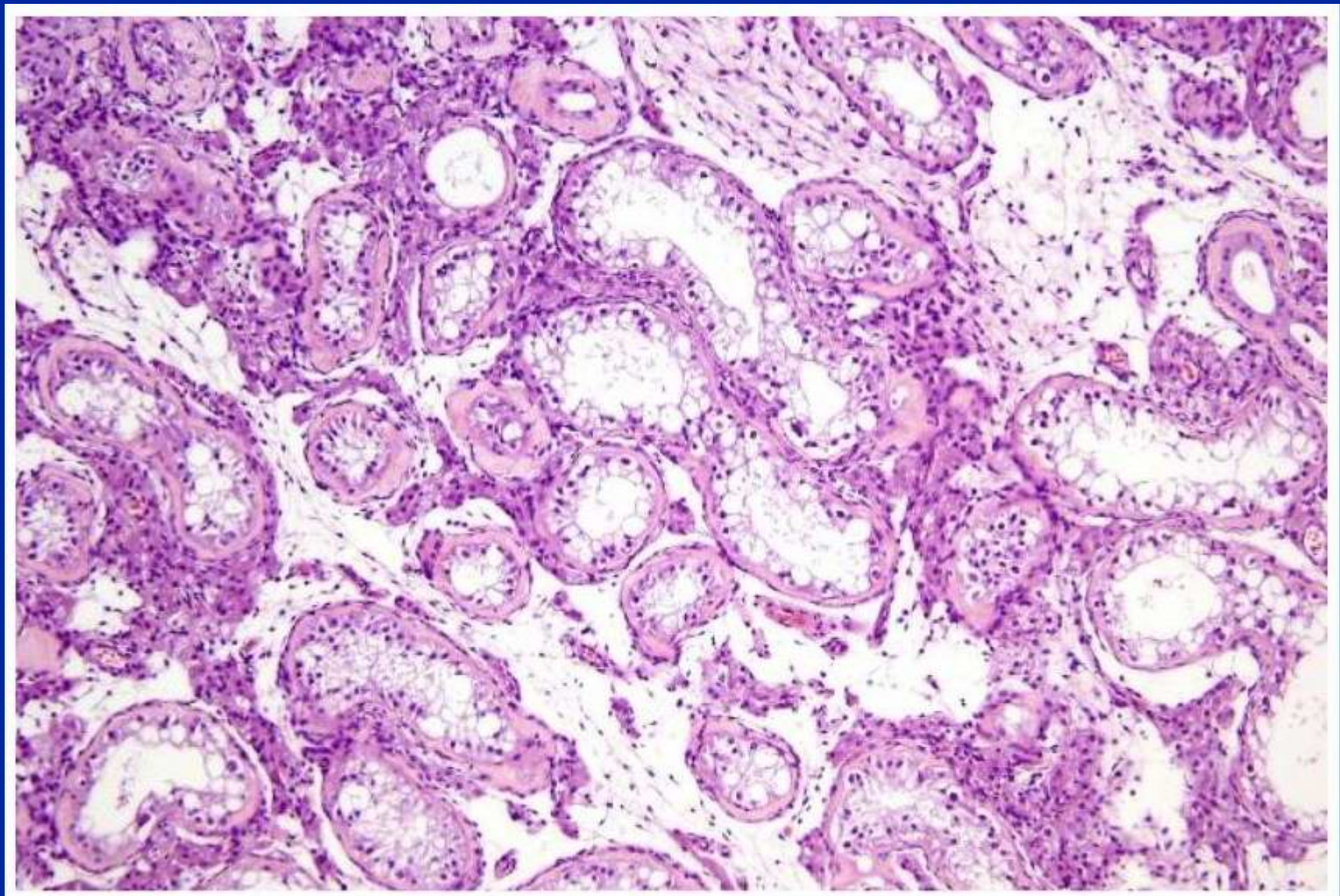


IGCNU in Rete

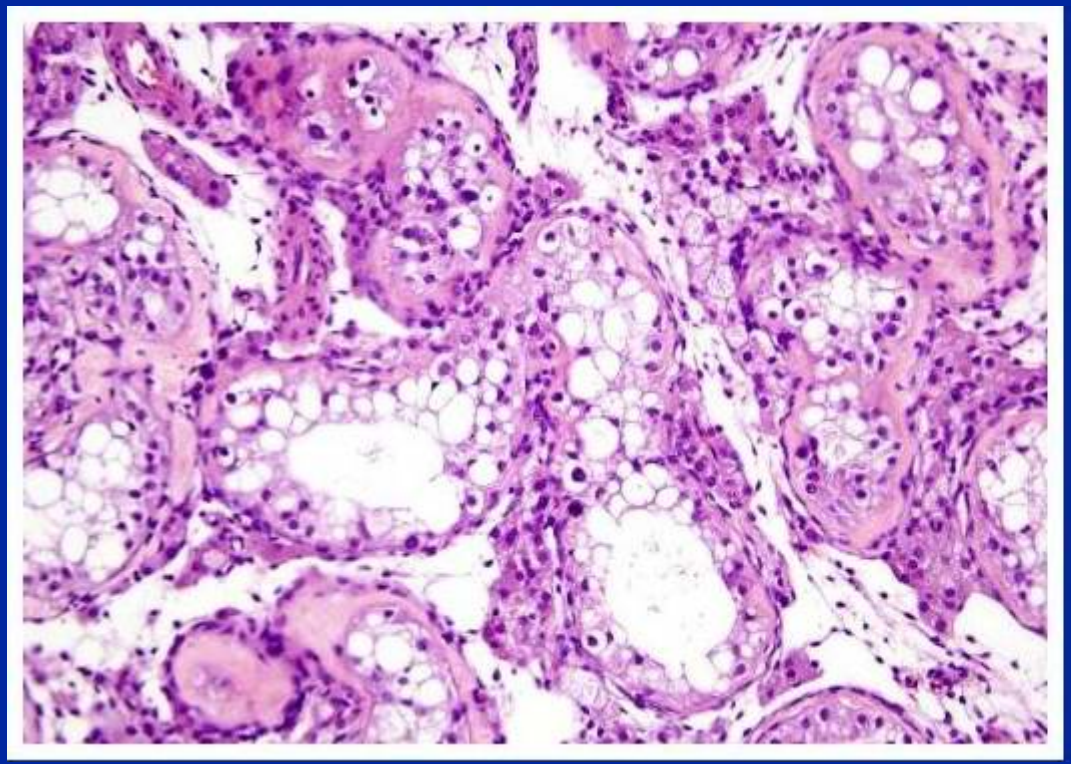


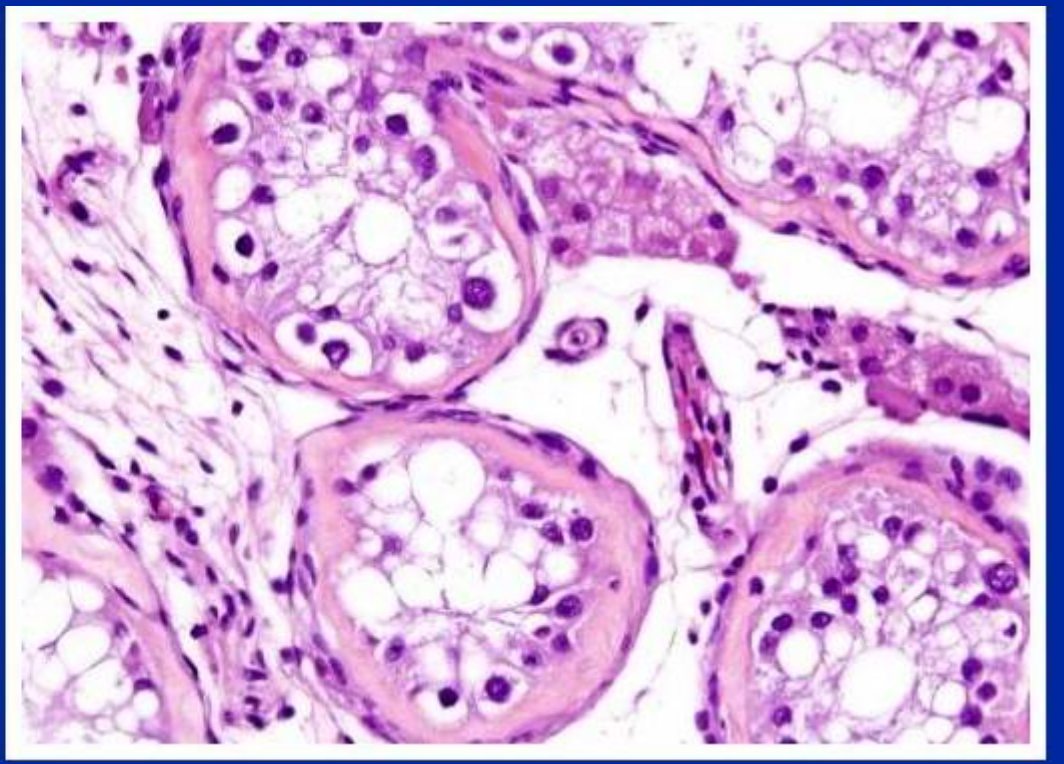
IGCNU in Rete Mimicking Emb Ca

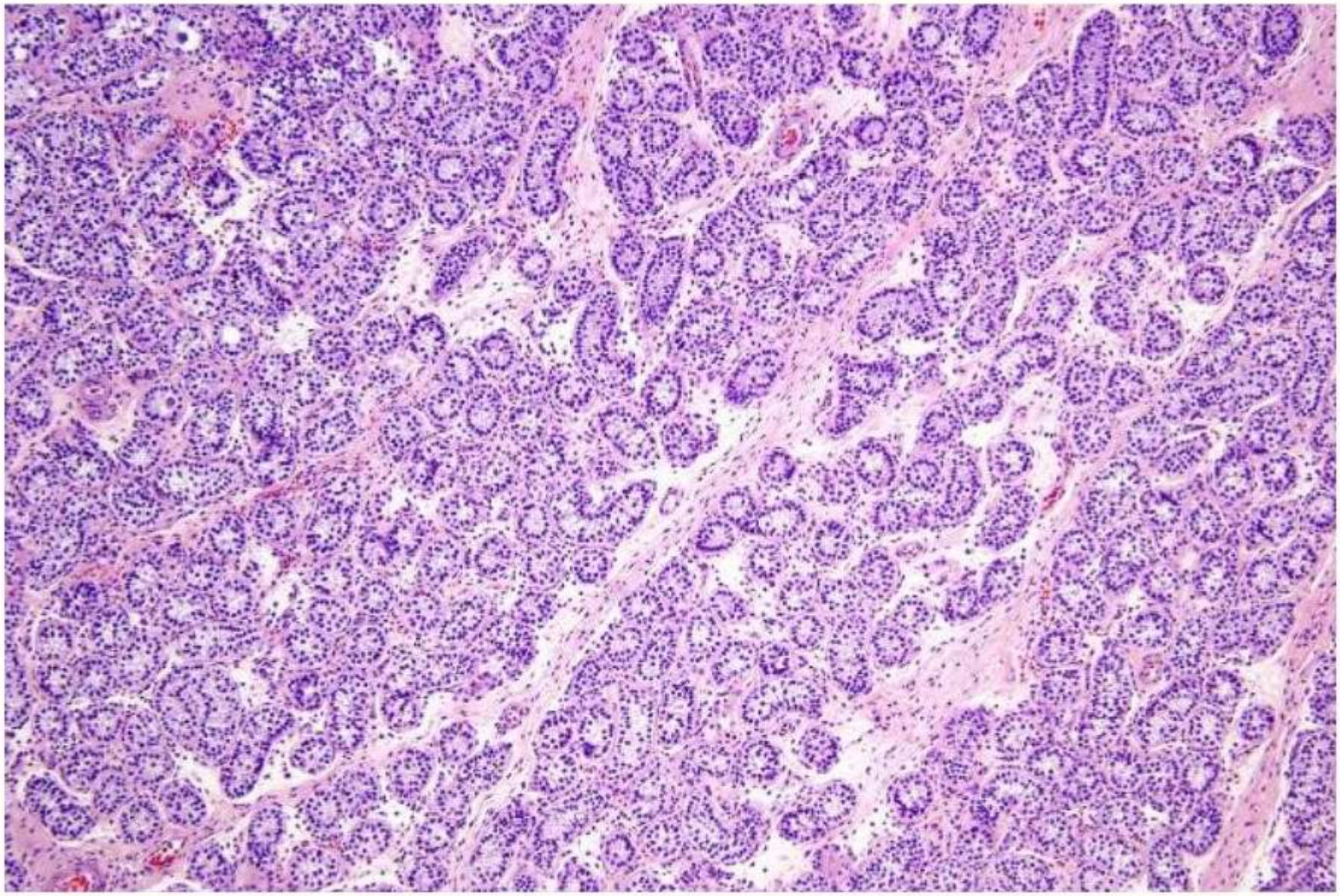




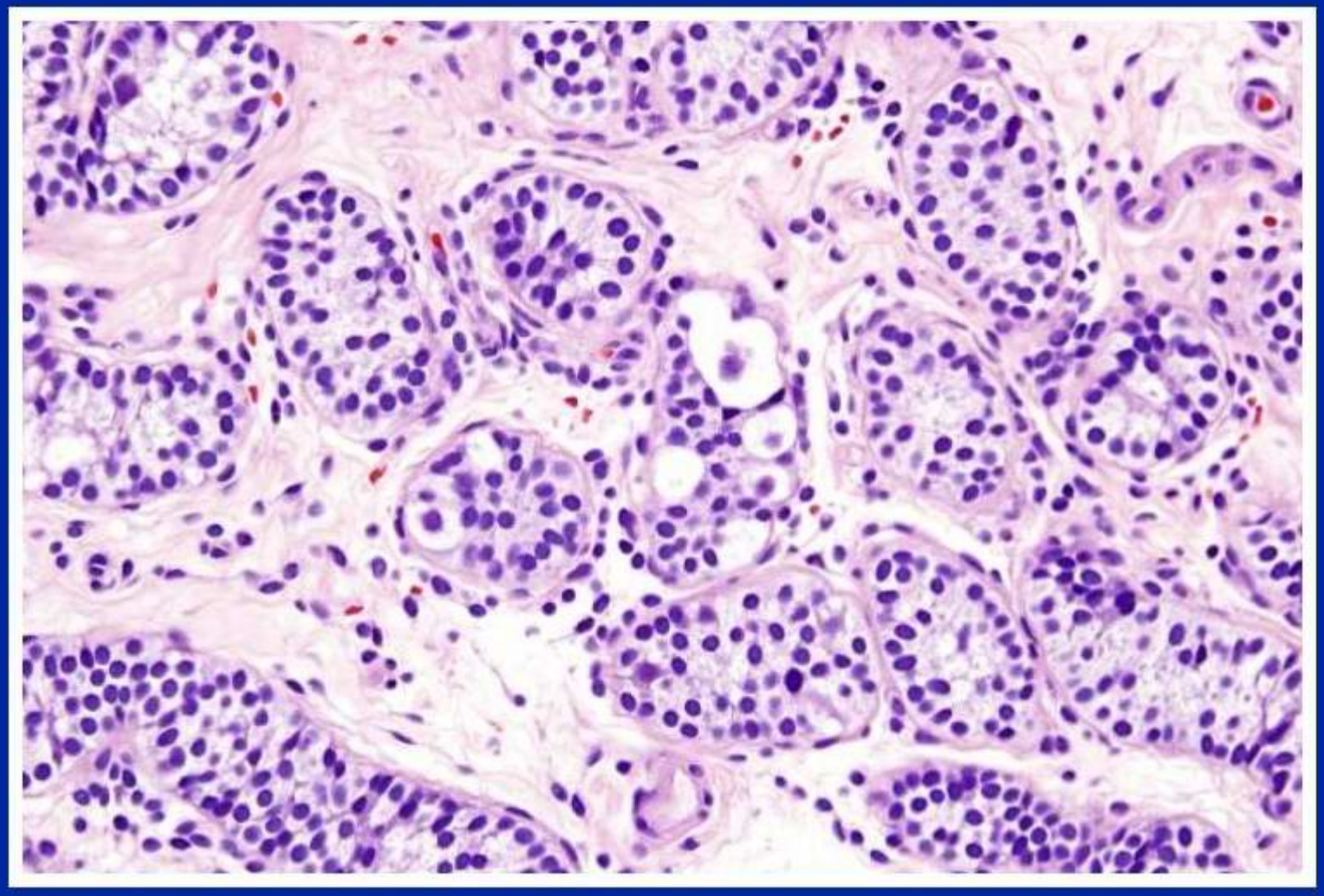
Sertoli Cell-Only Mimicking IGCNU

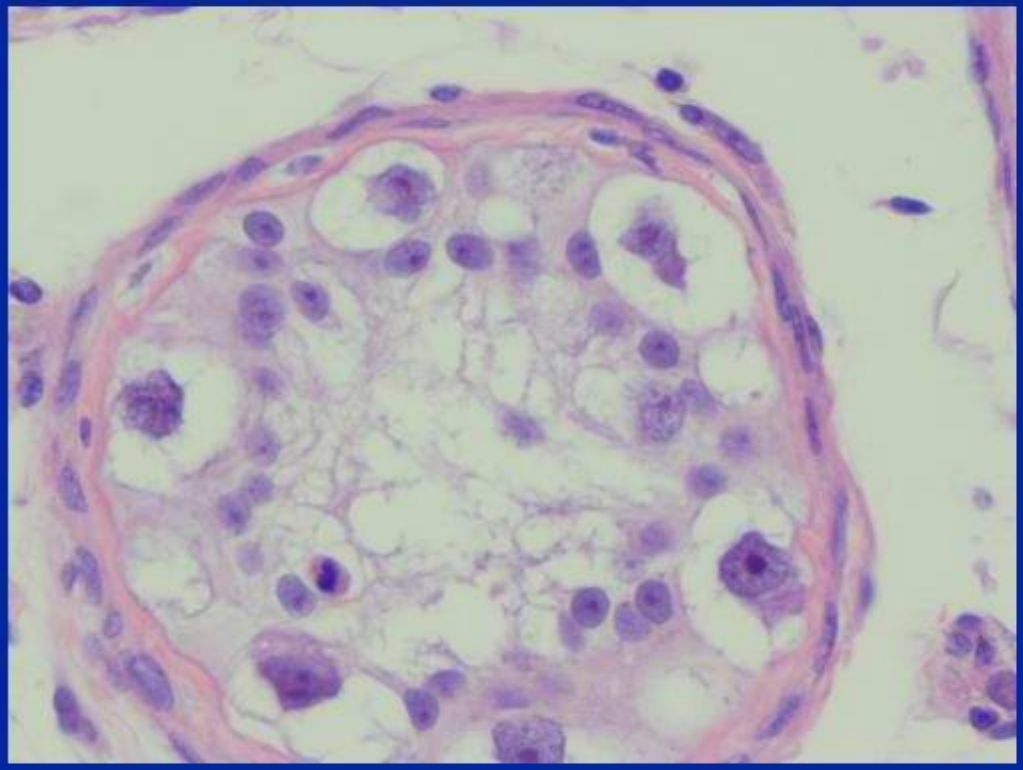


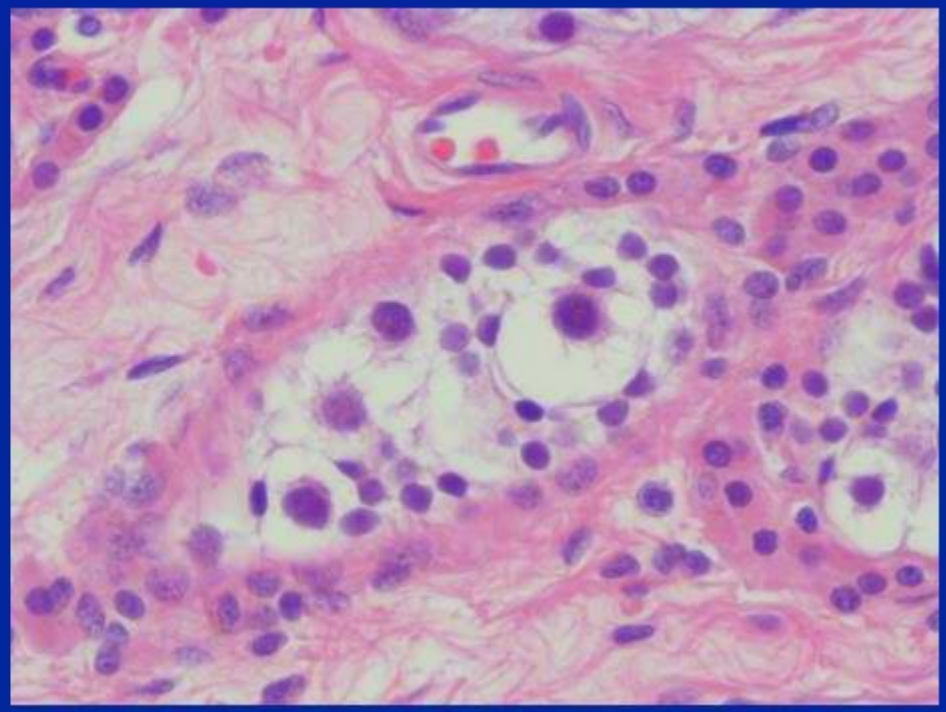


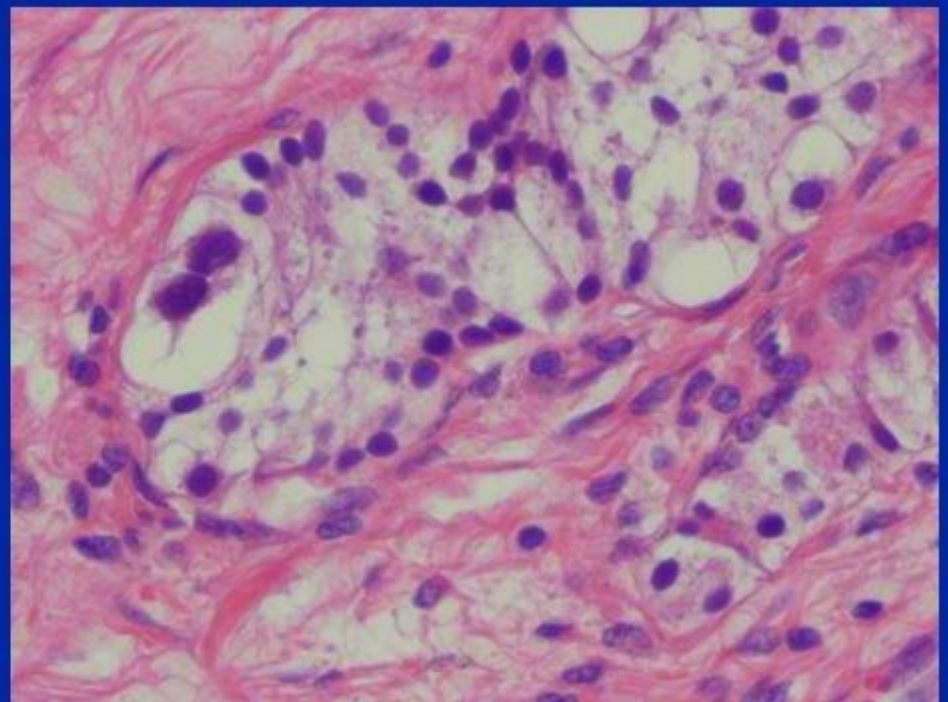


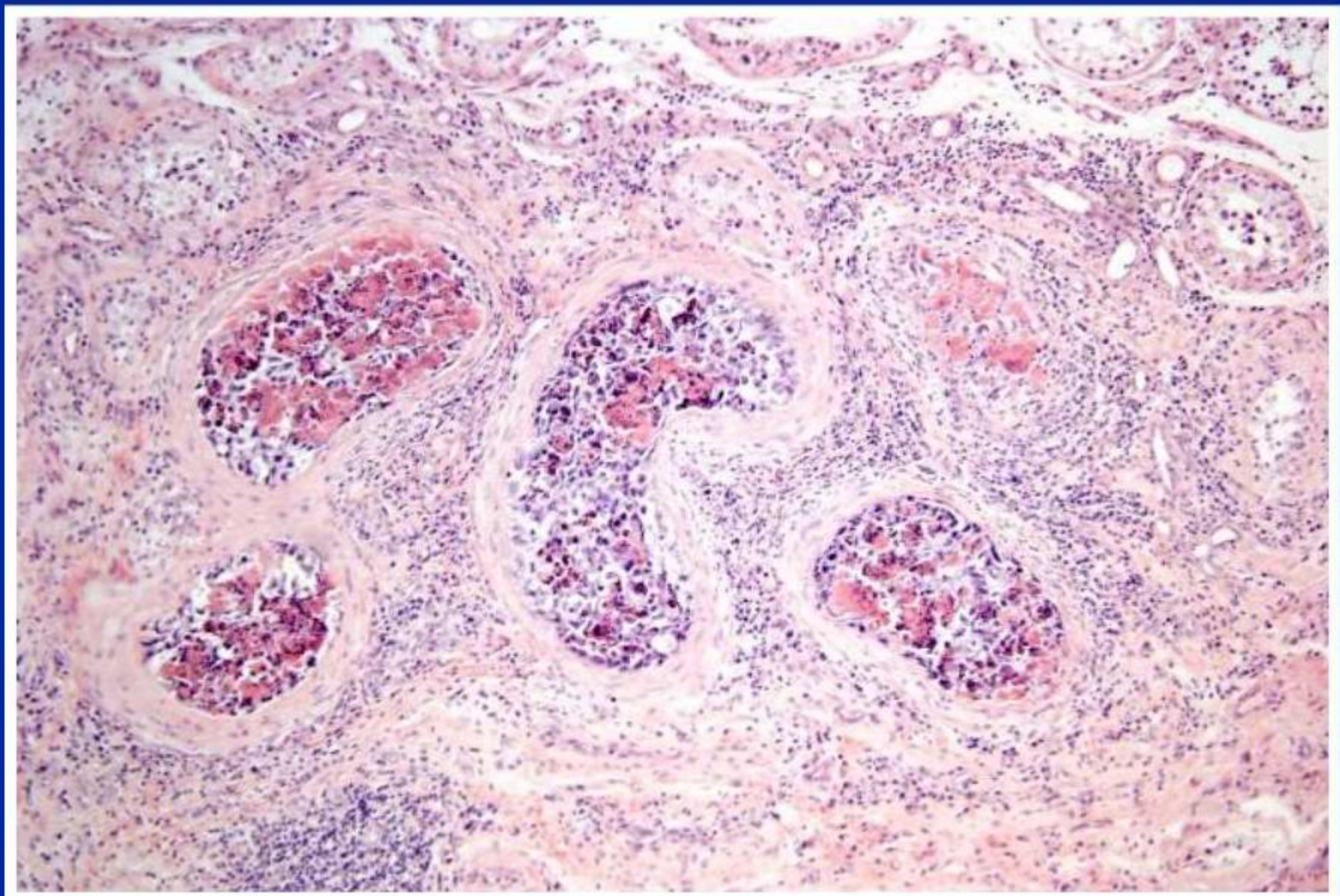
Prepubertal Testis Mimicking IGCNU



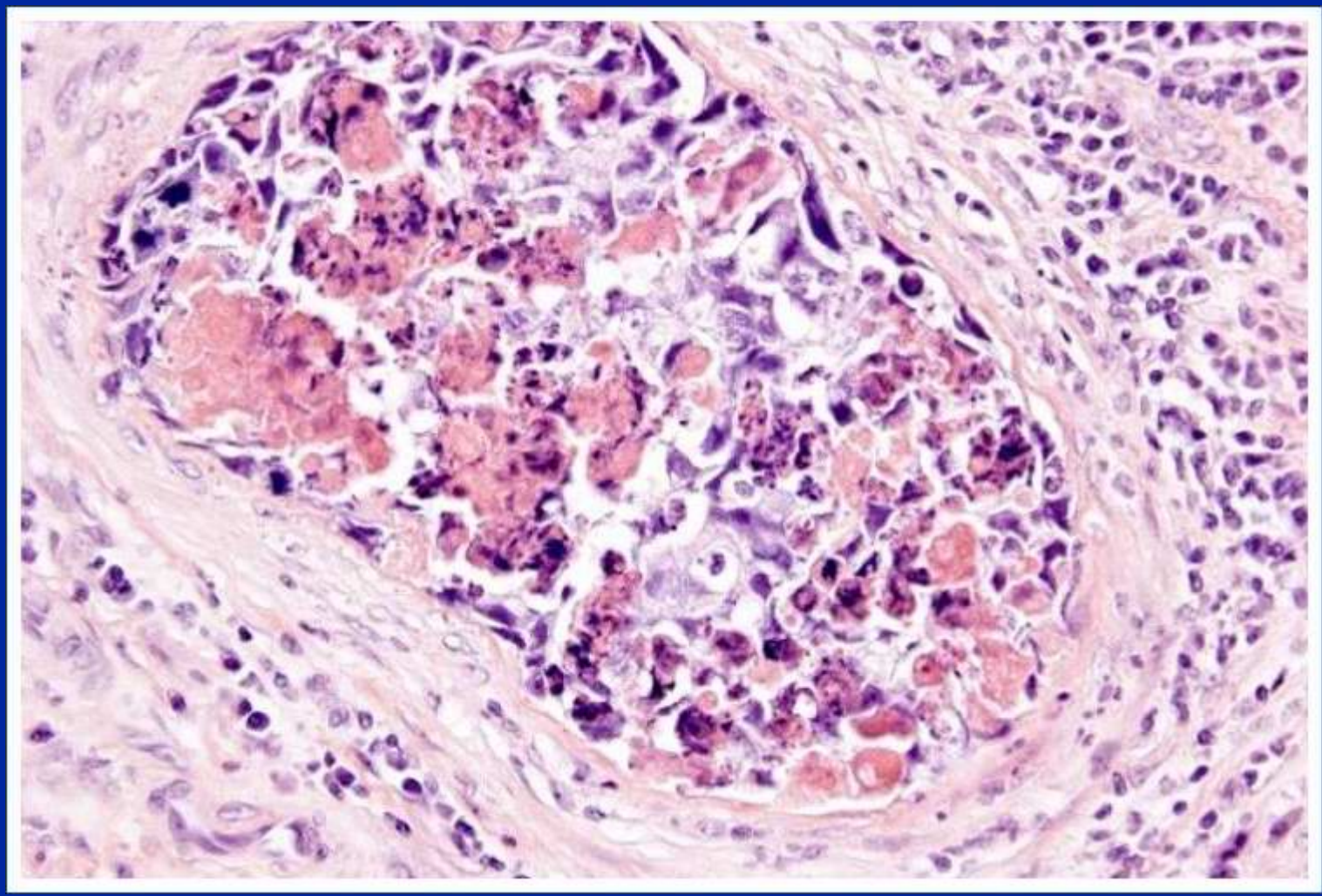


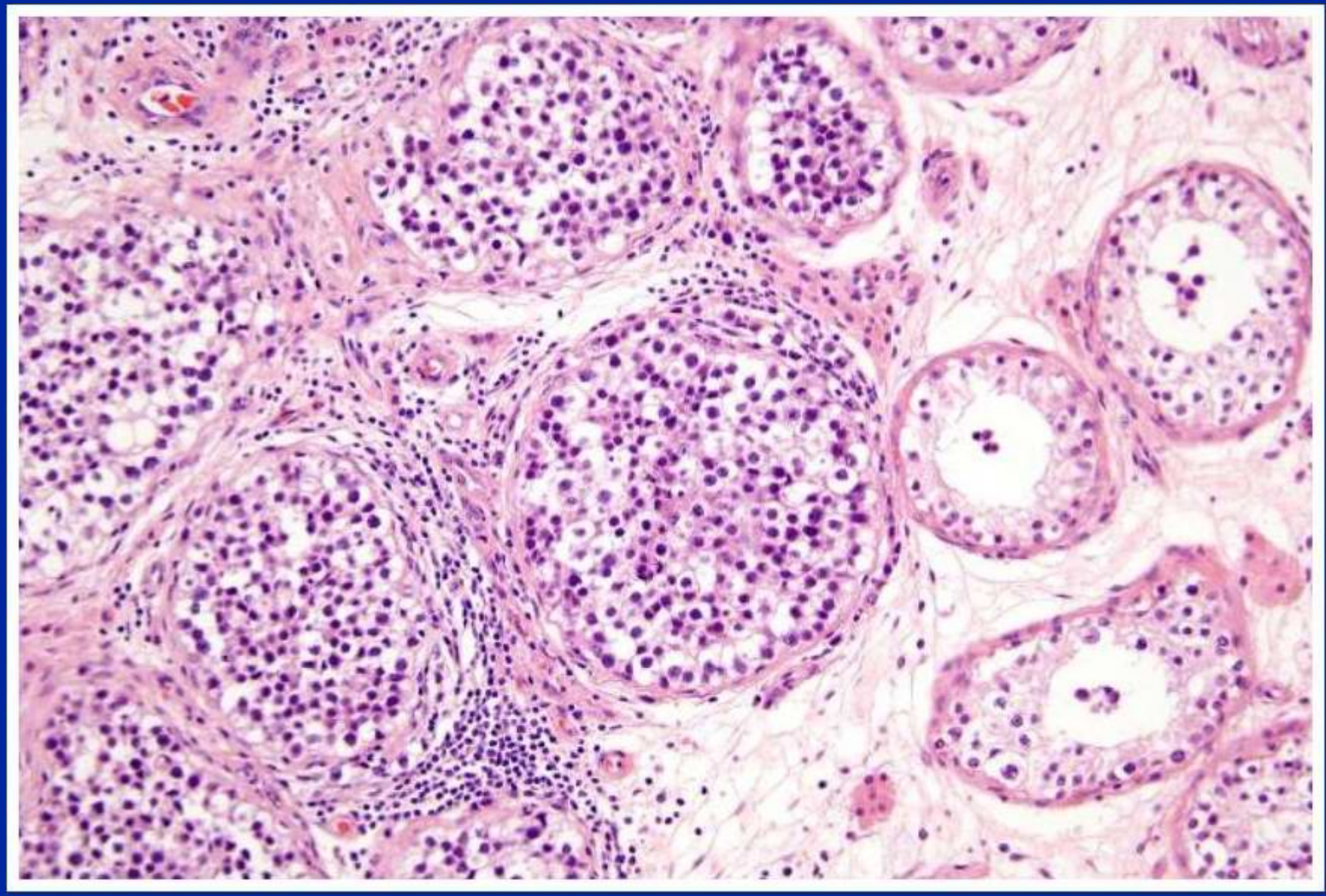




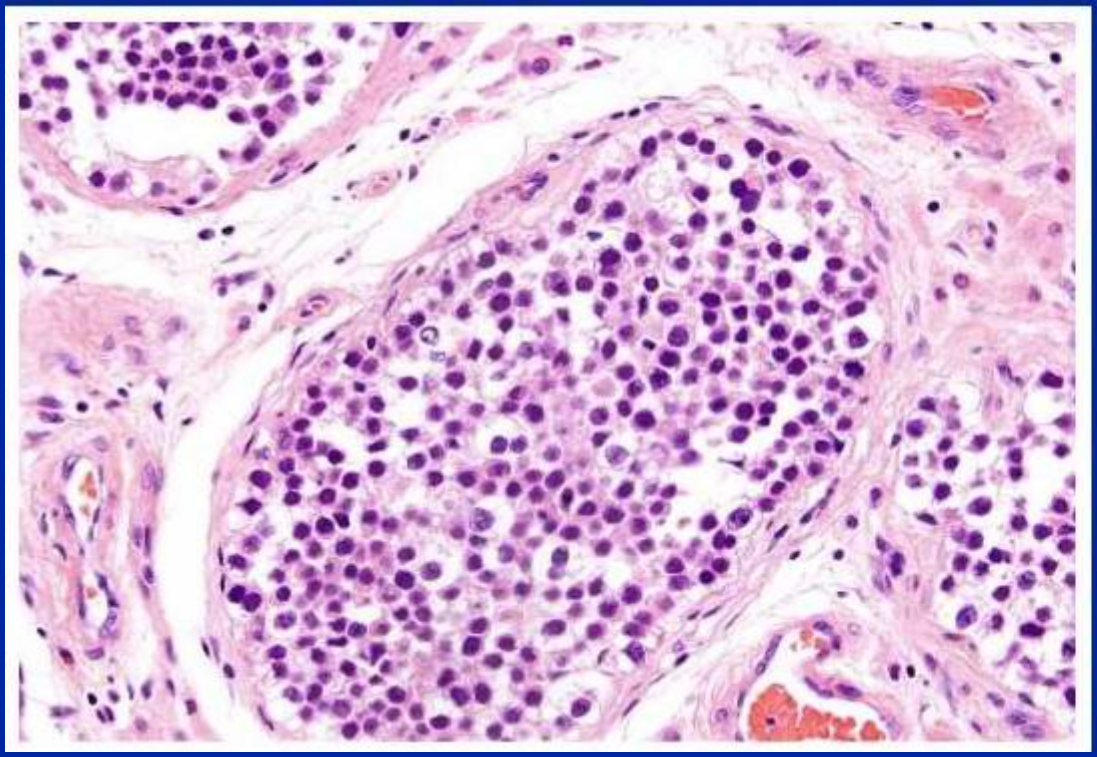


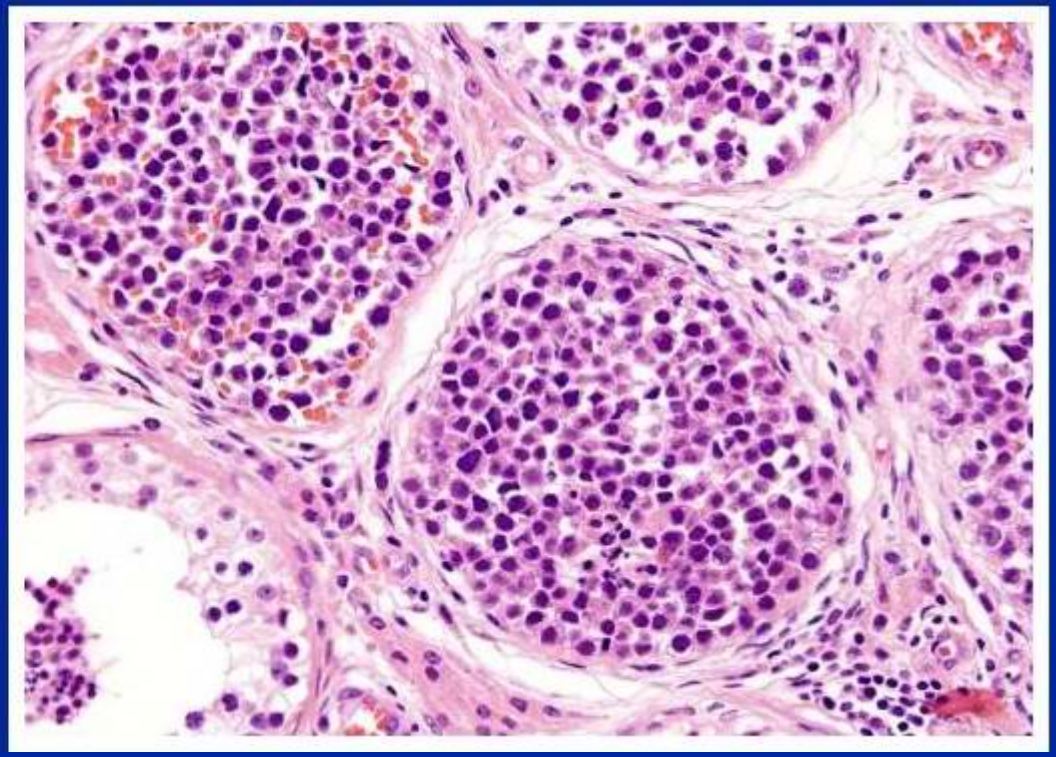
Intratubular Emb Ca





Intratubuular Seminoma





Overview

- Epidemiology & Risk Factors
- Genetics & Novel Markers
- Gender & Age Factors in Gonadal GCT
- IGCNU
- **Diagnostically “Problematic” Germ Cell Tumors (GCT)**

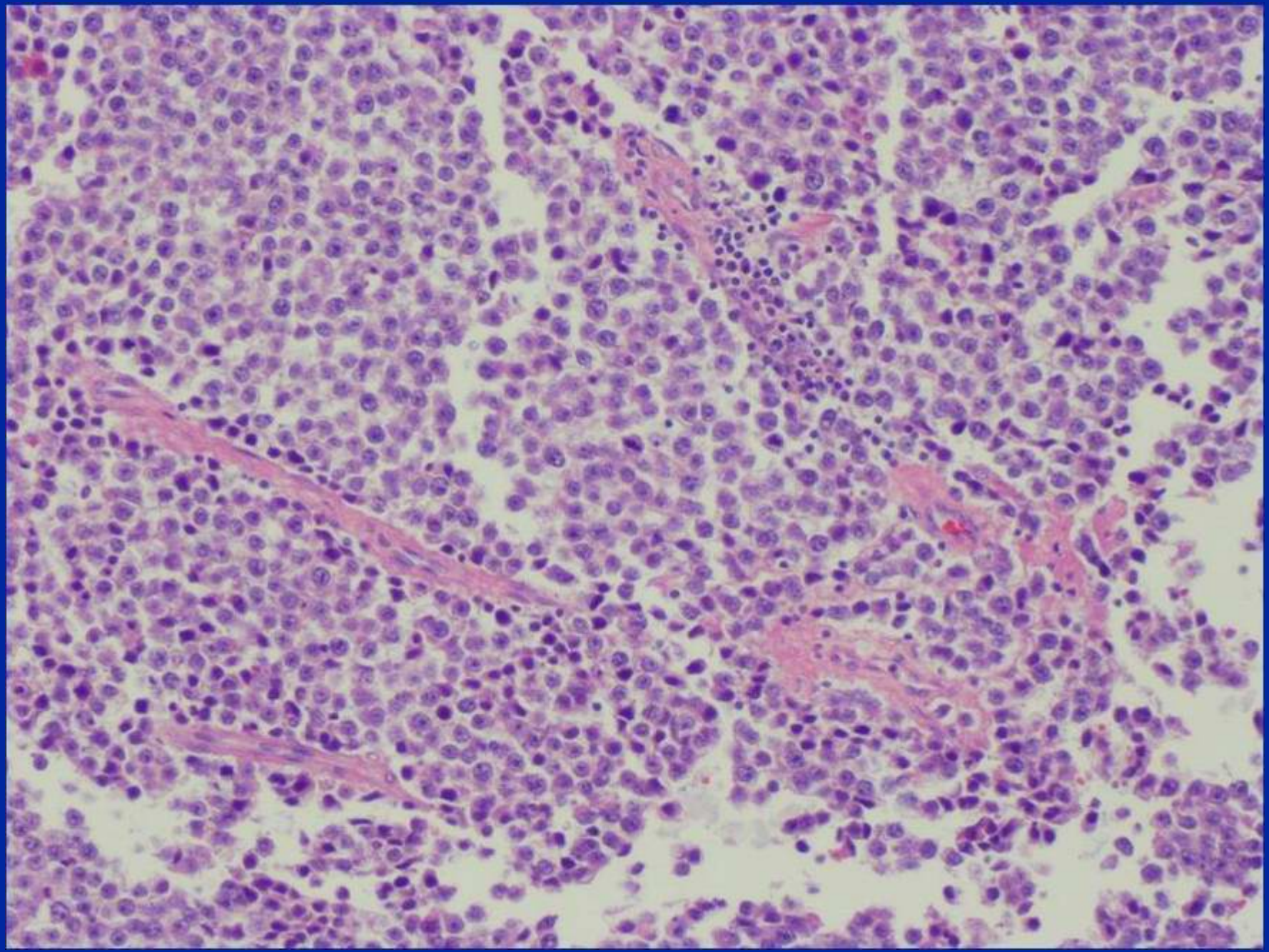
Germ Cell Tumors

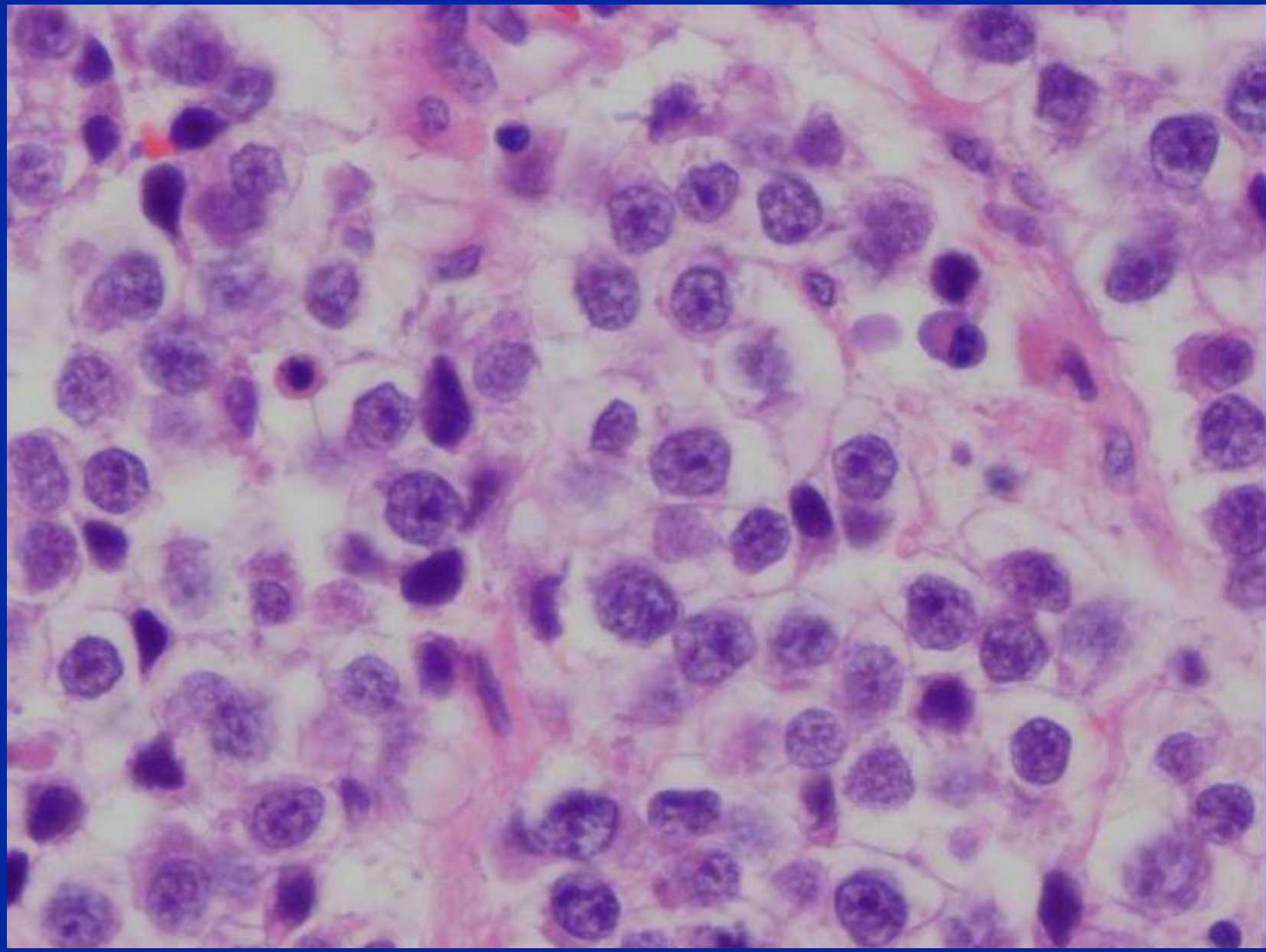
Classic Seminoma

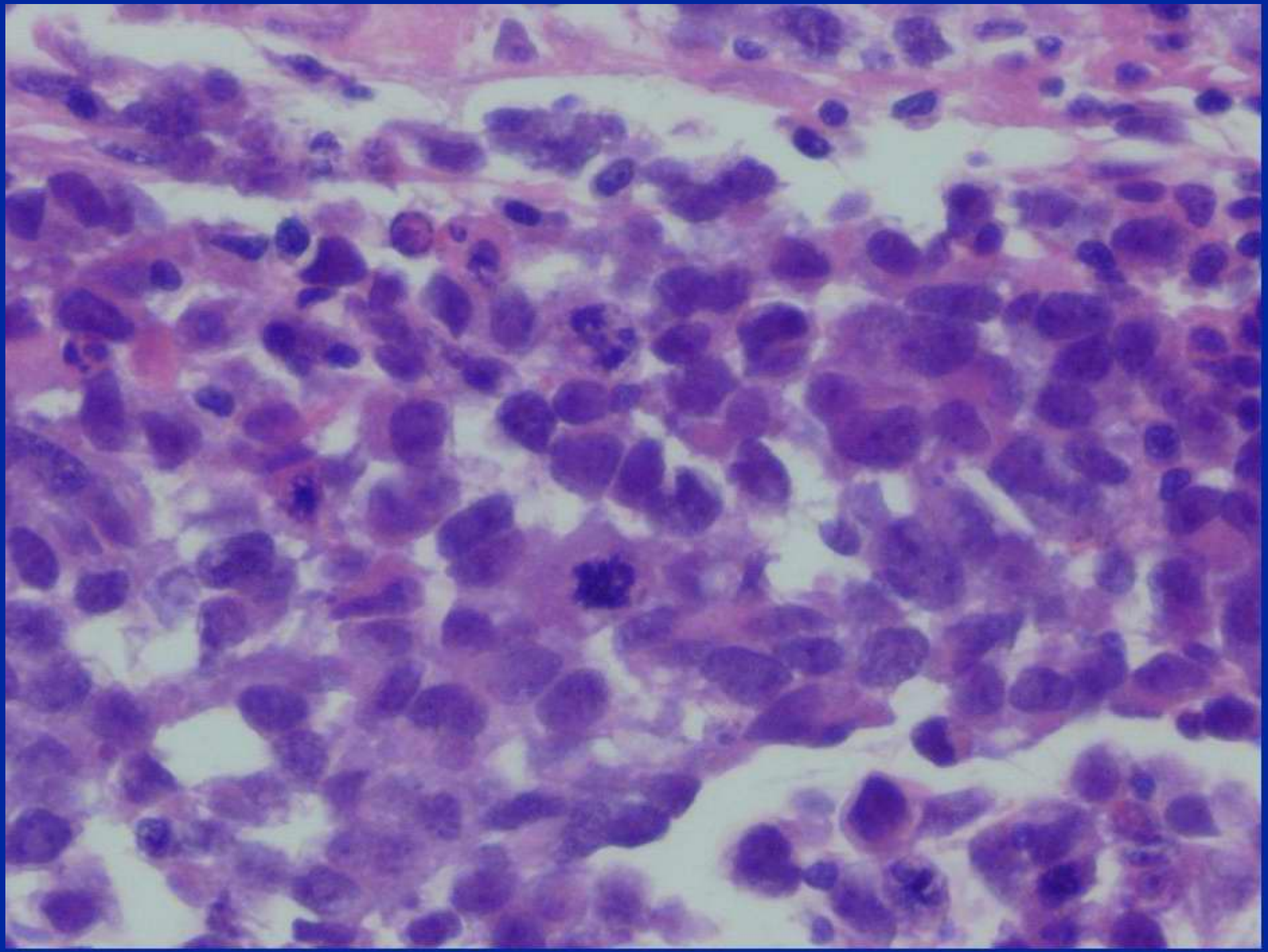
- Sheets of clear cells, well defined cytoplasmic borders, “squared-off” central large nuclei, amphophilic nucleoli, fibrous bands, granulomatous-lymphocytic host reaction.
- IHC: PLAP(+), C-kit (+), OCT3/4 (+), AE1/AE3(-).

- “Anaplastic” seminoma:
  - Terminology is now generally discouraged.
  - No Prognostic or Rx implications.
  - Poor fixation can lead to plasmocytic or “anaplastic” morphology.

- True focal transformation into an embryonal carcinoma component can be confirmed by a contrasting IHC staining pattern (AE1/AE3 + & CD30 +).
- MSKCC group: seminoma with atypia ?







Seminoma

"Problematic" Variants

- **Intertubular (Interstitial) Seminoma:**
  - can be underdiagnosed as orchitis.
  - grossly non palpable.
  - discovered during infertility W/U.
  - can be obscured by the host response or admixture with Leydig cells.
  - identifying an interstitial seminoma component will lead to a more accurate GCT size estimates for staging.

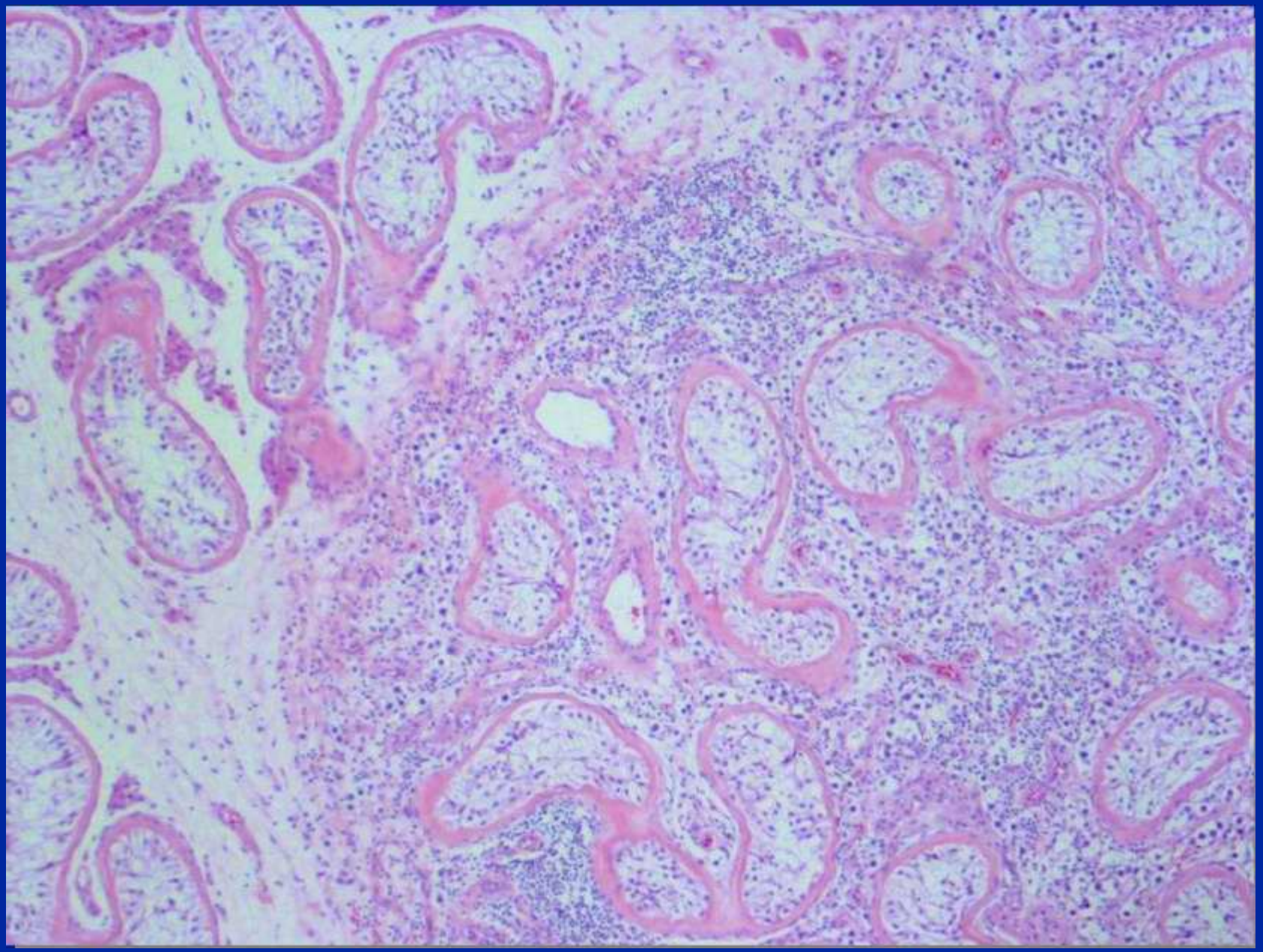
- **Microcystic Seminoma:**
  - Edema?
  - may mimic YST
  - lined by polygonal rather than flattened cells; exfoliated cells; inflammatory response
  - IHC panel: C-kit+, OCT3/4+, AFP-, AE1/AE3 -

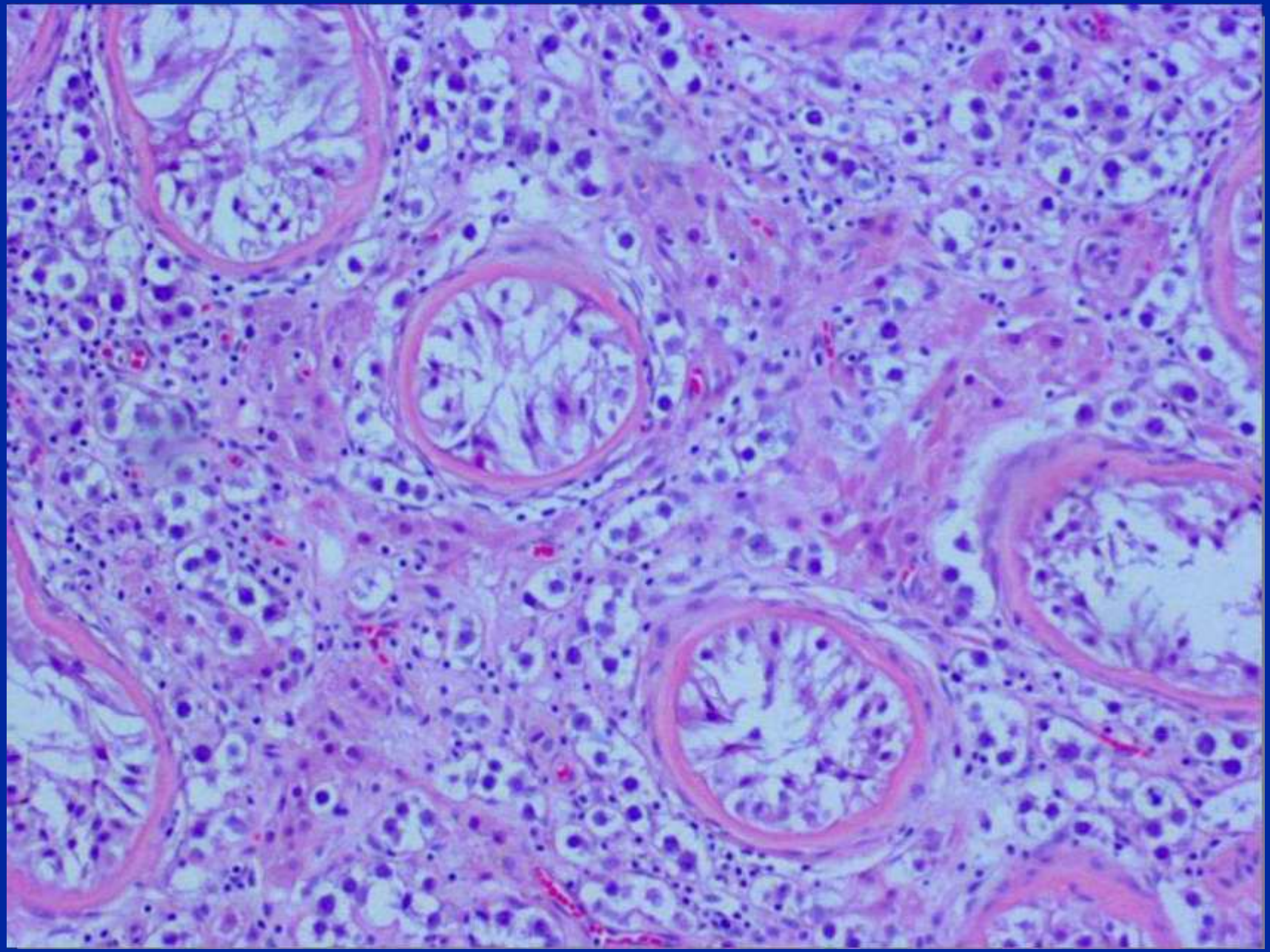
Seminoma

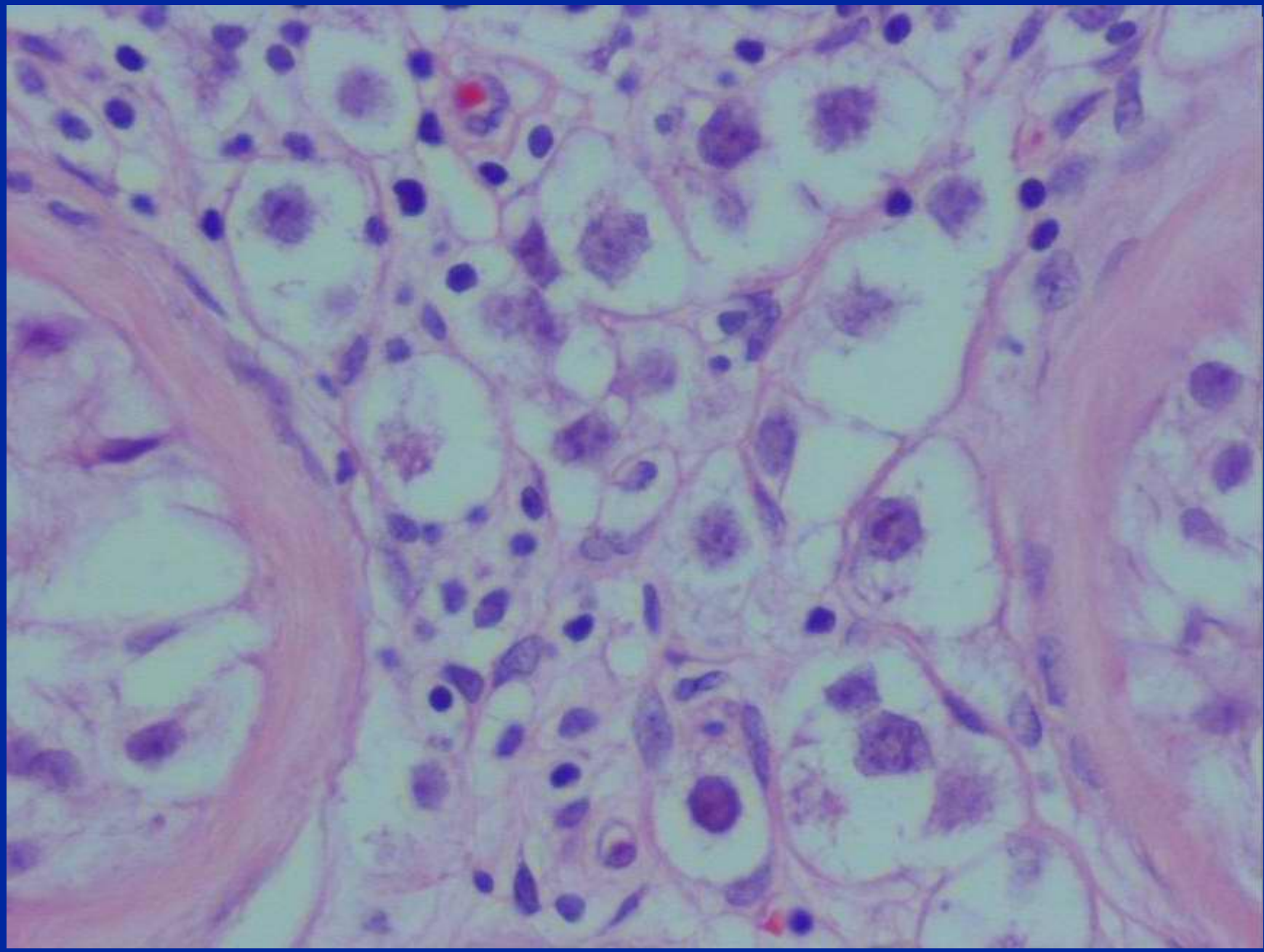
"Problematic" Variants

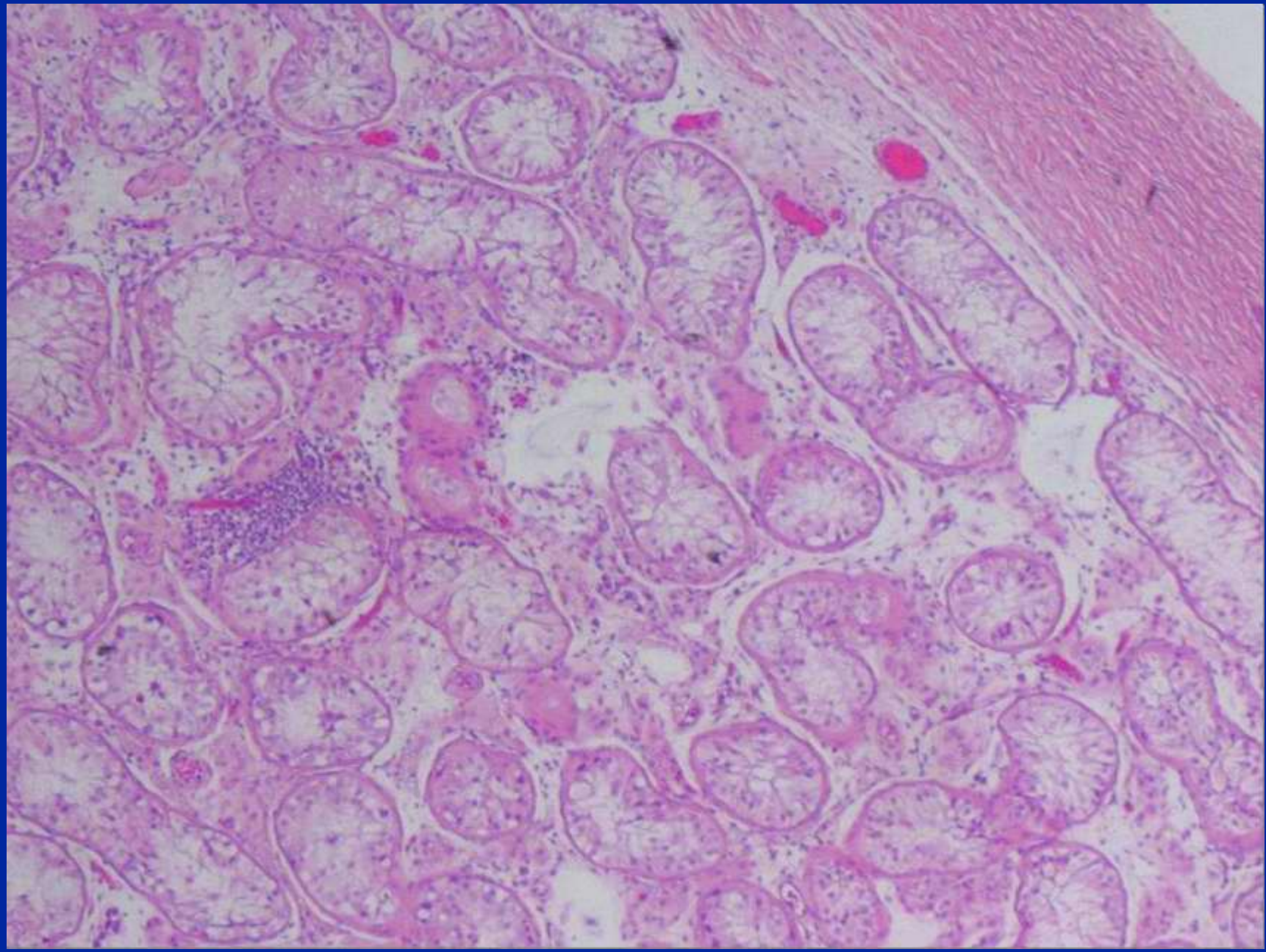
- **Tubular Seminoma:**
  - Can mimic Sertoli cell tumor
  - IHC panel: C-kit + ,OCT3/4 +, Inhibin -

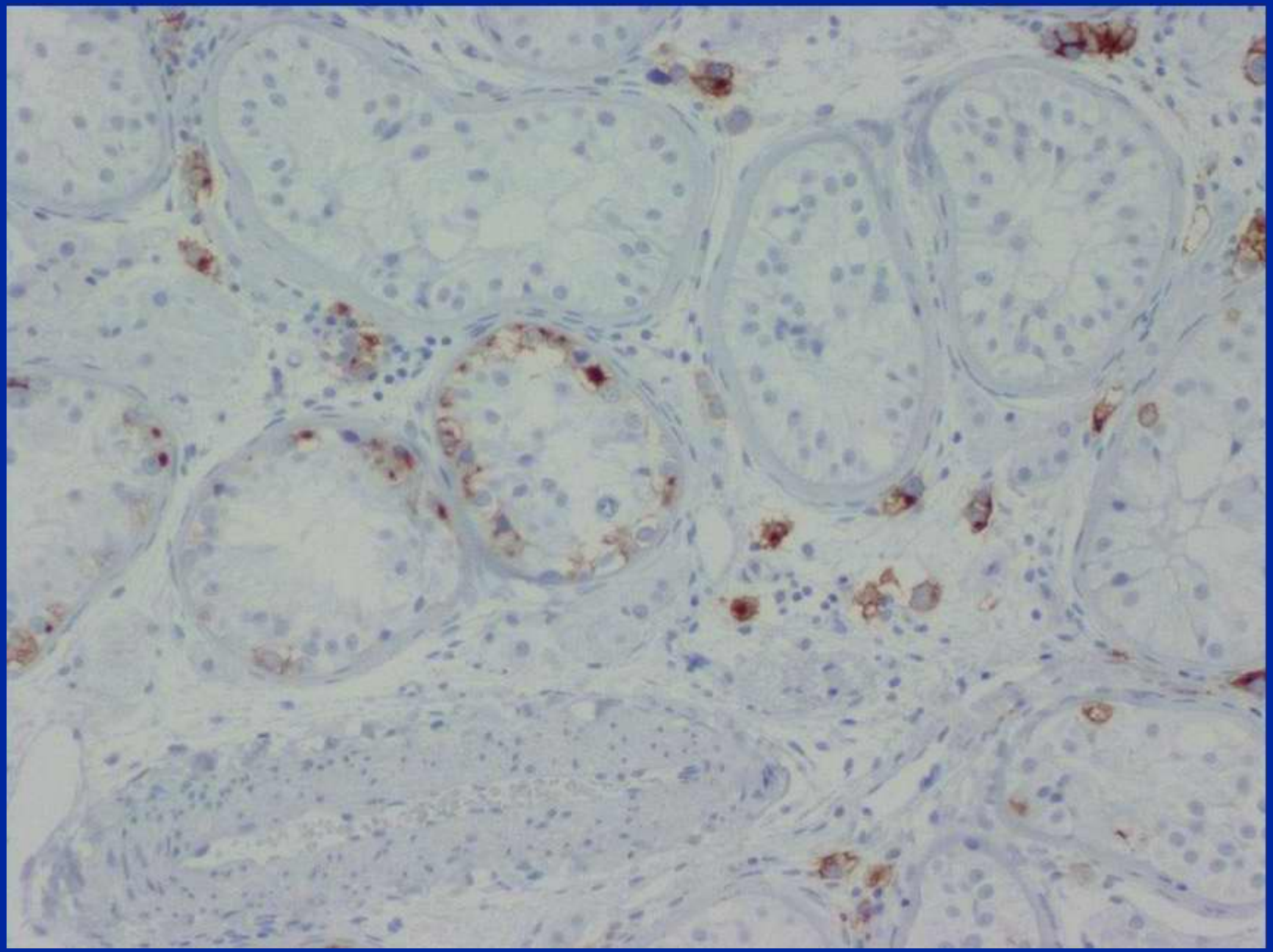
- **Seminoma with prominent syncitiotrophoblast:**
  - 5% of classic seminoma have scattered syncitiotrophoblasts.
  - when clustered (adjacent to areas of hemorrhage) may mimic a component of choriocarcinoma.
  - lack of an admixed cytotrophoblastic component.
  - only clinical relevance: help explain an associated hCG levels/hormonal manifestations

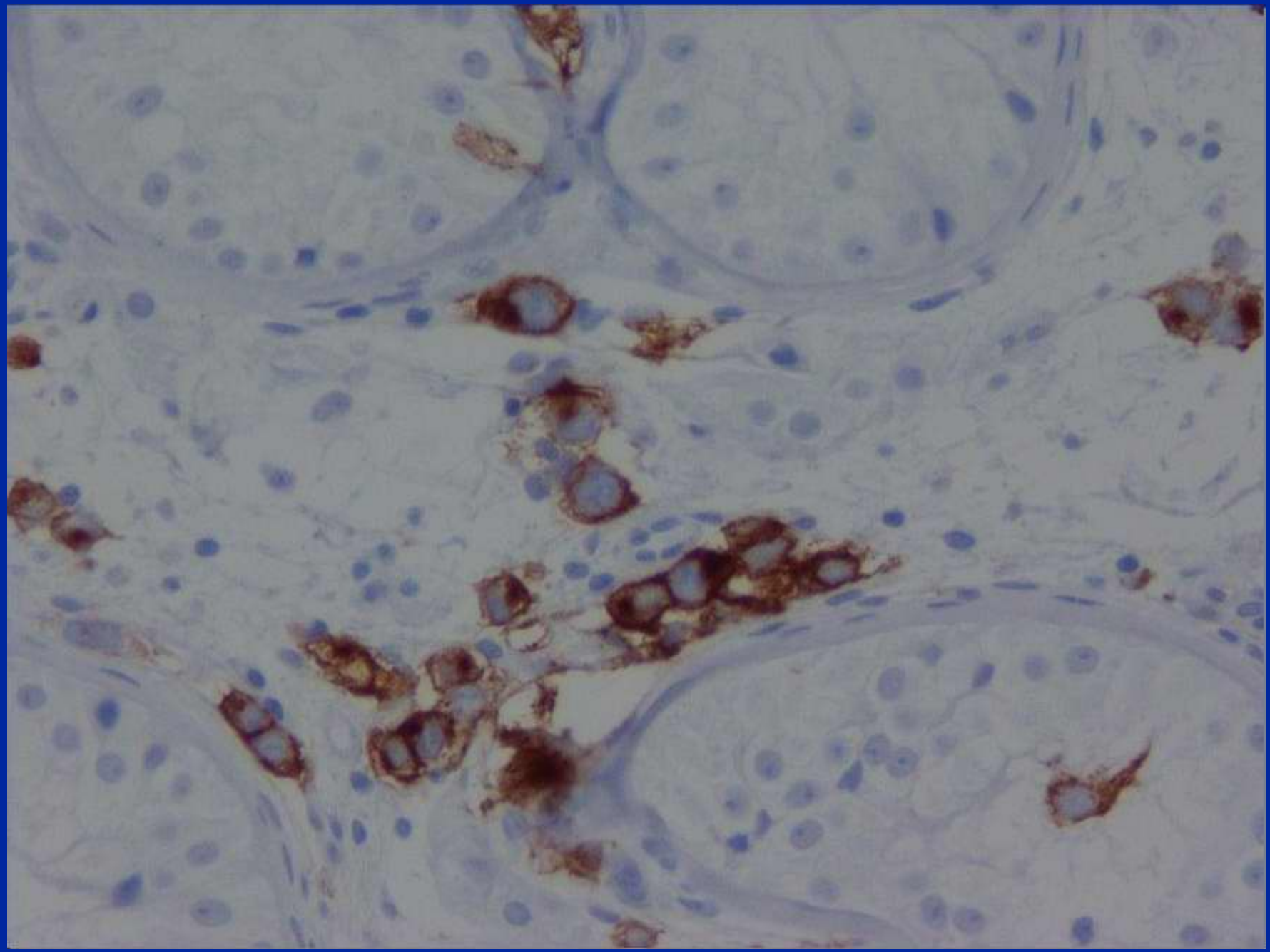


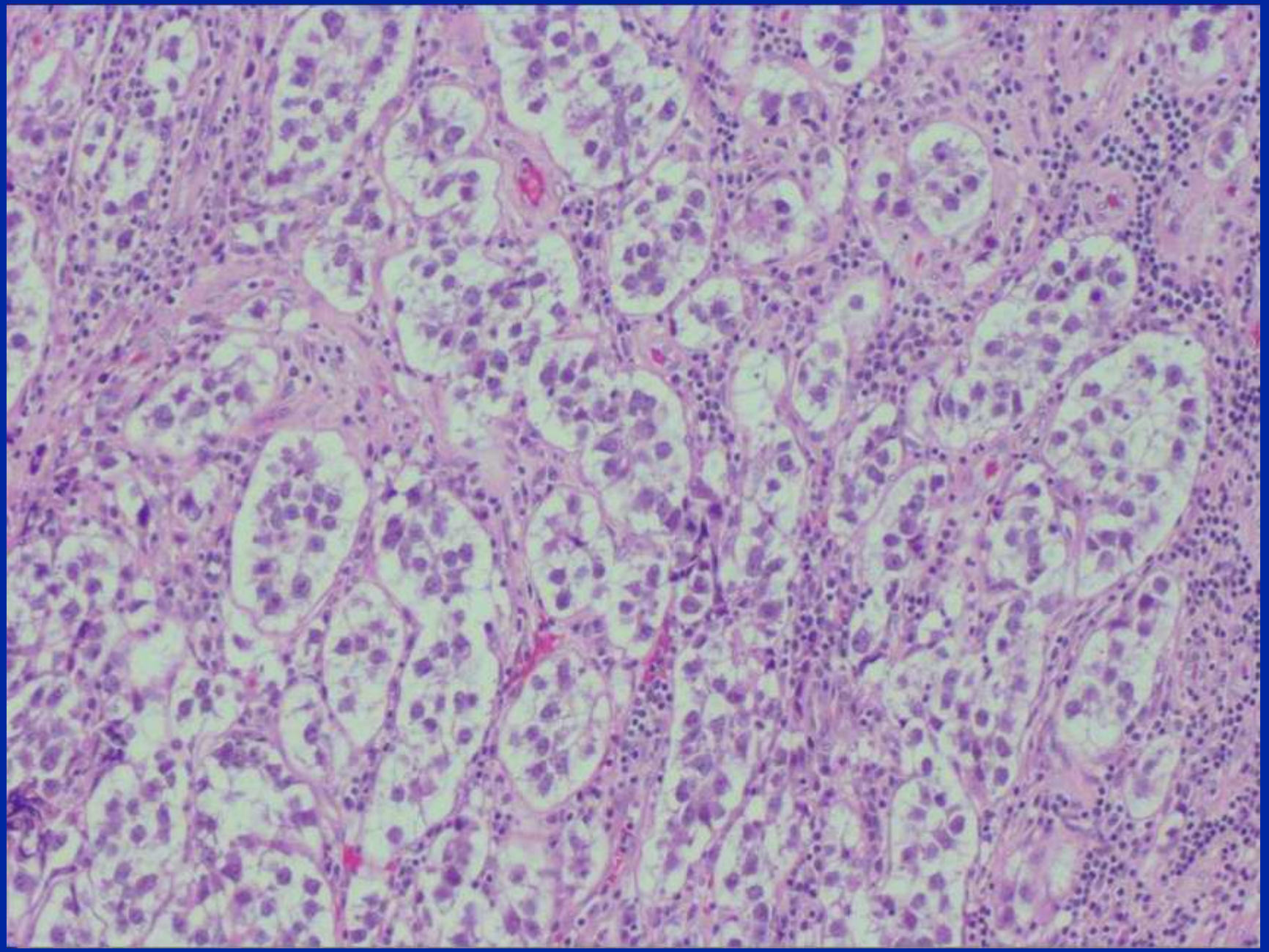


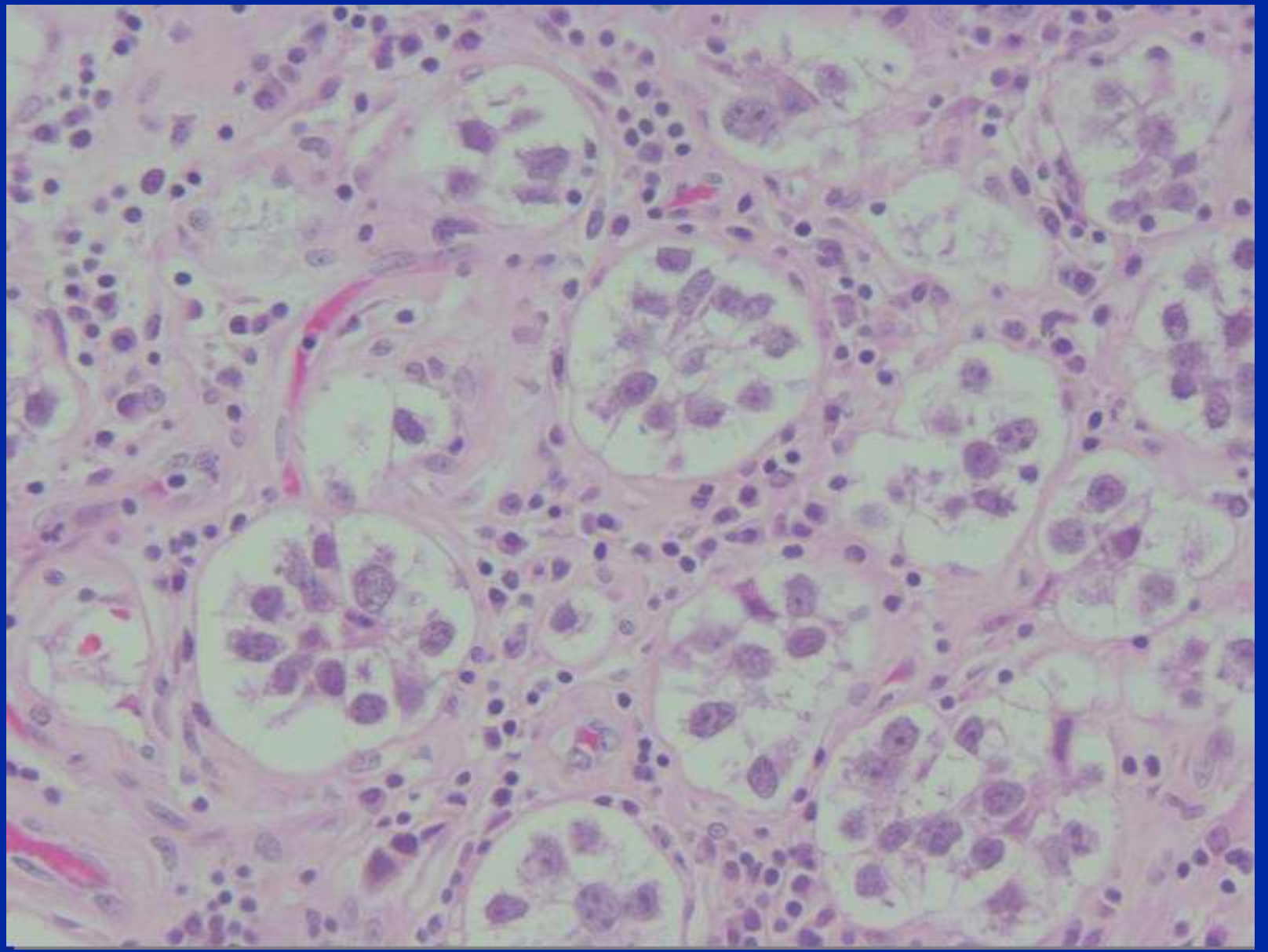


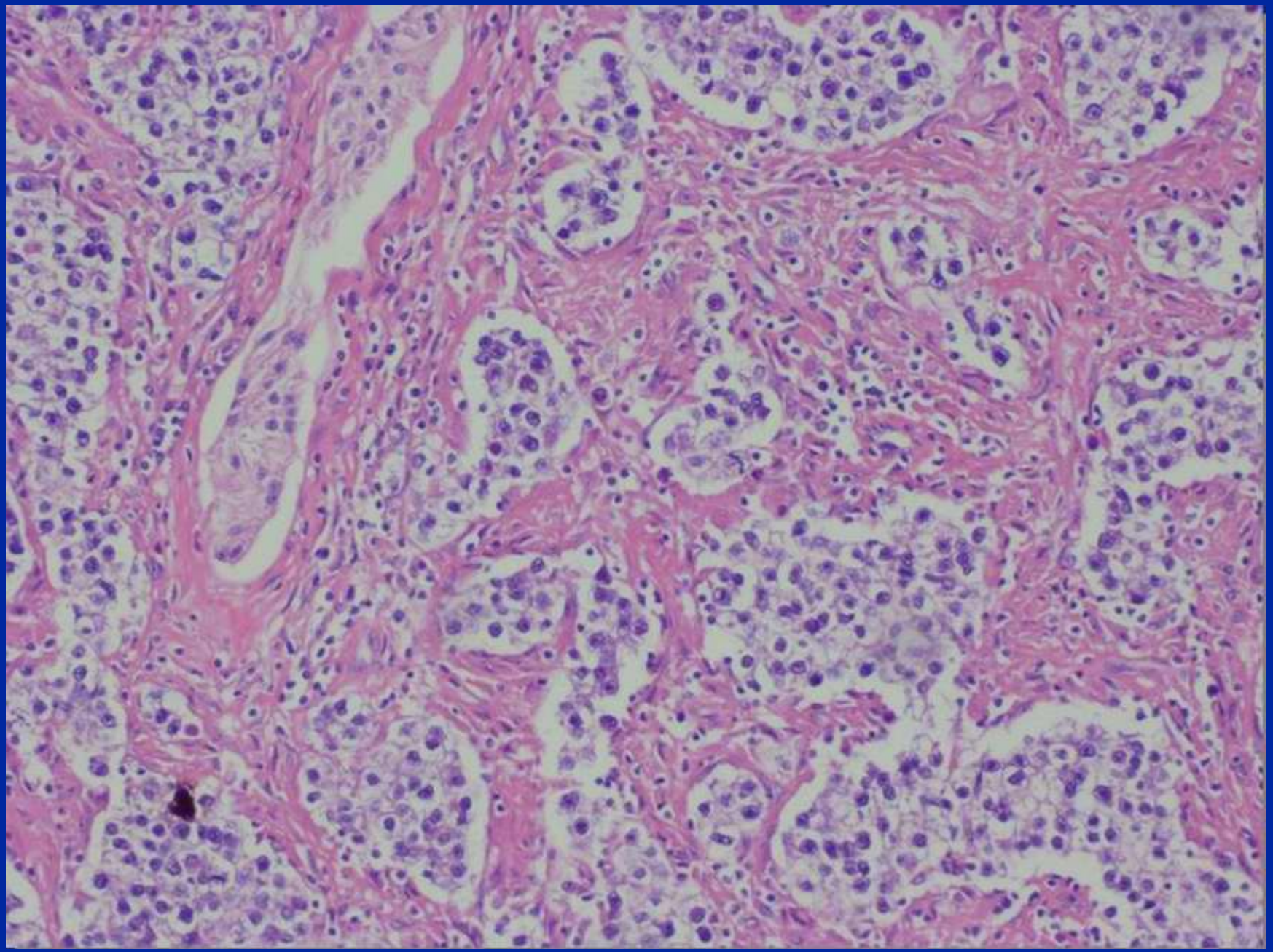


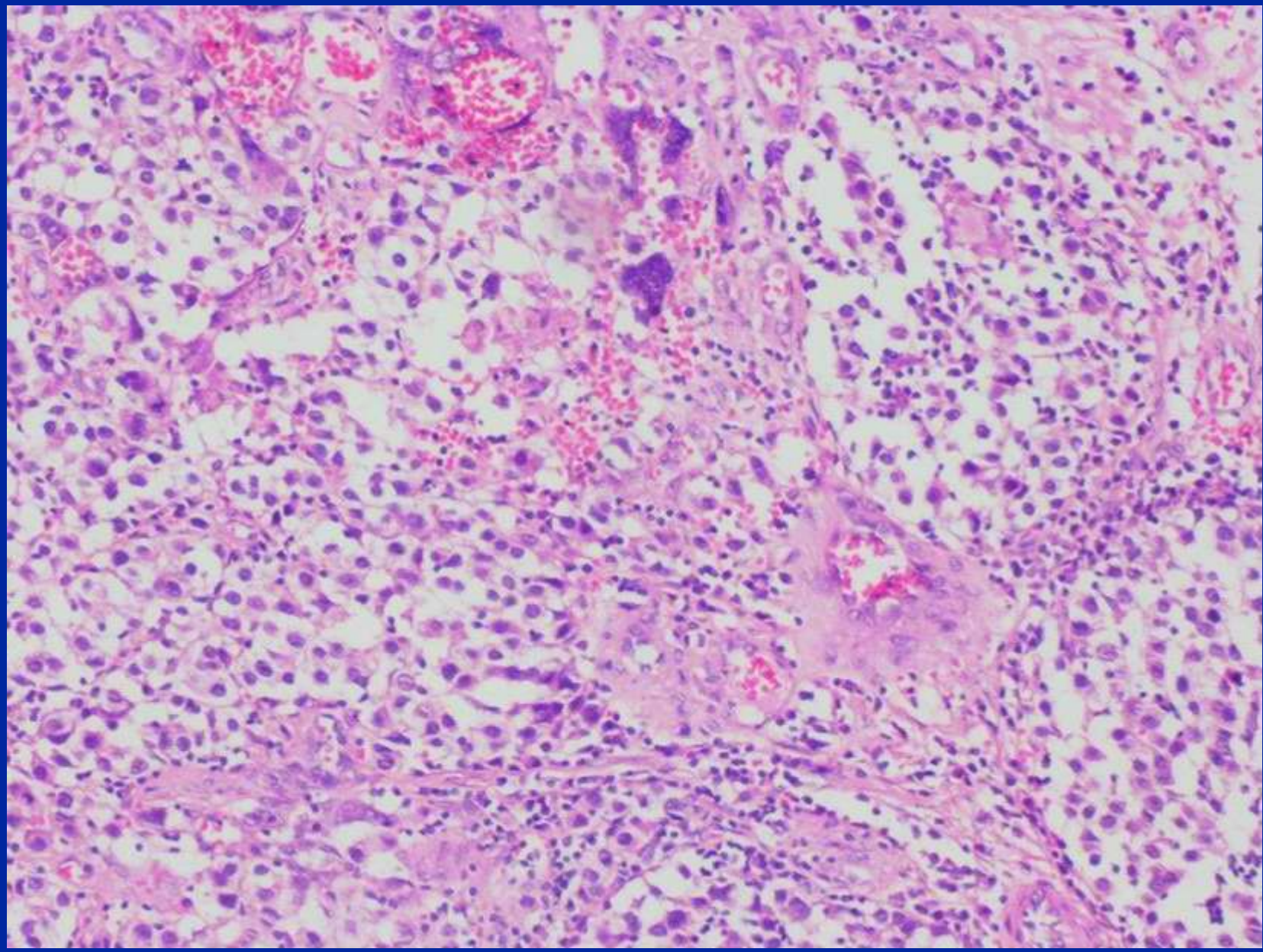


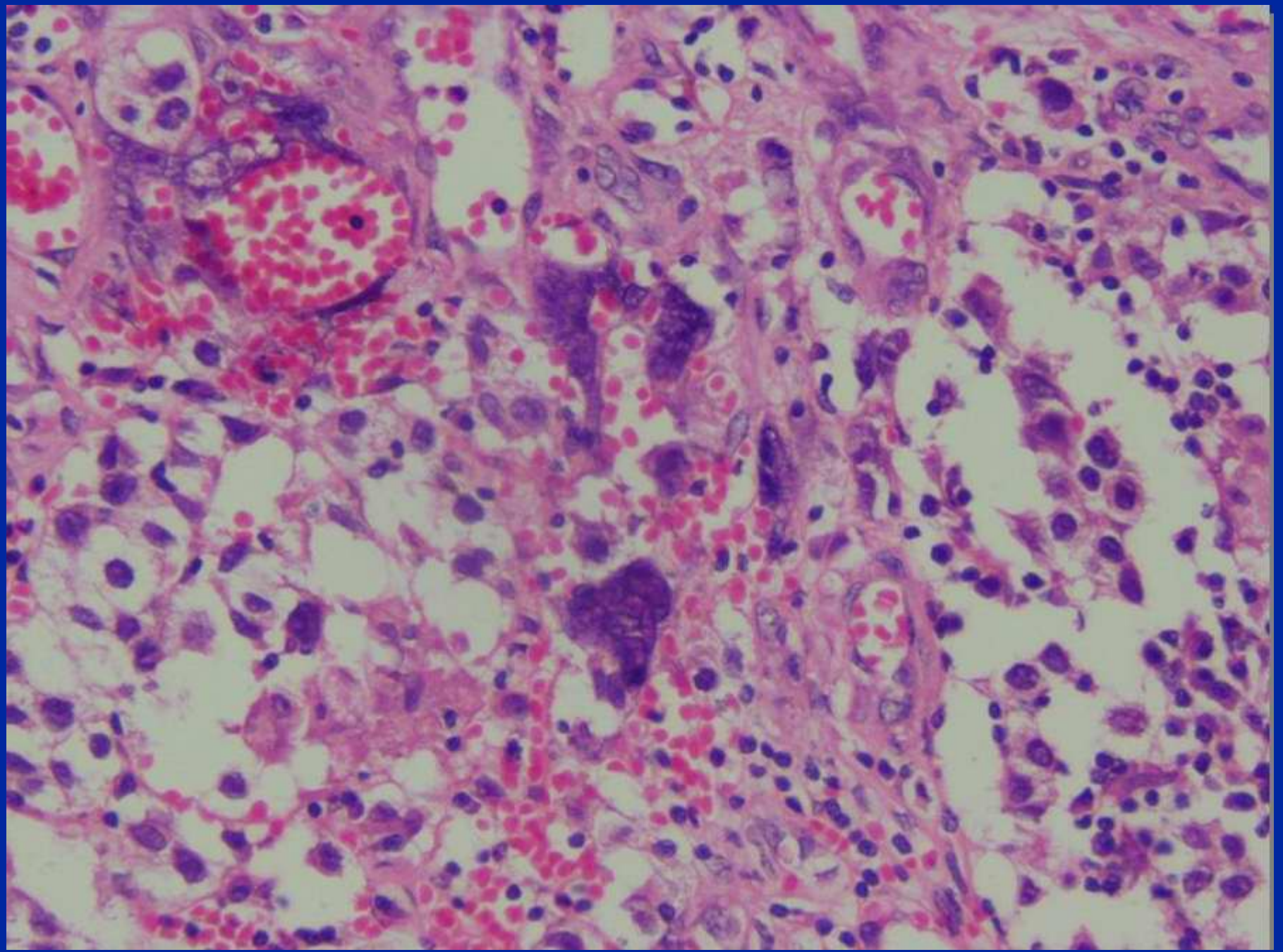












Germ Cell Tumors
Spermatocytic Seminoma

Clinical Features:

- Older patients (average age: sixth decade), rare before 30 years of age.
- Occurs only in testicular location.
- No association with cryptorchidism.
- No ovarian counterpart.
- EXELLENT prognosis.
- Rx: orchiectomy ONLY

- Aggressive behavior seen only in rare cases with associated “sarcomatous” component.

Germ Cell Tumors
Spermatocytic Seminoma

Histopathology

- Hallmark: cytologic polymorphism/round nuclear shape
- Three cell types : small, intermediate and large
- “spiremic” filamentous chromatin pattern.
- Can have brisk mitotic activity.
- Lack IGCNU component or lymphogranulomatous host response
- No other non-seminomatous GCT component.
- Rare examples with focal areas of a more monotonous large cells may raise DDX of Embryonal Ca.
- IHC: PLAP (absent to scant); OCT3/4 -; C-kit +/-; AE1/AE3-; CD30 -

Germ Cell Tumors
Spermatocytic Seminoma

Histopathology

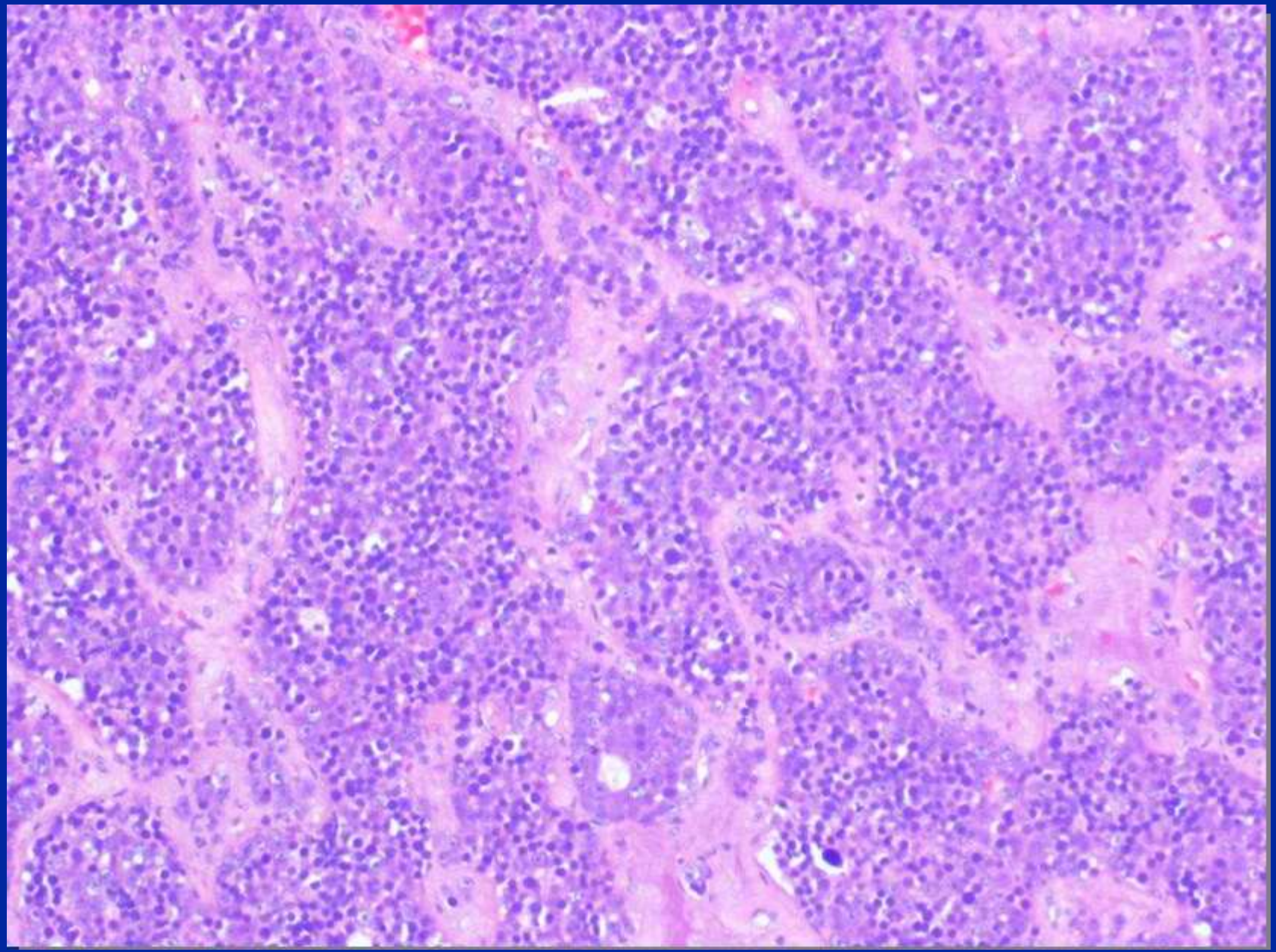
- **Spermatocyic Seminoma with sarcomatous component:**

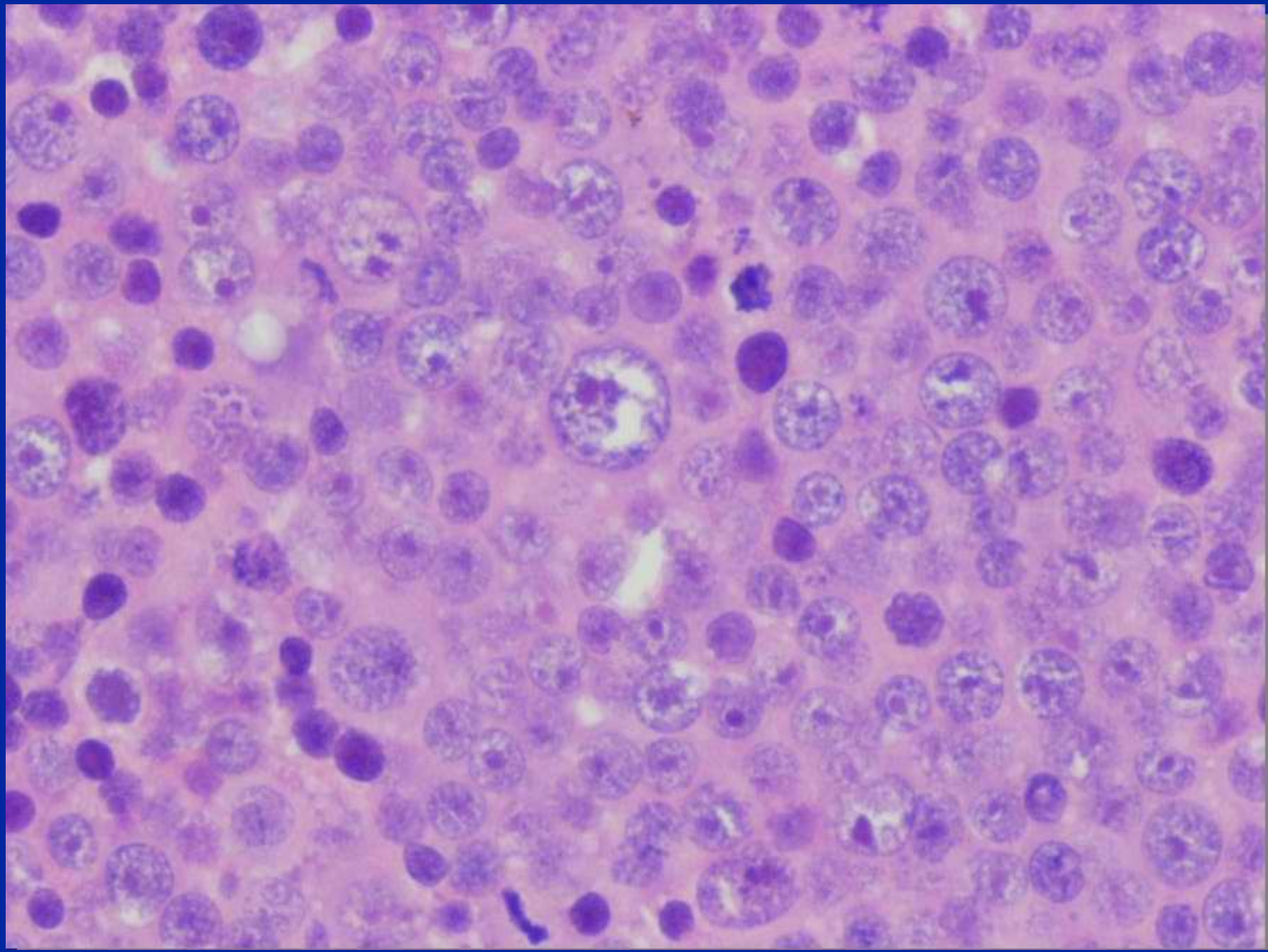
  - very rare occurrence(12 cases)
  - undifferentiated sarcoma
  - metastasis in 50% of cases
  - DDX: malignant transformation in other GCT

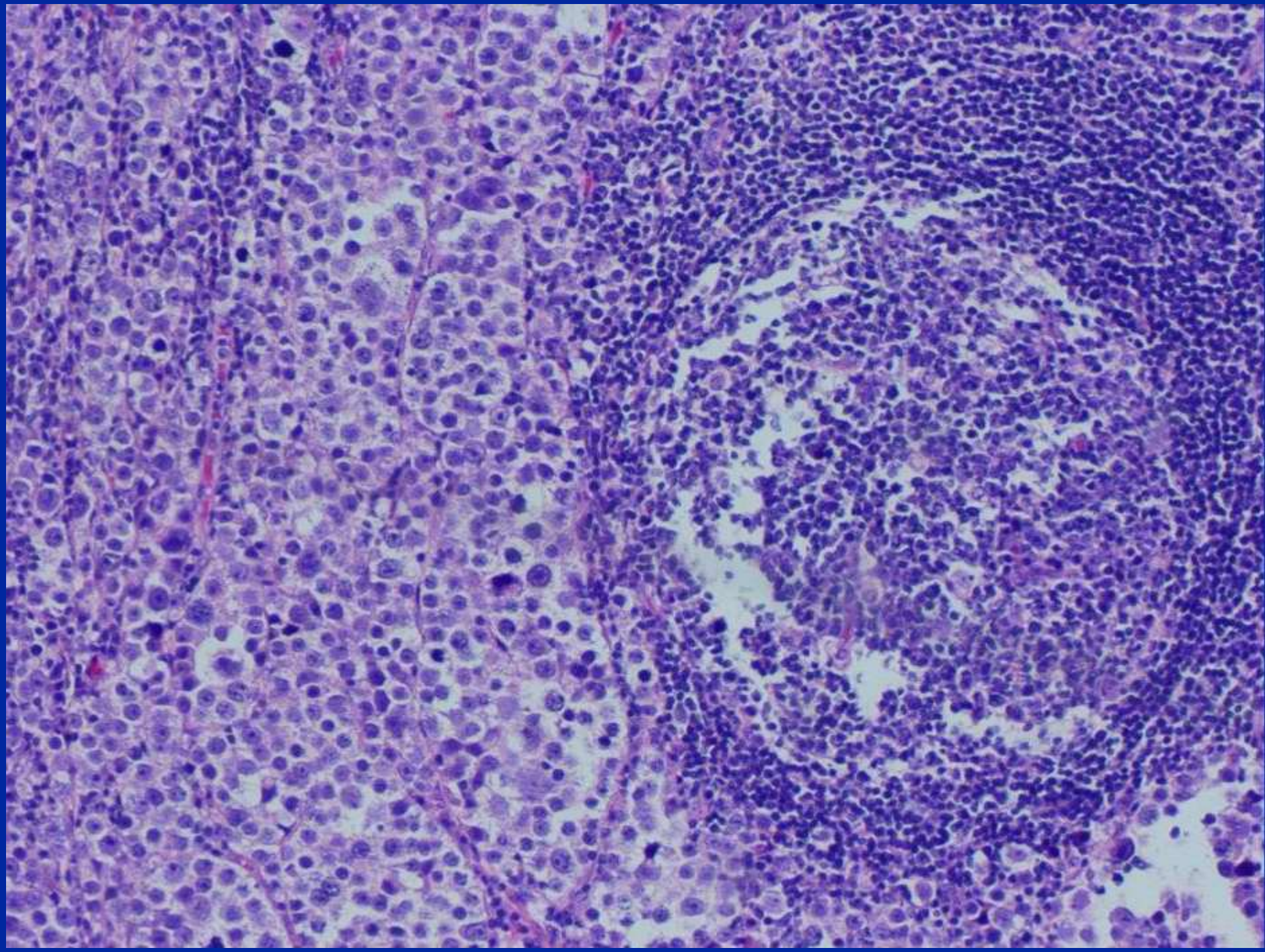
Germ Cell Tumors
Spermatocytic Seminoma

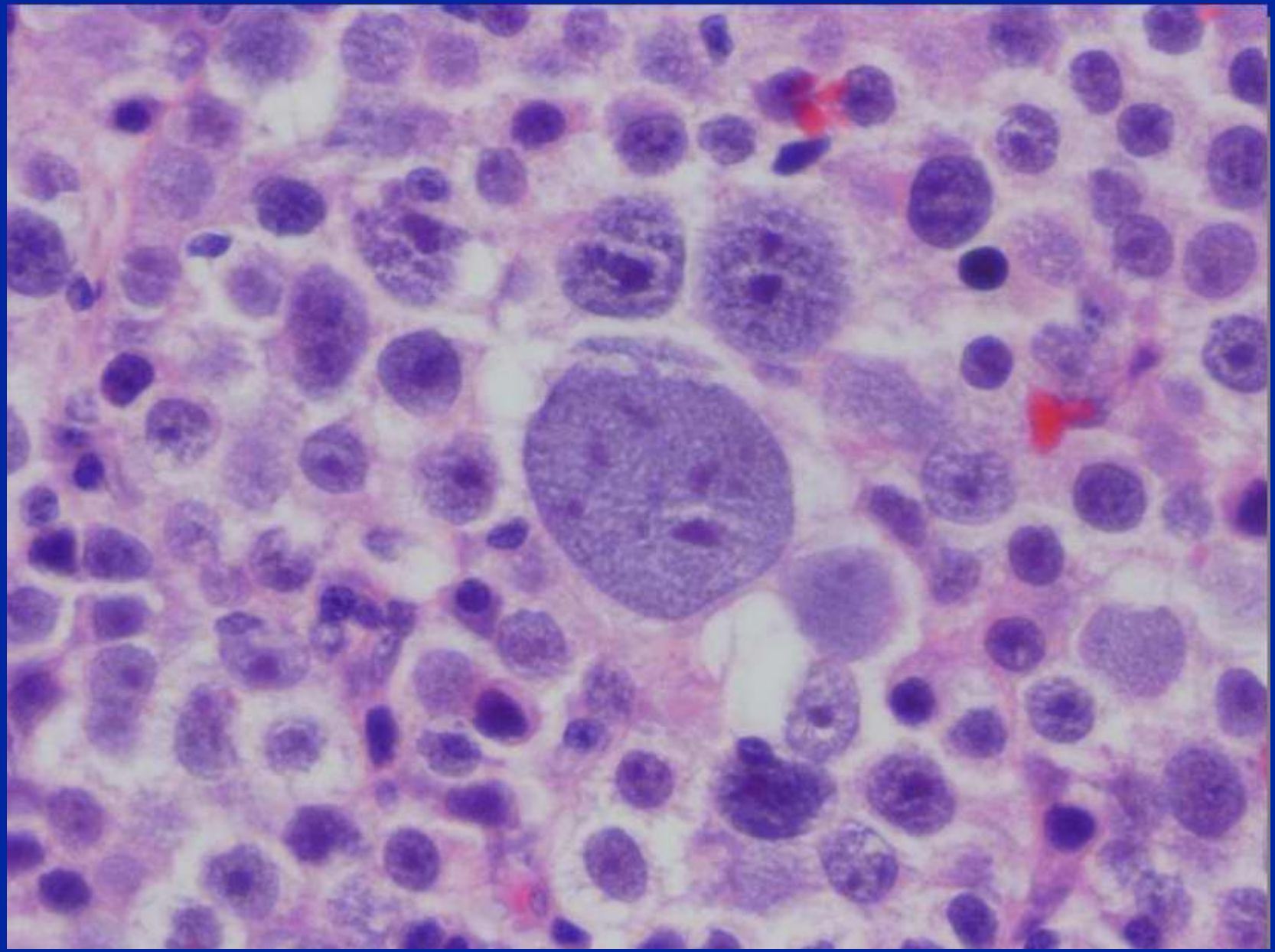
***Looijenga LH et al. Cancer Res. 2006*:**

- **Genomic analysis: karyotyping; SKI; array CGH and gene expression profiling.**
- **Spermatocytic Seminoma expressed markers specific to prophase meiosis I while Classic Sem. expressed stem cell markers such as OCT3/4 and CD133**
- **Support a "primary spermatocyte" origin for spermatocytic seminoma**
- **Chromosome 9 gains was the only consistently present karyotypic abnormality in S.Sem**
- ***DMRT1*: a male-specific transcriptional regulator on chromosome 9 likely candidate gene involved in S. Sem pathogenesis**



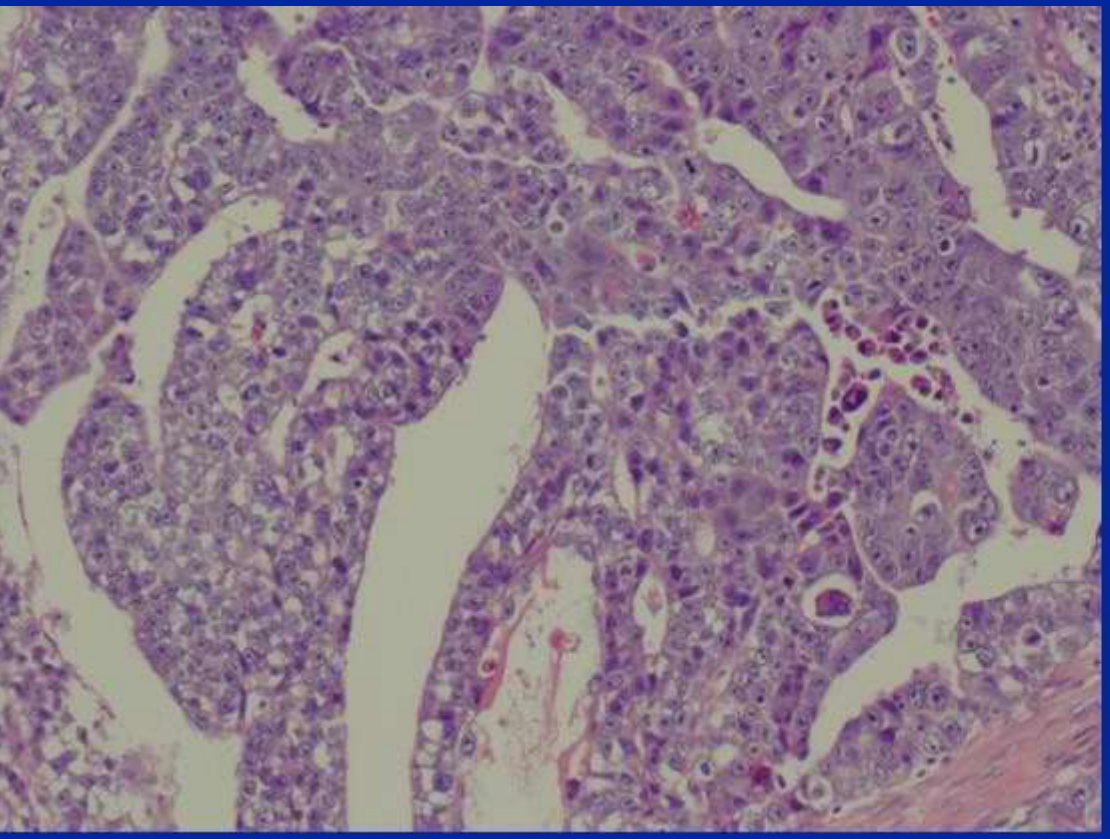


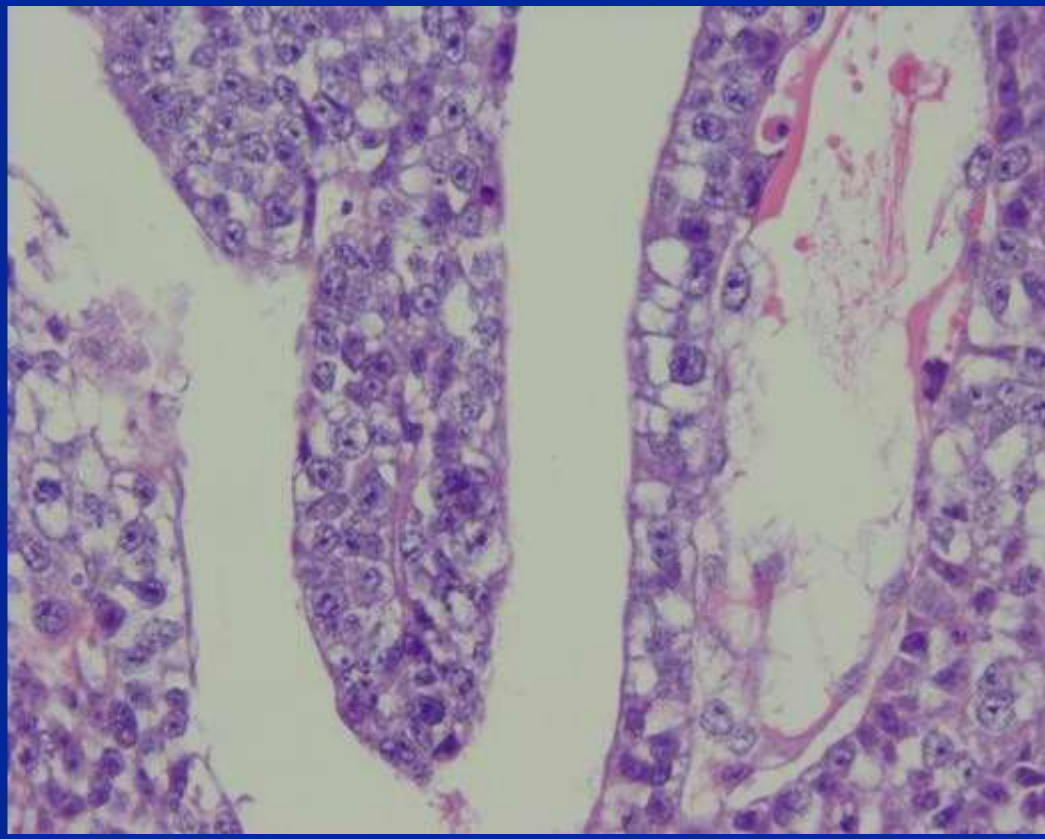


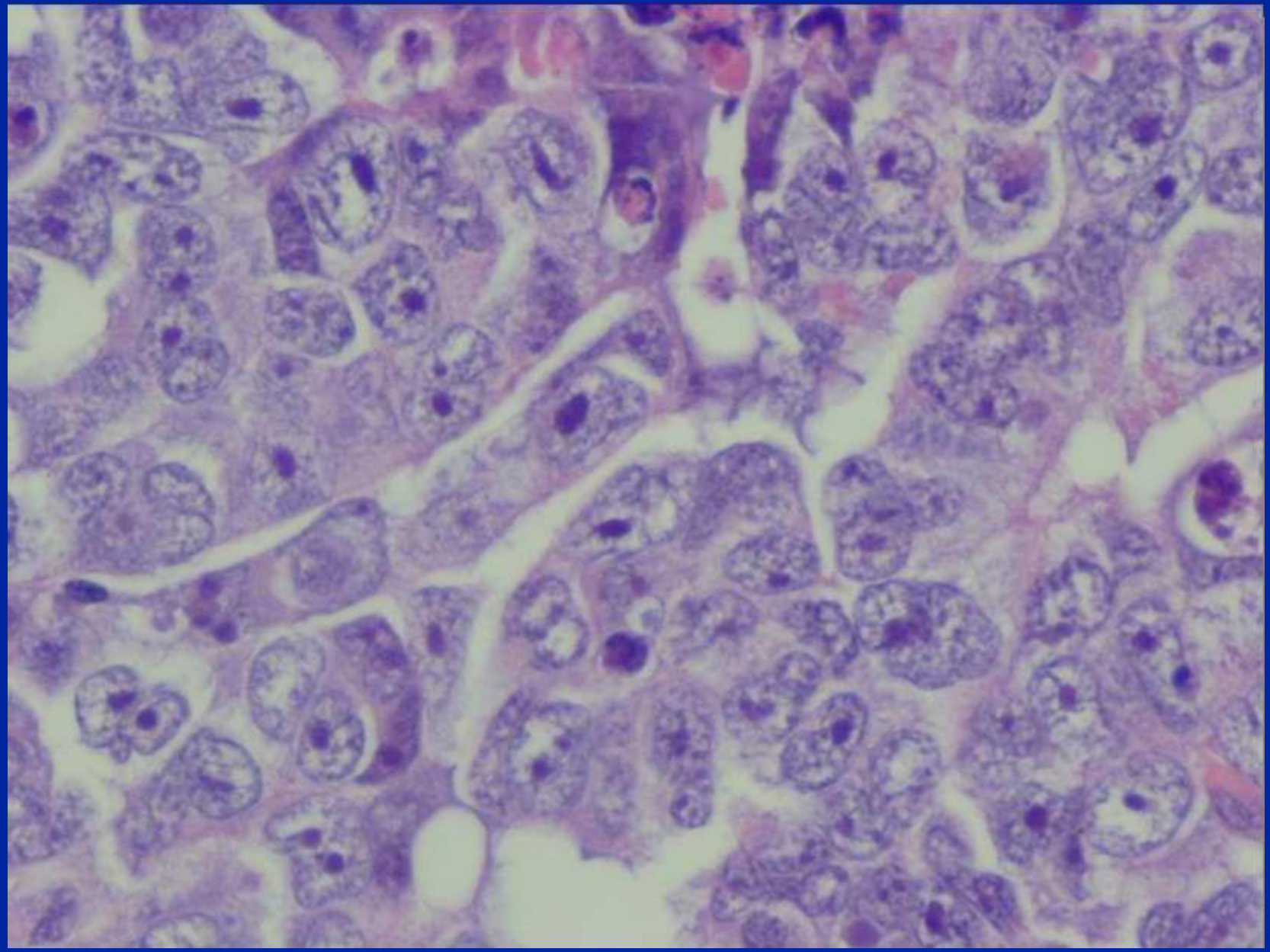


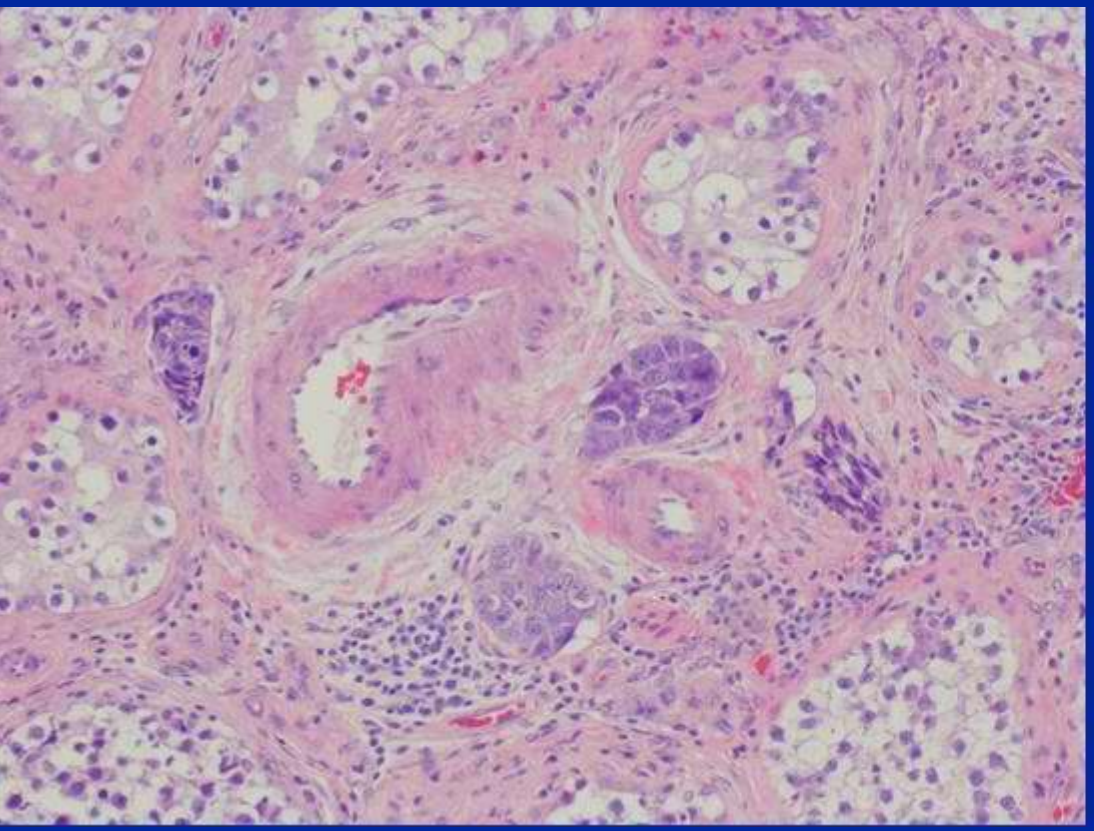
Embryonal Carcinoma

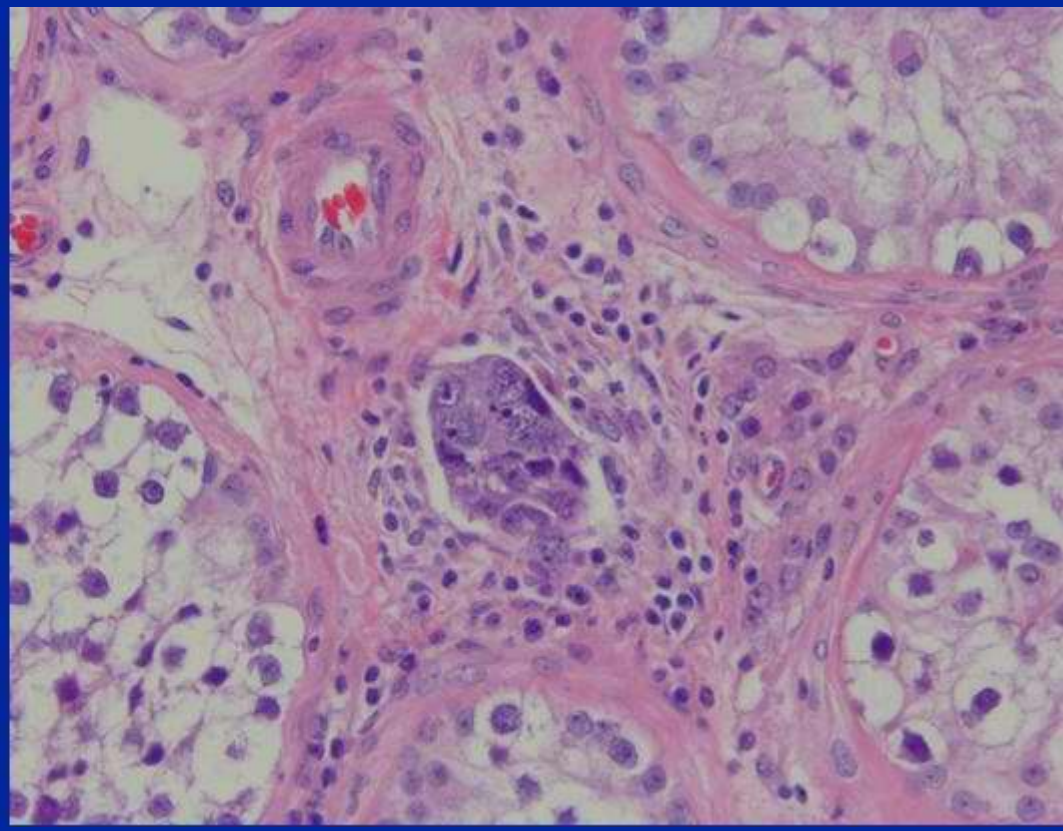
- Histology: solid sheets, tubular-papillary architecture, primitive cells with indistinct cell borders, marked nuclear atypia.
- IHC: AE1/AE3 (+), CD30 (+), C-kit (+/-), OCT3/4 (+), PLAP (+), AFP (+/-).
- Differentiating papillary areas of Emb. Ca. from YST: Cytologic features less primitive in YST.
- In extratesticular location (e.g. mediastinum) → DDX with metastatic adenocarcinoma. IHC: EMA (-), OCT3/4 (+), CD30 (+) is helpful.
- PGX: lymphovascular invasion. Percentage of embryonal in mixed GCT predictive of stage II (opposite of YST and Teratoma %)

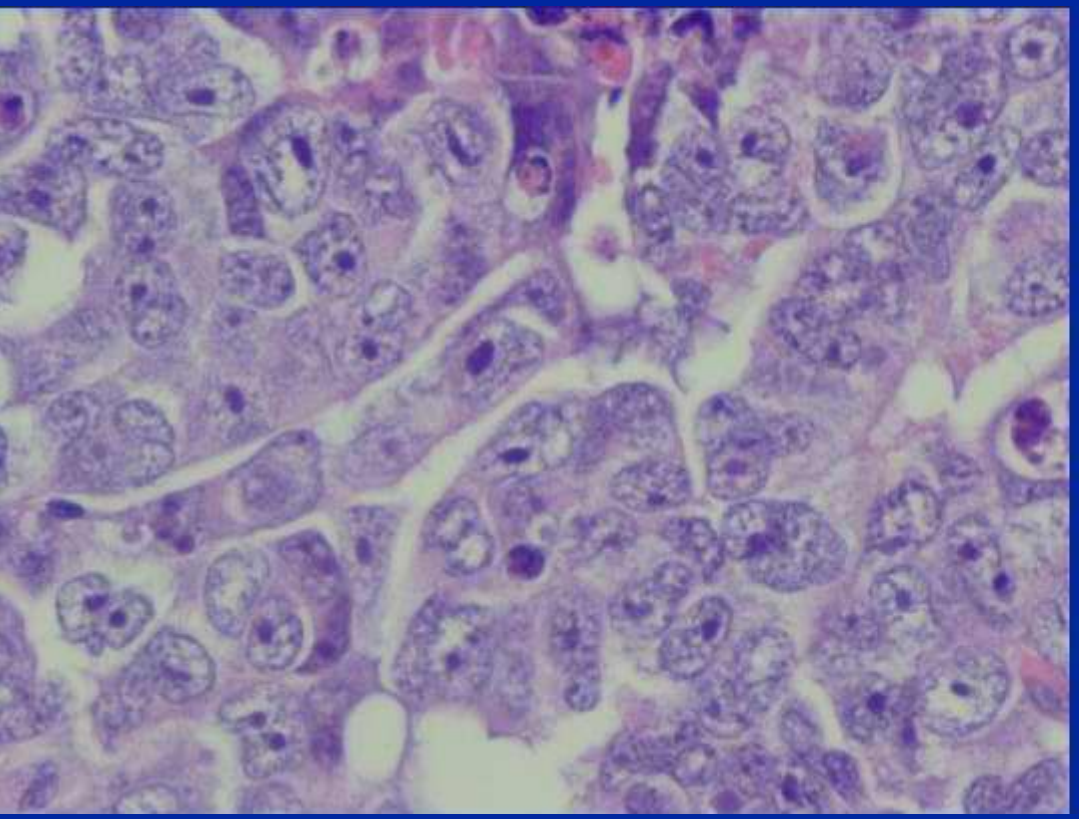




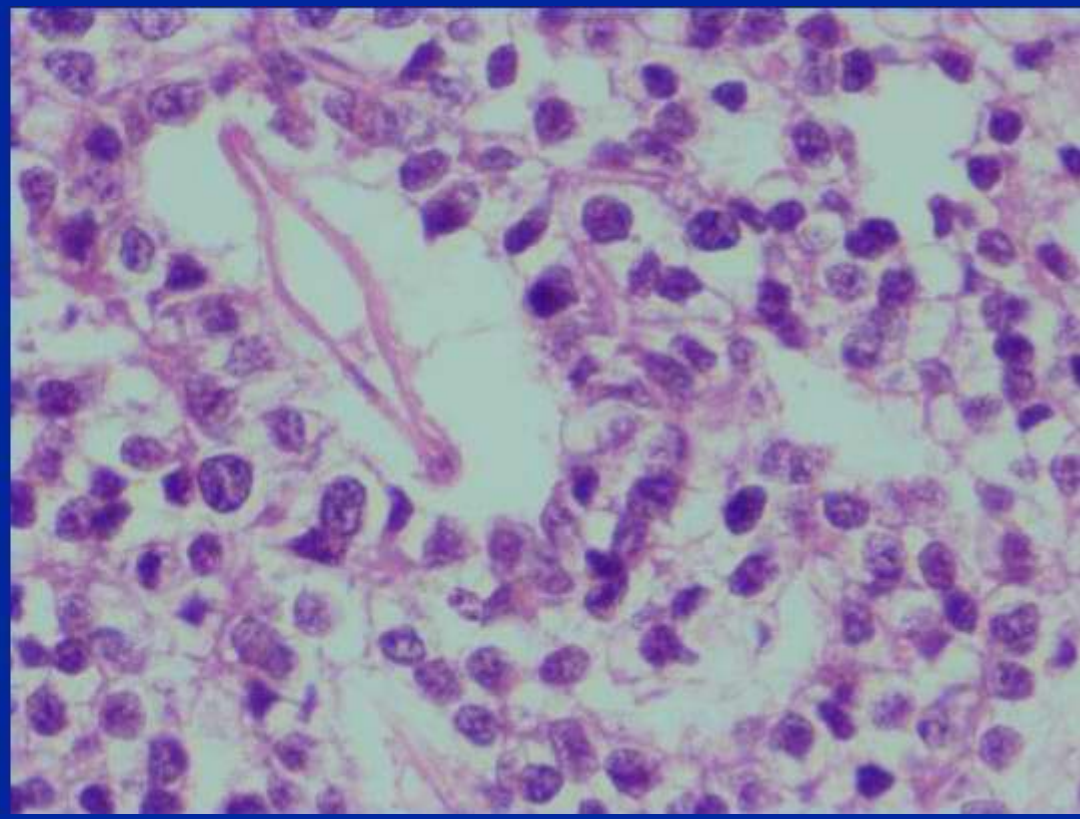








Emb Ca

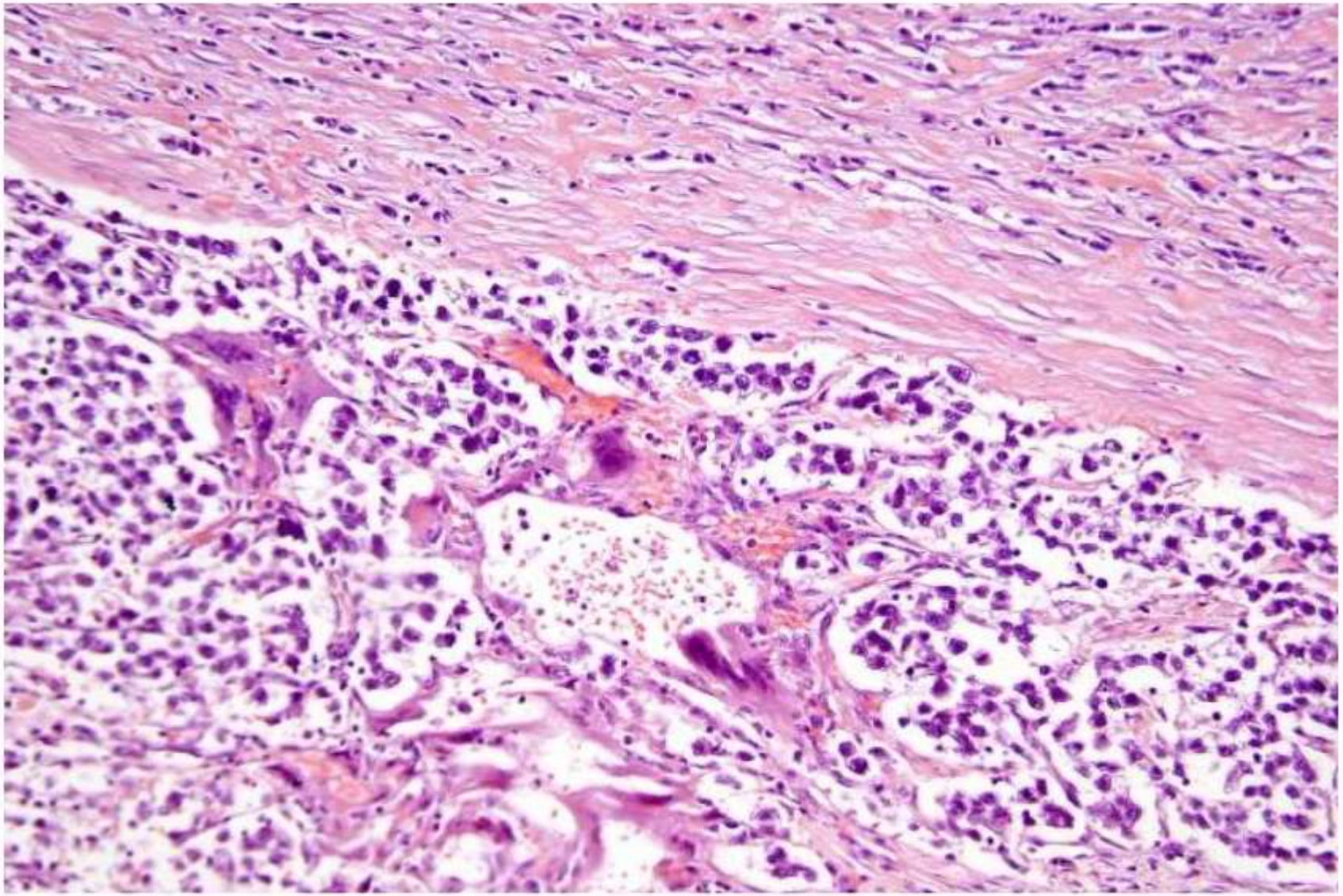


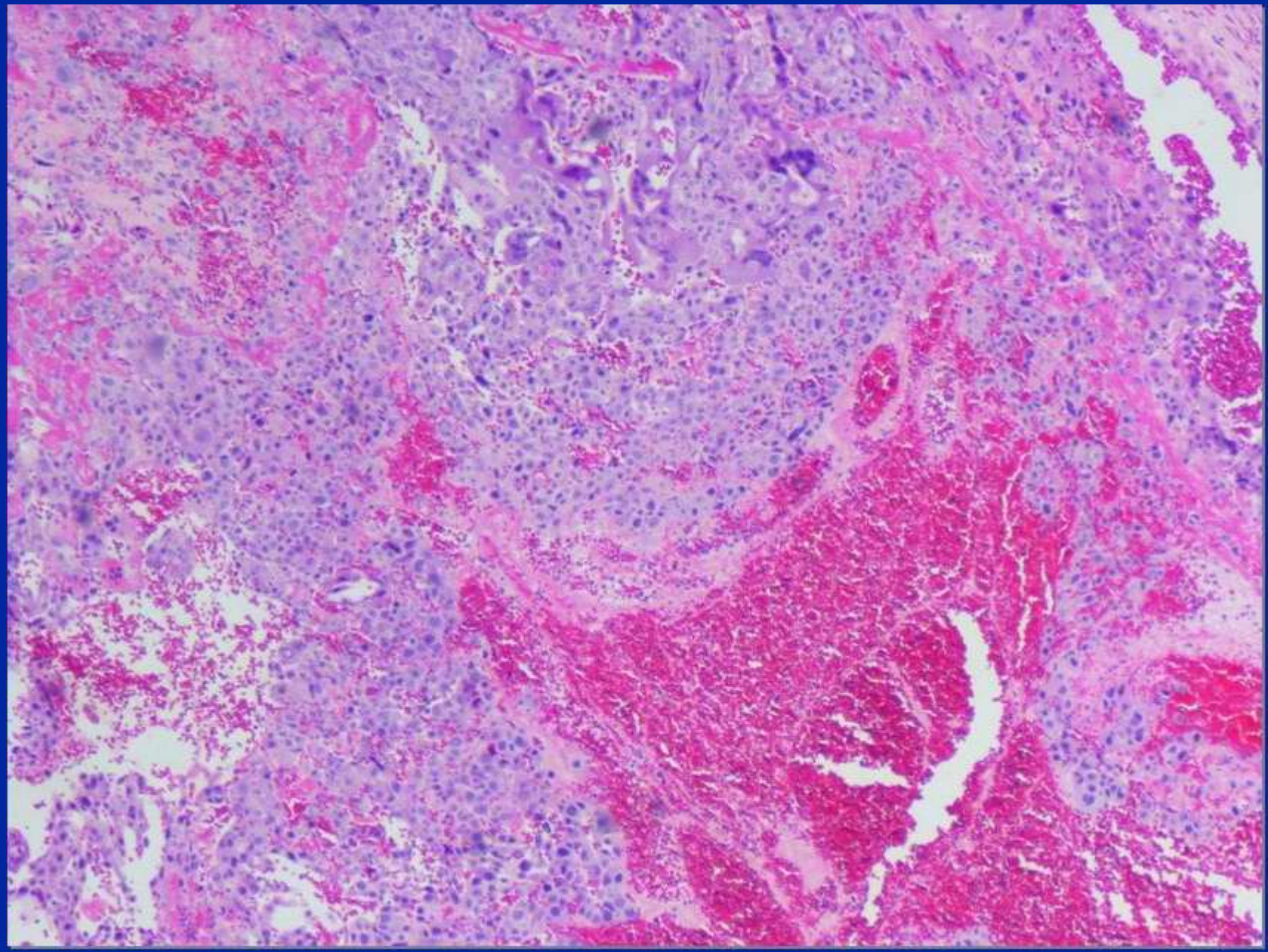
YST

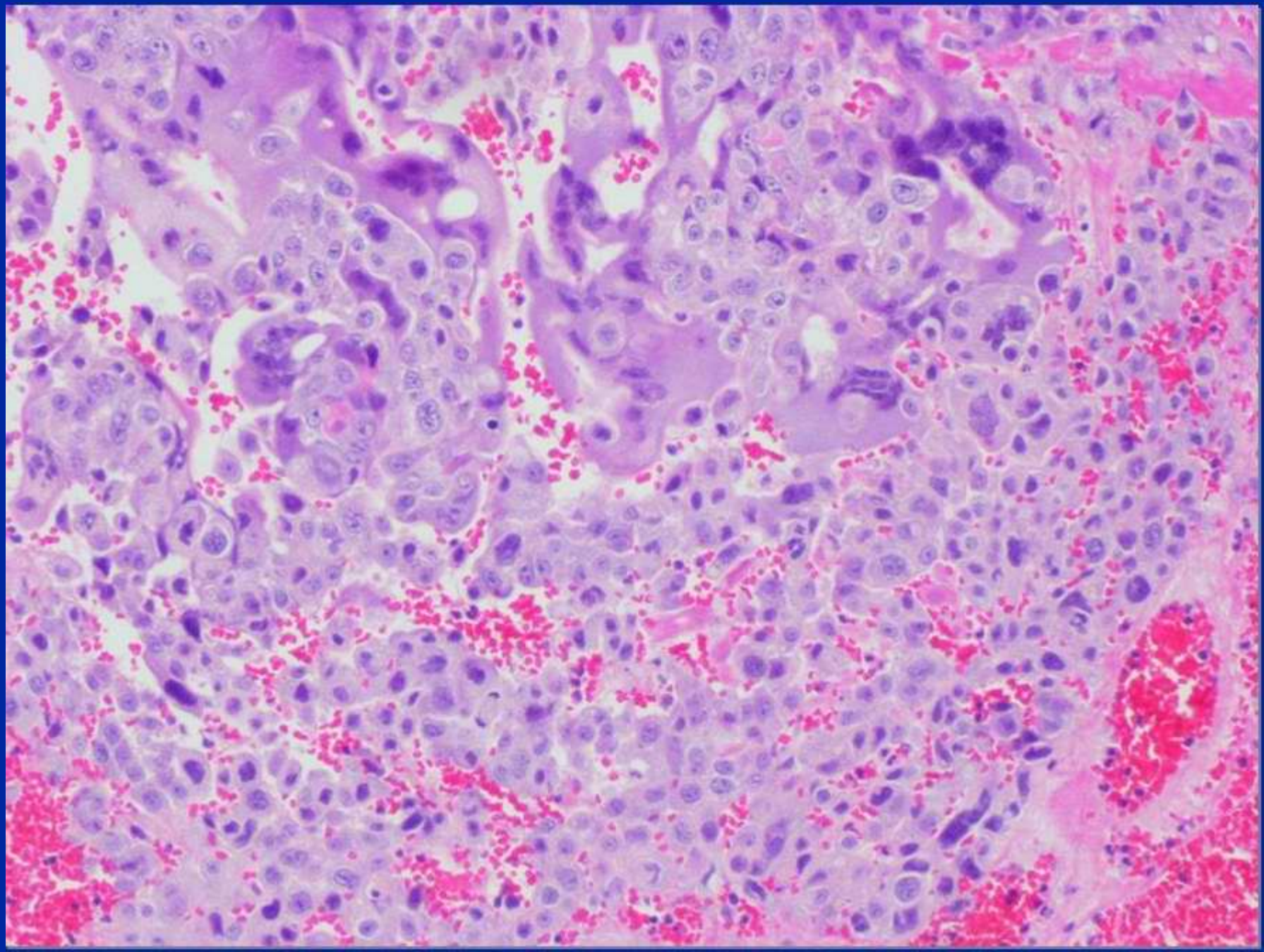
Choriocarcinoma

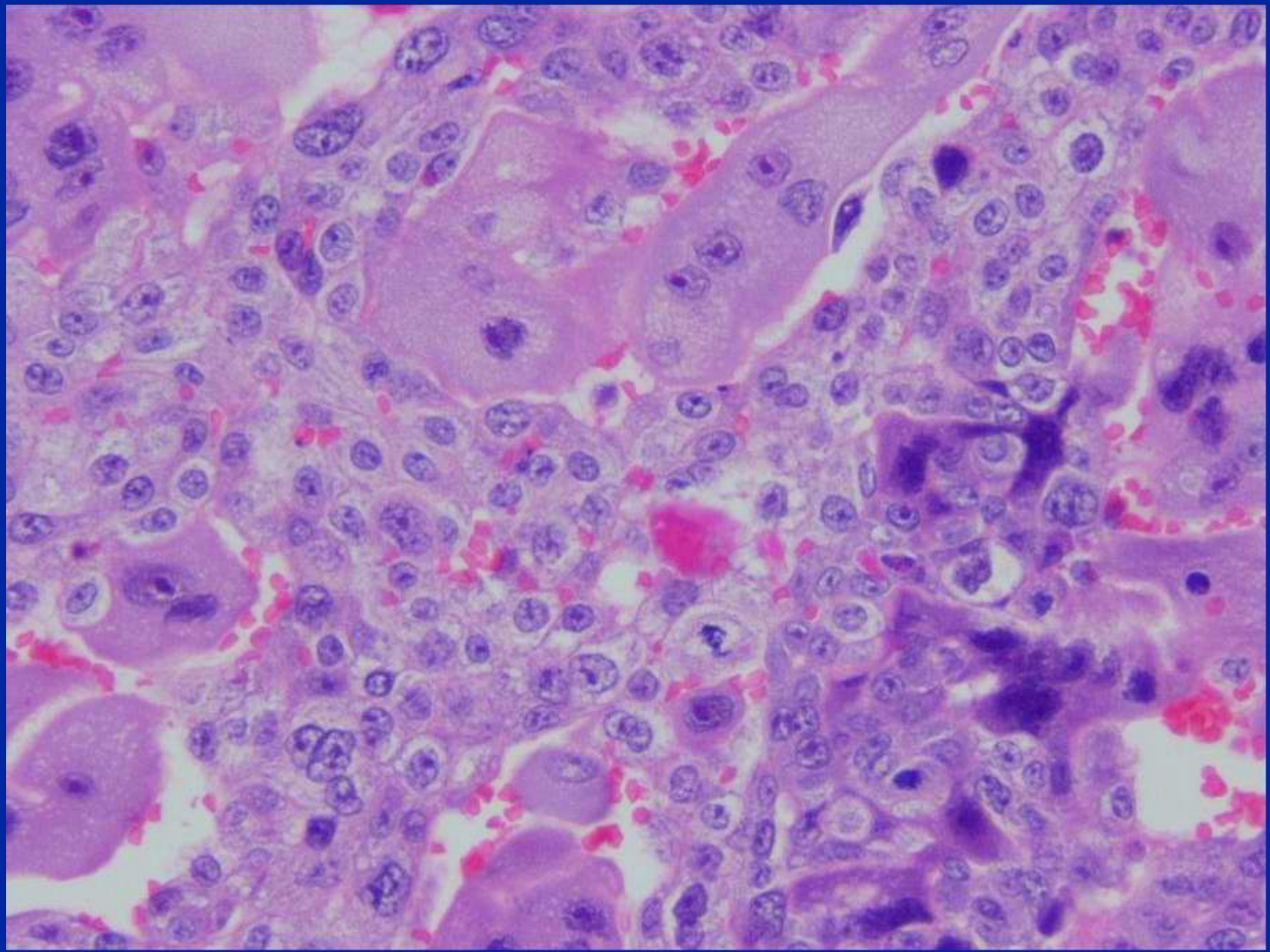
- Present in 8% of mixed GCT
- Very rare in pure form (1% of GCT)
- **Histology:** Plexiform admixture of syncitiotrophoblasts, cytotrophoblasts and intermediate trophoblasts.
- **DDX:** "syncitiotrophobalst only" elements associated with seminoma or mixed GCT **IS NOT CONSIDERED** a choriocarcinoma component.
- **IMHX:** hCG (+), CK (+), EMA (+/-), PLAP (+/-). Intermediate cells HPL (+)

- **PGX:**
  - presence of a minor element of choriocarcinoma in mixed GCT **DOES NOT AFFECT** prognosis.
  - pure choriocarcinoma usually presents with advanced stage & high hCG levels ⇒ worse prognosis.



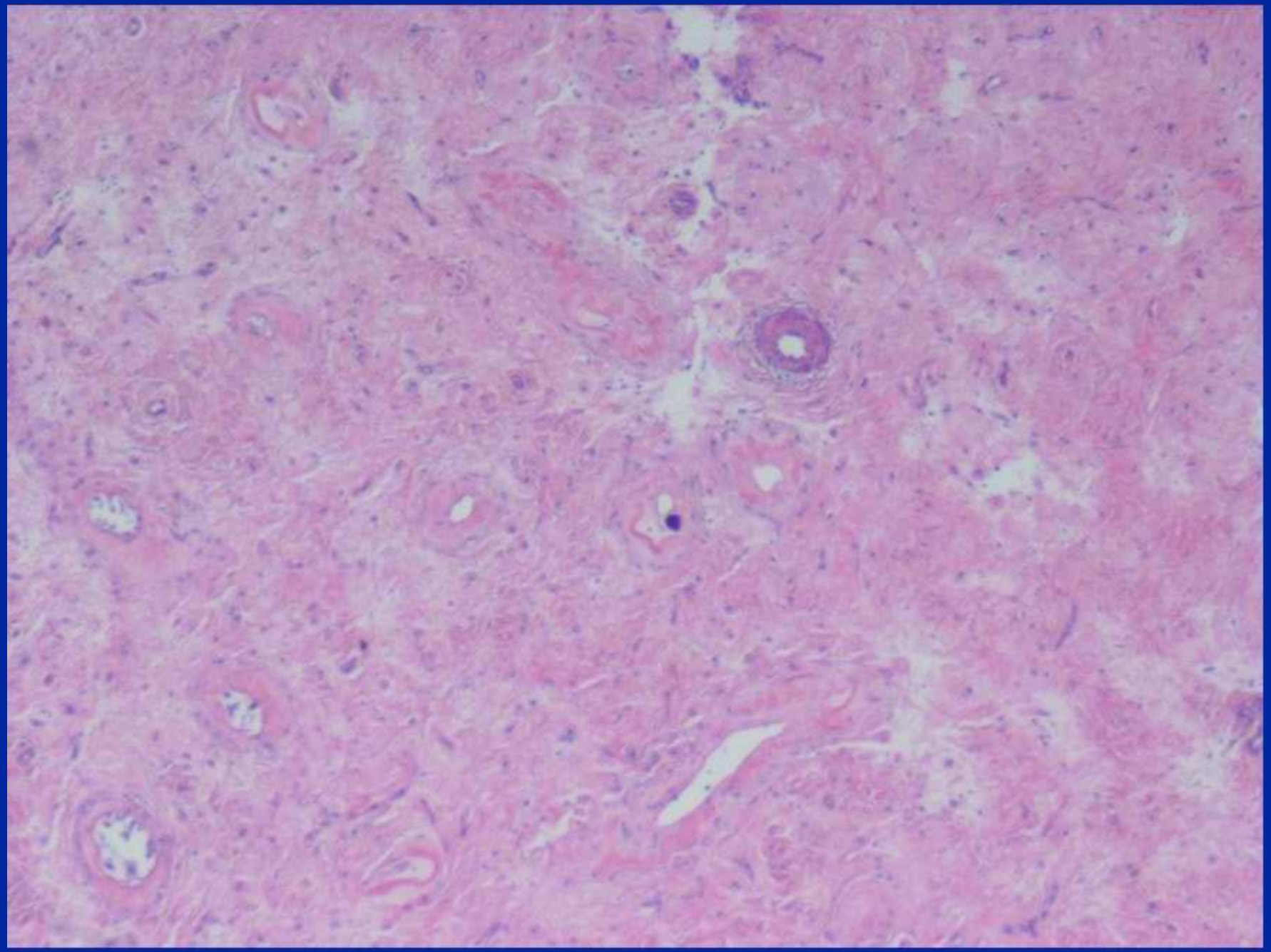


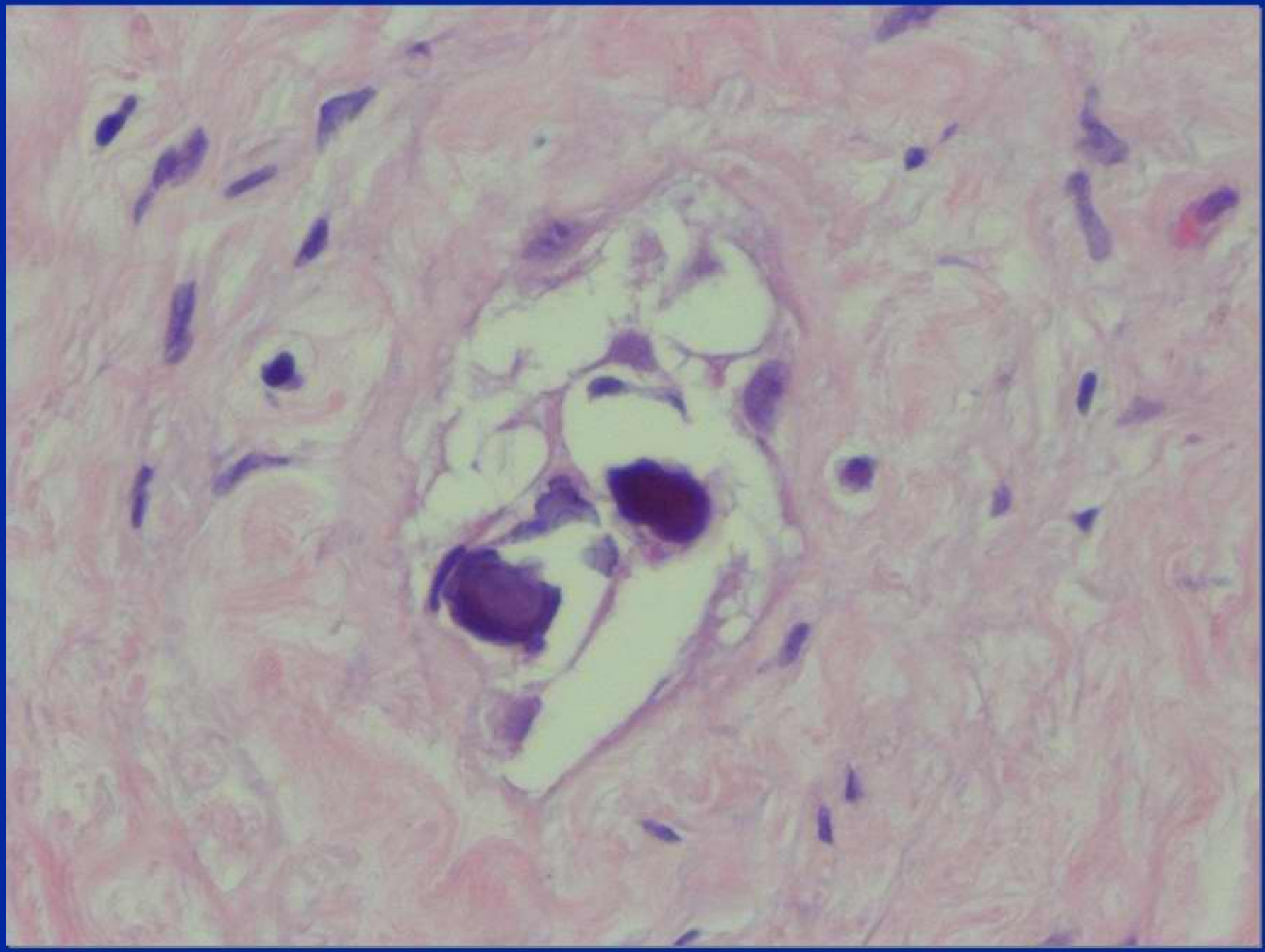


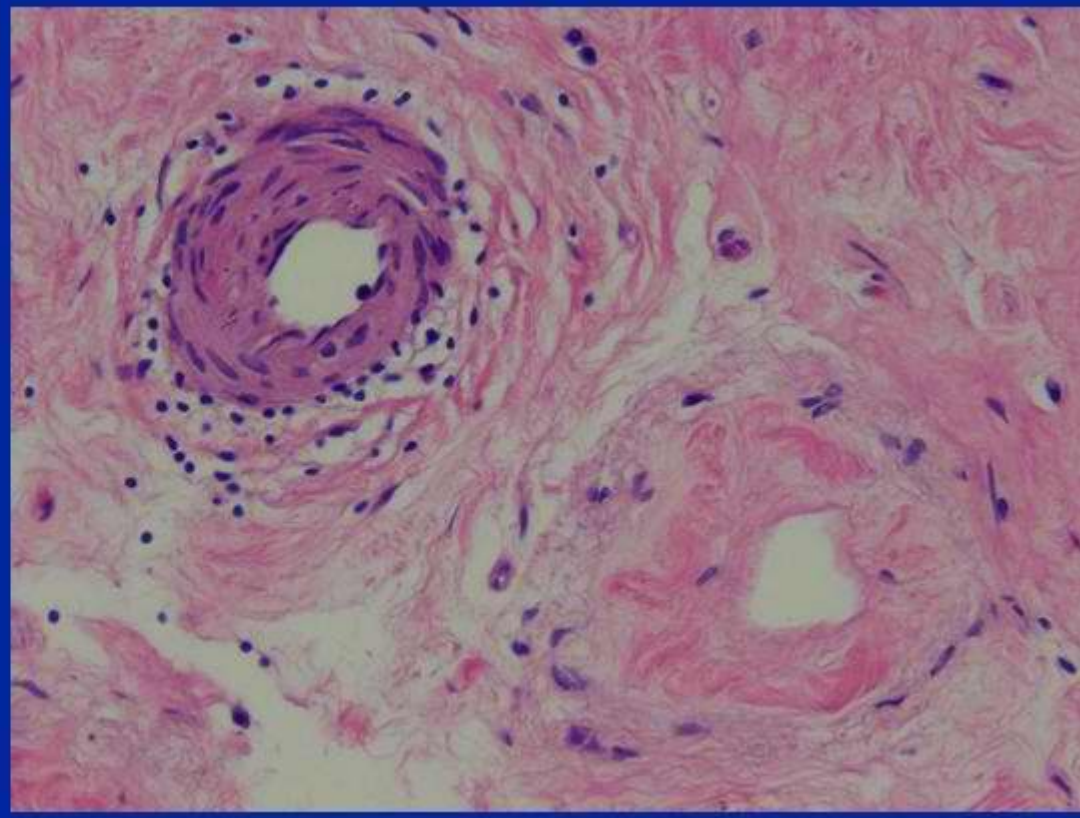


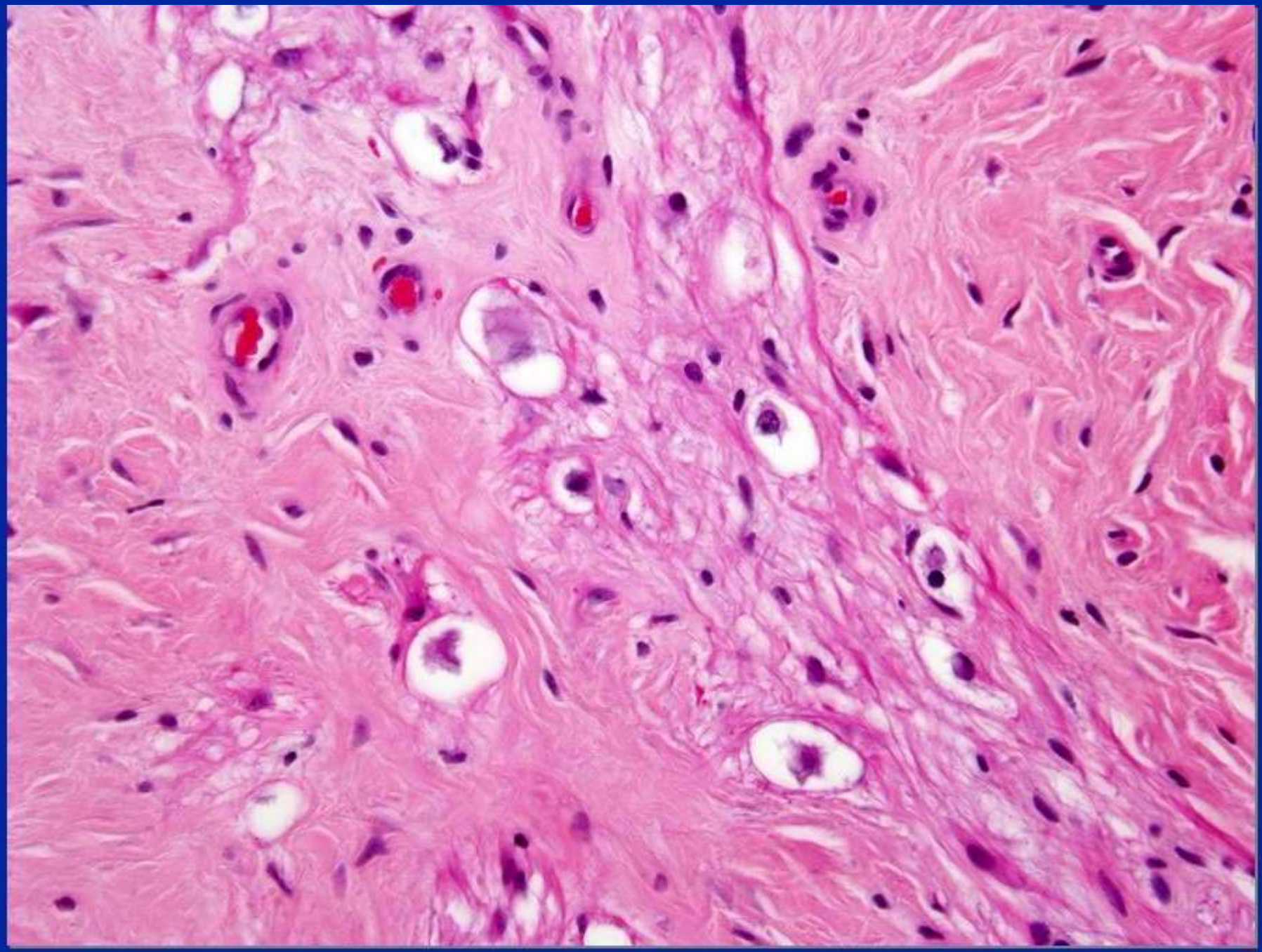
“Burnt-out” GCT

- Spontaneous regression.
- Most frequently occurs in choriocarcinoma but also in seminoma (responsible for most of cases) and embryonal ca.
- Can led to GCTx presenting as extratesticular metastasis with no clinically evident testicular primary
- **Histology:** Scar with inflammation, “ghost hyalinized tubules”, intratubular calcifications, hematoxyline bodies, may have residual partially viable tumor, IGCNU.









THANK YOU !!!!!