

Histopathology of cryptorchid testis

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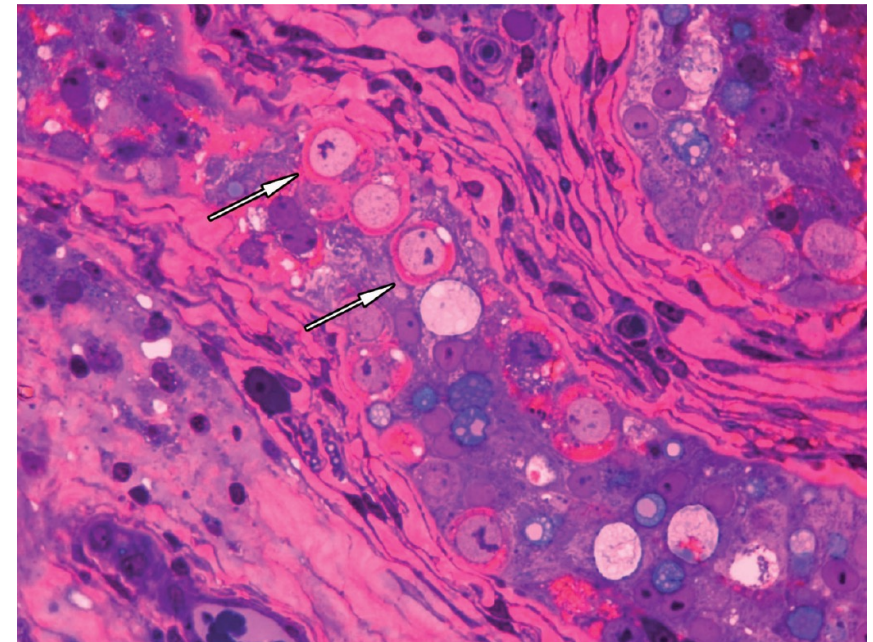
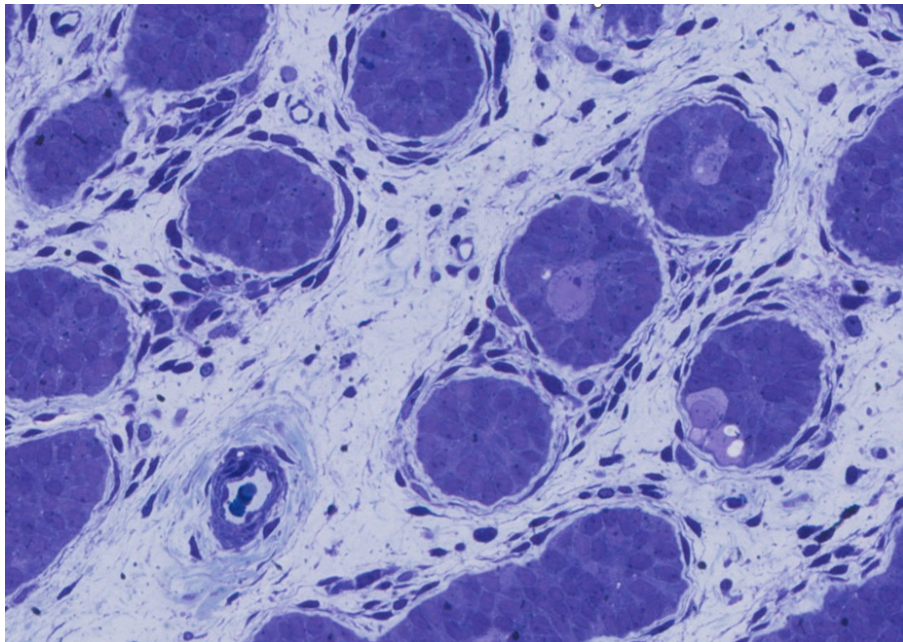
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- Cryptorchid testis: histological findings
- Cryptorchidism and testicular tumour development



Cryptorchid Testis

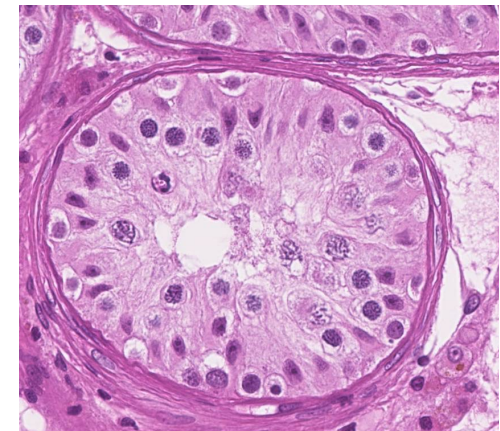
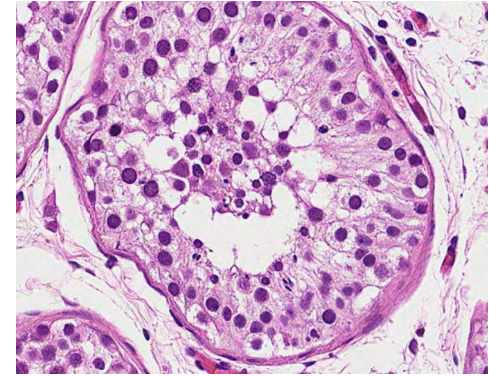
Histology: Key findings

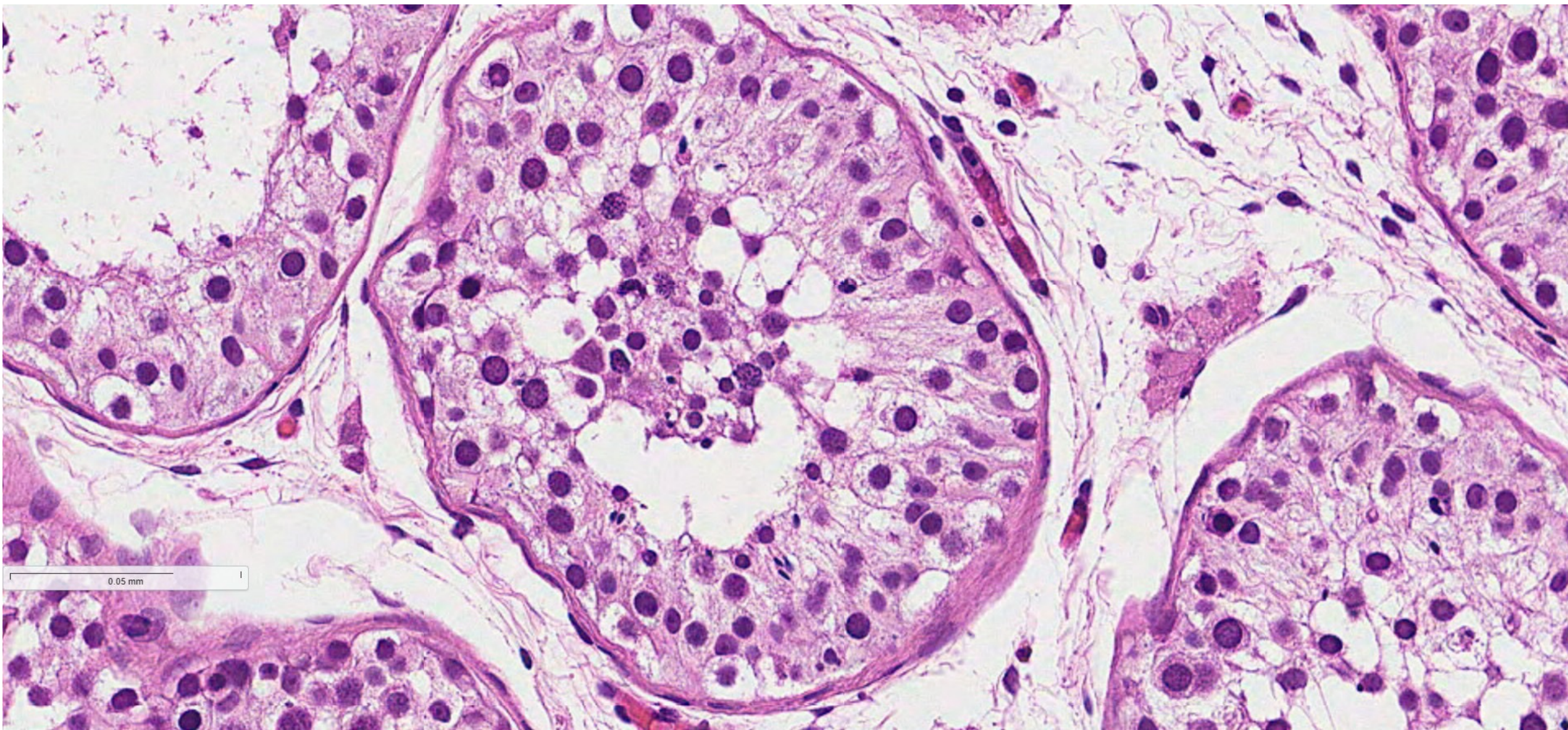
- Depletion of germ cells
 - Resulting from impaired maturation of gonocytes accompanied by interstitial fibrosis
 - Absence (decrease in the number) of Ad spermatogonia
- Developmental arrest of Sertoli cells, and a diminished number of Sf (fetal) and Sb (polarized) Sertoli cells
- Leydig cell atrophy
 - A hallmark of cryptorchid testis
 - Supports endocrinopathy as etiological factor in cryptorchidism

Cryptorchid Testis

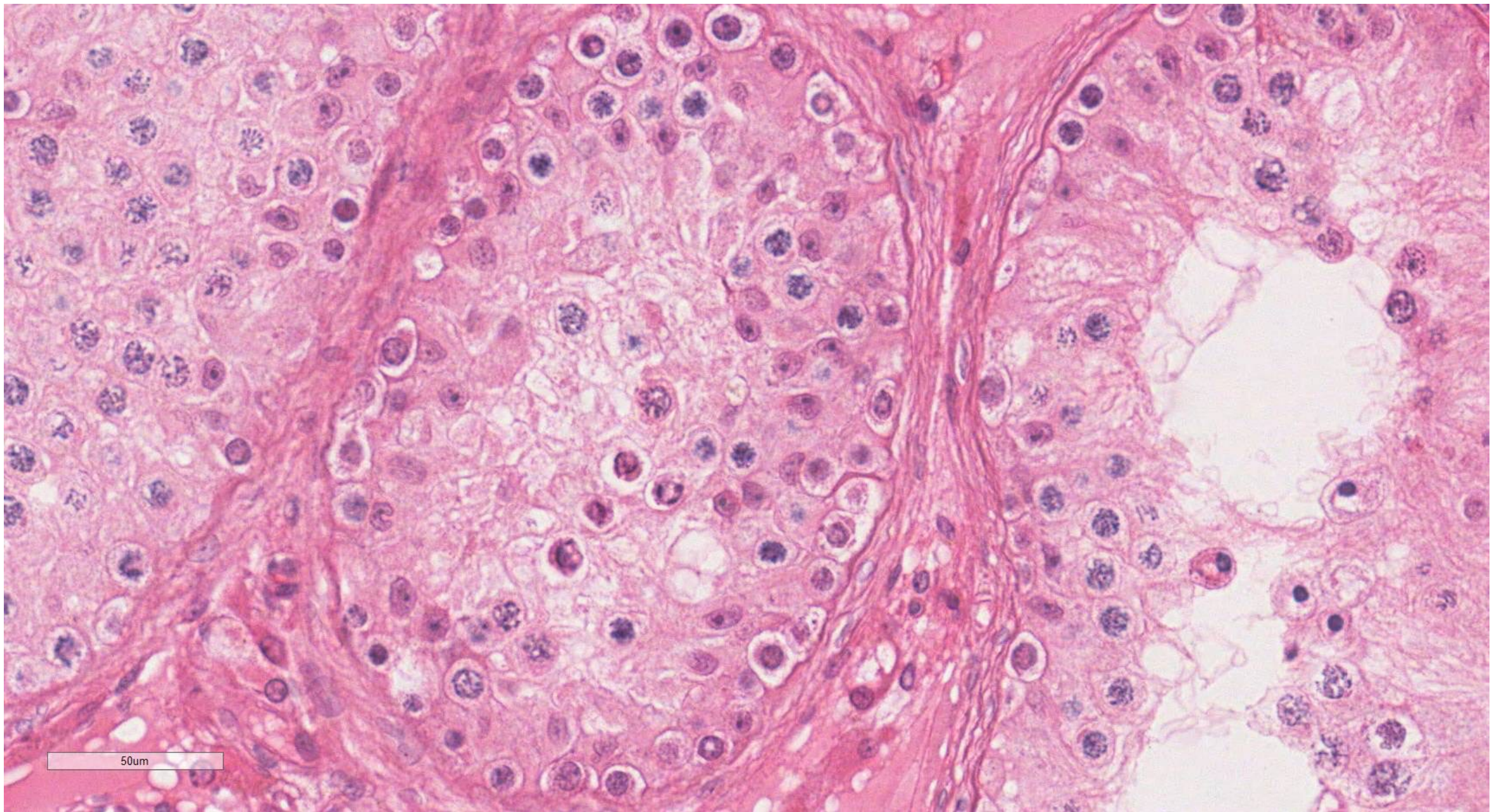
Histology: Methodological aspects of slides preparation by usual techniques

- Fixative
 - 10% Neutral Buffered Formalin (NBF)
 - most common fixative in pathology labs, economical, good tissue preservation for routine examination
 - It can cause artifacts (shrinkage and sloughing of cells), obscuring the tissue's cellular lineage and assessment of spermatogenesis
 - Buin's (mixture of picric acid, formaldehyde, and acetic acid)
 - Preserve testicular tissue architecture better than formalin
 - It has known health and safety issues. Not ideal for long-term archival purposes
 - Stieve's (mixture of formalin, mercuric chloride, acetic acid)
 - Improved Staining, Good Nuclear Morphology
 - Environmental concerns and regulations regarding mercury disposal
- Paraffin embedding
- Thickness of sections: 3 μm
- H&E staining
- Routine practice in most histological laboratories

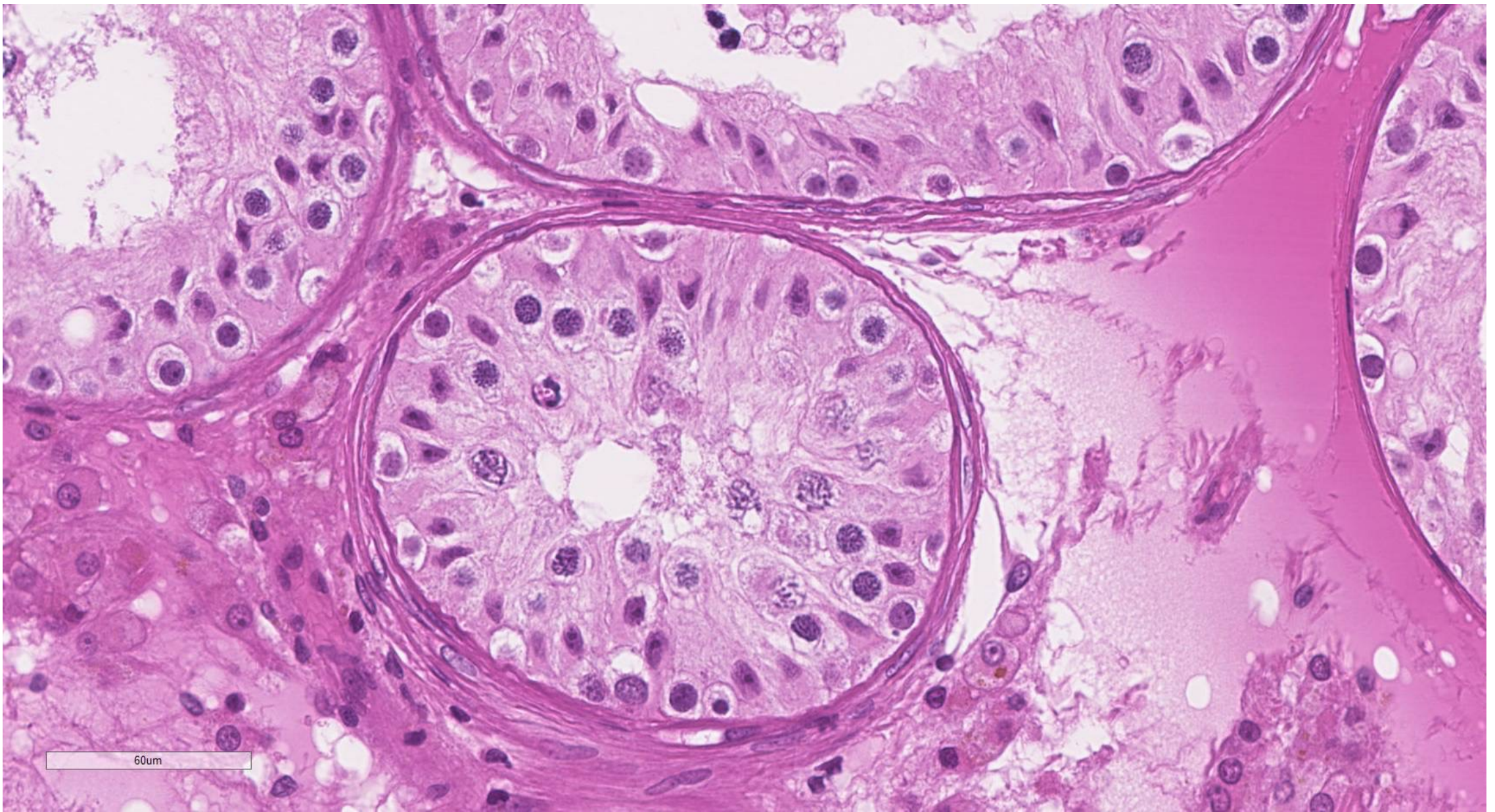




Histological picture from testis biopsy (15 years old male): Formalin fixed, Paraffin embedded tissue. H&E staining, 40x. Marked shrinkage artifacts are evident within the germinal epithelium. Proper identification of Ad spermatogonia is barely possible



Histological picture from testis biopsy (33 years old male with azoospermia): Formalin fixed, Paraffin embedded tissue. H&E staining, 40x

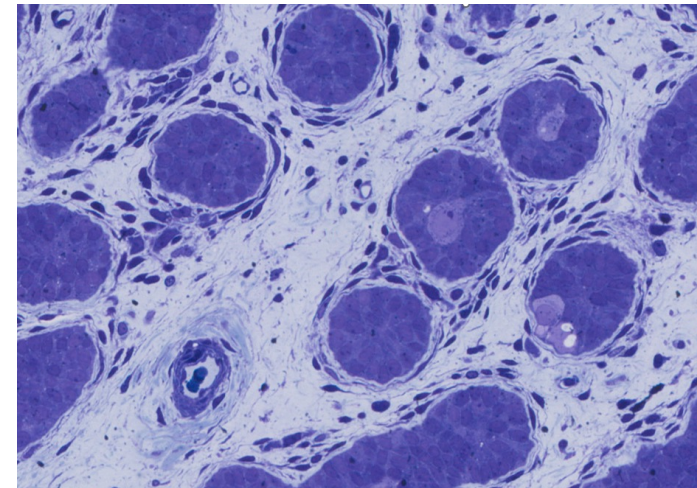


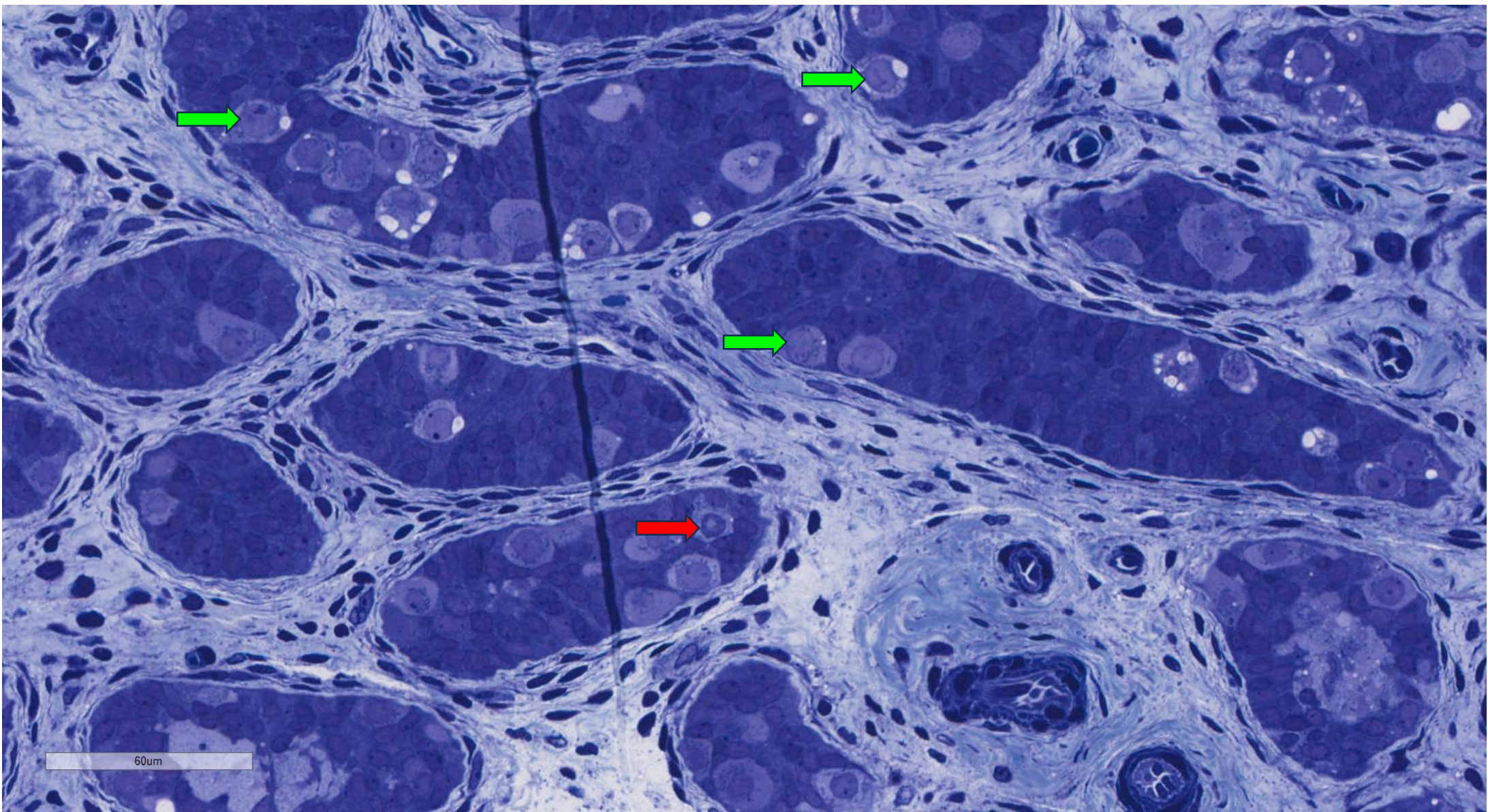
Histological picture from testis biopsy (30 years old male with azoospermia): Buin's fixed, Paraffin embedded tissue. H&E staining, 40x. Better preserved architecture of germinal epithelium. Proper identification of Ad spermatogonia is barely possible

Cryptorchid Testis

Histology: Methodological aspects of slides preparation by „Semithin sections“ technique

- 2% Glutaraldehyde fixative
 - primary fixative for electron microscopy, preserving detailed cellular and tissue structures at high resolution
 - rapid fixation (faster than formaldehyde), allowing for the quick stabilization delicate structures
- EPON resin embedding
- Semithin sections (0,5-1 μm)
 - Using ultramicrotome
- Mounted onto standard glass slides
- Toluidine blue staining
 - according to a standard histochemical protocol
- **Better for identification of Ad spermatogonia**



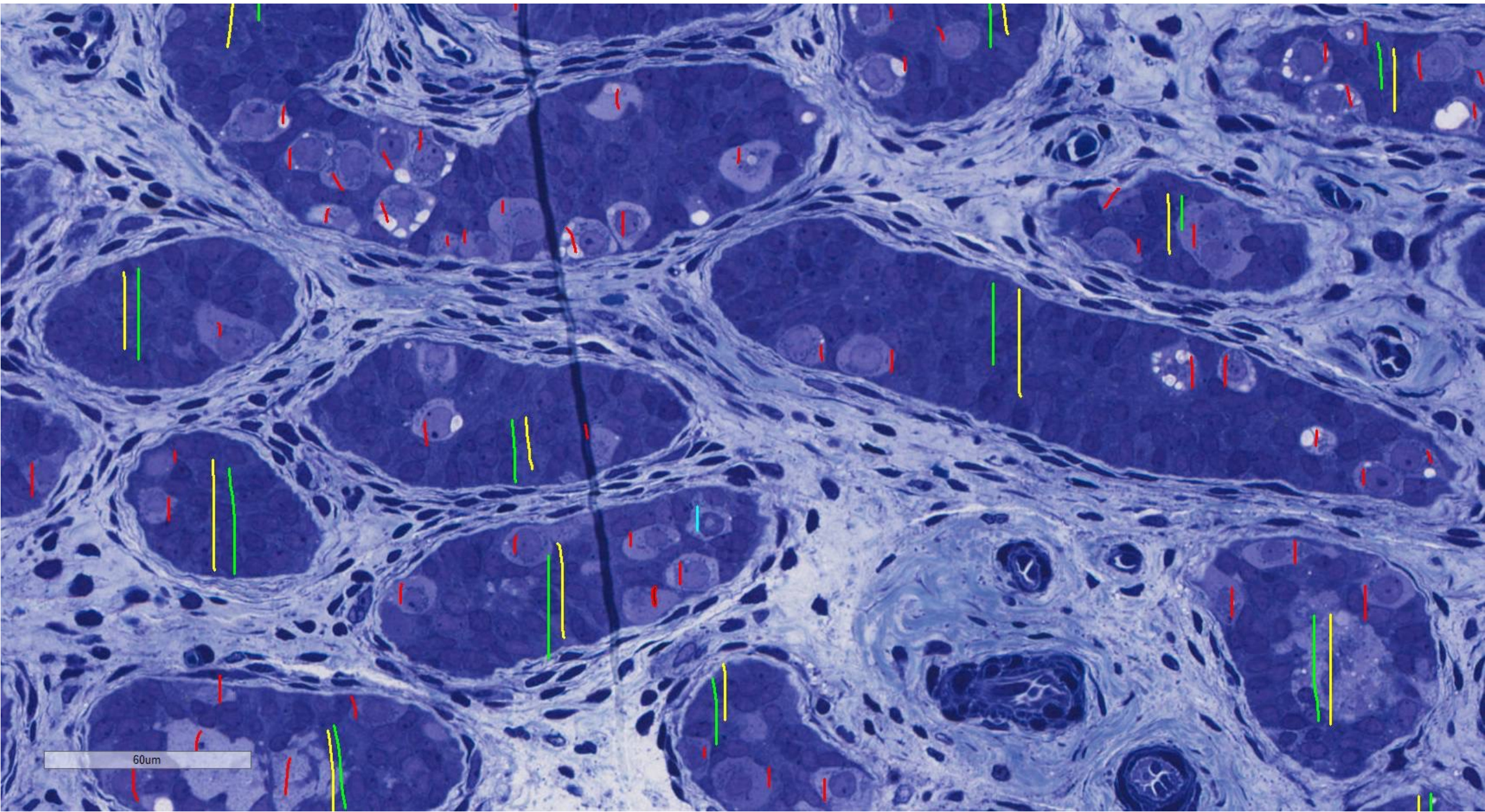


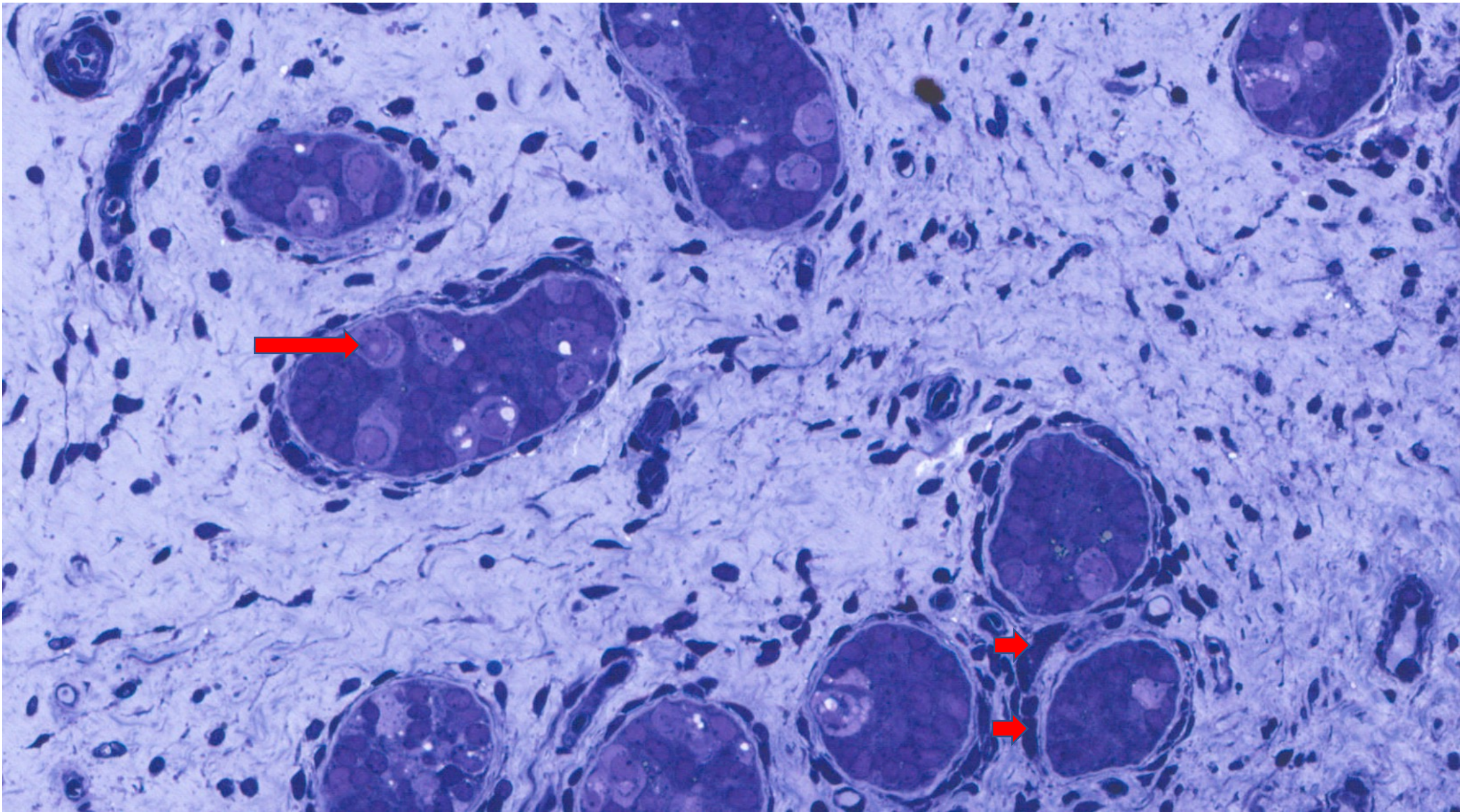
Histological picture from testis biopsy (11 months old boy with cryptorchidism): „Semithin methodology“. Glutaraldehyde fixated, EPON resin embedded tissue. Toluidine blue staining, 40x. Tubules filled with Sertoli cells and Spermatogonia (green arrows). Ad spermatogonia (with characteristic intranuclear vacuole-like focus of rarefaction in the chromatin) observed (red arrow)

Cryptorchid Testis

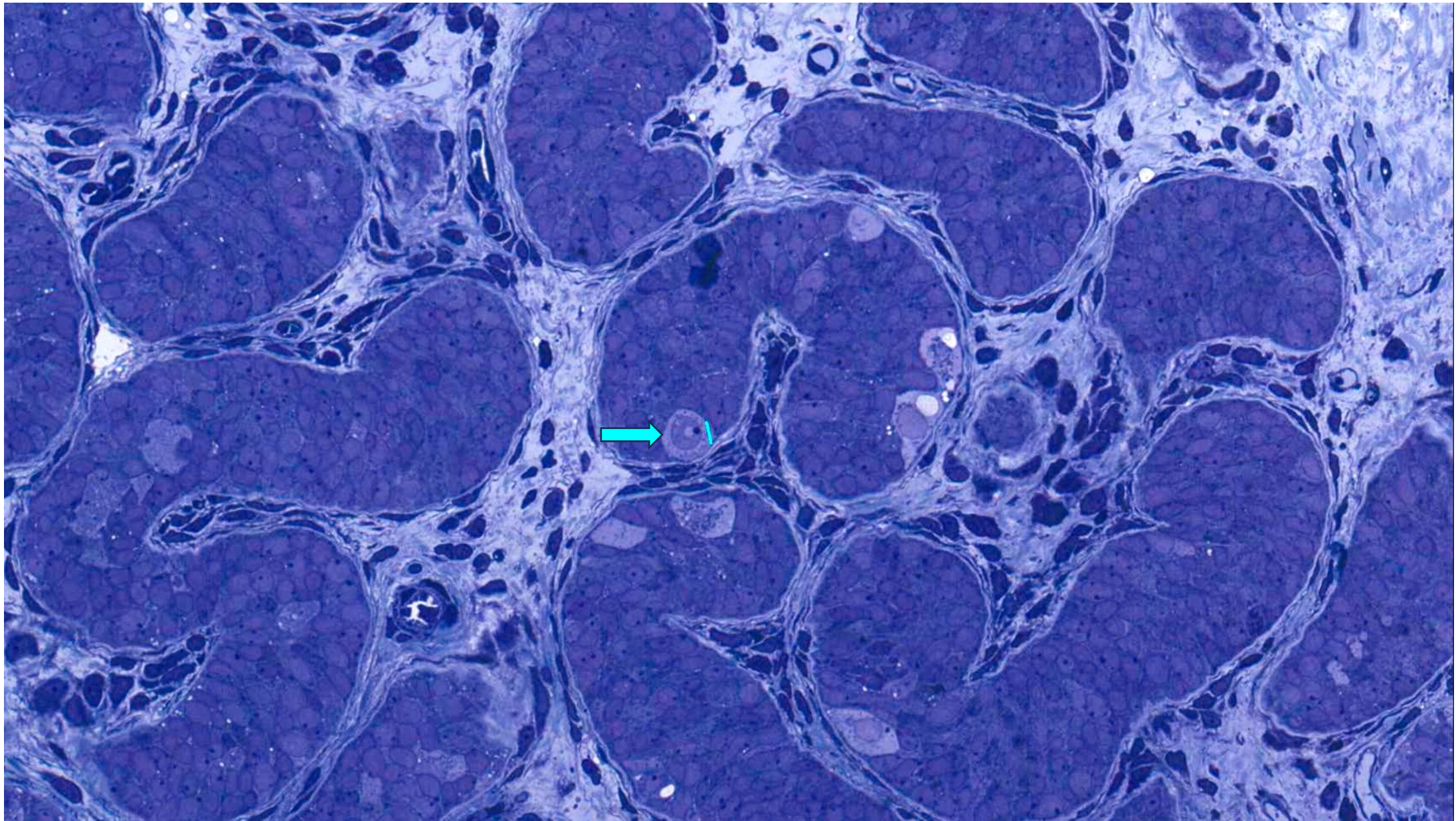
Histology: Semithin methodology

- Absolute parameters
 - total number of seminiferous tubules ($\Rightarrow$ 200)
 - number of tubules containing spermatogonia
 - total spermatogonial count
 - **number of Ad spermatogonia**
- Relative parameters
 - tubular fertility index/TFI (ratio of spermatogonia-containing tubules to the total number of tubules)

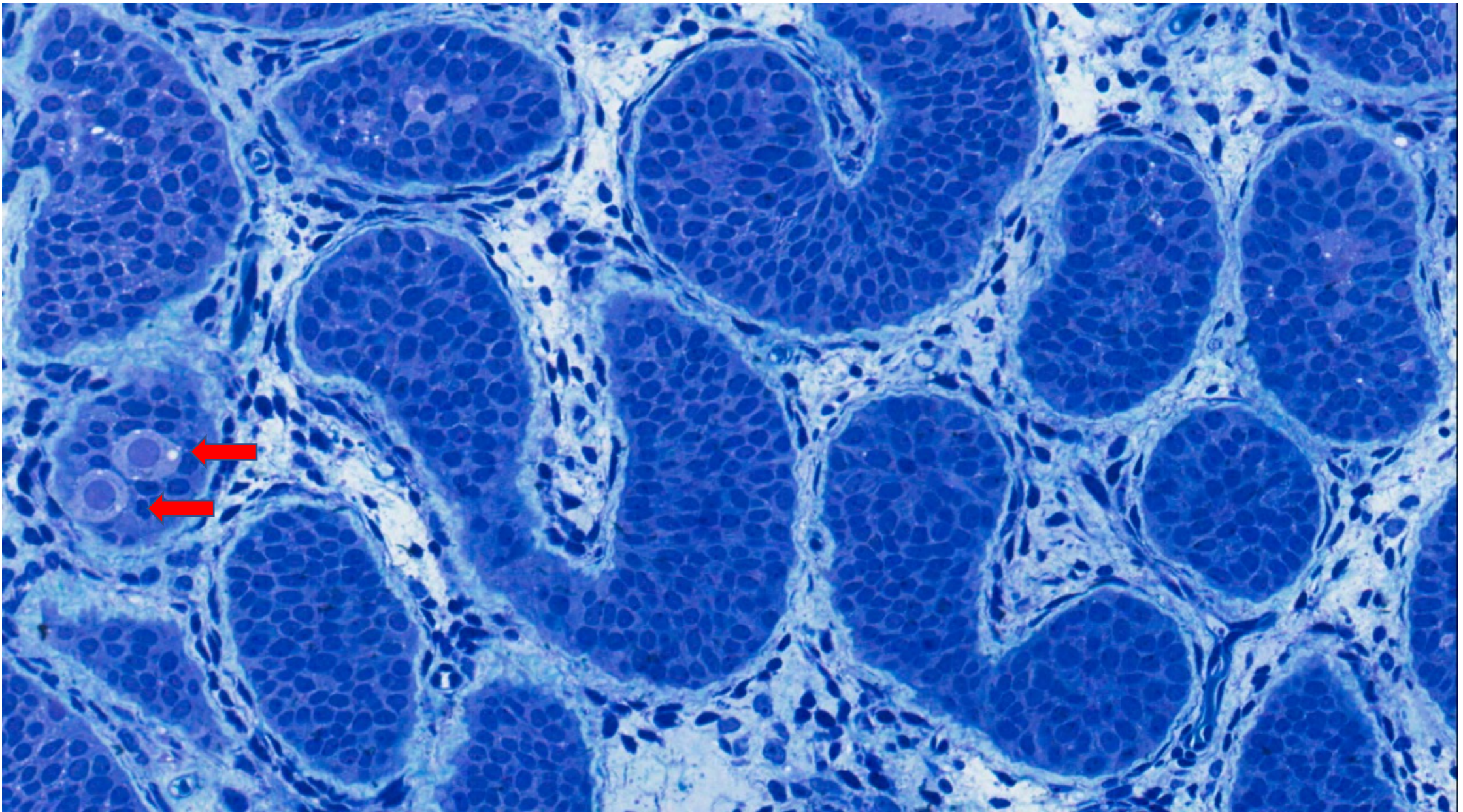




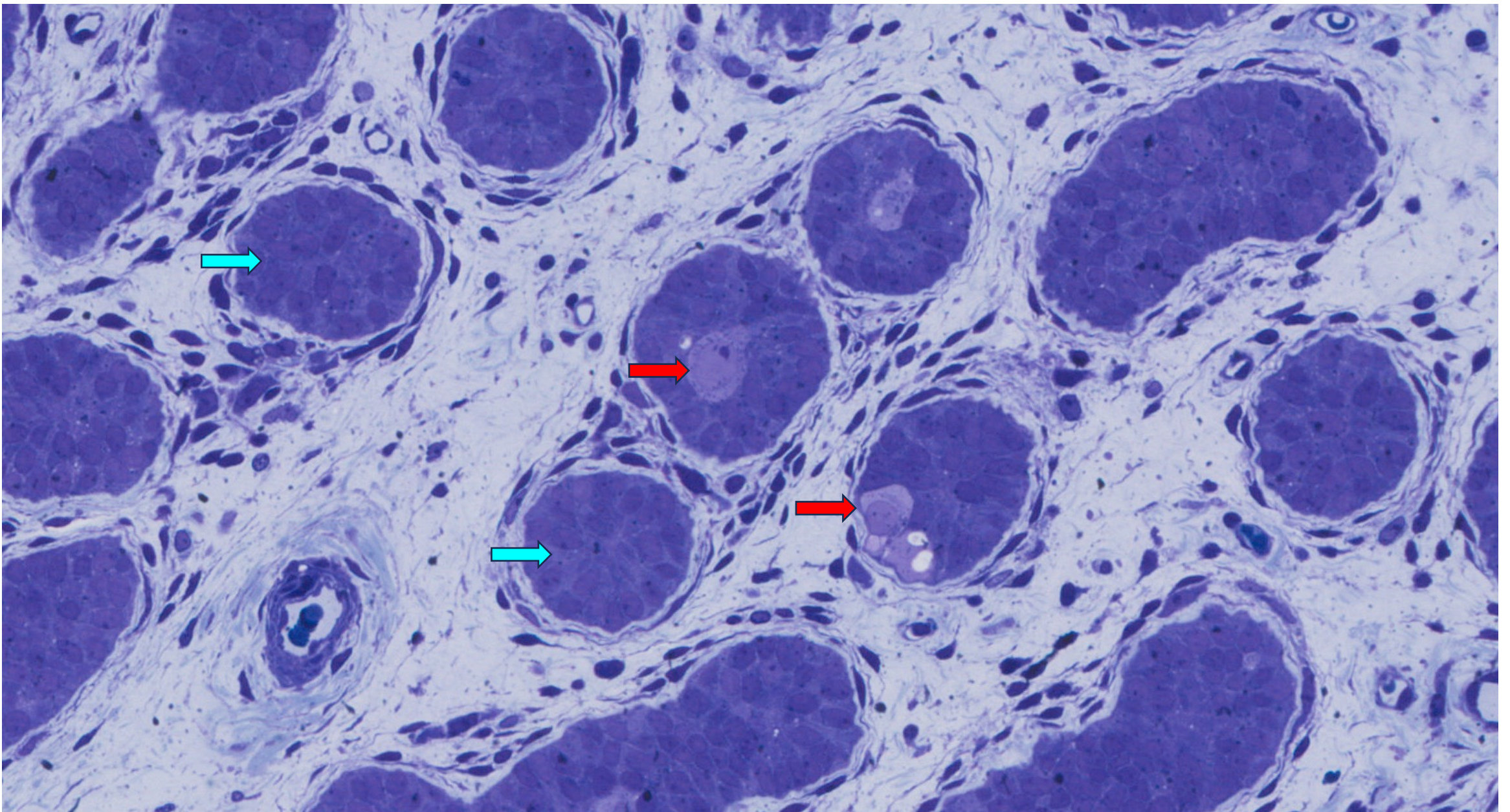
Low Infertility Risk. All tubular cross-sections have spermatogonia, some - Ad spermatogonia (long arrow). Broad interstitium with several atrophic Leydig cells (short arrows) (Tol, 40x)



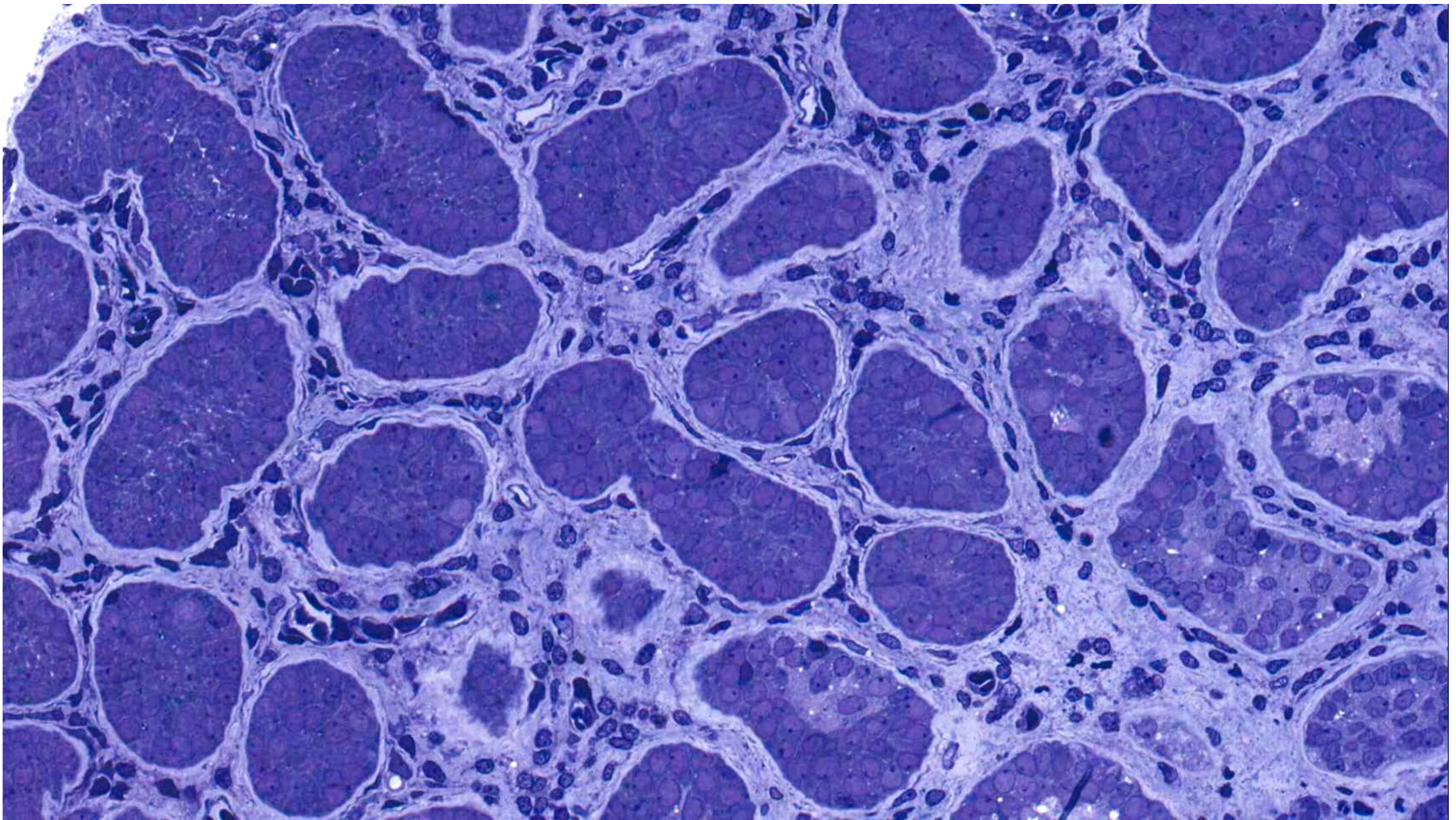
Low Infertility Risk. Spermatogonia in some tubular cross-sections. One Ad spermatogonia (light blue arrow) (Tol, 40x)



High Infertility Risk. Severe germ cell depletion (arrow), no Ad spermatogonia. Most of the tubules contain only Sertoli cells (Tol, 40x)



High Infertility Risk. Diffuse decrease of tubular density. Severe germ cell depletion (red arrows). No Ad spermatogonia. Most of the tubules contain only Sertoli cells (light blue arrows) (Tol, 40x)



High infertility risk. All tubules contain only Sertoli cells, with no spermatogonia observed (Toluidine blue, 40x).

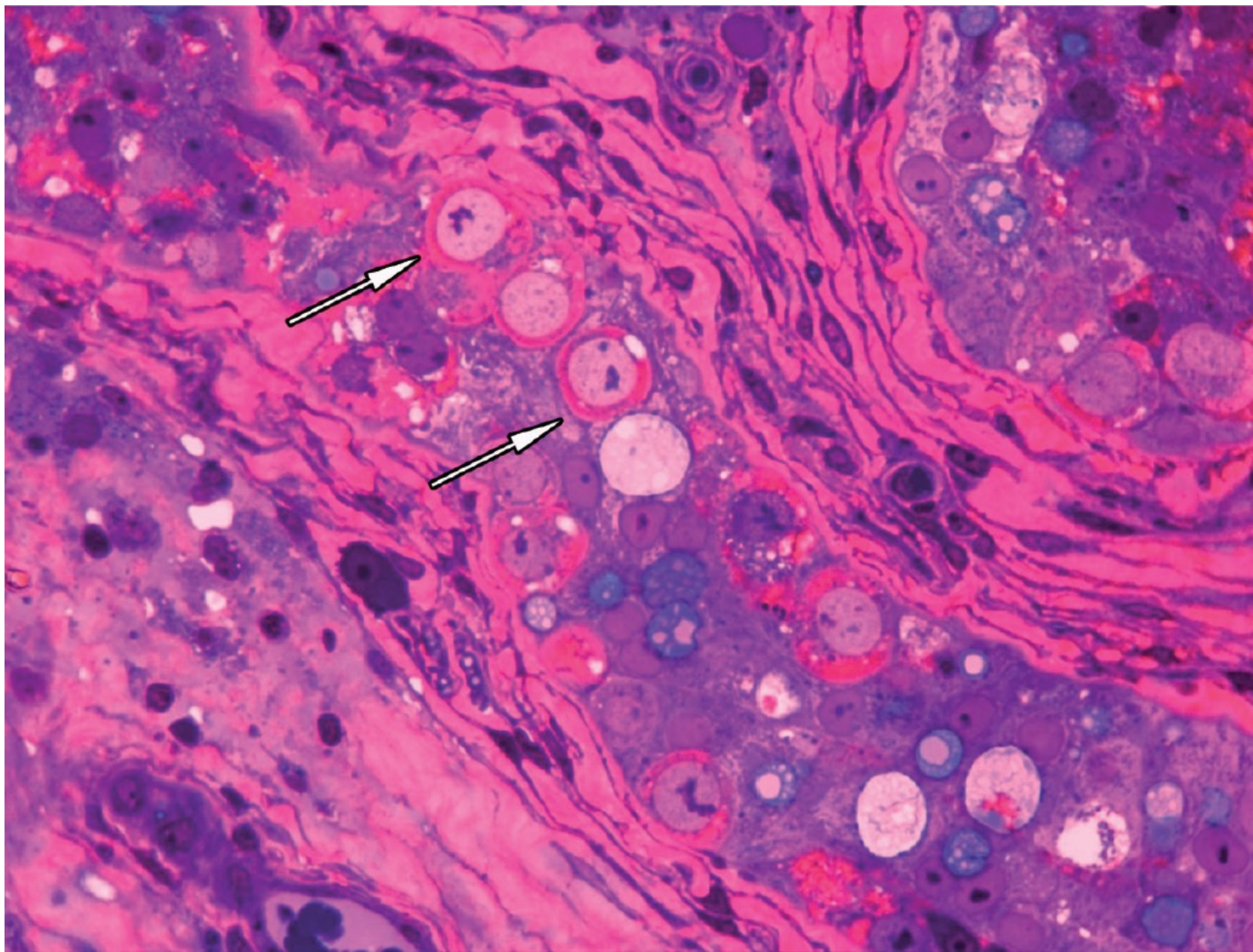
Cryptorchidism and Tumour development

Complications of cryptorchidism

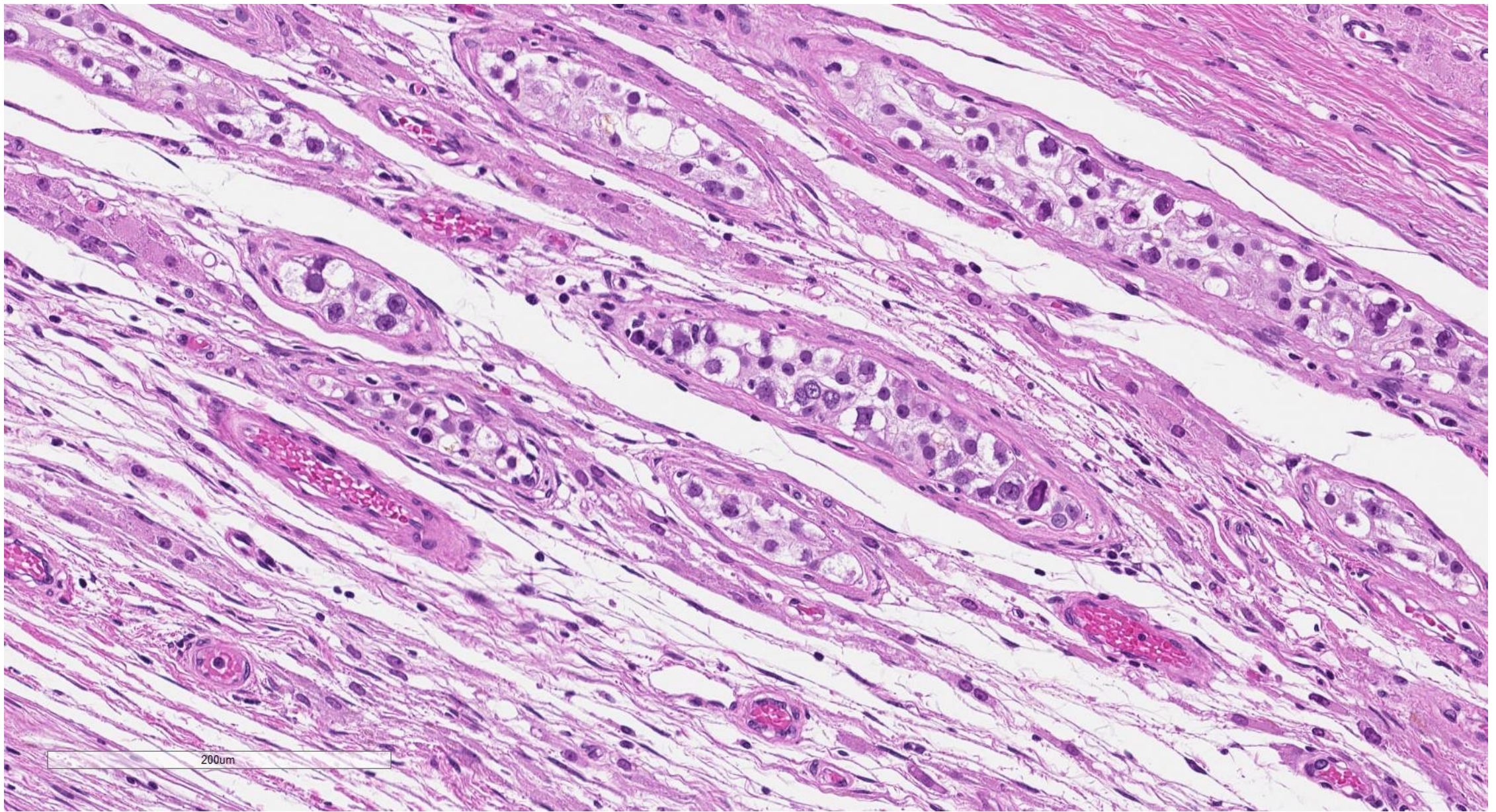
- The most frequent
 - Infertility
 - testicular torsion
 - iatrogenic atrophy
 - psychological problems (derived from an empty scrotum)
 - **Testicular cancer**

Cryptorchidism and Tumour development

- The cancer risk of undescended testis is higher than the general population
 - Risk increase with bilateral Cryptorchidism
- Germ cell neoplasia in situ (GCNIS)
 - common precursor of all seminomas and most adult non-seminomatous germ cell tumours of the testis
 - Not recommended: carcinoma in situ of the testis (CIS); intratubular germ cell neoplasia, unclassified (ITGCNU); testicular intraepithelial neoplasia
 - 2-3% of adult cryptorchidic testis
 - Half of cryptorchid patients with GCNIS develop testicular cancer within five years and 70% within seven years
 - multinucleated spermatogonia - association with increased cancer risk
- Orchidopexy does not decrease the risk of cancer
 - allows for earlier detection
 - The prognosis of germ cell tumors in intra-abdominal testes is similar to that of tumors in normally-descended testes



Cryptorchid testis displaying large and glycogen-rich GCNIS/CIS cells in an atrophic tubule (arrow). Laczko-staining of semithin EPON-resin section (x63)



Testicular biopsy (with conventional FFPE slide preparation technique) with Germ cell neoplasia in situ/GCNIS in germinal epithelium (H&E, 20x)

Key notes

- Ad spermatogonia represent the stem cell population that constitutes the reservoir for normal spermatogenesis
- Accurate identification and quantification of Ad spermatogonia in testicular biopsies by light microscopy require highly specific fixation and tissue preparation techniques, with the „semithin section“ methodology being the leading one
- The presence of Ad spermatogonia in testicular biopsy specimens serves as a prognostic indicator of preserved fertility potential