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ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

GUIDANCE DOCUMENT ON HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 2) FOR THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)

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Guidance Document on Histopathology Techniques and Evaluation (Part 2)

Environment Directorate

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT

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FOREWORD

The project to develop a Larval draft Amphibian Growth and Development Assay (LAGDA) was initiated by Japan and the United States and included in the work plan of Test Guidelines Programme in 2009.

The Integrated Summary Report and first draft TG were submitted to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) in 2014, followed by subsequent commenting rounds in 2014. The draft guidance document on amphibian histopathology was prepared to accompany the draft Test Guideline and help users of the test become more proficient in applying tissue sampling and preparation techniques, evaluation techniques and in the interpretation of the slides.

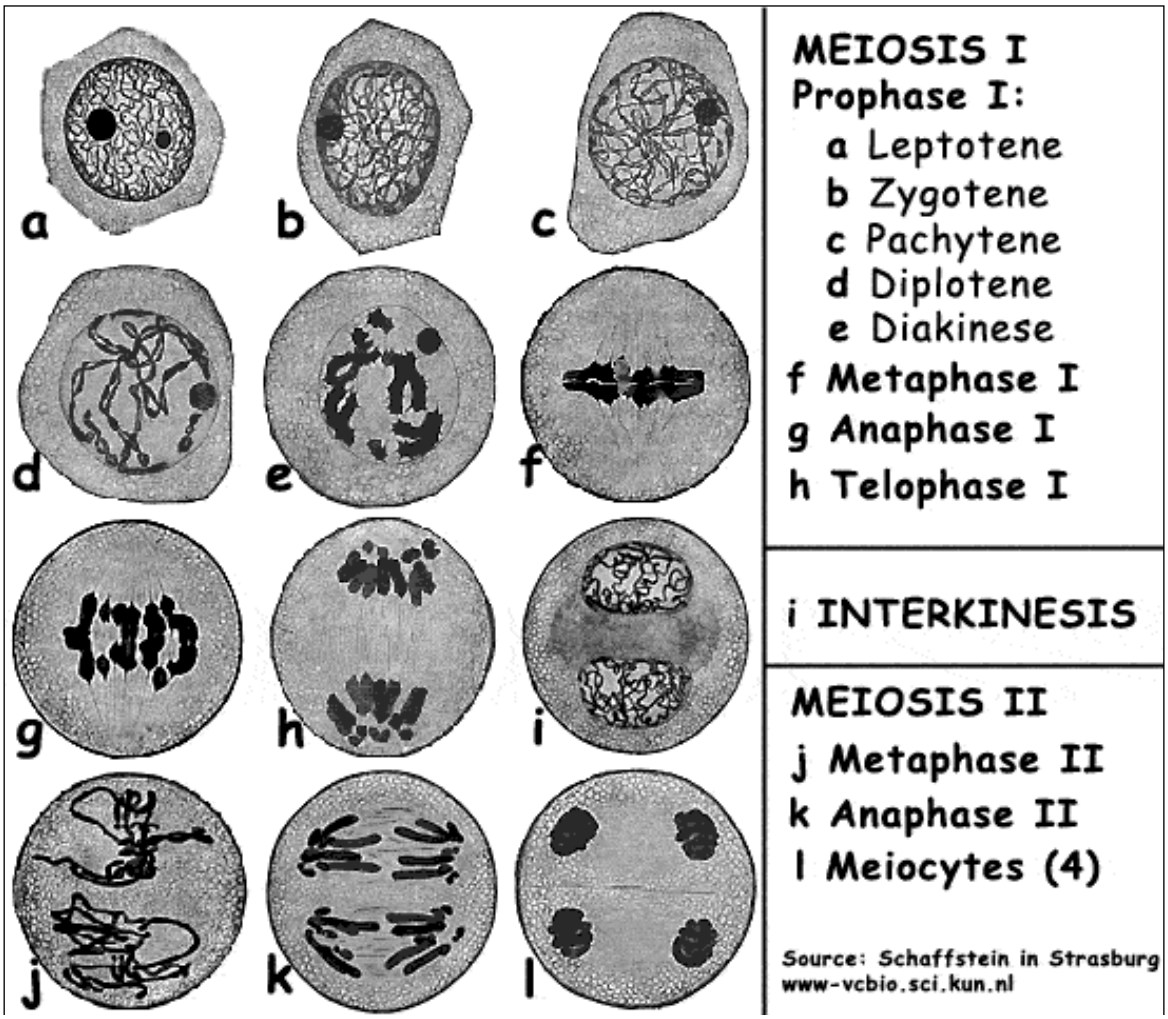
The guidance document on amphibian histopathology techniques and evaluation was approved by the WNT at its 27th meeting in April 2015. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to the declassification of the guidance document on 10th July, 2015.

This document presents **Part 2** of the guidance document which in total consists of three parts.

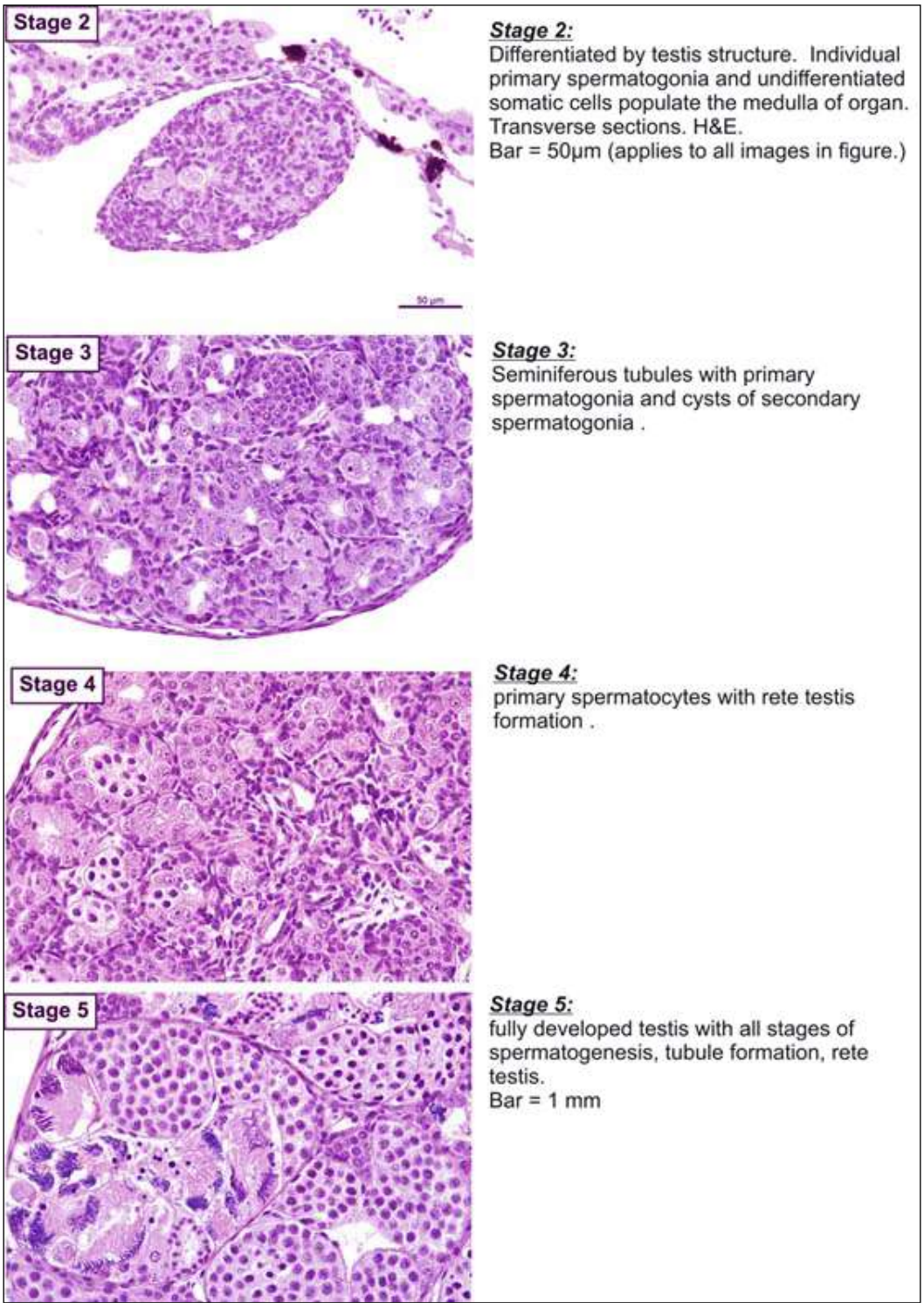
This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

GUIDANCE DOCUMENT ON HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 2)

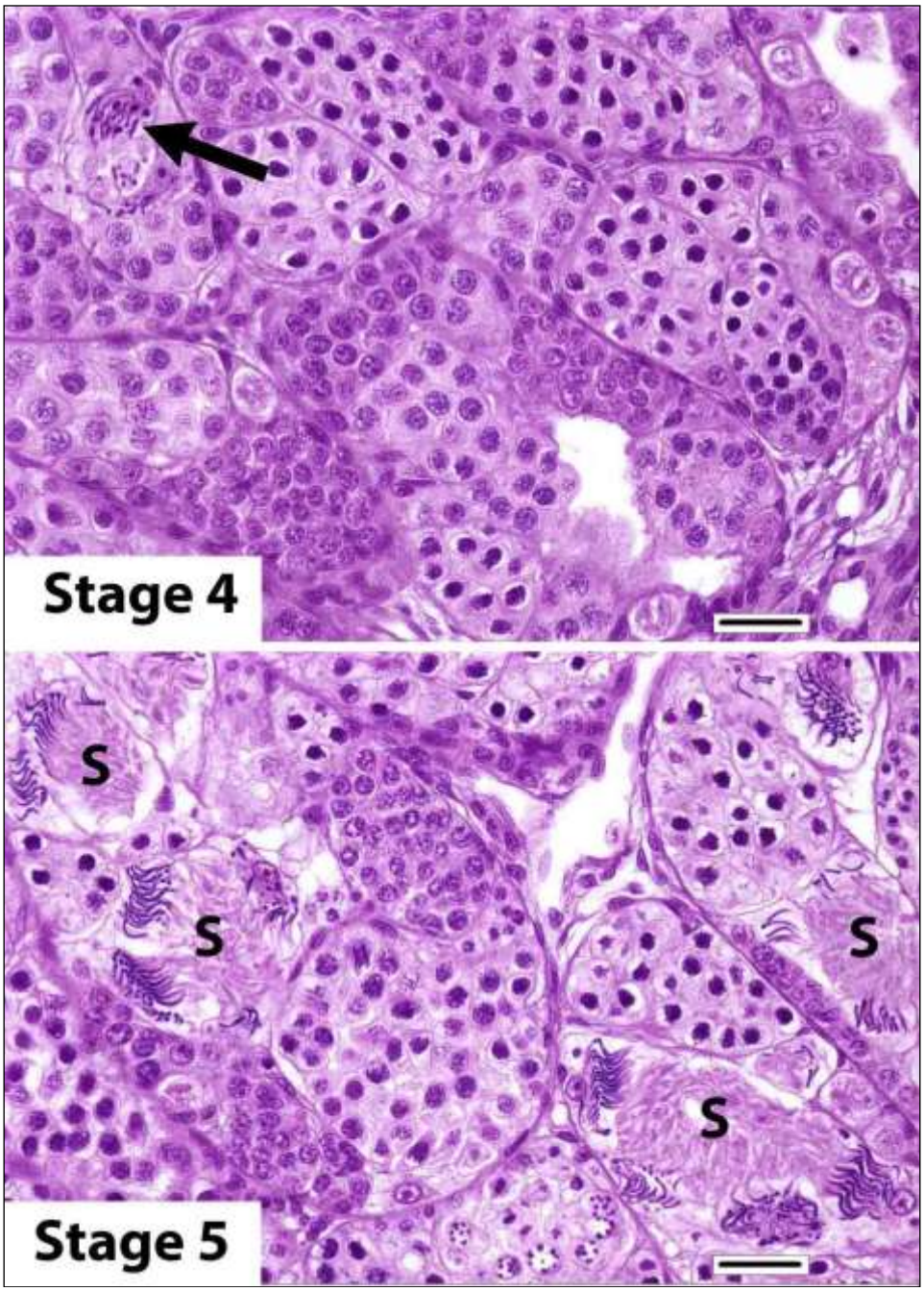
FOR THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)



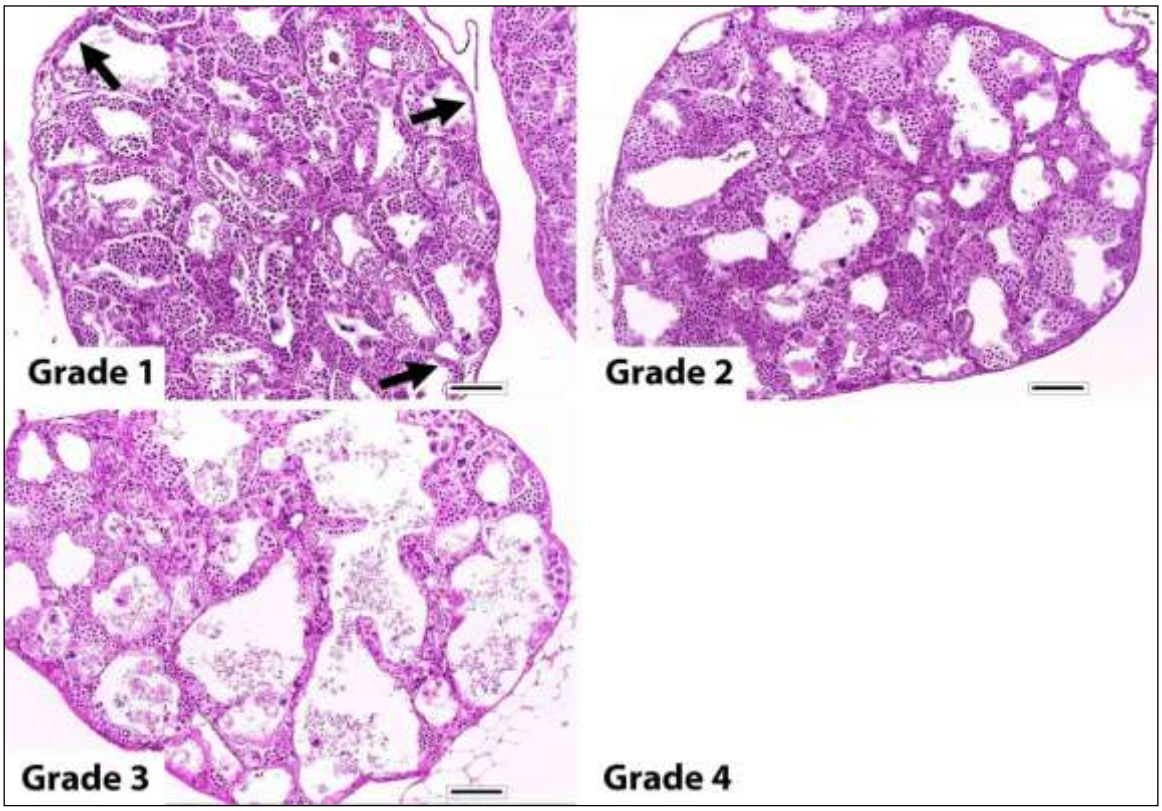
Meiotic Phases in the Vertebrate Testis. Schematic diagram included for reference.



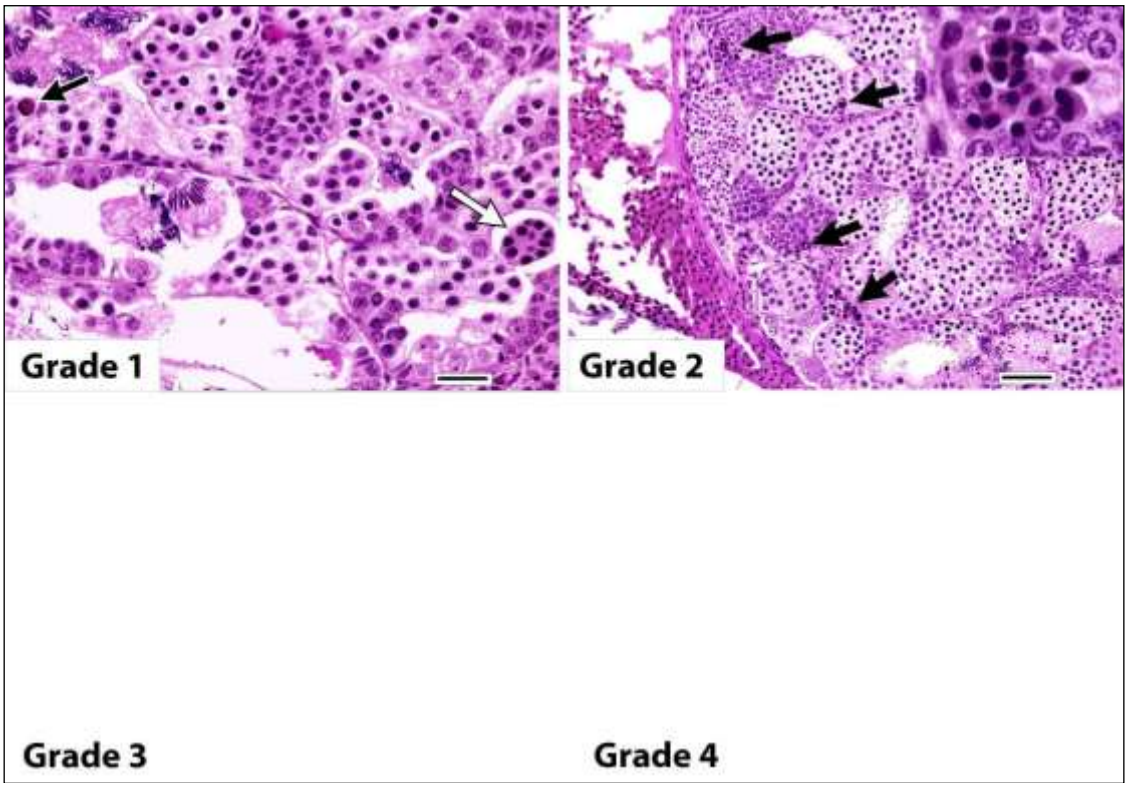
Staging of the Testis. Stage numbers increase with increasing maturity of the testis. Control males 10 weeks post NF stage 62 (LAGDA termination) typically have Stage 4 or 5 testes.



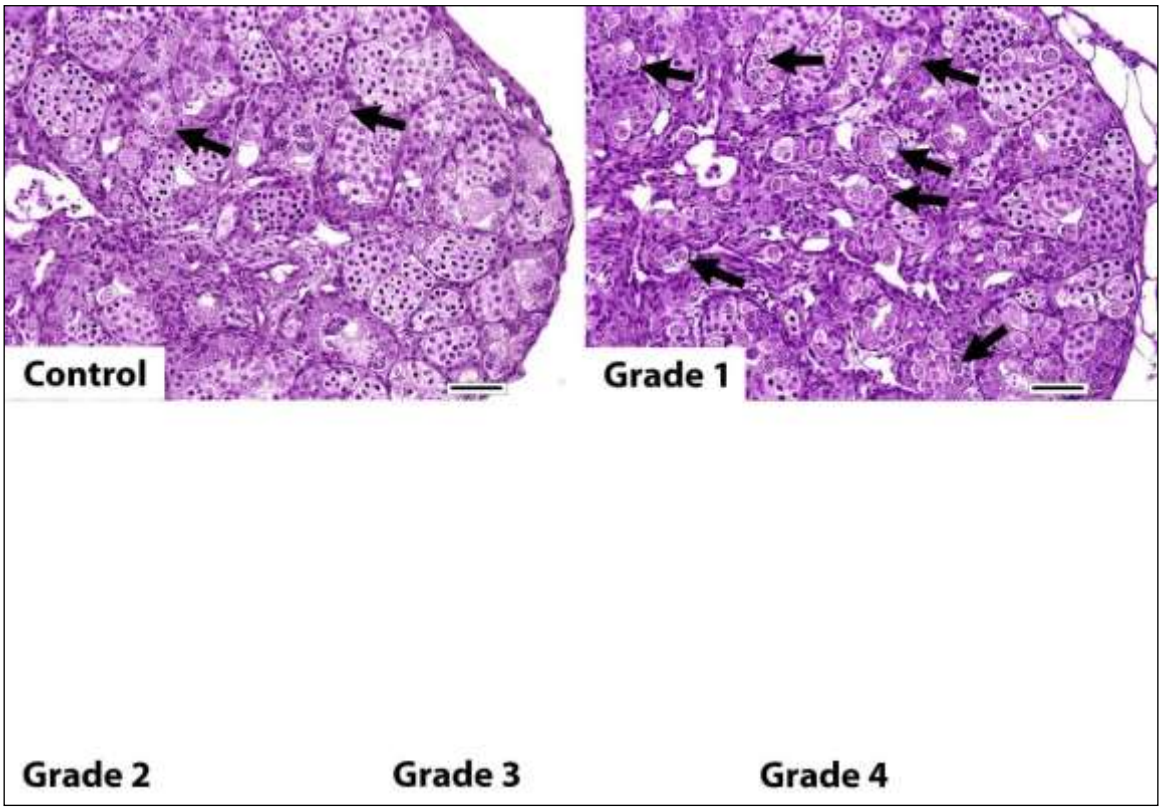
Stage 4 Versus 5 Testes, Higher Magnification. Stage 4 testes can have elongating spermatids (arrow), but spermatocysts that contain spermatozoa are rare or not apparent. Conversely, spermatozoa (S) are abundant in Stage 5 testes. Bar = 25 microns.



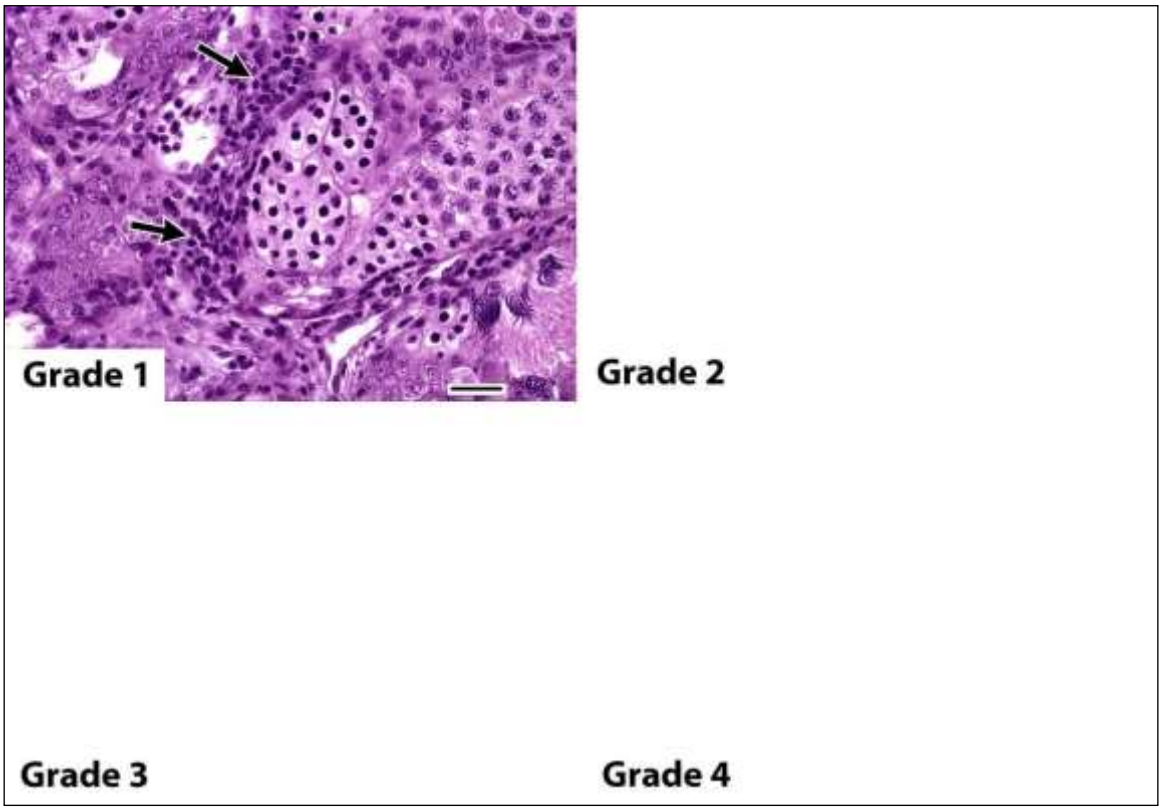
Germinal Epithelium Thinning and Dilation. This finding is characterized by focal to diffuse attenuation of the testicular germinal epithelium (arrows), with expansion of the adjacent tubular lumen. Germ cell degeneration is also evident. In a previous report, similar changes were termed “dilated testis tubules” (Wolf et al., 2010). Bar = 100 microns.



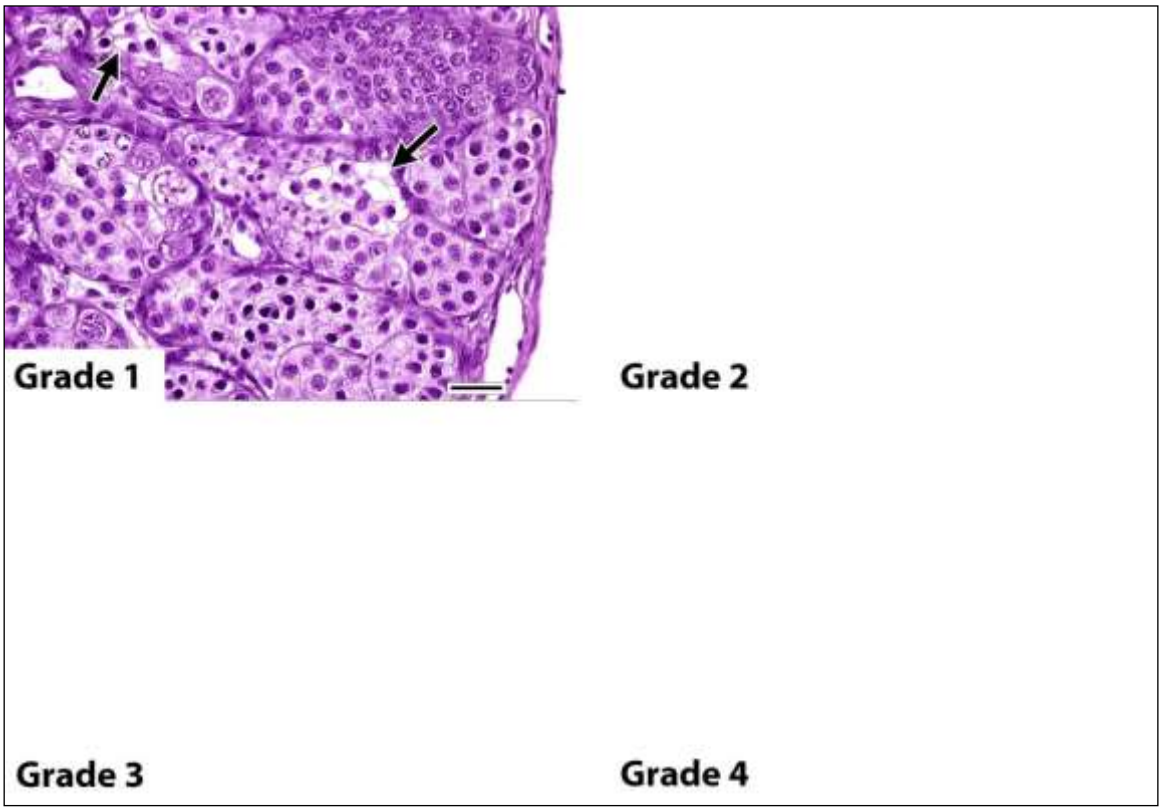
Testis, Germ Cell Degeneration. This finding is characterized by the scattered presence of individual or clustered apoptotic germ cells (black arrows and inset), or germ cell syncytia (white arrow), within the germinal epithelium. Affected testes may also have increased numbers of exfoliated cells and cellular debris in tubular lumina. Bar = 25 microns (Grade 1), 50 microns (Grade 2).



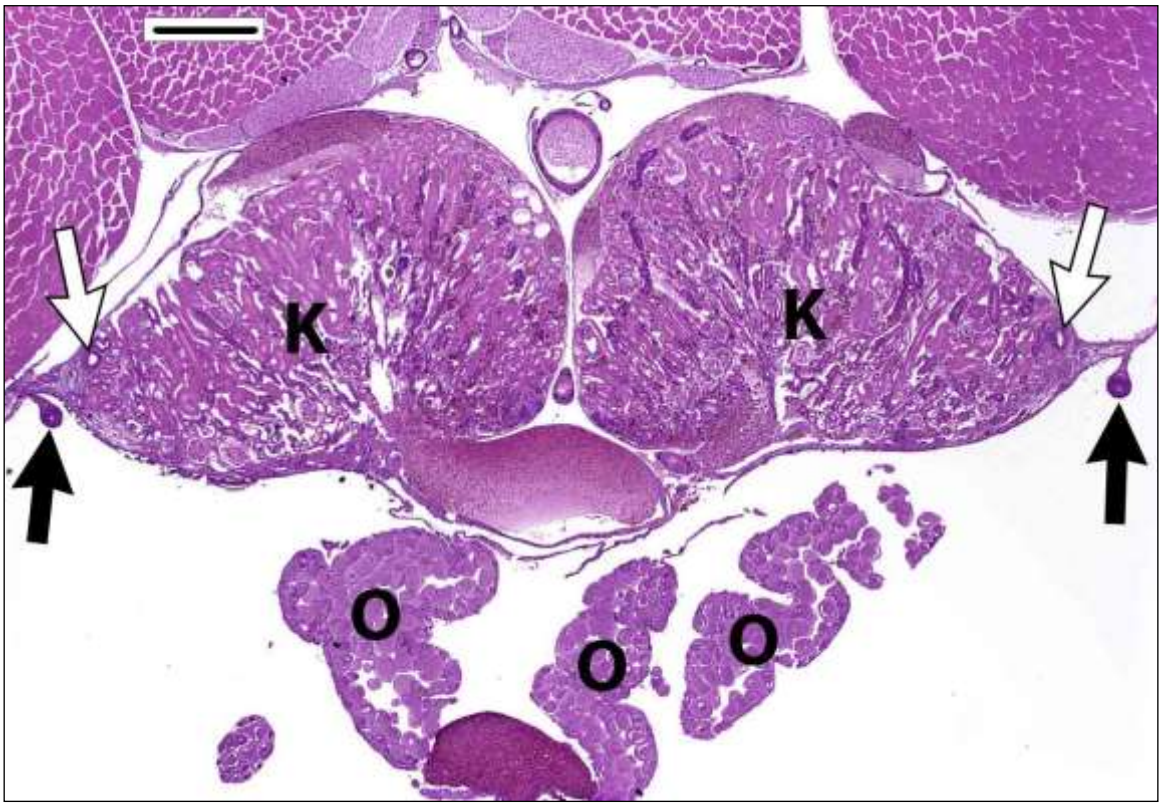
Spermatogonia, Increased. This finding is characterized by a greater frequency of spermatogonia (arrows) in the testes of affected frogs as compared to those of the average control frog. Bar = 50 microns.



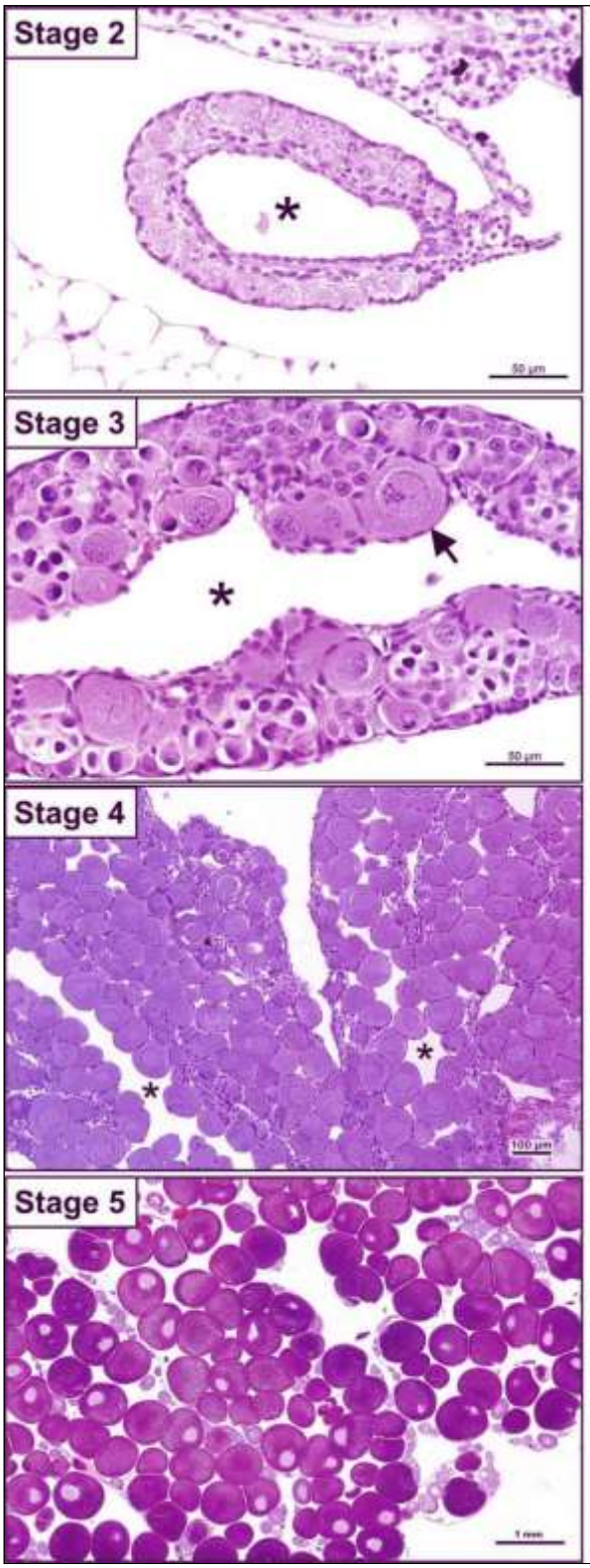
Mononuclear Cell Infiltrates. Mononuclear cell infiltrates (arrows), which appear to consist primarily of lymphocytes, are relatively common in the testes. Bar = 25 microns.



Germ Cell Vacuolation. This finding is characterized by increased amounts of clear cytoplasm in clusters of zygotene-phase primary spermatocytes. Bar = 25 microns.



Normal Ovaries, Oviducts and Wolffian Ducts. The ovaries (O) are attached to the peritoneal surface of the kidneys (K) by suspensory ligaments. The black arrows indicate the oviduct (Müllerian duct) remnants (Stage 2 oviducts in this case), whereas the white arrows indicate the Wolffian ducts. Bar = 500 microns.



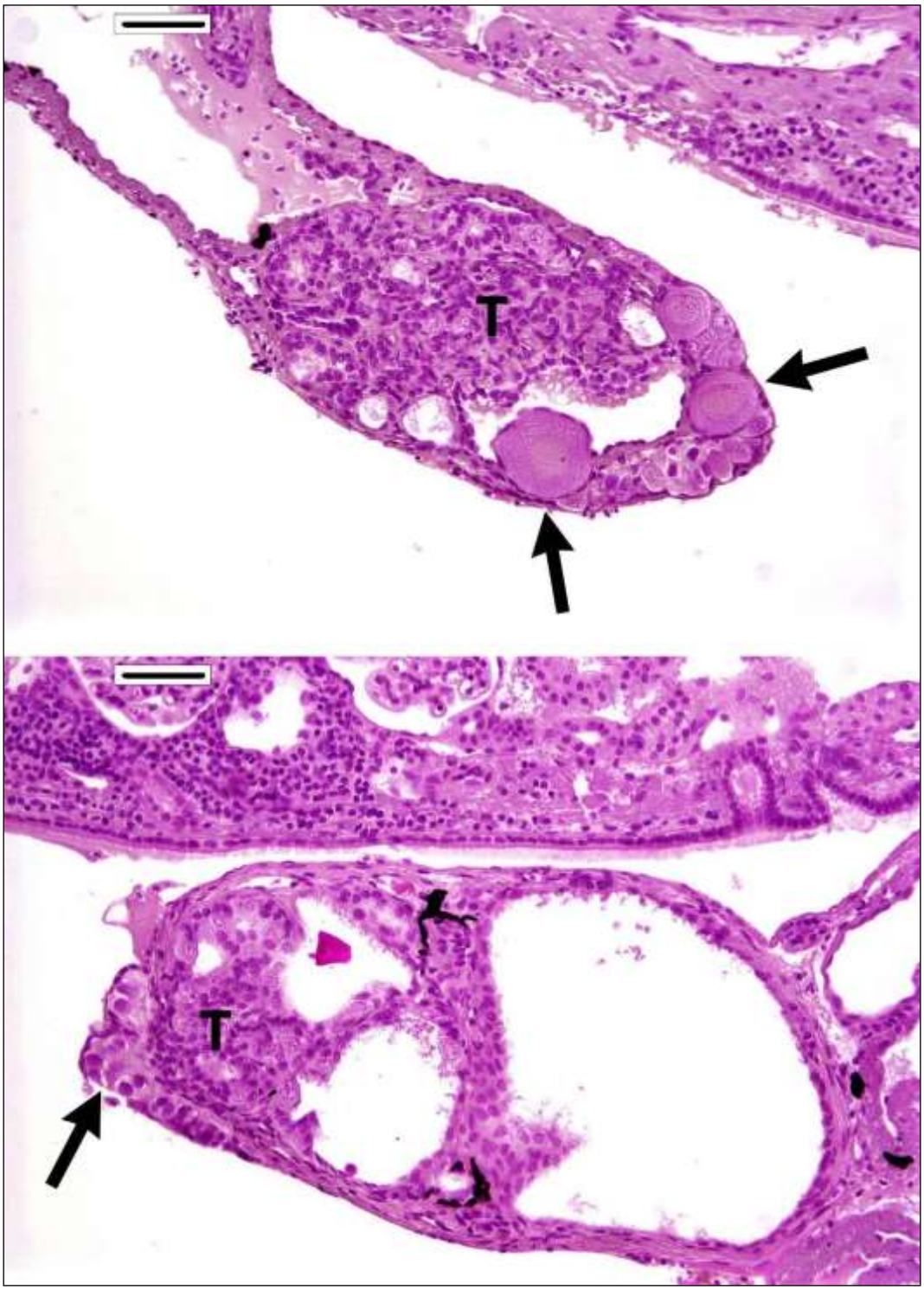
Stage 2:
Gonad identifiable as an ovary based on the presence of a discontinuously open lumen lined with epithelial cells. The germ cells within the cortex (the region outside the epithelial layer) consist of primary oogonia, cysts of primary mitotic oogonia, secondary oogonia, and very early meiotic oocytes. Transverse sections. H&E.
Bar = 50µm
Asterisk*= Ovarian lumen/Ovarian cavity

Stage 3:
First appearance of diplotene oocytes in cortex. Structure and shape of the ovary similar to stage 1, but the appearance of diplotene oocytes is the first morphological difference between male and female germ cells. The most prevalent germ cell types at this stage are cysts of secondary oogonia and cysts of leptotene-pachytene primary meiocytes.
Bar = 50µm
Asterisk*= Ovarian lumen/Ovarian cavity
Arrow = Diplotene oocyte

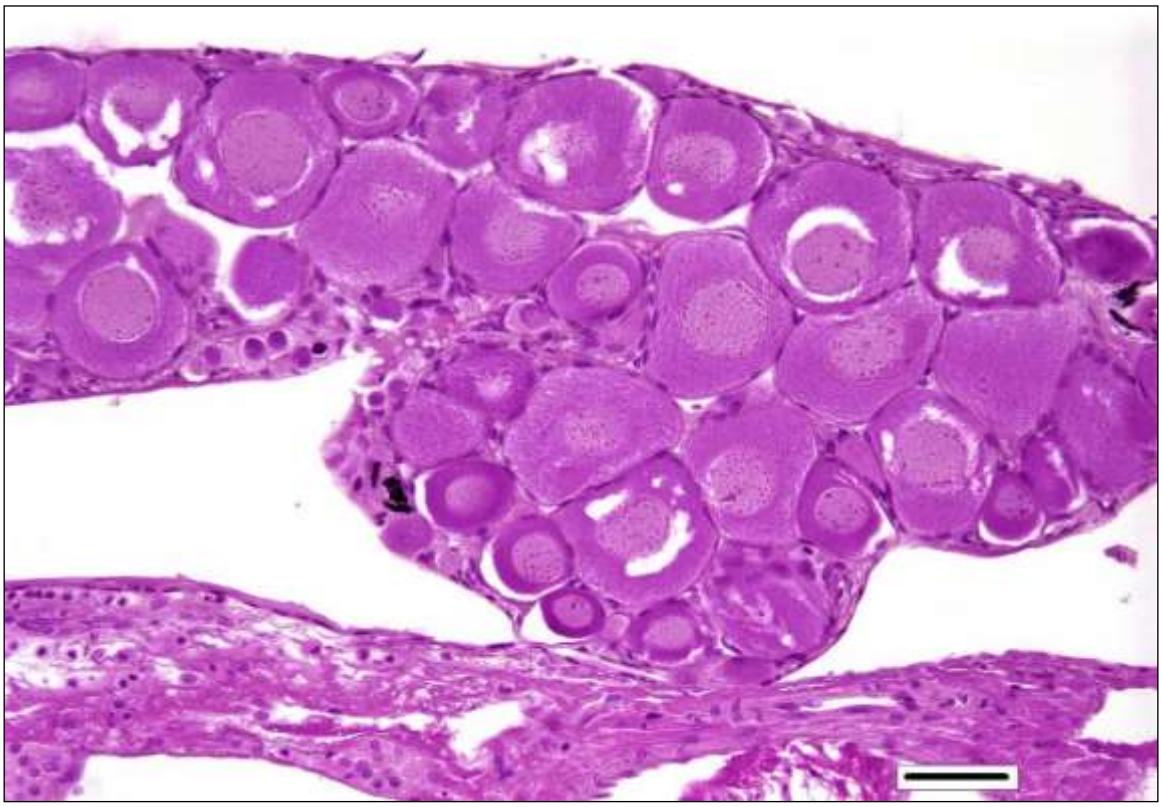
Stage 4:
Pre-vitellogenic (Dumont Stage I) diplotene oocytes are the most prevalent germ cell type observed by area and absolute cell counts. The central lumen is proportionately smaller while the whole ovary grows greatly in size and volume due to the growth of the oocytes. Cysts in earlier stages of oogenesis become fewer in number and are located along the periphery of ovary.
Bar = 100µm
Asterisk*= Ovarian lumen/Ovarian cavity

Stage 5:
Ovary consists almost entirely of vitellogenic oocytes (Dumont Stage IV). Pre-vitellogenic diplotene oocytes can be found along the periphery of the ovary and germ patches of primary and secondary oogonia are difficult to locate.
Bar = 1 mm

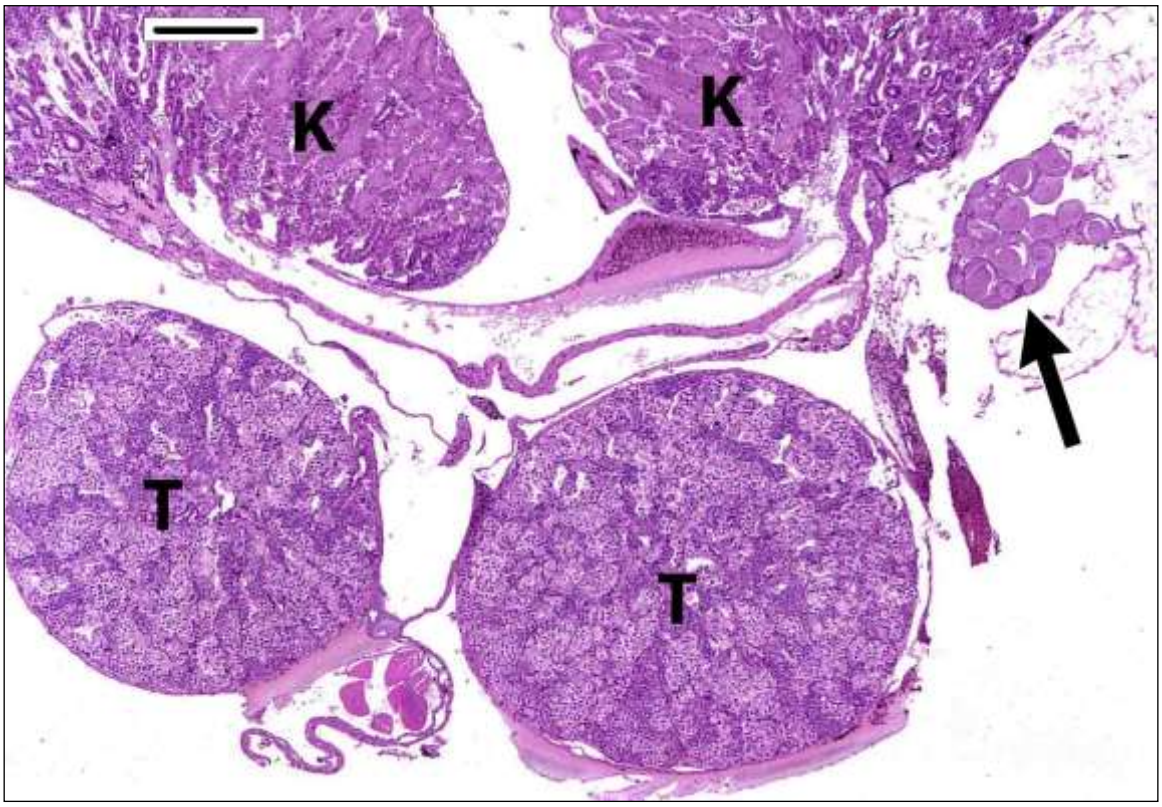
Staging of the Ovary. Control females 10 weeks post NF stage 62 (LAGDA termination) typically have Stage 3 or 4 ovaries.



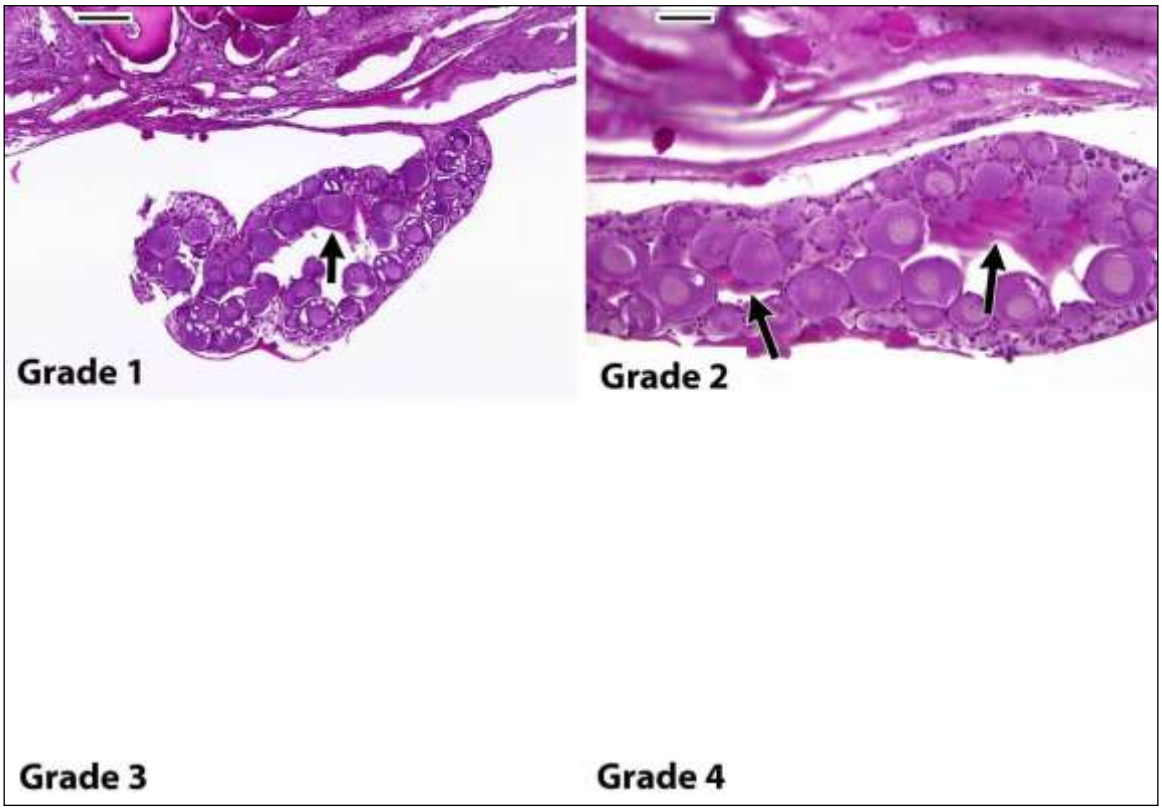
Mixed Sex Gonads. The gonads contain both testicular (T) and ovarian (arrows) components. Each of these gonads received a Phenotype Score of 2. Bar = 50 microns.



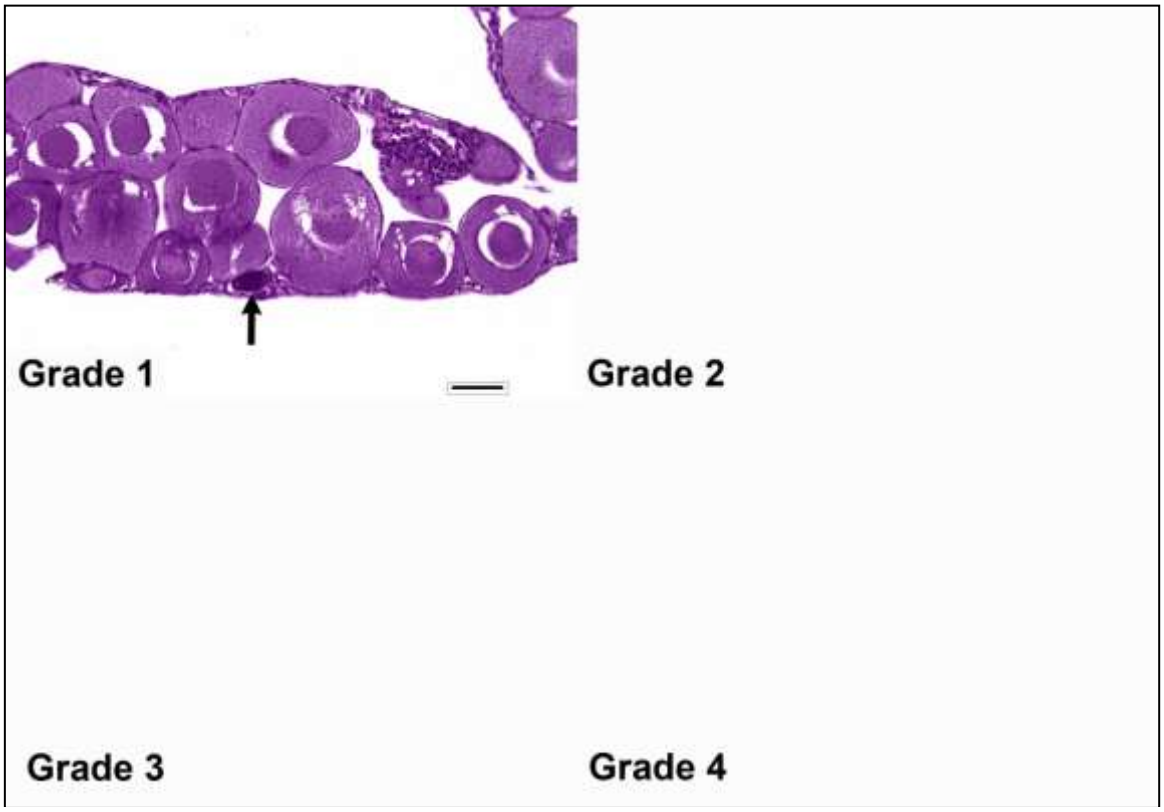
Complete Sex Reversal. This normal appearing ovary was a treatment-related phenotypic finding in a genetic male frog. This ovary would receive a Gonad Phenotype Score of 5. Bar = 50 microns.



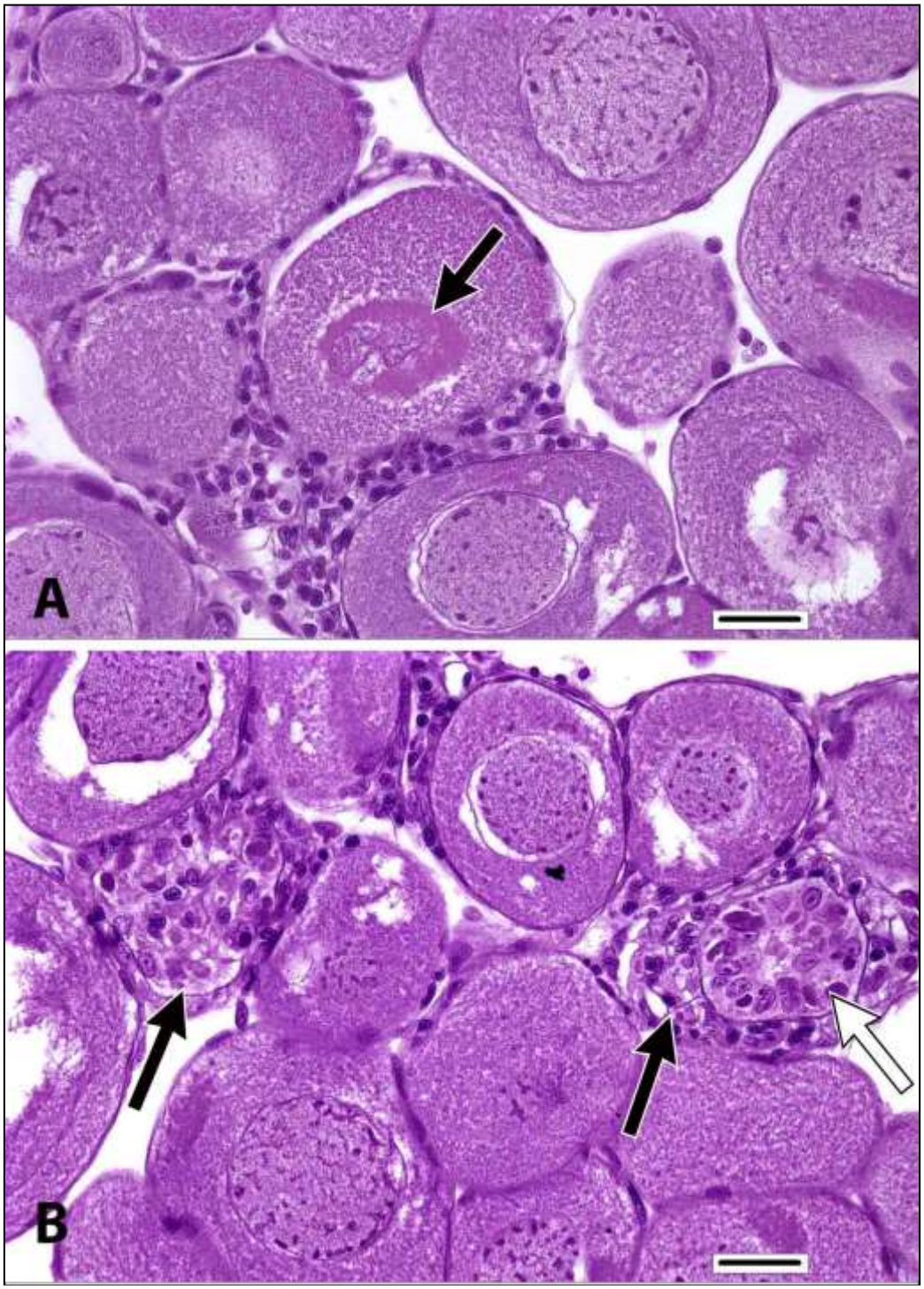
Hermaphroditism. In addition to left and right testes (T), this animal had a small amount of ovarian tissue (arrow) within the abdominal mesentery. K = kidney. Bar = 250 microns.



Proteinaceous Fluid. Proteinaceous fluid (arrows) appears as homogenous dark pink material within the central and subcapsular regions of the ovarian interstitium. Bar = 100 microns (Grade 1), 50 microns (Grade 2).



Oocyte atresia. Spontaneous oocyte atresia appears to be relatively uncommon in the Stage 3 or 4 ovaries of most untreated control frogs. Bar = 50 microns.



Oocyte atresia, Additional Examples. A. In this example, early oocyte degeneration is characterized by a loss of nuclear detail and increased nuclear density. B. In this later stage of atresia, oocyte remnants are being scavenged by nests of macrophages (black arrows). The white arrow indicates what appear to be hypertrophic granulosa cells from an atretic follicle. Bar = 25 microns.