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GUIDANCE DOCUMENT ON HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 3) FOR THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)

Series on Testing & Assessment
No. 228

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Guidance Document on Histopathology Techniques and Evaluation (Part 3)

Environment Directorate

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT

Paris 2015

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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 The draft guidance document on amphibian histopathology was prepared to accompany the draft Test Guideline and help users of the test become more proficient in applying tissue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.

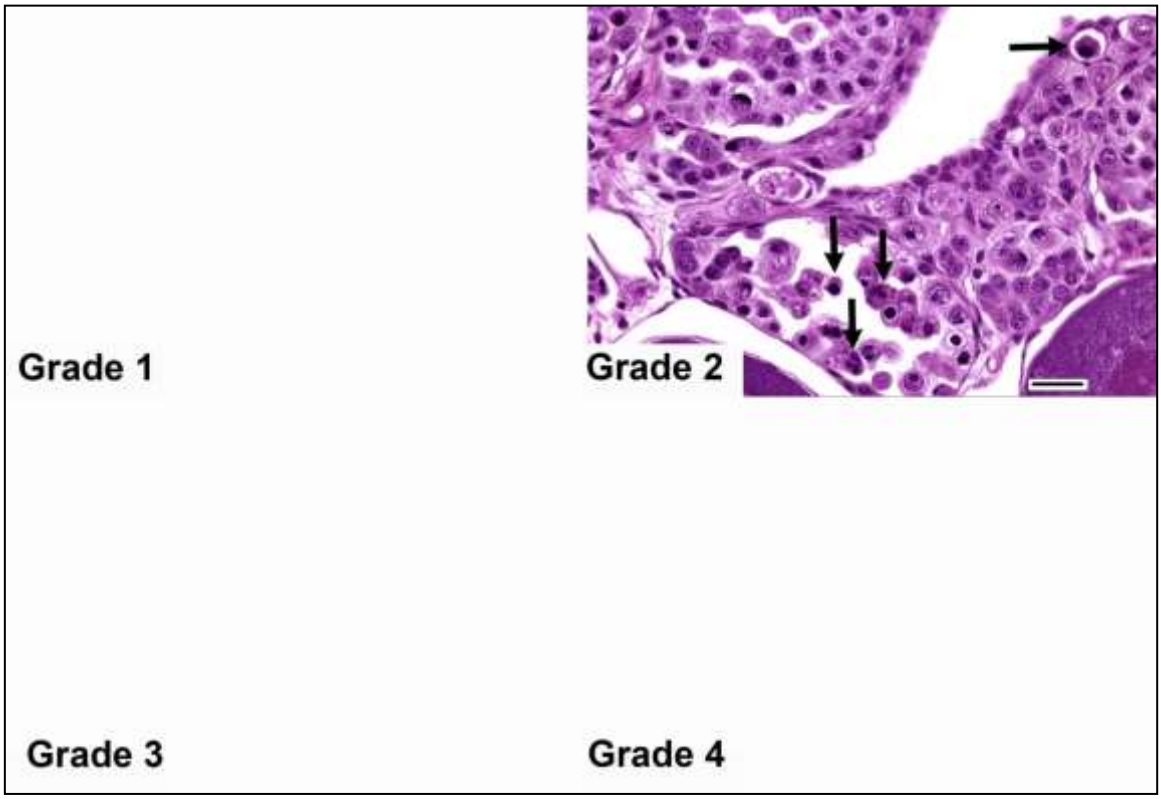
The guidance document on amphibian histopathology techniques and evaluation was approved by the WNT at its 27th meeting in April 2015. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to the declassification of the guidance document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

This document presents **Part 3** 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.

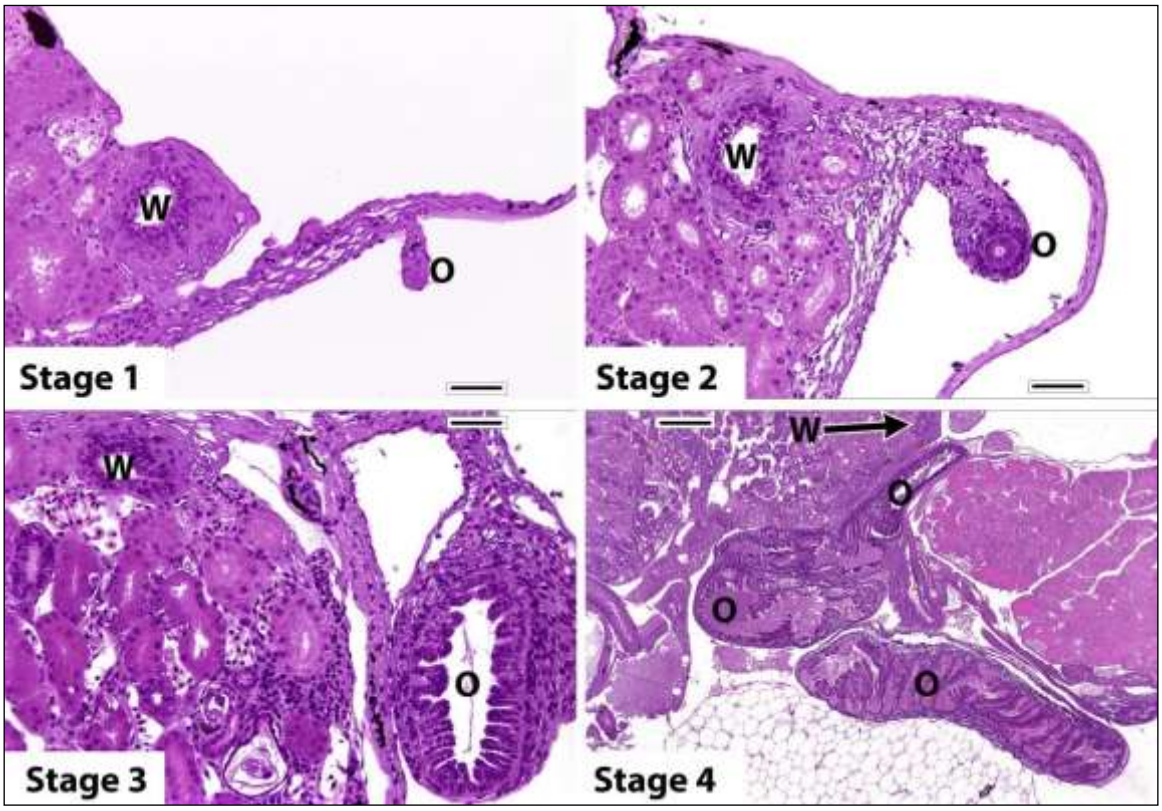
GUIDANCE DOCUMENT ON HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 3)

FOR THE LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)

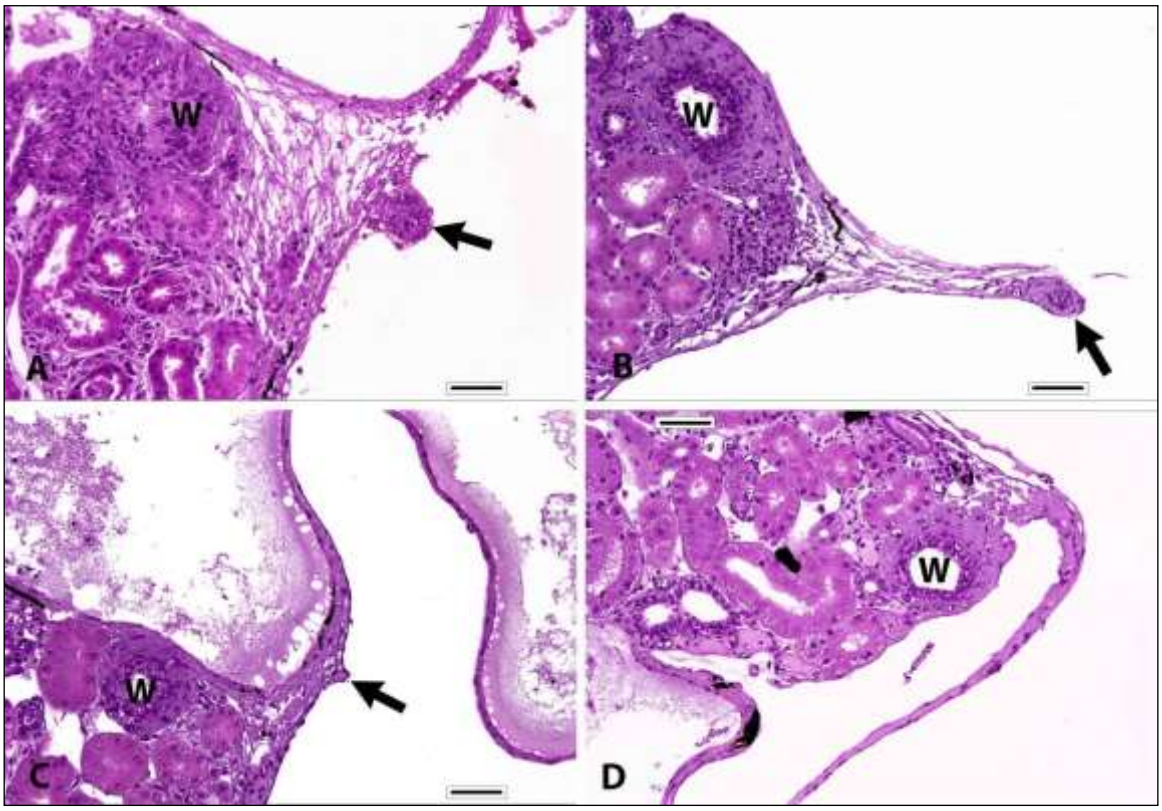


Ovary, Germ Cell Degeneration. The term “germ cell degeneration” is used rather than “atresia” to characterize the *in vivo* deterioration of stem cells or oogonia, or the deterioration of phenotypically undifferentiated cells in early stage gonads. Ovarian germ cell degeneration in the form of apoptotic-like cells (arrows) was observed as a treatment-related response in the Grade 2 image. Bar = 25 microns.

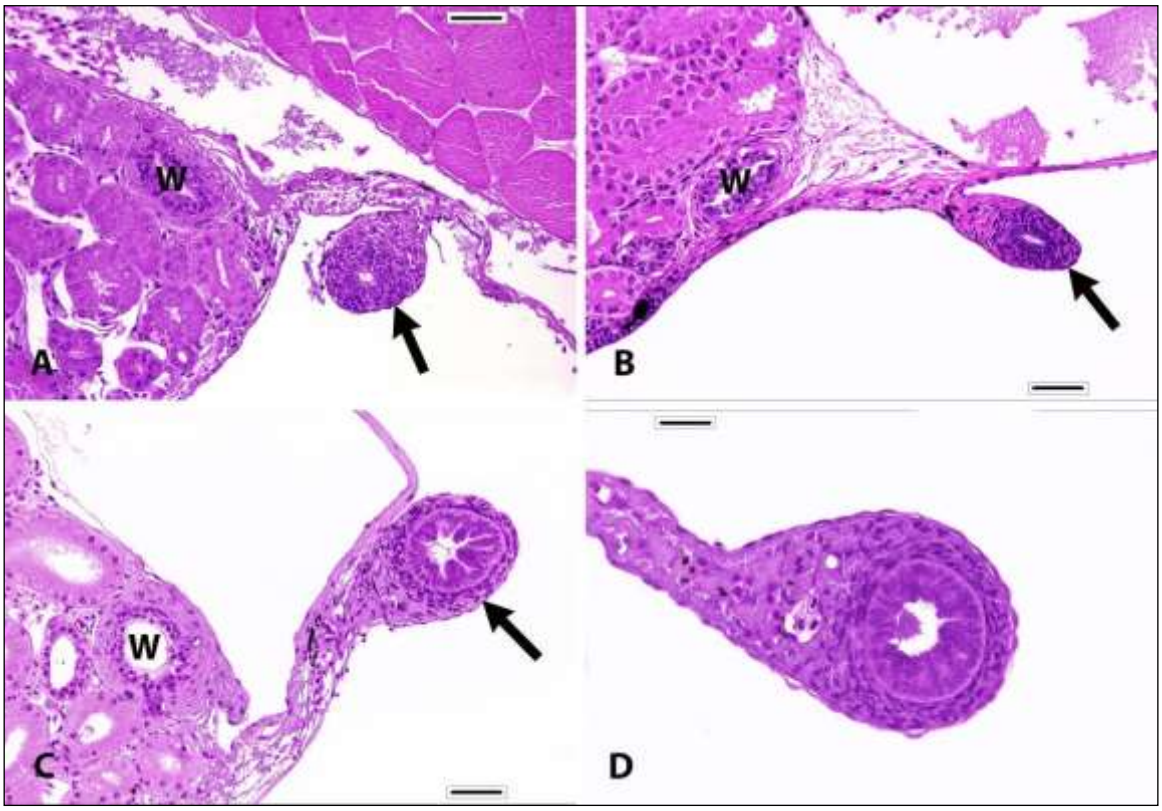
(iv) **Gonadal Ducts**



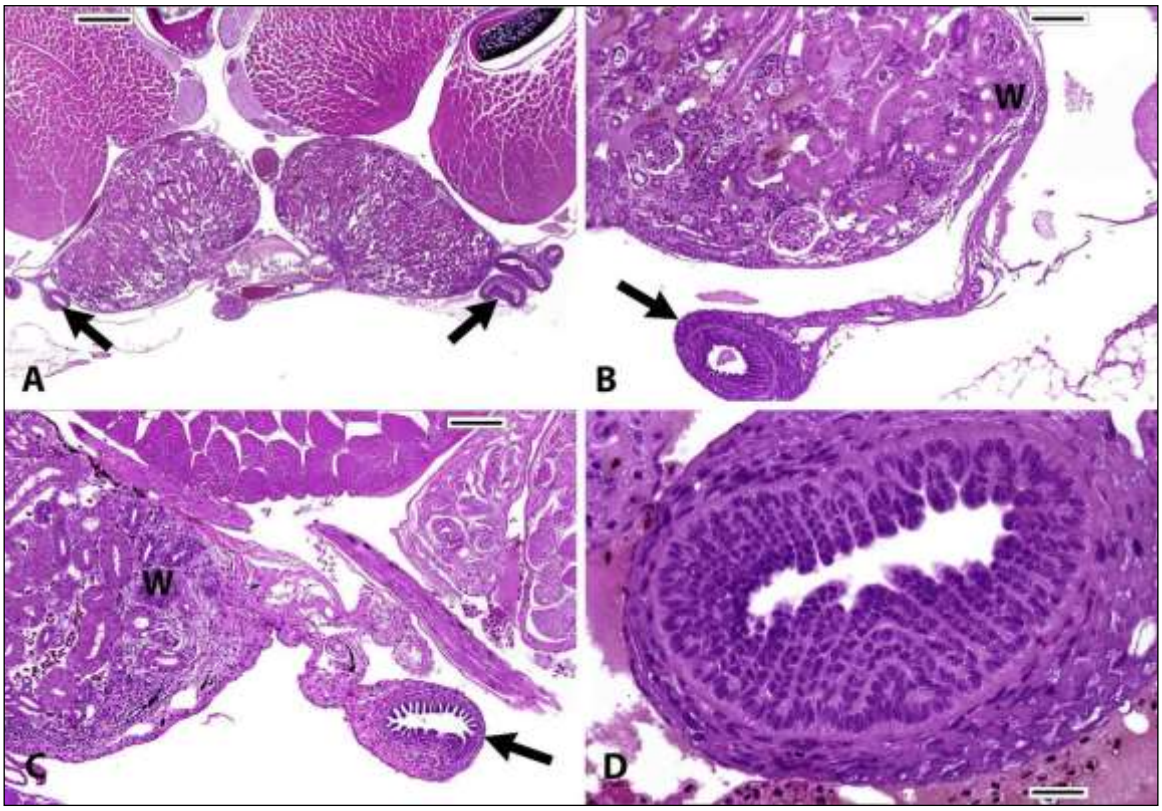
Oviduct Staging. As the oviduct (Müllerian duct) matures in the female, it becomes progressively larger, with an expanded lumen and a more convoluted mucosal lining. Conversely, oviducts regress over time in male frogs. Control male frogs typically have Stage 1 or 2 oviducts 10 weeks post NF stage 62 (LAGDA termination), and the average stage in males is usually less than the average stage in same-aged females of the same study. In borderline cases, the size of the oviduct relative to the Wolffian duct is a useful criterion for differentiating between Stage 2 and Stage 3 oviducts. Bar = 50 microns (Stages 1, 2, and 3), 100 microns (Stage 4).



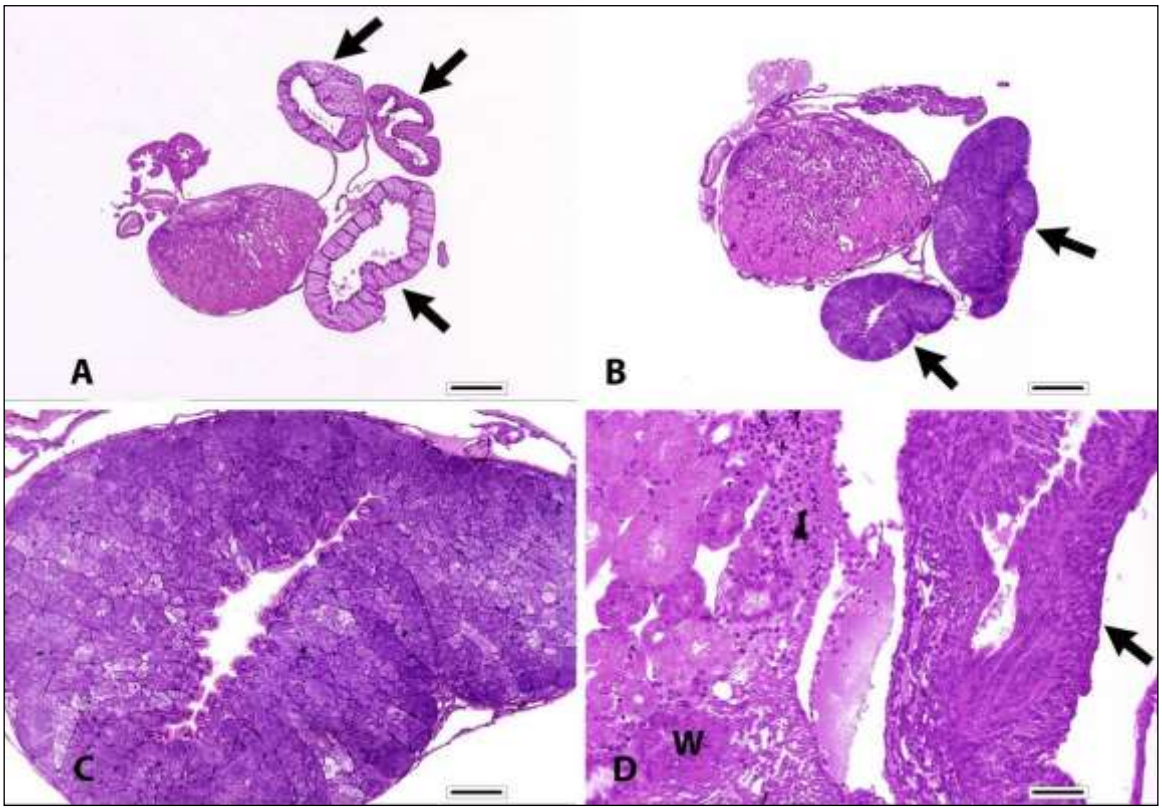
Oviduct Staging, Additional Examples of Stage 1 Oviducts. Stage 1 oviducts (arrows) range from small fibrous protuberances, to suspensory ligaments in which there is no apparent residual oviduct (e.g., Fig. D). These oviducts do not have lumens or mucosal linings. Bar = 50 microns.



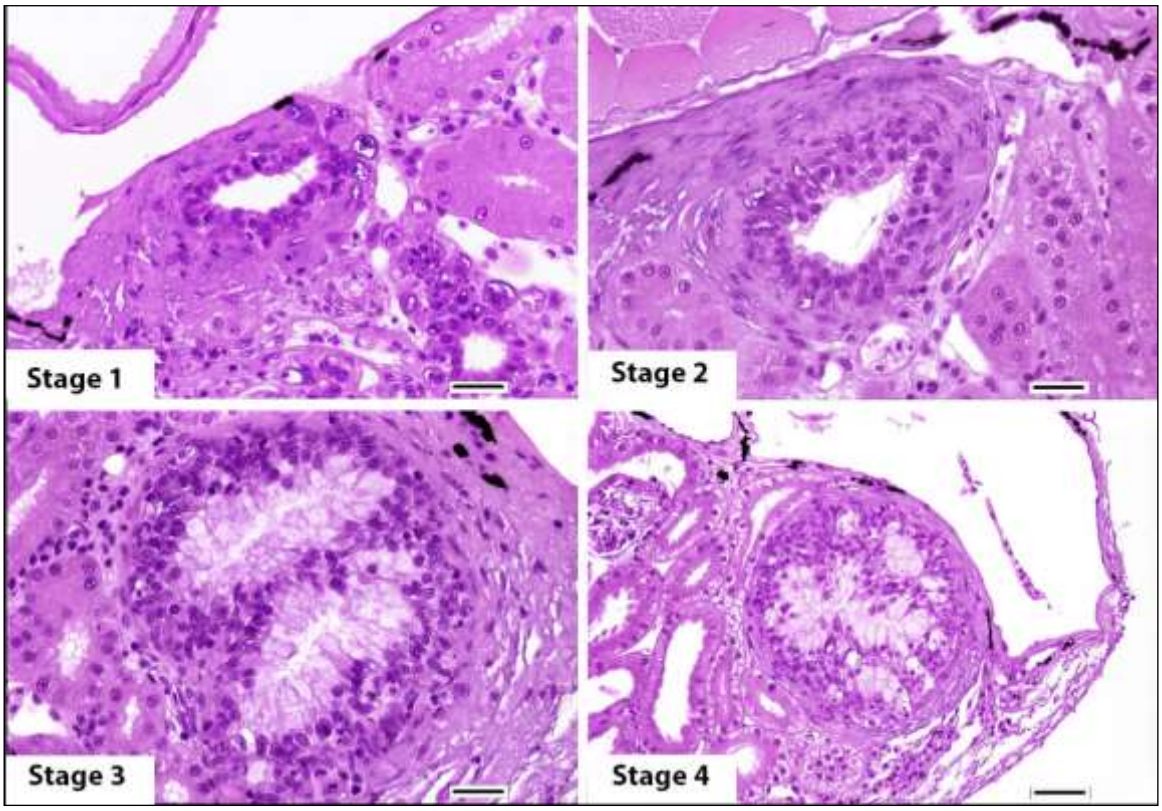
Oviduct Staging, Additional Examples of Stage 2 Oviducts. Stage 2 oviducts (arrows), which are comparable in size to the Wolffian ducts, all have a visible lumen and are lined by one to a few layers of mucosal epithelial cells, with or without slight mucosal folding. Bar = 50 microns (Figs. A-C), 25 microns (Fig. D).



Oviduct Staging, Additional Examples of Stage 3 Oviducts. Stage 3 oviducts (arrows), which greater than 1.5 times the size of Wolffian ducts, have intricate mucosal folding. Bar = 500 microns (Fig. A), 100 microns (Figs. B and C), 25 microns (Fig. D).

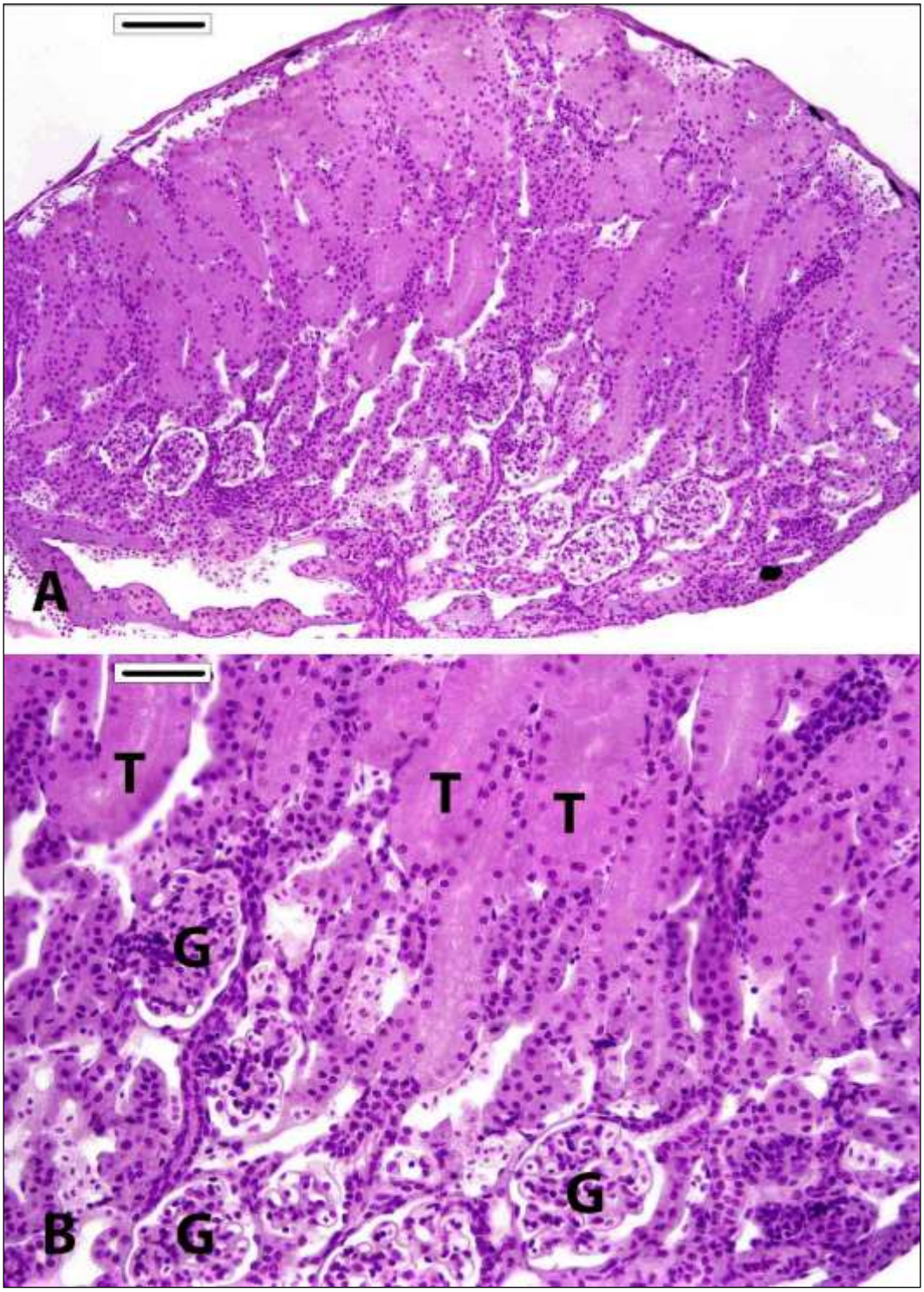


Oviduct Staging, Additional Examples of Stage 4 Oviducts. Stage 4 oviducts (arrows) are markedly larger than Wolffian ducts, and the mucosal epithelium is forming, or has formed, large glandular structures. Fig. D is enlarged relative to the other figures. The oviduct in Fig. D represents the borderline between Stages 3 and 4. Bar = 500 microns (Figs. A and B), 100 microns (Fig. C), 50 microns (Fig. D).

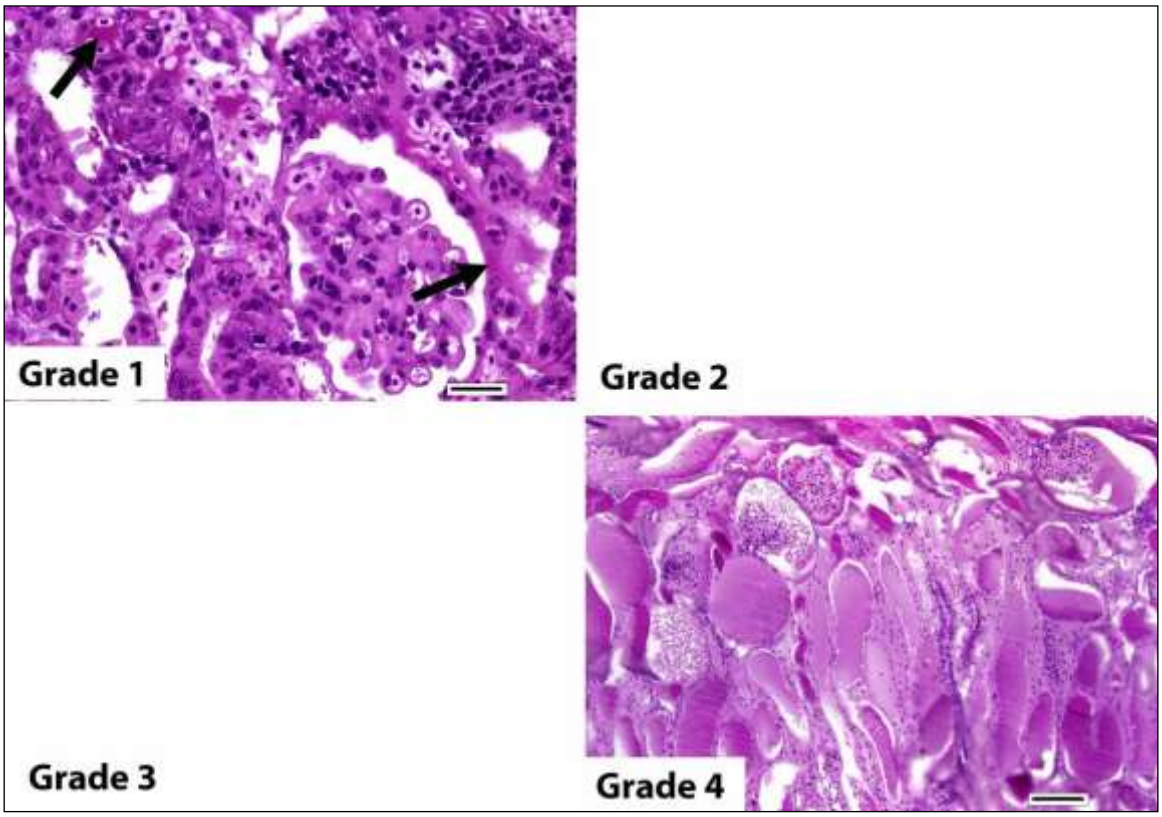


Wolffian Duct Staging. The Wolffian ducts, which are located within the lateral extremities of the right and left kidneys, also function as ureters in *Xenopus* spp. With maturity, the Wolffian ducts become progressively larger, although not to the extent of oviducts in female frogs. The mucosal lining of the ducts also becomes thicker and contains more mucous cells with age. Generally, mean Wolffian duct scores are slightly higher in control males than in control females of the same study. Bar = 25 microns (Stages 1-3), 50 microns (Stage 4).

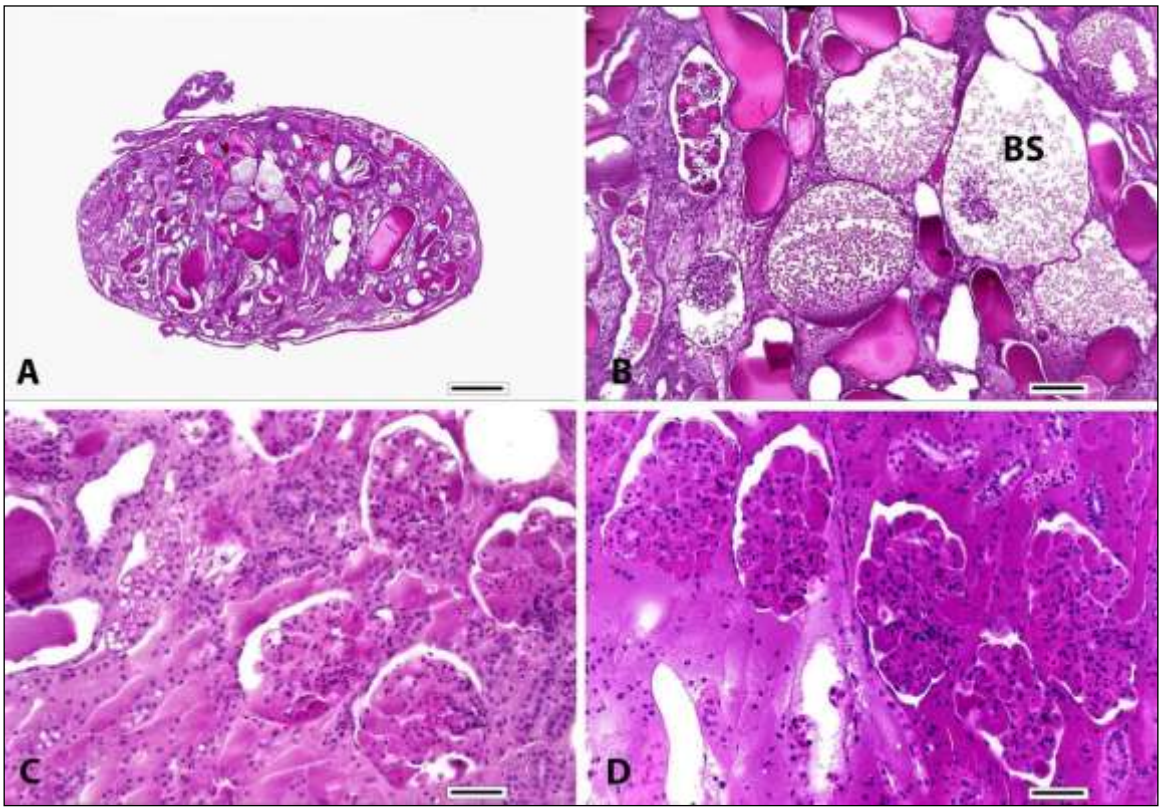
(v) **Kidneys**



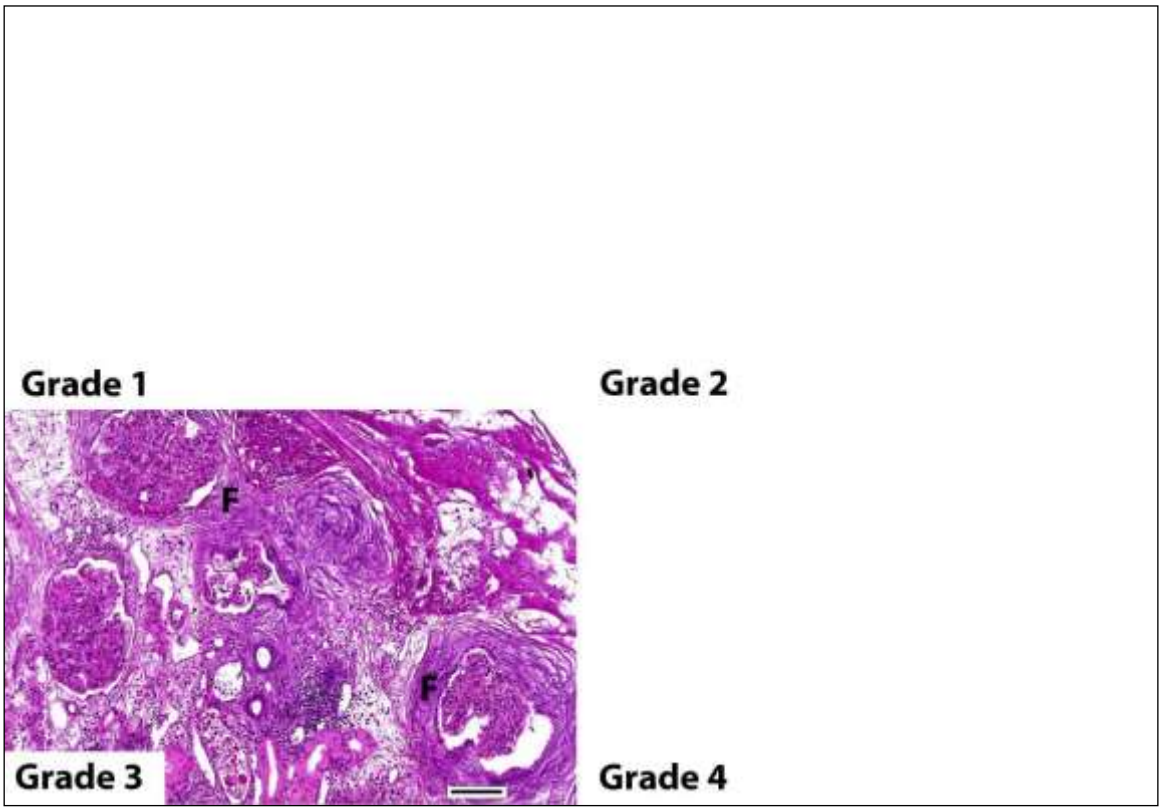
Normal Kidney from a Control Frog. Glomeruli (G) are located in the ventral third of the kidneys, whereas proximal tubules (T) occupy the dorsal portions. Bar = 100 microns (Fig. A), 50 microns (Fig. B).



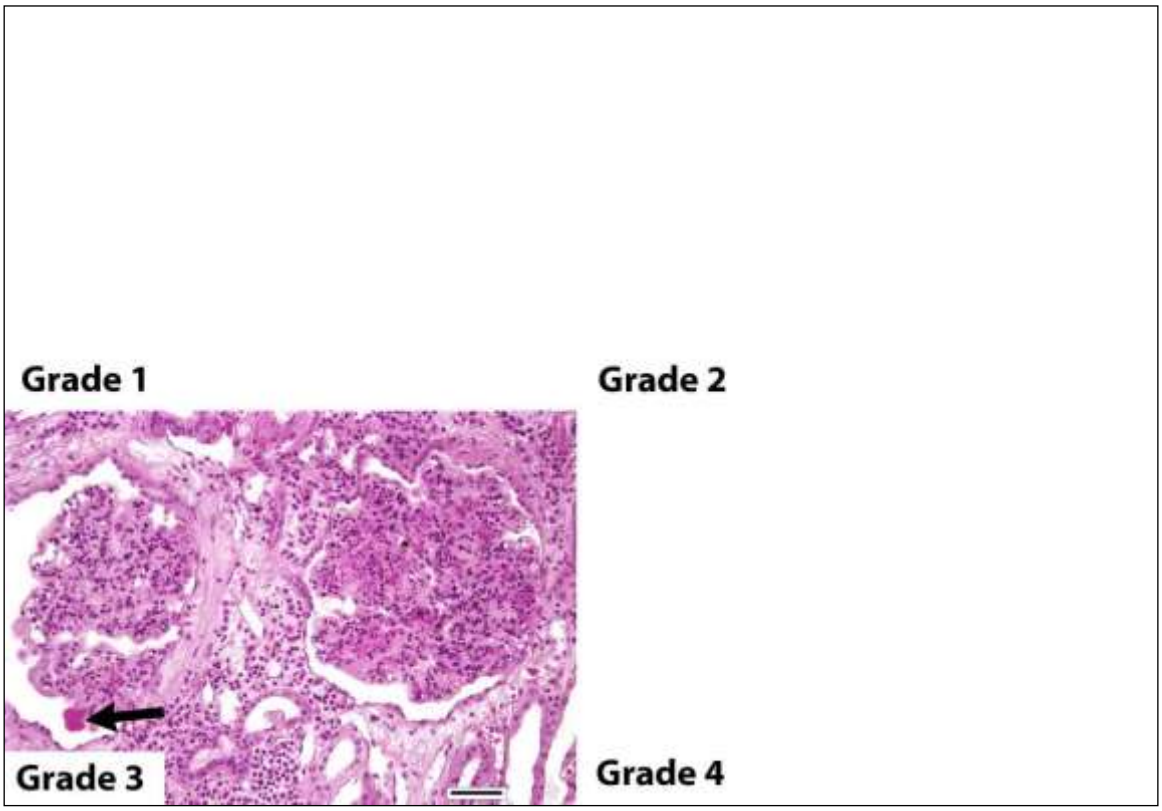
Proteinaceous Fluid. Proteinaceous fluid (arrows) appears as homogenous dark pink material within the renal interstitium, blood vessels, tubules, and/or Bowman's spaces. Bar = 25 microns (Grade 1), 100 microns (Grade 4).



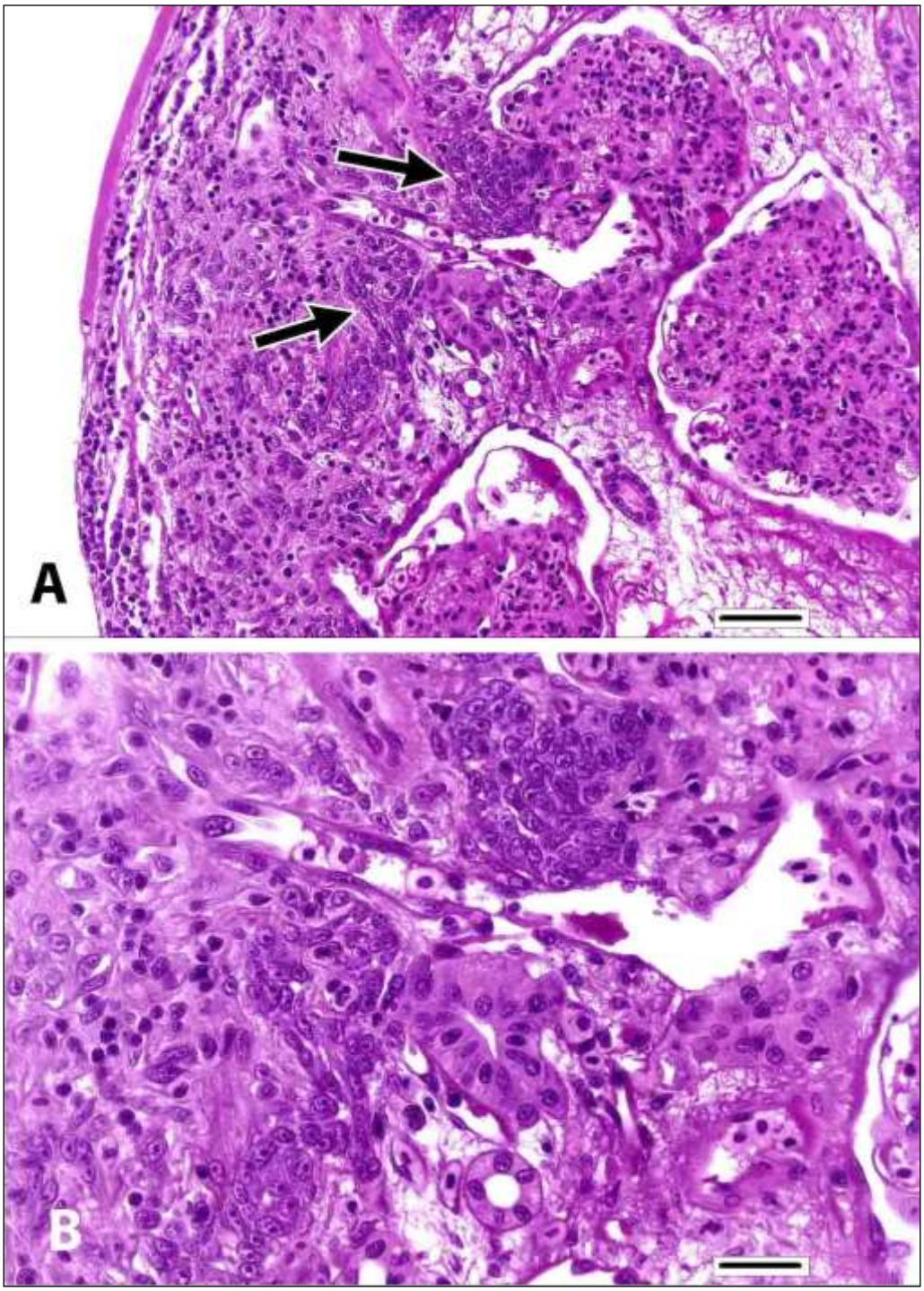
Proteinaceous Fluid, Grade 4, Additional Examples. Tubules, glomeruli, and the renal interstitium are flooded by proteinaceous fluid in these examples. BS = dilated Bowman's space. Bar = 500 microns (Fig. A), 100 microns (Fig. B), 50 microns (Figs. C and D).



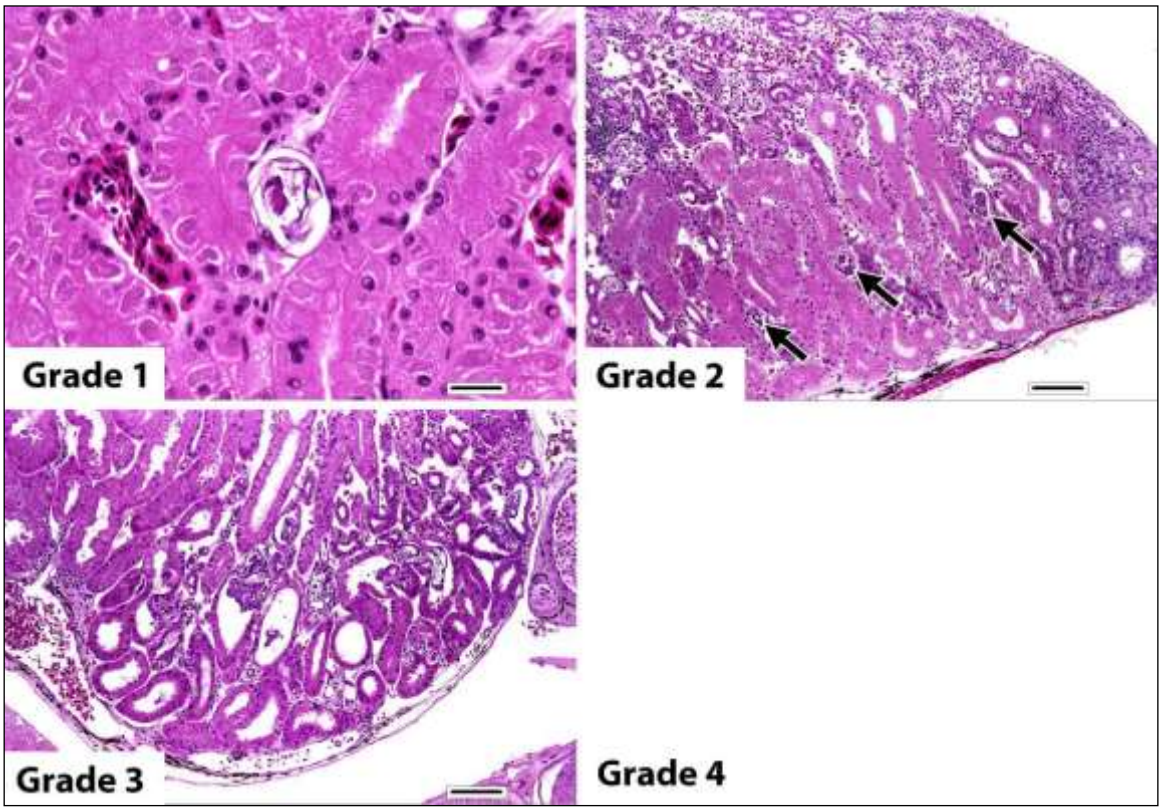
Fibrosis. This finding, which is characterized by excessive amounts of fibrous connective tissue (F) within the renal interstitium, usually occurs as a sequel to chronic inflammation or other long standing parenchymal damage. Bar = 100 microns (Grade 3).



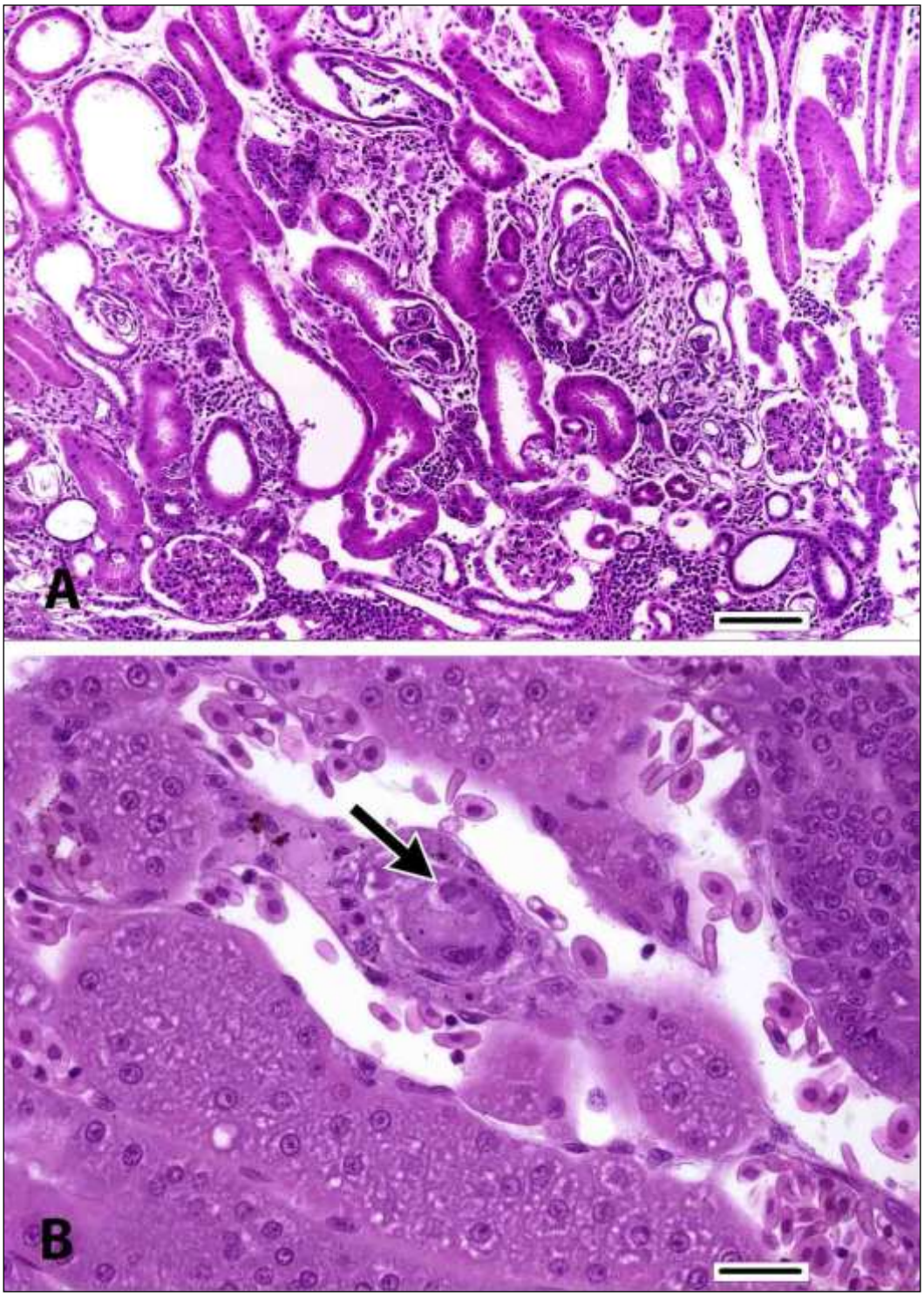
Glomerulomegaly and Glomerular Hypercellularity. This finding can be a consequence of low level chronic glomerular damage. The arrow indicates a small deposit of proteinaceous material within a glomerular capillary. Bar = 20 microns (Grade 3).



Regenerative Blast Cell Hyperplasia. This finding, which is another consequence of chronic renal damage and tubular loss, is characterized by streaming proliferations of cells with large, hyperchromatic (dark) nuclei (arrows). Figure B is a higher magnification of Figure A. This particular case was assigned a severity score of Grade 2 (mild). Bar = 50 microns (Fig. A), 25 microns (Fig. B).



Mineralization with Tubular Dilation. Mineralization appears to be a common background finding in laboratory reared *X. laevis*, and the severity of this finding appears to vary from facility to facility. Husbandry factors (e.g., issues involving feed and/or water composition) are suspected causes. The occurrence of mineralization is associated with focal to diffuse renal tubular dilation, presumably due to obstruction of urine flow. Tubular dilation and mineralization are usually graded separately. In a given frog, the severity grade for tubular dilation is typically one grade less than that of mineralization (see example below). Bar = 25 microns (Grade 1), 100 microns (Grades 2 and 3).



Mineralization, Additional Examples. Figure A represents another case of Grade 3 mineralization and Grade 2 tubular dilation. Figure B illustrates a small amount of mineral (arrow) within a multinucleated giant cell macrophage. Bar = 100 microns (Fig. A), 25 microns (Fig. B).

(f) **References.**

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