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Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (MEOGRT) - Part 3

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Series on Testing and Assessment

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Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (MEOGRT) – Part 3

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Paris 2015

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Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

FOREWORD

The project to develop a Medaka One Generation Reproduction Test (MEOGRT) was initiated by Japan and the United States and included in the work plan of Test Guidelines Programme in 2000, originally under the name; Medaka Life Cycle (MLC)/Multi-generation Test (MMT).

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 2013, with subsequent commenting rounds in 2013 and 2014. The draft guidance document on Medaka histopathology was prepared to accompany the draft Test Guideline and help users of the test become more proficient in applying tissue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 Medaka 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.

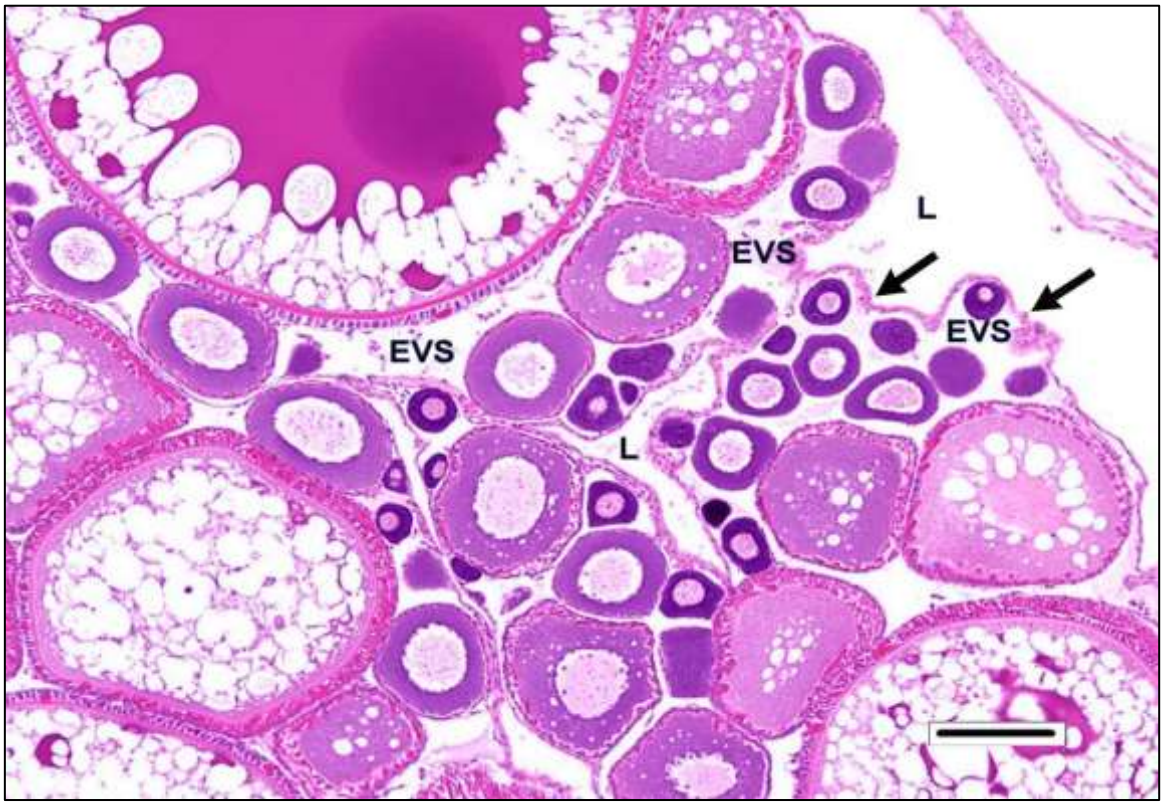
This document presents **Part 3** of the guidance document which in total consists of four parts.

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

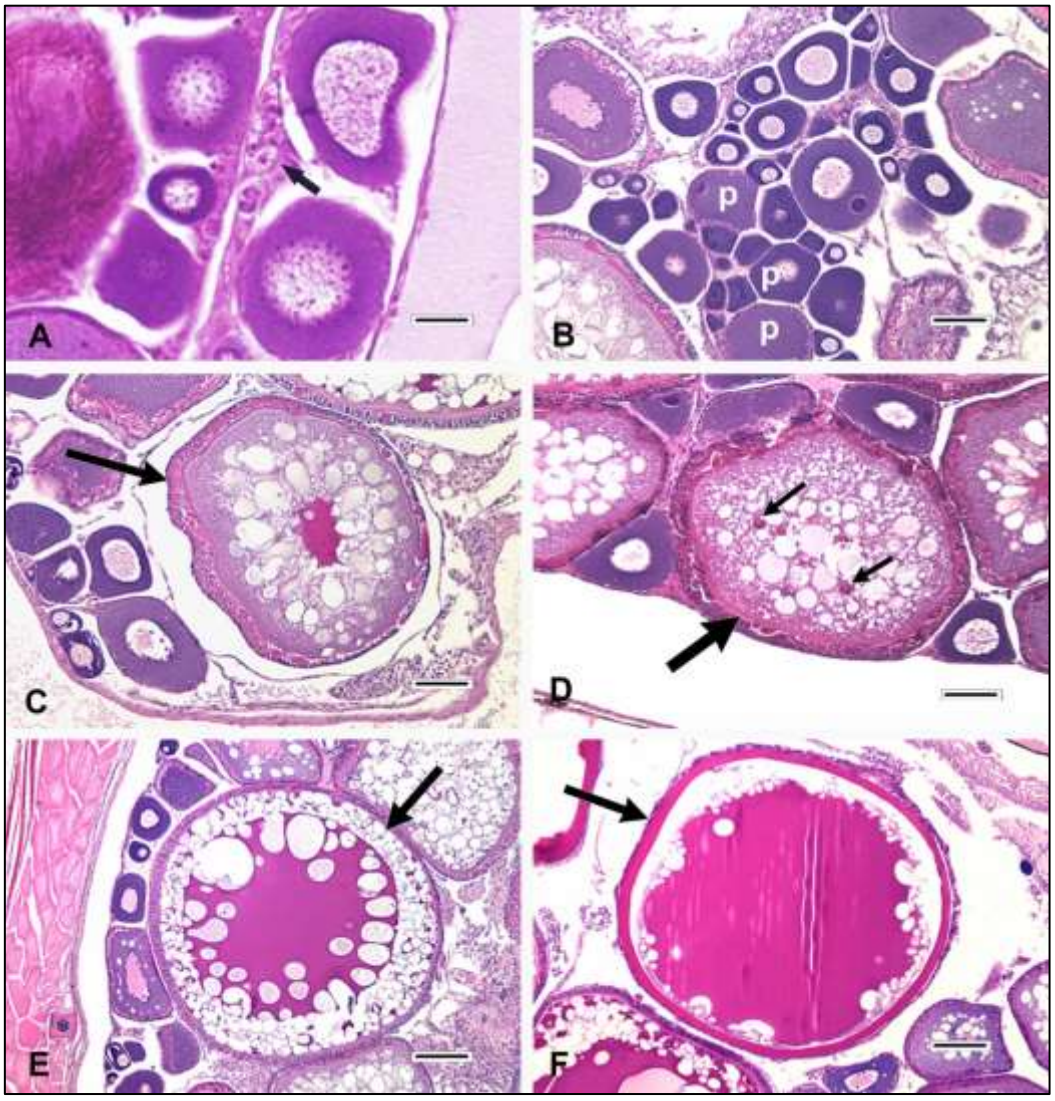
GUIDANCE DOCUMENT ON MEDAKA HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 3)

FOR THE

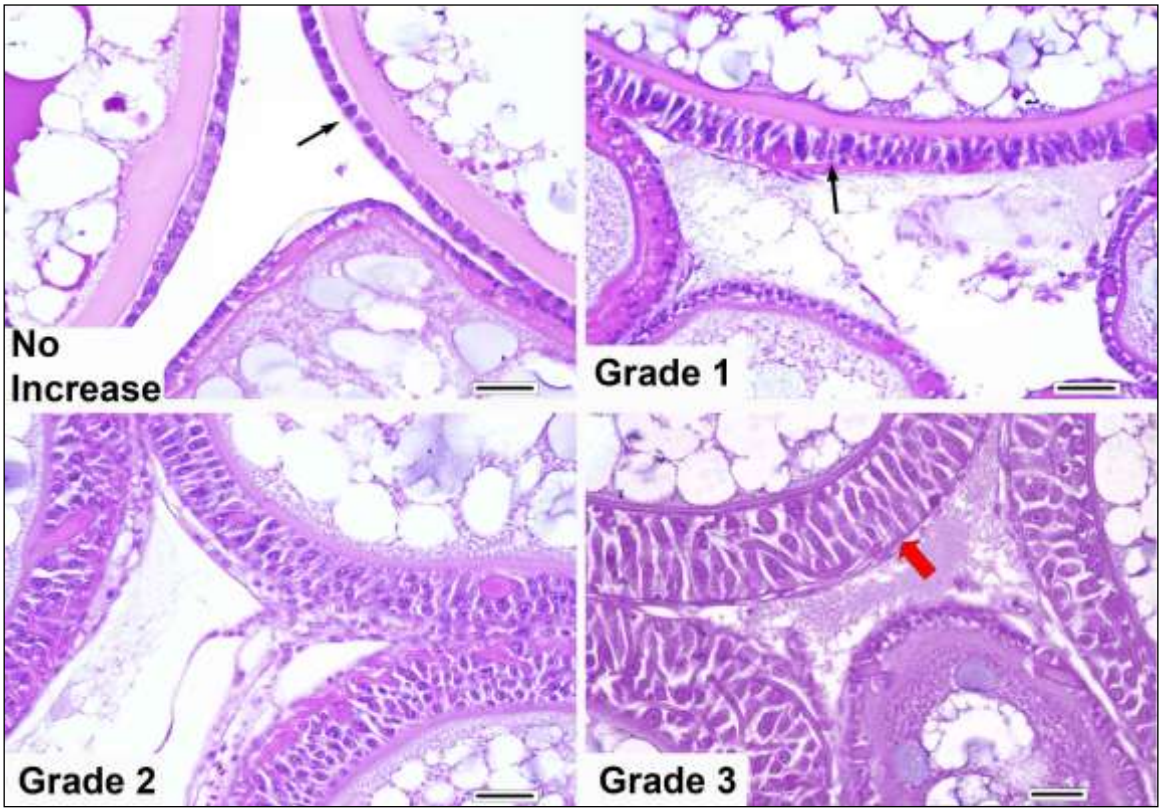
MEDAKA EXTENDED ONE-GENERATION REPRODUCTION TEST (MEOGRT)



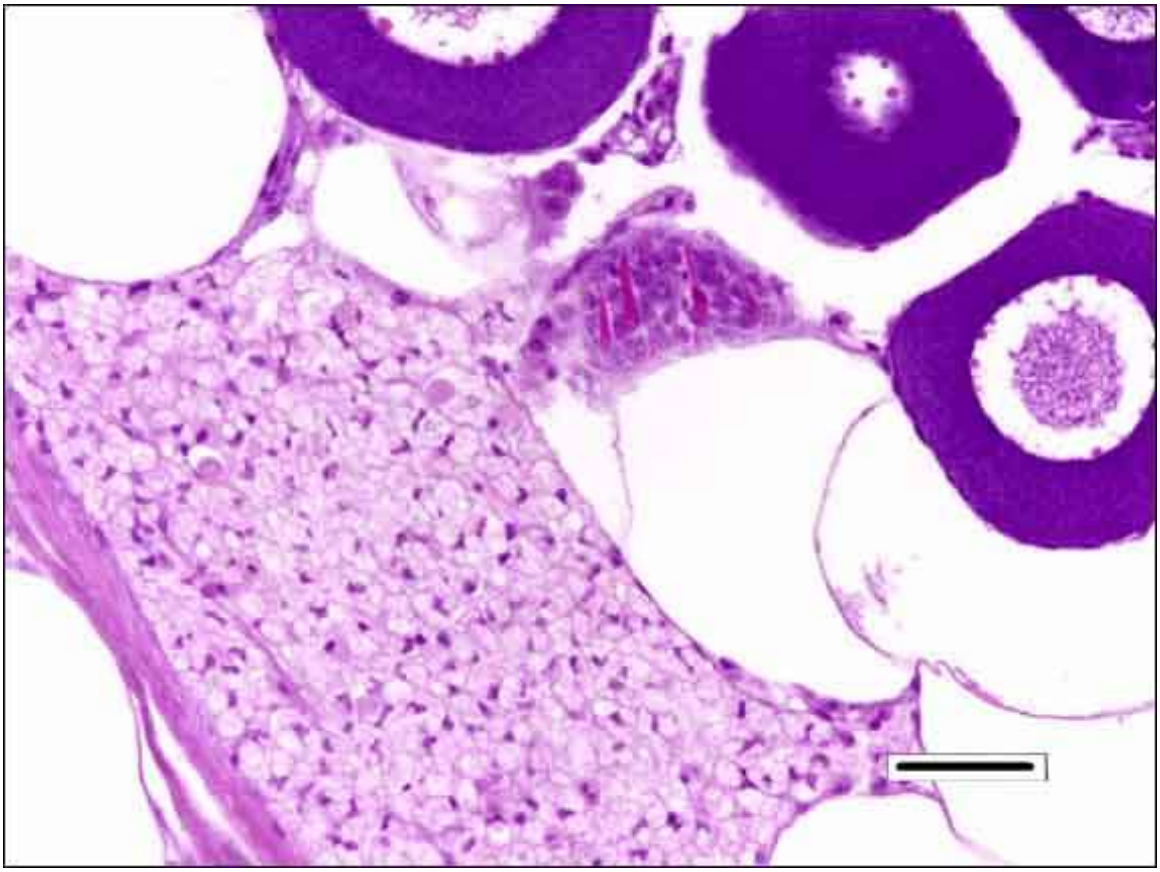
Ovary, germinal epithelium. Normal ovary from an adult female. Arrows indicate the germinal epithelium which, at this magnification, is a membranous structure that separates the ovarian lumen (L) from the extravascular space (EVS) of the ovarian stroma. The germinative parenchyma of the ovary, the membrane bound germinal epithelium constitutively contains oogonia, pre-follicular and pre-thecal cells, epithelial cells, and occasionally small chromatin nucleolar (primary growth) oocytes (Norberg et al., 1999; Parenti and Grier, 2003). The germinal epithelium separates the ovarian lumen from the stroma, the latter of which often contains perinucleolar, cortical alveolar, and vitellogenic follicles within a variably-apparent extravascular space. Bar = 100 µm.



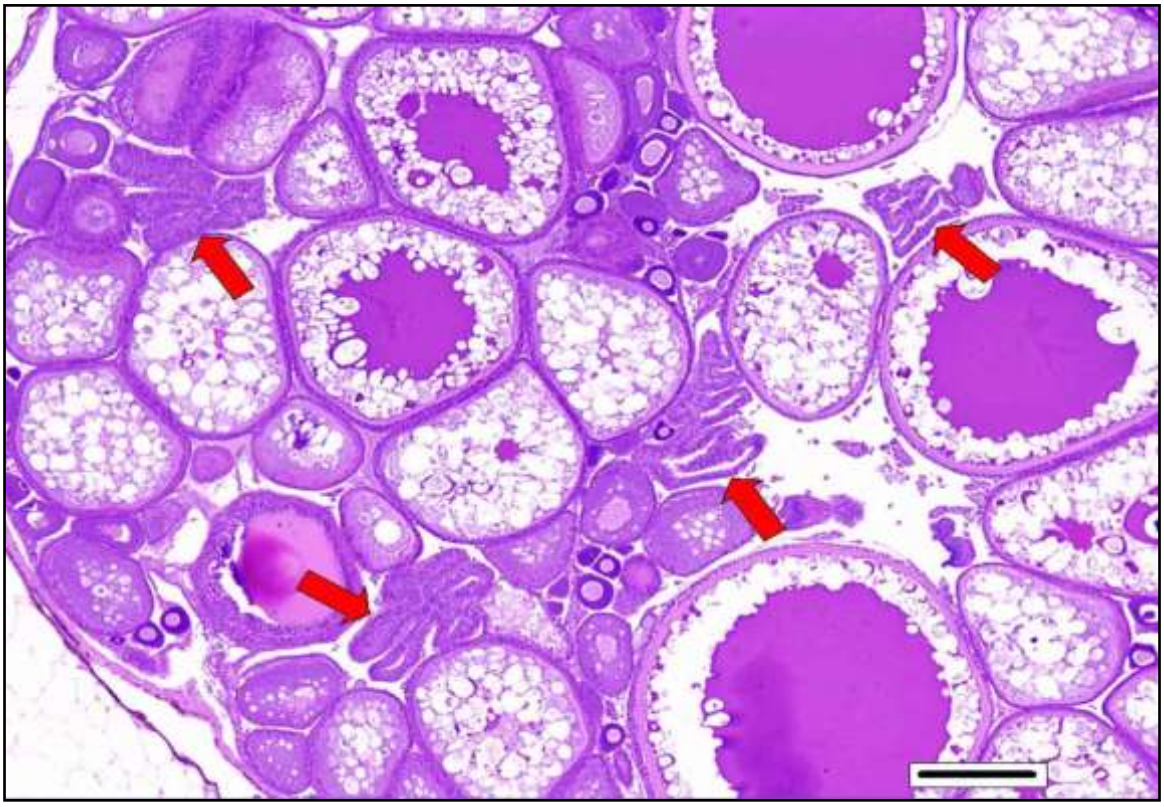
Ovary, oogenic cell types*.** A: **Oogonia** (arrow). Unlike mammalian oogonia, which traditionally are considered to be non-proliferative following the early post-natal period, piscine oogonia continue to divide in juvenile and adult fish. The smallest of the oocytic cells, oogonia reside within the ovarian germinal epithelium, usually in comparatively low numbers. Oogonia are characterized by a relatively large nucleus with small or inapparent nucleolus, and minimal amounts of cytoplasm. B: **Perinucleolar phase oocytes** (p). Concomitant with oocyte growth, the nucleus (germinal vesicle) increases in size and multiple nucleoli appear, generally at the periphery of the nucleus. The cytoplasm stains uniformly dark, although late perinucleolar oocytes may have small clear or amphophilic vacuoles in the cytoplasm. These cells tend to be abundant in normal adult ovaries. C: **Cortical alveolar oocytes** (arrow). Generally larger than perinucleolar oocytes, this phase is characterized by the appearance of cortical alveoli (yolk vesicles) within the ooplasm. The cortical alveoli are technically not yolk, as they do not provide nourishment for the embryo (Selman and Wallace, 1989). The chorion becomes distinctly evident in this phase, the nucleus becomes reduced, and the perifollicular cells are more easily visualized. D: ***Early vitellogenic oocytes (large arrow). Larger than cortical alveolar oocytes, these cells are characterized by the centralized appearance of spherical, eosinophilic, vitellogenic yolk granules / globules (small arrows). The nucleus has moved to the periphery of the cell and dissolved. E: **Late vitellogenic oocytes** (arrow). These cells are characterized by an increased accumulation of yolk material that fuses into a central liquid mass which displaces the cortical alveolar material to the periphery of the cytoplasm. F: **Mature spawning follicle** (arrow). In this phase of development, vitellogenesis has reached its peak, the cell has become larger and more hydrated, and ooplasm consists almost entirely of yolk. Because of the transient nature of these cells in fractional spawning fish, mature / spawning oocytes are uncommonly observed. Bar = 25 µm (A), 50 µm (B through D), 100 µm (E and F).



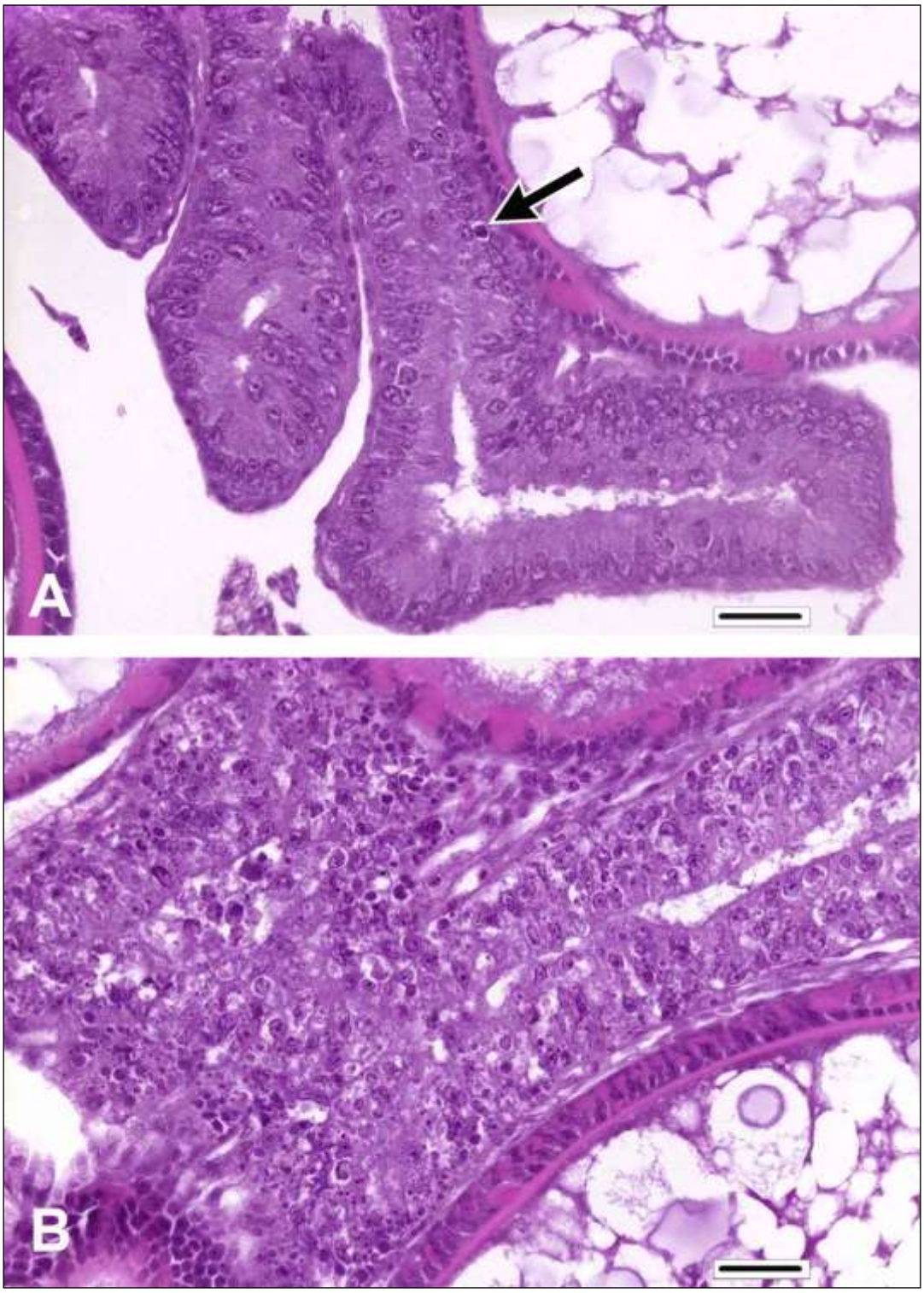
Ovary, perifollicular cell hyperplasia / hypertrophy, grading. Exposure to aromatase inhibitors (e.g., fadrozole, prochloraz) has been associated with these perifollicular cell changes in medaka ovaries. A similar effect has also been linked with exposure to the non-aromatizable androgen, trenbolone (unpublished data). This finding is characterized by an increase in the height and number of granulosa cells, which gives this cell layer a "pseudostrafied" appearance in extreme cases. A common coexisting change in affected medaka has been decreased yolk formation. Because perifollicular cells (i.e., granulosa cells) are thought to be involved with aromatase production in fish (Nagahama, 1987; Devlin and Nagahama, 2002), it is possible that the increased number and size of these cells is a compensatory mechanism aimed at restoring aromatase to levels required for vitellogenesis. It is important to note that: 1) normal perifollicular cells may appear hypertrophic in tangentially-sectioned oocytes, and 2) perifollicular cell changes are best identified by comparisons made with concurrent control fish. Bar = 25 µm (all).



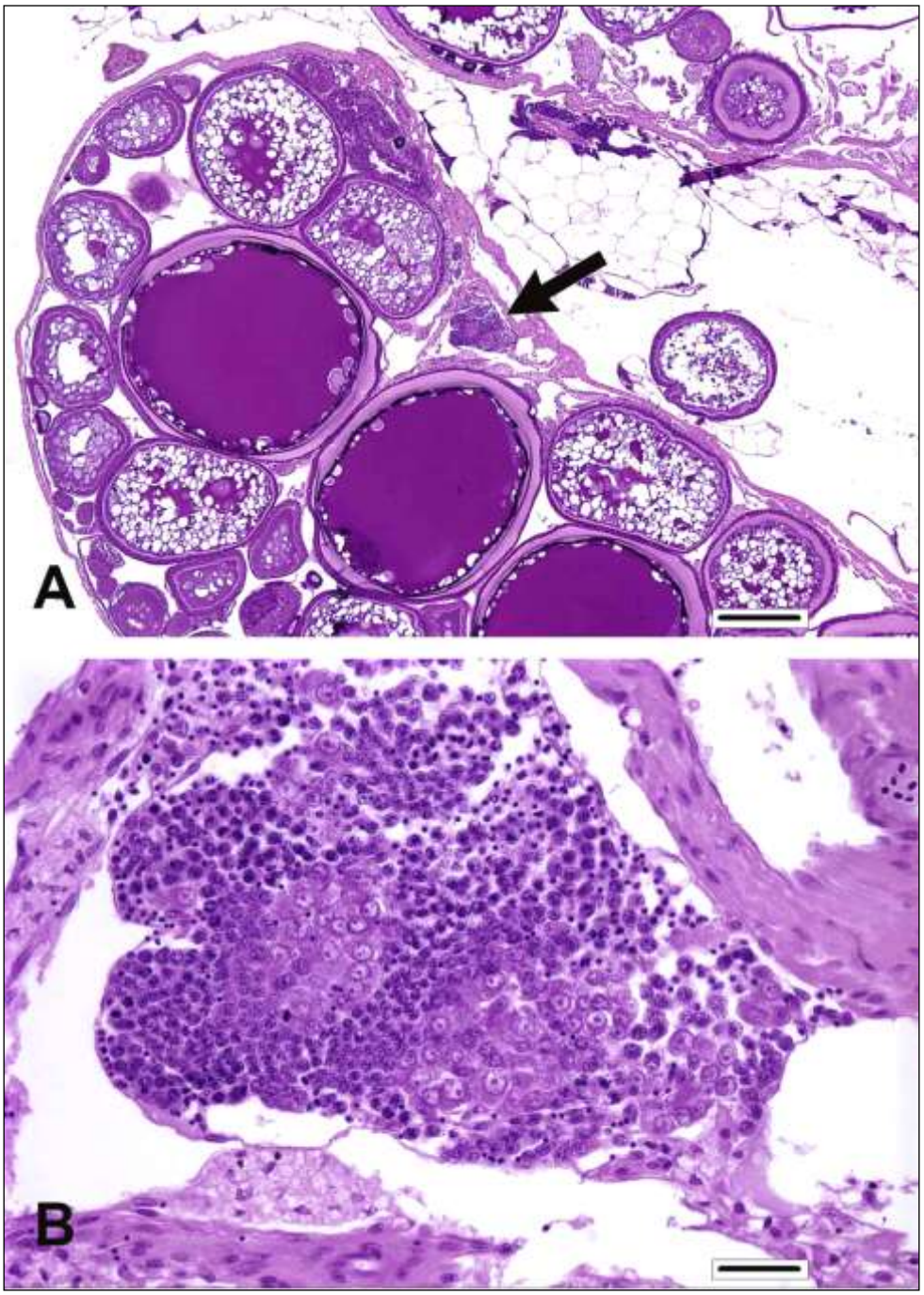
Ovary, pigmented macrophage aggregate (histiocytic cells) in the ovary of an adult female. These aggregates are present constitutively in the interstitium of the ovary, and rarely in the testis. Cells comprising pigmented macrophage aggregates (PMA) have small condensed eccentric or peripheralized nuclei and various brown, yellow, red, or gold pigment granules (lipofuscin, ceroid, hemosiderin, and/or melanin) that often impart a slightly crystalline appearance to their comparatively abundant pale cytoplasm. In the normal ovary, these macrophage aggregates are likely involved in the processing of breakdown products associated with atresia of unspawned oocytes. It has been demonstrated that macrophage aggregates may become larger and/or more numerous following exposure to certain toxicants or infectious agents (Blazer et al., 1987). Whenever possible, macrophage aggregates should be distinguished from granulomatous inflammation. Granulomatous inflammation, which is a reaction to the presence of pathogens or foreign substances, is characterized by the presence of epithelioid macrophages, with or without multinucleated giant cells, additional inflammatory cells, and necrosis. Distinguishing PMA from inflammation is not always easy, as pigmented macrophage aggregates may become incorporated into areas of granulomatous inflammation. Bar = 25 µm.



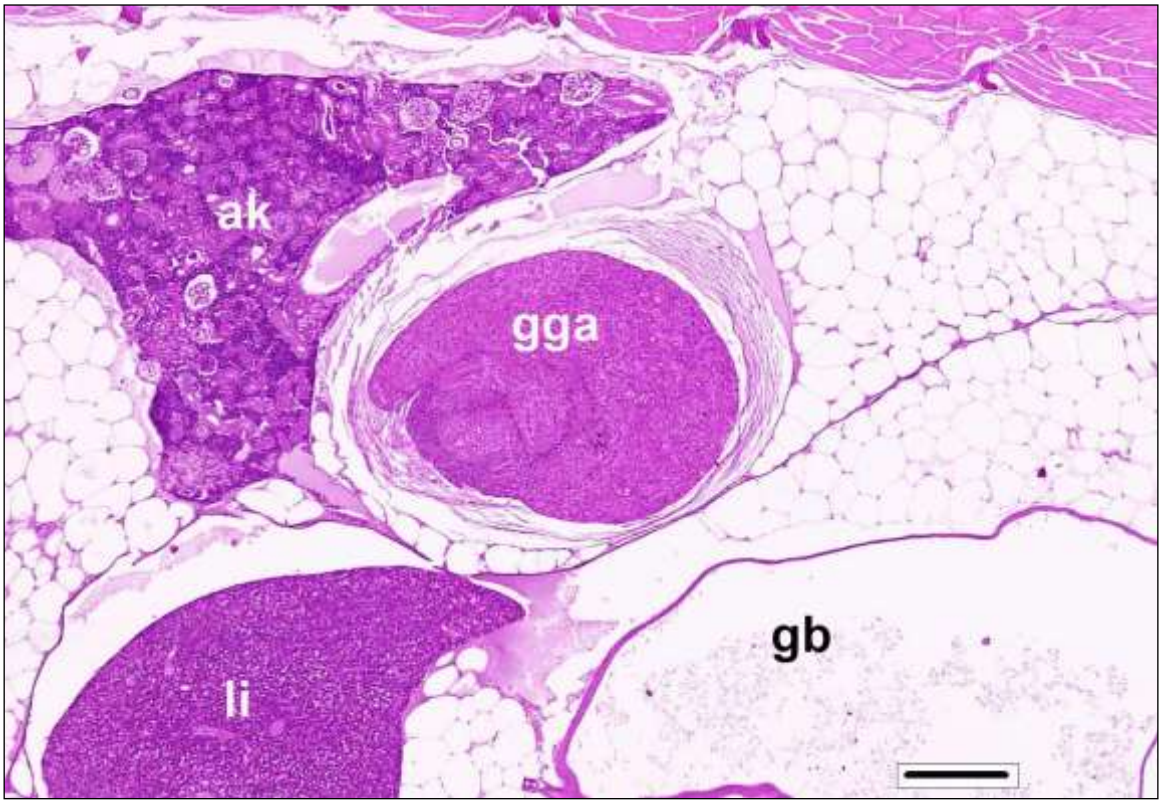
Ovary, post-ovulatory follicles. A number of post-ovulatory follicles (POF), indicating recent spawning, are evident in in this ovary from an adult female (arrows). Following release of an oocyte (i.e., spawning), the perifollicular sheath, which is a membranous structure lined by granulosa cells, theca cells, and surface epithelium, collapses into a POF. Consequently, POFs are most likely to be seen in Stage 2 and Stage 4 ovaries, and they are rarely present in Stage 3 ovaries. The granulosa cells of POFs are much larger than those of intact follicles. Mammalian terms such as "corpus lutea" and "Graafian follicles", are probably inappropriate, due to structural and functional differences between those entities and piscine POFs. POFs should be differentiated from collapsed atretic follicles, the latter of which contain ooplasmic debris. Post-ovulatory follicles are graded according to the maximum number per ovary section as follows: Grade 1 = 3-5 POF; Grade 2 = 6-8 POF, and Grade 3 = 9 or greater POF. Bar = 250 µm.



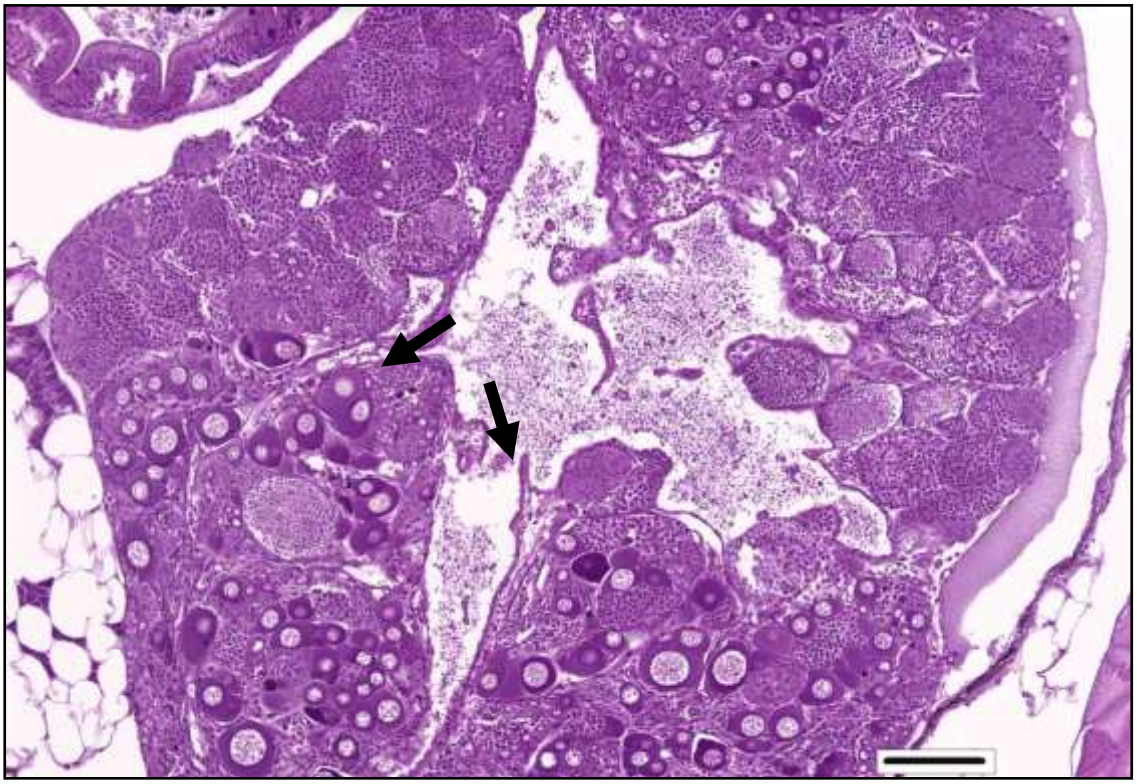
Ovary, post-ovulatory follicles, accelerated involution. A: Typical post-ovulatory follicle, in which only occasional apoptotic-like cells (arrow) are present. B: In this ovary from a compound-treated fish, post ovulatory follicles contained myriad apoptotic cells. Bar = 25 µm (A and B).



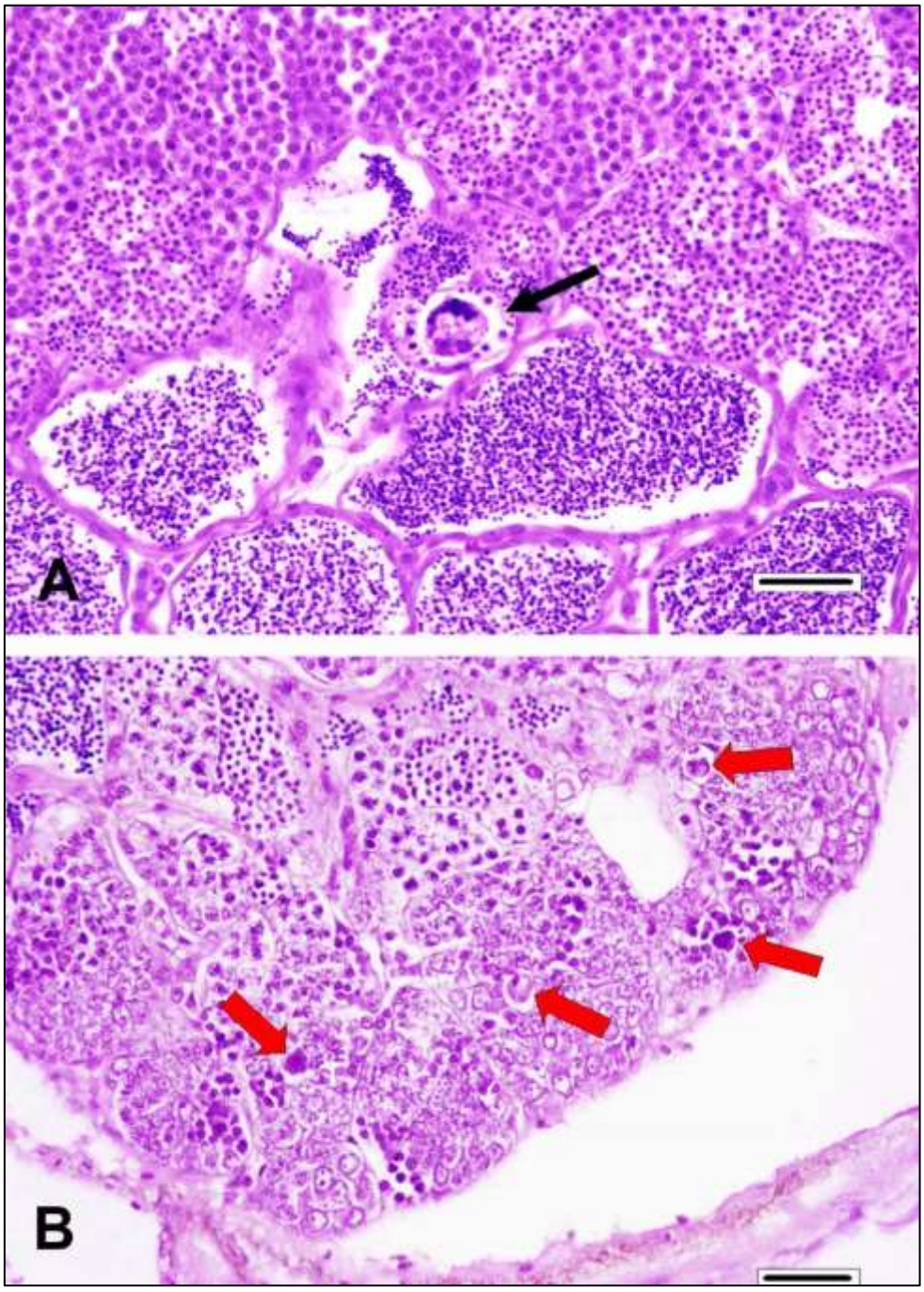
***Ovary, spermatogenesis*.** A and B: Ovary from an adult female control in which ovarian spermatogenesis (arrow) was not a treatment-related finding. In B, spermatogenic cells of various phases are represented. This change is characterized by the presence of non-neoplastic spermatogenic cells, usually immature, within the ovary. There is little or no evidence of lobular or tubular testicular architecture. Care should be taken to distinguish ovarian spermatogenesis from mitotically dividing oogonia; a key feature of ovarian spermatogenesis is the presence of multiple spermatogenic phases. Ovarian spermatogenesis must also be distinguished from inadvertent carryover of spermatogenic tissue during the trimming or microtomy process. It should be recognized that ovarian spermatogenesis may not always indicate masculinization. In some situations it may represent incomplete conversion of a genotypic male to the female phenotype. Bar = 250 µm (A), Bar = 25 µm (B).



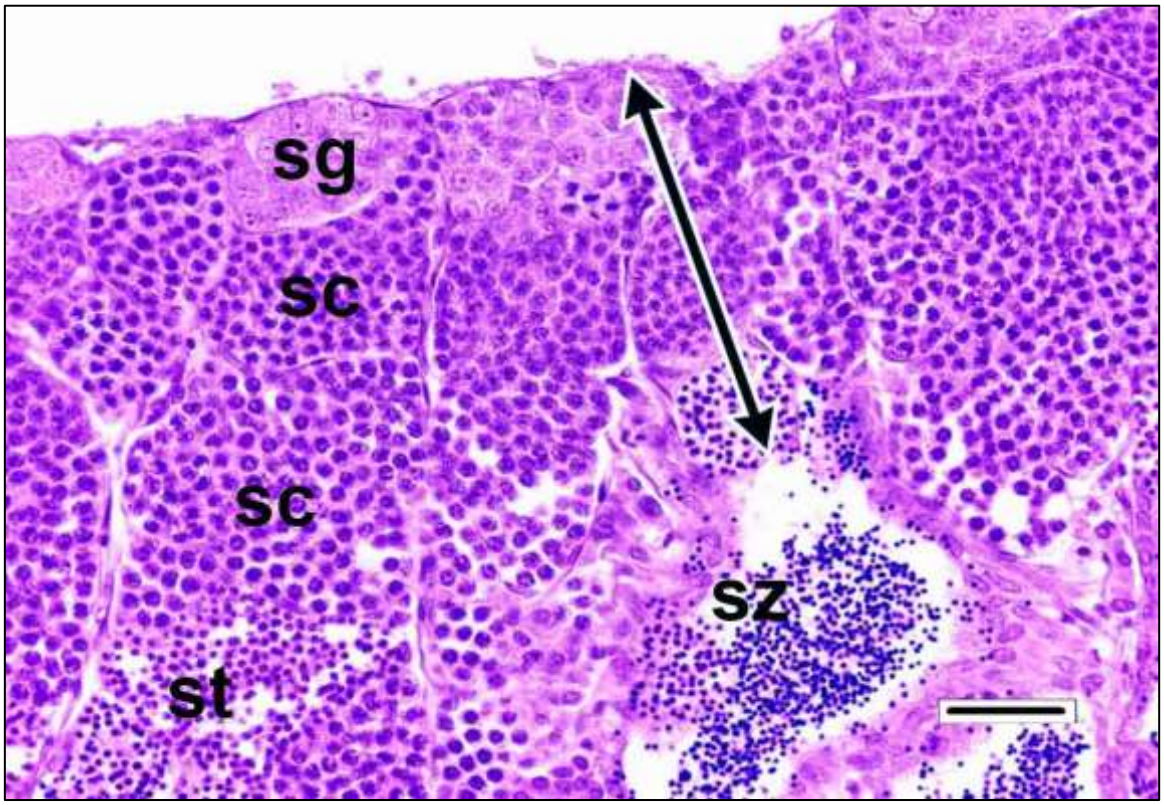
Swim bladder, gas gland adenoma. Gas gland adenomas (gga) of the swim bladder are uncommon, but not rare, neoplasms in medaka. Thus far, this appears to be an incidental finding in toxicology studies. Anecdotal evidence suggests that these lesions may be associated with congenital deformities of the spine and/or swim bladder, resulting in pneumatic duct patency, swim bladder inflammation (pneumocystitis), and tumor formation. Related lesions include hyperplasia of the swim bladder gas gland epithelium (increased amounts of epithelium without the formation of a distinct mass), and gas gland adenocarcinomas (locally invasive tumors with cytologic pleomorphism). ak = anterior kidney, li = liver, gb = gallbladder. Bar = 250 µm.



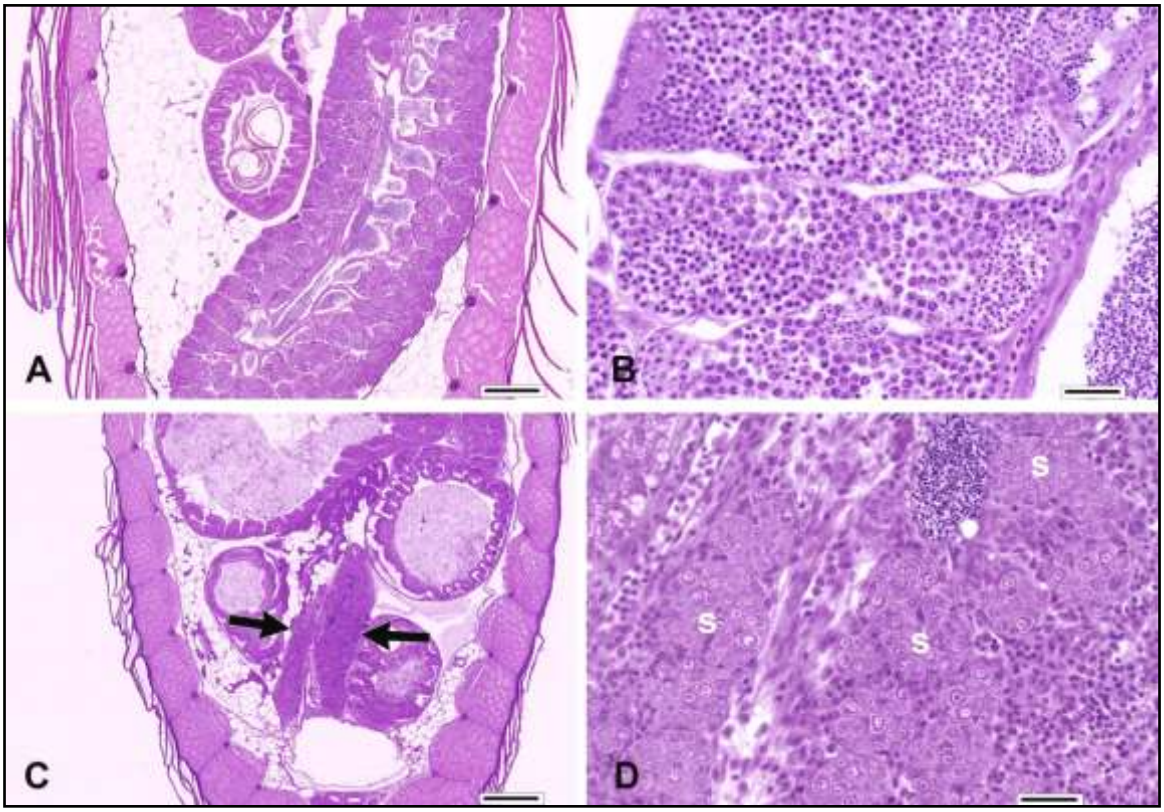
Testis, asynchronous development. This finding is characterized by the presence of distinctly different populations (i.e., range of developmental stages) of gametogenic cells in different regions of a gonad, or the aberrant positioning of gonadal cell populations. In this particular case, an 8-week old male had been exposed for approximately eight weeks to 27 µg/L 4-*tert*-octylphenol. In addition to the presence of numerous testis-ova, the efferent duct system is abnormally irregular, and spermatogonia-containing spermatocysts (arrows) are located in an atypical position adjacent to the ducts (asynchronous development). Bar = 100 µm.



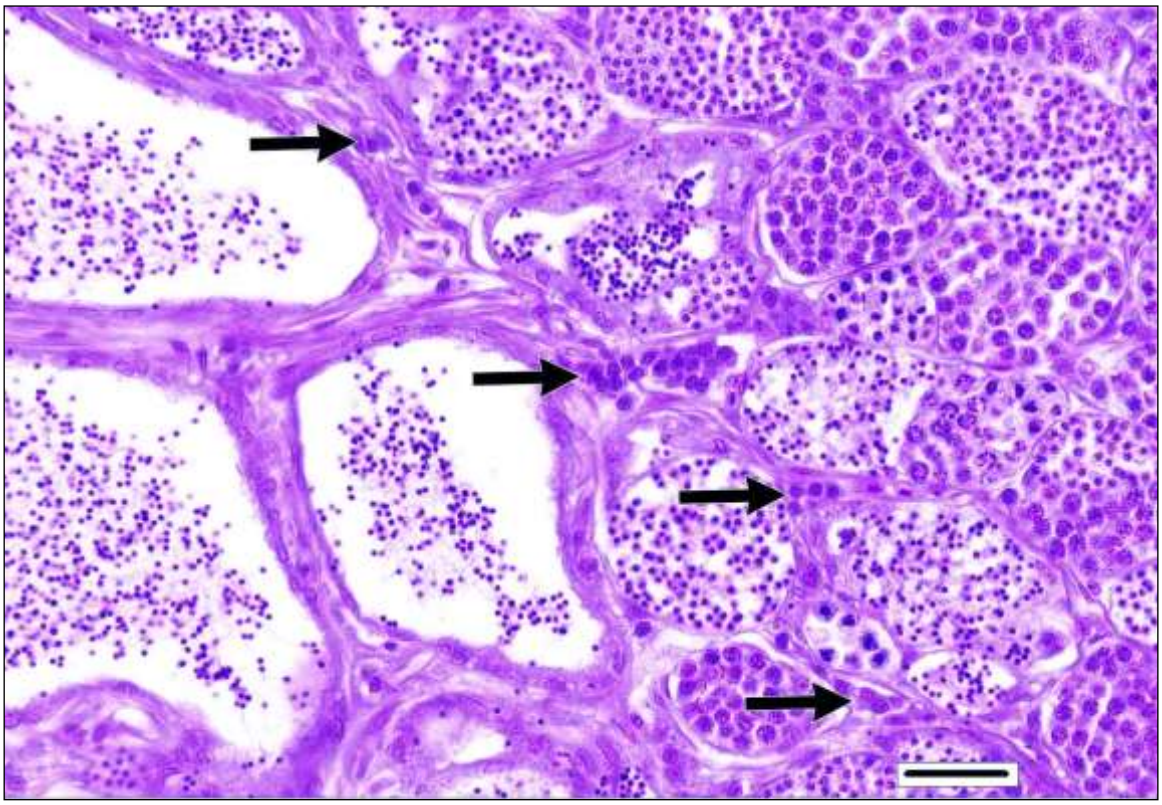
***Testis, degeneration, increased*.** Examples of degenerative findings in the testis include: 1) individual or clustered apoptotic germ cells; 2) vacuolated germ cells; 3) multinucleated (syncytial) cells in the germinal epithelium or testicular lumen. Apoptotic germ cells are characterized by cell shrinkage, nuclear condensation, and fragmentation into spherical, membrane-bound bodies, which are often phagocytized by neighboring cells. Typically, there is no associated inflammation associated with these cells. Low numbers of degenerating germ cells are commonly found in the testes of control males. Extensive testicular degeneration may lead to localized or generalized loss of the germinal epithelium. A: Germ cell syncytium (arrow) in the testis of a control male. B: Moderate testicular degeneration characterized by the presence of numerous apoptotic cells within the germinal epithelium (arrow). Moderate to severe testicular degeneration may also occur occasionally in untreated males. Bar = 25 µm.



Testis, germinal epithelium. Normal testis from an adult male medaka. The double arrow indicates width of germinal epithelium, which extends from the tunica albuginea to the efferent duct. Germ cell maturation occurs from the periphery inward. sg = spermatogonia, sc = spermatocytes, st = spermatids, sz = spermatozoa. Bar = 25 µm.



Testis, hypoplasia. A and B: Normal testis in an adult male. C and D: Hypoplastic testis (arrows) from an 8-week-old male exposed to 450 mg/L 4-n-amylaniline for approximately 8 weeks. The hypoplastic testis is not only small, it is poorly formed, consisting primarily of nests of spermatogonia with no clear efferent duct system. Indicating underdevelopment, this condition may be associated with interstitial fibrosis and increased prominence of interstitial cells in affected areas of the testis. Hypoplasia may be chemically induced, or it can occur spontaneously in rare instances. Bar = 250 µm (A and C), 25 µm (B and D).



***Testis, interstitial (Leydig) cells*.** Testis from a 16-week old control male. These androgen-producing cells have dense, dark round or oval nuclei with little detail and moderate amounts of variably-evident, faintly vacuolated cytoplasm. Compared to germinal cells, interstitial cells are usually present in low numbers, usually as single cells or small aggregates, scattered irregularly throughout the interlobular interstitium. Although they may resemble spermatocytes, interstitial cells are only present in intertubular areas. Bar = 25 µm.