Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

19-Nov-2015

English - Or. English

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

Cancels & replaces the same document of 22 September 2015

Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (MEOGRT) - Part 2

Series on Testing & Assessment
No. 227

JT03386573

ENV/JM/MONO(2015)36/PART2
Unclassified
English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. 227

Guidance Document on Medaka Histopathology Techniques and Evaluation for the Medaka Extended One-Generation Reproduction Test (MEOGRT) - Part 2

IOMC INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT

Paris 2015

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

This publication is available electronically, at no charge.

Also published in the Series on Testing and Assessment (link):

**For this and many other Environment,
Health and Safety publications, consult the OECD's
World Wide Web site (www.oecd.org/chemicalsafety/)**

or contact:

**OECD Environment Directorate,
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.

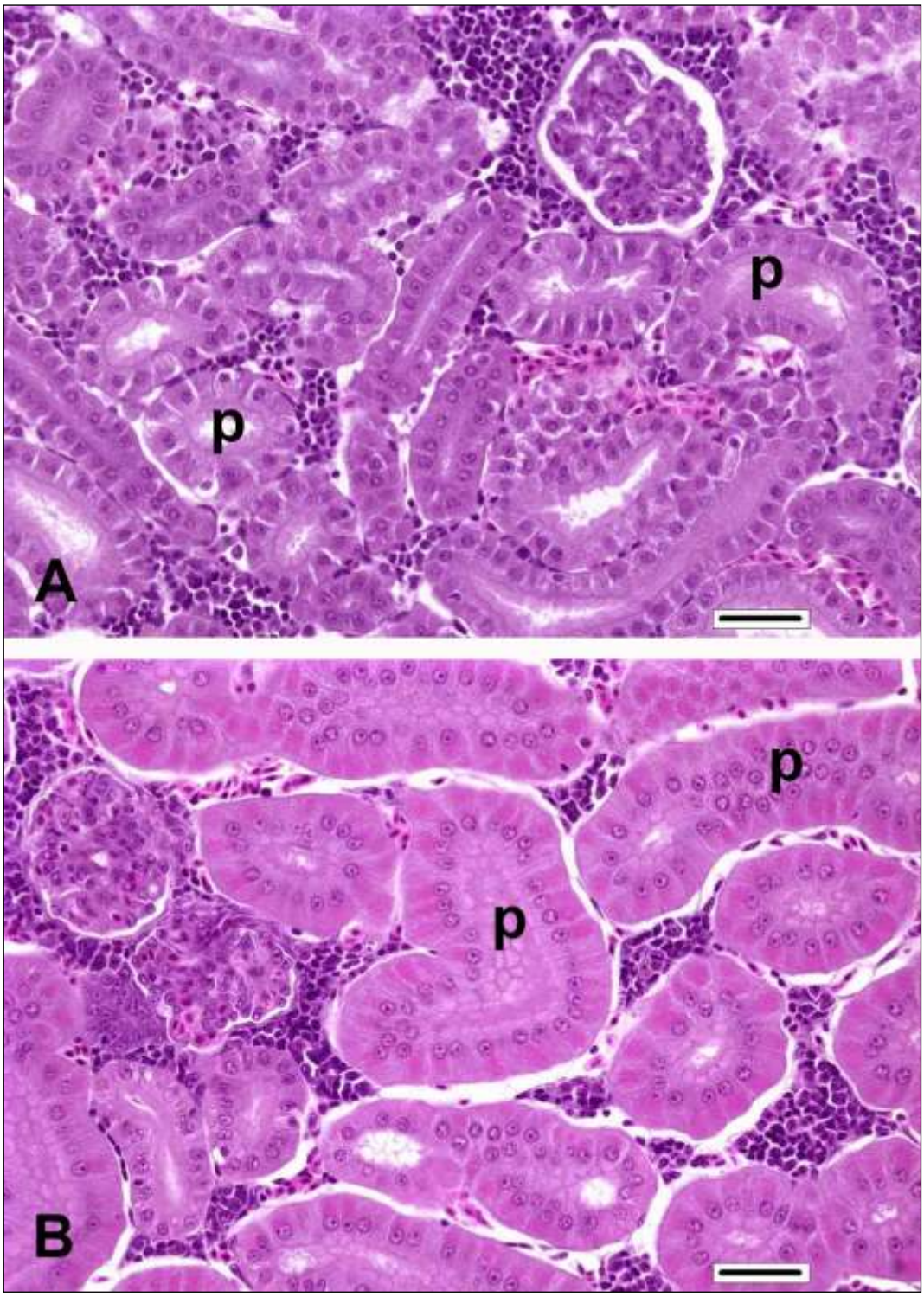
This document presents **Part 2** of the guidance document which in total consists of four parts.

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

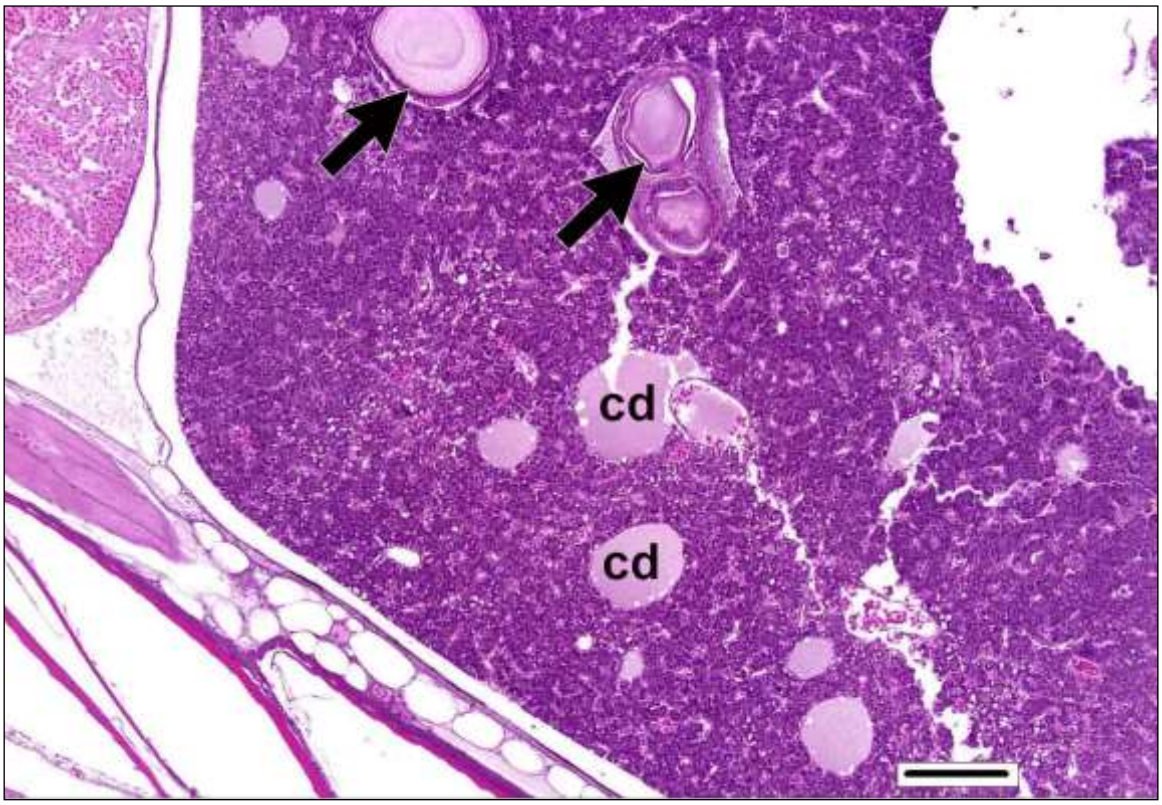
GUIDANCE DOCUMENT ON MEDAKA HISTOPATHOLOGY TECHNIQUES AND EVALUATION (PART 2)

FOR THE

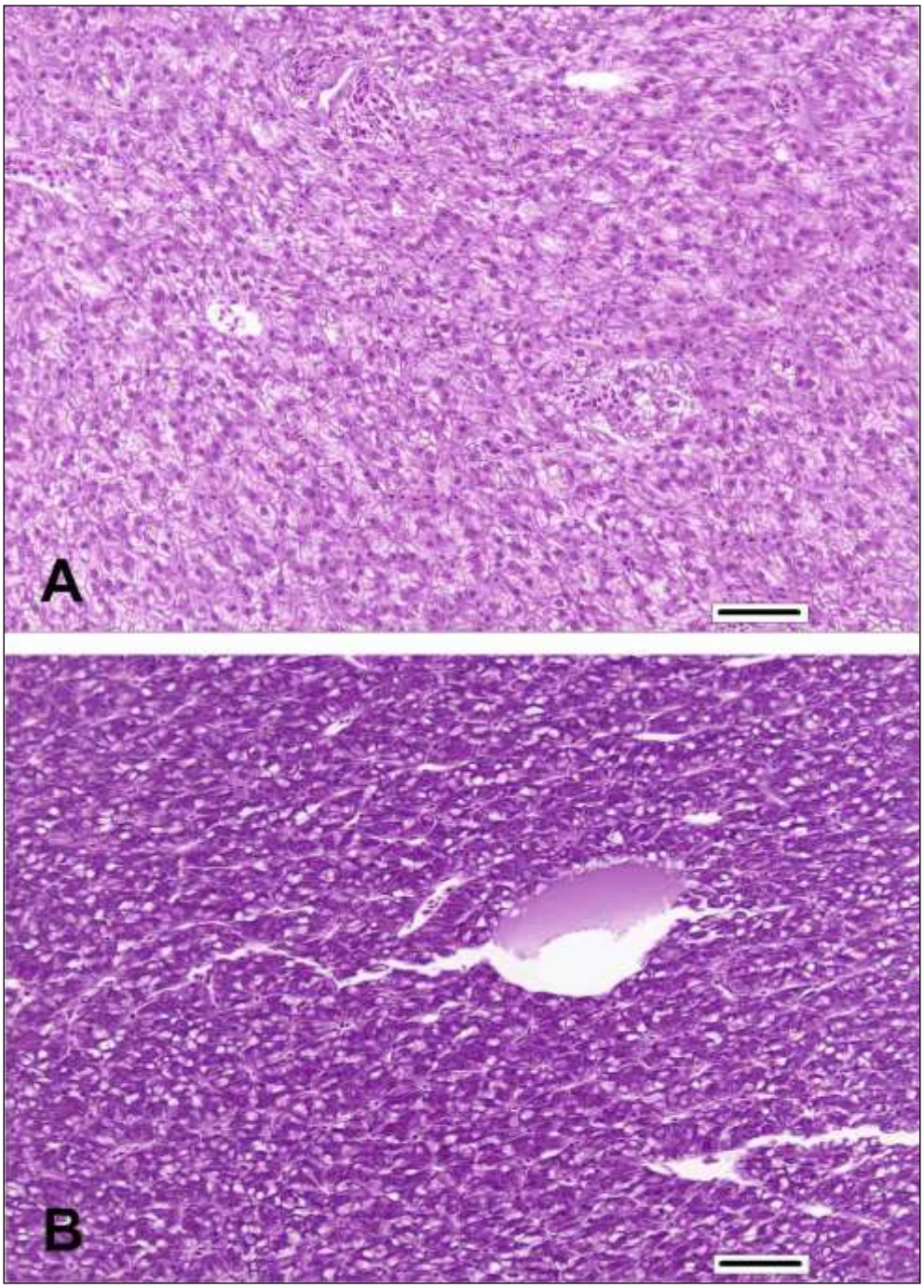
MEDAKA EXTENDED ONE-GENERATION REPRODUCTION TEST (MEOGRT)



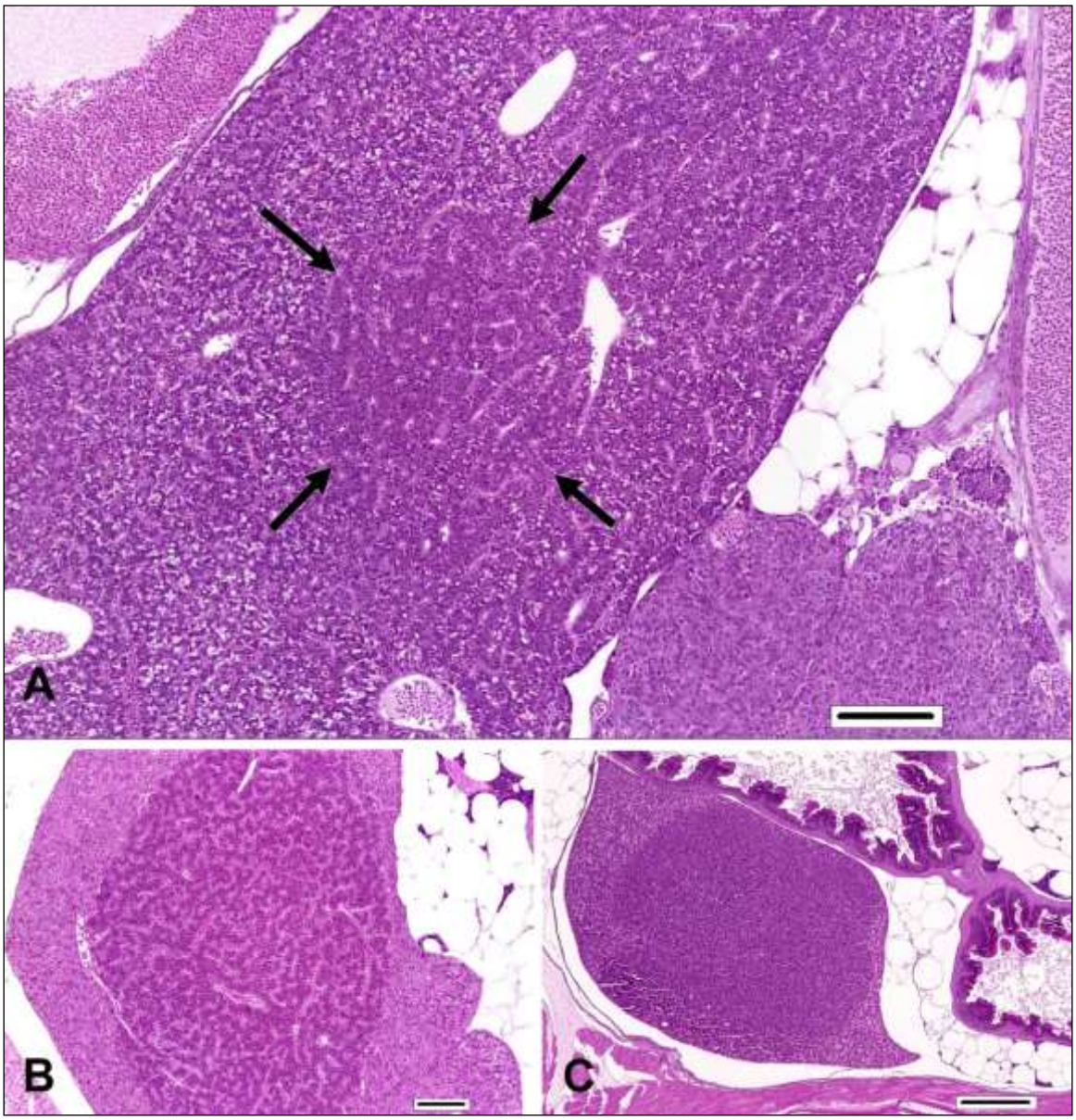
Kidney, tubular eosinophilia. A: Renal tissue from an adult female. Relative to the male (B), epithelial cells of the proximal tubules (p) are smaller and have more basophilic cytoplasm. B: Renal tissue from an adult male. The plump epithelial cells of the proximal tubules (p) have very fine granular eosinophilic material in their basal cytoplasm. Severity grading of tubular eosinophilia is as follows: Grade 1 = essentially no eosinophilia; Grade 2 = small amount of eosinophilia; Grade 3 = abundant eosinophilia. The kidney in B was scored Grade 3. Bar = 250 µm.



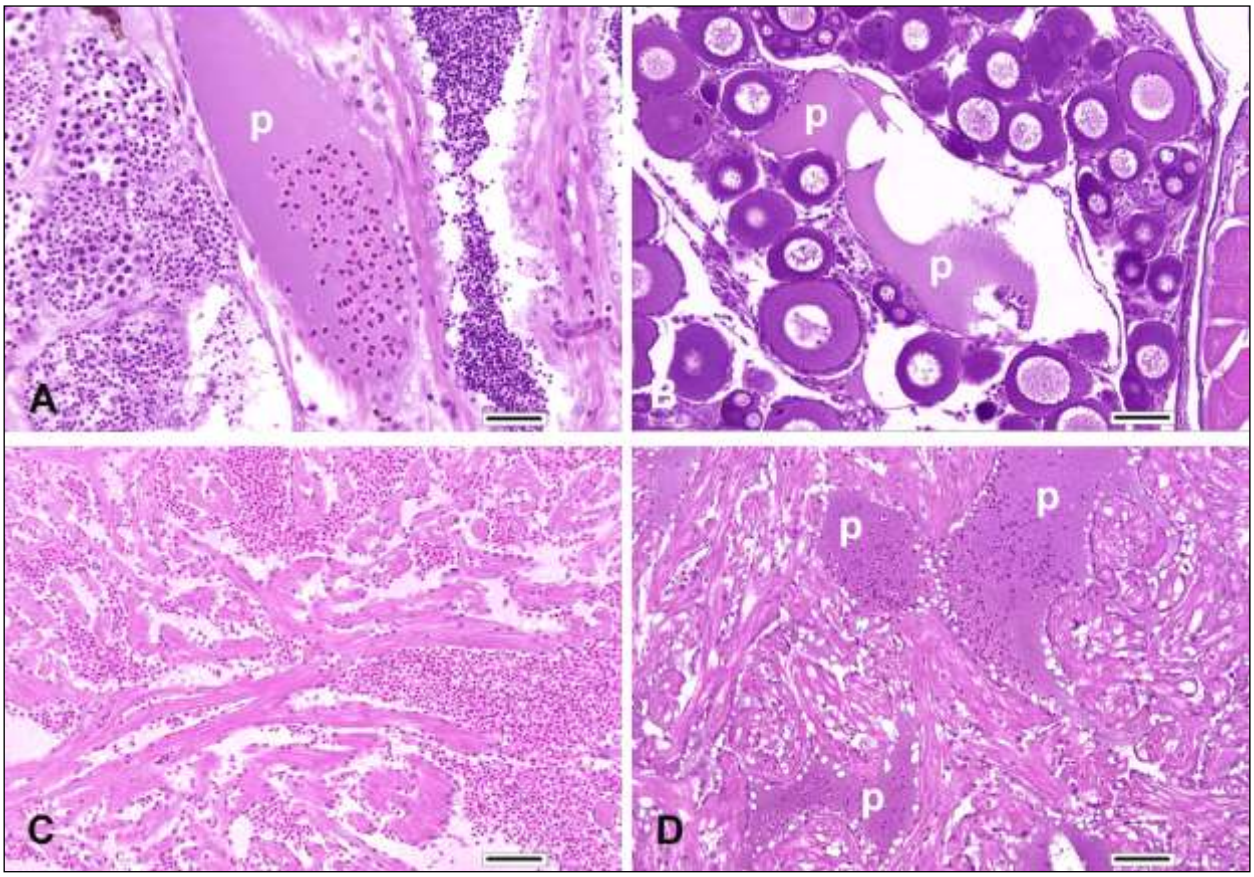
Liver, cystic degeneration. Liver from an adult female medaka. Cystic degeneration (cd) is characterized by various numbers of single or multilocular, roughly spherical, fluid-filled spaces that are scattered throughout the hepatic parenchyma. Individual lesions may or may not be associated with blood vessels, but the cysts themselves are not lined by endothelial cells. Based on morphologic criteria, such lesions have also been termed hepatic cysts or spongiosis hepatis, although empirical evidence suggests that these merely represent different stages in the progression of cystic degeneration. Cystic degeneration tends to be relatively common in medaka, and especially older females. This particular liver also features bile duct concretions (arrows). Bar = 100 µm.



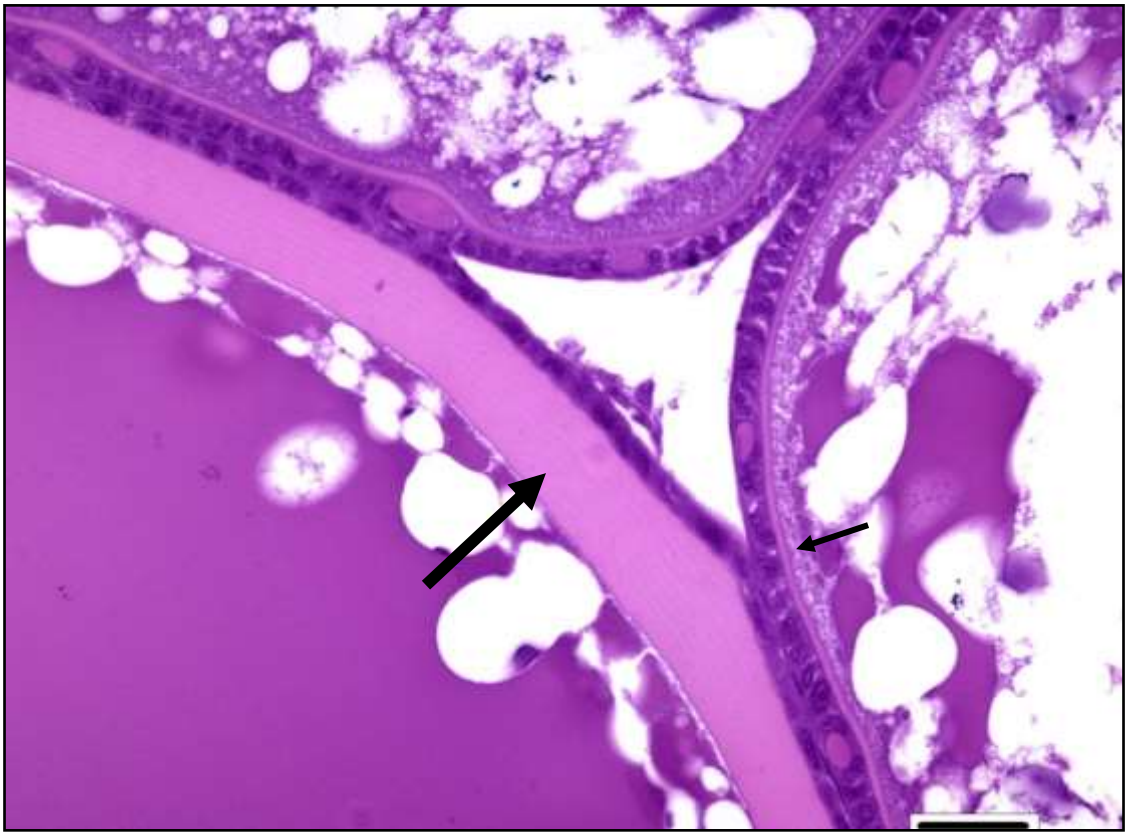
Liver, hepatocyte basophilia, increased. A: Liver from an adult control male. B: Liver from an adult male exposed to 100 µg/L 4-*tert*-octylphenol, an estrogenic substance. There is a diffuse increase in hepatocyte basophilia, a loss of cytoplasmic vacuolization, and hepatic blood vessels contain proteinaceous fluid. A generally diffuse increase in hepatocyte cytoplasmic basophilia has been observed in male fish that have been exposed to compounds that are able to interact with hepatic estrogen receptors, including E2 and 17β-methyldihydrotestosterone (Wester et al., 2003). This increase in basophilia, which is correlated with increased vitellogenin production, presumably mimics the heightened metabolic state (e.g., increased endoplasmic reticulum) that is required for the production of vitellogenin in the reproductively-active female fish. Bar = 50 µm.



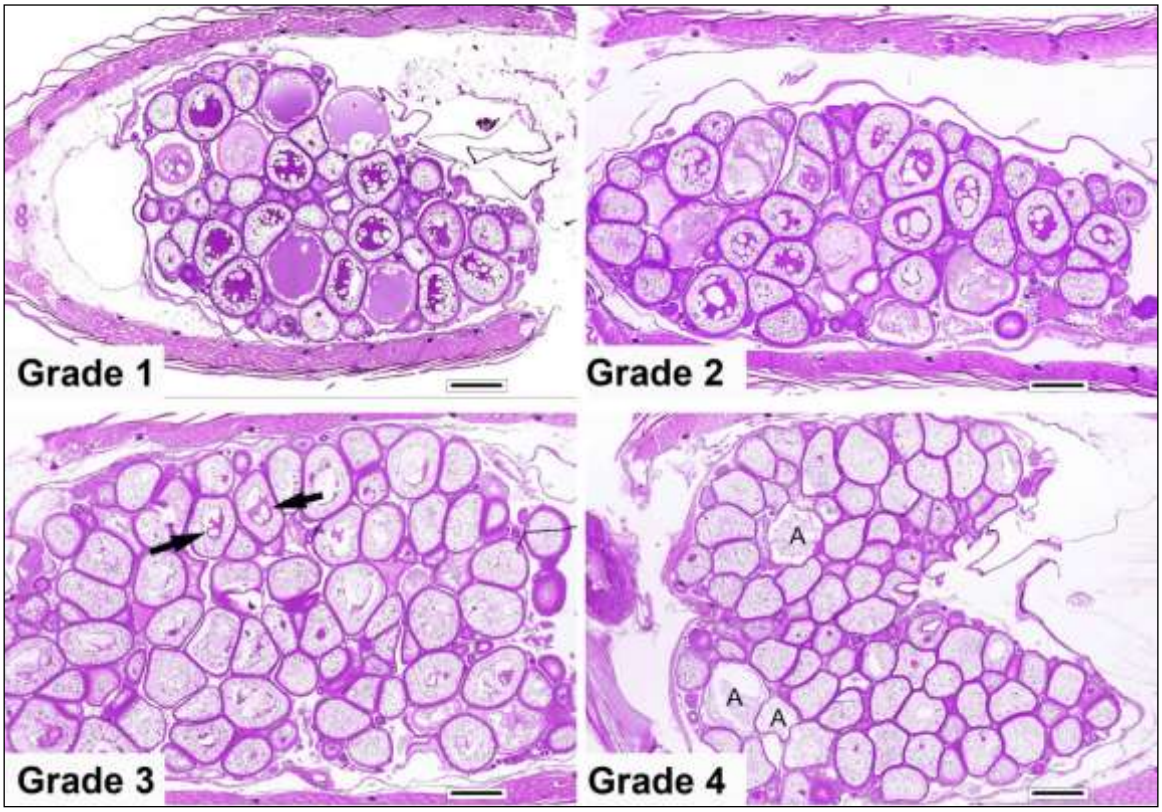
Liver, primary proliferative lesions. A: **Focus of hepatocellular alteration** (altered focus). This is a non-neoplastic, but likely pre-neoplastic, lesion that can be observed as a spontaneous or induced finding. Morphologic characteristics include changes in hepatocyte size and color relative to the surrounding liver parenchyma, and blending with unaffected hepatic tubules at the periphery of the lesion. B and C: **Hepatocellular adenomas**. Morphologic characteristics include distinct margins, peripheral compression of unaffected hepatic tissue, little cytologic atypia (relative to carcinomas), and generally larger size than foci. Hepatocellular carcinomas are less common but can occur also. Bar = 100 µm (A and B), 250 µm (C).



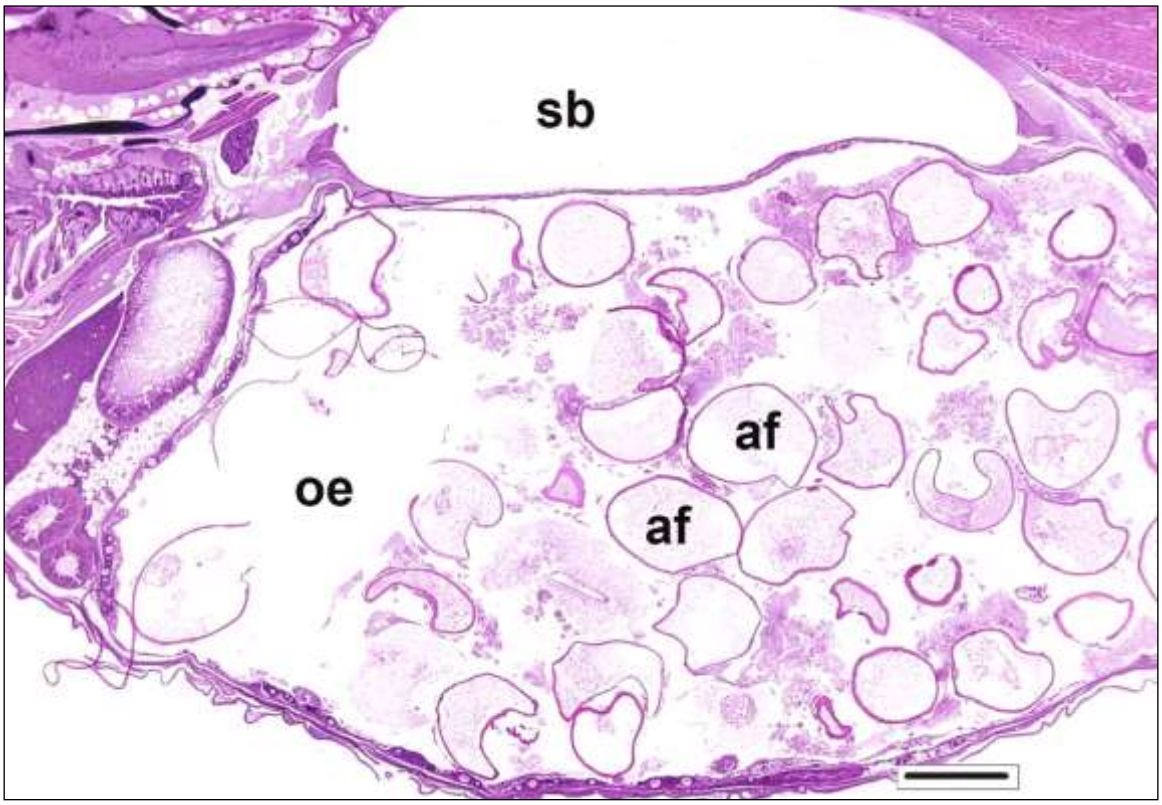
Multiple tissues, proteinaceous fluid. This finding is characterized by the presence of homogeneous dark pink translucent material, presumably vitellogenin, within the vascular and/or interstitial compartments of the testis, ovary, and other tissues in fish that have been exposed to estrogenic substances. A: Intravascular proteinaceous fluid (p) in the testis of an adult male exposed to 17β-estradiol at 100 ng/L for 4 weeks. B: Intravascular proteinaceous fluid (p) in the ovary of an adult female exposed to 4-*tert*-octylphenol at 90 µg/L for eight weeks. C: Heart from an untreated control fish. D: Heart with intravascular proteinaceous fluid (p). Bar = 25 µm (A), 50 µm (B, C, and D).



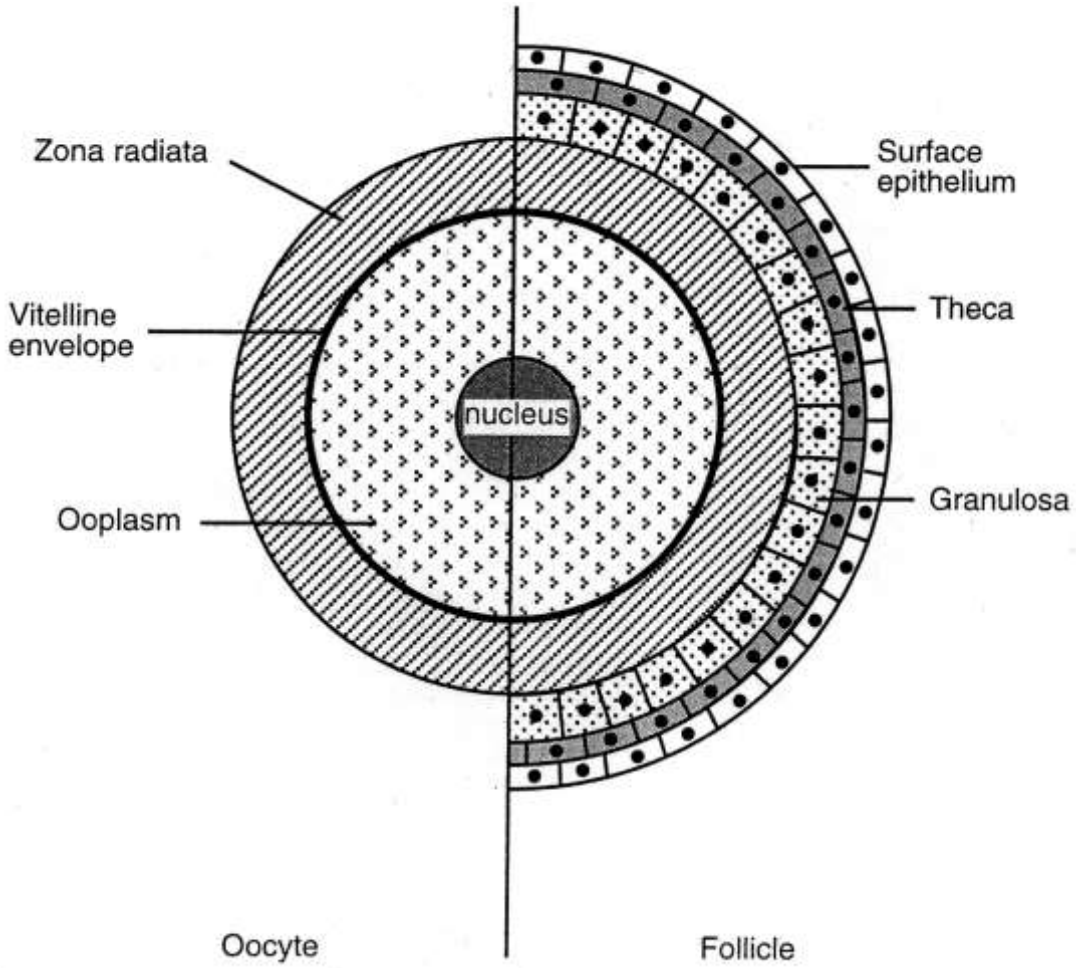
Ovary, chorion. Usually pale to dark eosinophilic and refractile, the chorion is the thick external layer of an oocyte that surrounds the ooplasm. The terms zona radiata and vitelline envelope have been used synonymously. In mature, unspent follicles, the chorion is noticeably surrounded by perifollicular cells (granulosa cells, theca cells, and surface epithelial cells). As viewed by light microscope, the chorion is often minimally apparent or inapparent prior to the cortical alveolar phase of oocyte development. Note the vast difference in thickness between the chorion of a cortical alveolar oocyte (small arrow) and the chorion of a mature vitellogenic oocyte (large arrow). H&E, Bar = 25 µm.



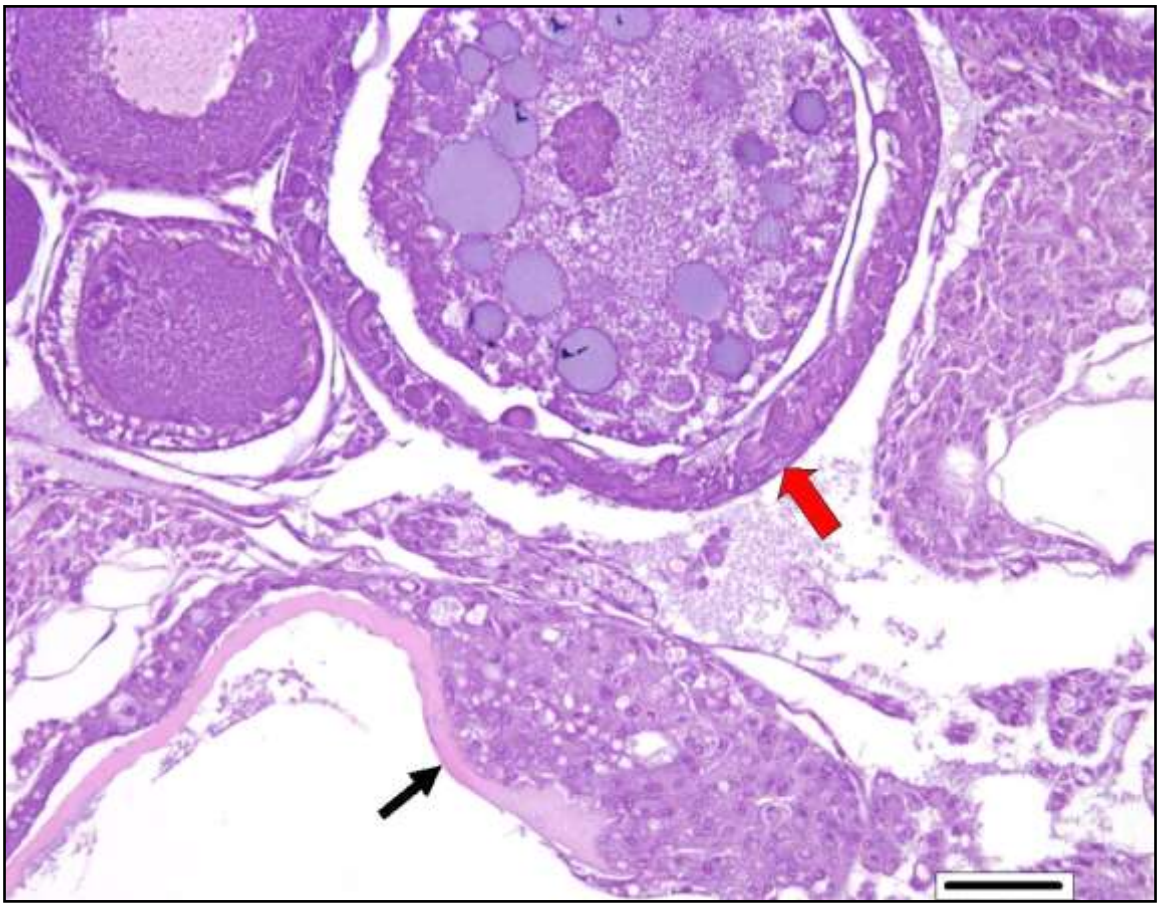
Ovary, decreased yolk formation, grading. This finding is characterized by a progressive decrease in the quality (i.e., the yolk becomes more watery) and amount of yolk in vitellogenic-sized follicles. In Grade 3 ovaries, follicles contain only a scant amount of yolk (arrows), whereas in Grade 4, yolk is essentially not visible. Affected oocytes often have cortical alveoli (yolk vesicles) that are fragmented or dissipated. Unlike oocyte atresia, the vitelline membrane (chorion) of affected oocytes is often smooth and contiguous. However, decreased yolk formation is often accompanied by at least a low degree of oocyte atresia (A). This type of change has been observed following exposure to aromatase inhibitors such as prochloraz and fadrozole, and the non-aromatizable androgen trenbolone. H&E, Bar = 500 µm.



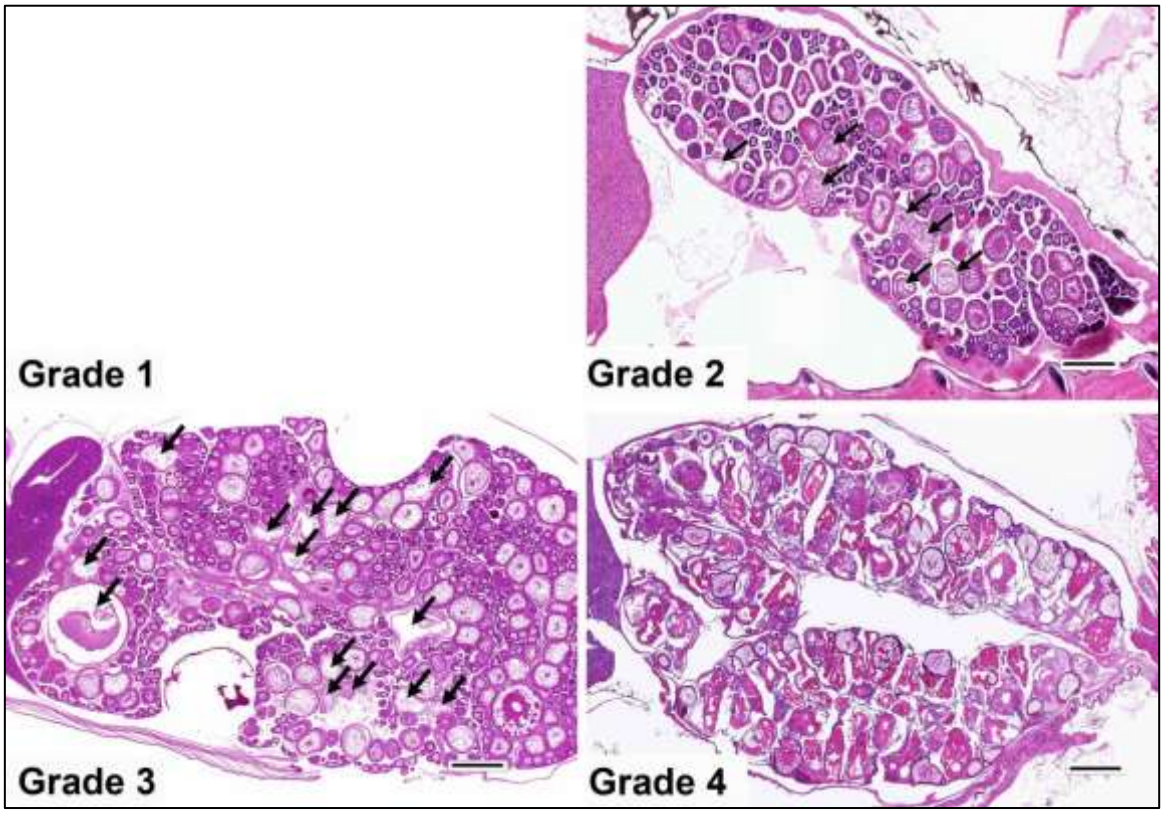
Ovary, edema. This ovary is markedly enlarged due to abundant ovarian edema (oe), which is represented by excess clear space within the ovary. Numerous atretic follicles (af) are also present. The cause was undetermined in this case. sb = swim bladder. Bar = 800 µm.



***Ovary, follicle*.** Diagram from Tyler and Sumpter, 1996. The functional unit of the ovary, this term generally refers to an oocyte plus its surrounding sheath of perifollicular cells (granulosa cells, theca cells, and surface epithelium cells) (Tyler and Sumpter, 1996). However, there are subtypes of follicles in which the oocyte is not present or may be difficult to appreciate; these include post-ovulatory (spent), empty, and atretic follicles. A **post-ovulatory follicle** (the follicle has ruptured to release an oocyte during spawning) is collapsed and often has enlarged (hypertrophic) granulosa and theca cells. Conversely, an **empty follicle** (in which the oocyte has been dislodged from the histologic section as a post-mortem artifact) generally retains the shape of the oocyte and may or may not have enlarged granulosa and theca cells. An **atretic follicle** must be distinguished from both spent follicles and empty follicles; the presence of at least some ooplasmic material (often heterochromatic) within a follicle indicates that it contains an atretic oocyte.



***Ovary, follicular atresia*.** Ovary from an adult female. The larger red arrow indicates a cortical alveolar oocyte that is atretic, whereas the smaller black arrow denotes a large fragment of chorion that is partially surrounded by macrophages and hypertrophic perifollicular cells. Essentially, degradation and resorption of an oocyte at any point in development, including unspawned senescent oocytes, atresia can be the result of either physiological or pathological processes. For consistency, the term atresia should generally be used in preference to the term "degeneration" et al. when referring to oocytes. Histopathologically, atresia is often characterized by clumping and perforation of the chorion, fragmentation of the nucleus, disorganization of the ooplasm, and/or the uptake of yolk materials by perifollicular cells. Because even severe oocyte atresia can be observed as an apparently spontaneous finding in one or more control females, it is important to compare populations rather than individuals, and putative effects in studies with low animal numbers should be interpreted with caution. Although increased oocyte atresia is a non-specific finding that is not limited to EDC exposure, it may contribute to an indication of causality in a "weight-of-evidence" approach. The following is an example of a severity grading scheme for increased oocyte atresia: Not remarkable = <3 atretic oocytes per ovary section; Grade 1 = 3 to 5 atretic oocytes per section; Grade 2 = 6 to 9 atretic oocytes per section; Grade 3 = greater than 9 atretic oocytes per section, but less than the vast majority; and Grade 4 = the vast majority of oocytes in a section are atretic. Bar = 50 µm.



***Ovary, follicular atresia grading*.** Severity grading for follicular atresia is based on the maximum number of atretic follicles per ovary section as follows: Grade 1 = 3-5, Grade 2 = 6-8, Grade 3 = 9 or greater, but less than the vast majority, Grade 4 = the vast majority of follicles are atretic. Bar = 750 µm (Grades 2 through 4).