

EDMVAC Plenary Meeting

Washington, D.C.
April 27, 2005



Jeffrey C. Wolf, DVM, DACVP
Experimental Pathology Laboratories (EPL®)



Topics for this Presentation

- Introduction



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- Introduction
- Phase 1A Paris meeting, in brief



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- Phase 1A Paris meeting, in brief
- Phase 1B:
 - Overview of U.S. studies

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 - U.S. histopathology results by chemical

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 - U.S. histopathology results by chemical
 - Heidelberg meeting
 - Goals, objectives, and agenda

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 - U.S. histopathology results by chemical
 - Heidelberg meeting
 - Goals, objectives, and agenda
 - Consensus findings

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 - Consensus findings
 - Assessment of inter-laboratory consistency



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 - Goals, objectives, and agenda
 - Consensus findings
 - Assessment of inter-laboratory consistency
 - Conclusions, recommendations, and other considerations



Introduction

- A veterinary pathologist who specializes in the examination fish tissues from tox studies



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- I don't design or conduct live-animal studies



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- In 2003, I was asked to participate in a review of the Phase 1A histopathology results



Phase 1A Review, Paris, 2003

- Histopath results reviewed by a panel consisting of 11 pathologists and other scientists representing 6 countries in N. America, Europe, and Asia



Chimeras on Notre Dame Cathedral



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 - Very poor correlation of results among different laboratories



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- Bottom Line of Histopath Results:
 - Very poor correlation of results among different laboratories -- **Why??**
 - Slide quality varied from adequate to unreadable
 - Lack of uniform diagnostic criteria
 - Lack of standardized terminology for diagnoses



Chimeras on Notre Dame Cathedral

Phase 1A Review (cont.)

- Remedy: Develop guidance document for Phase 1B

U. S. ENVIRONMENTAL PROTECTION AGENCY
MID-CONTINENT ECOLOGY DIVISION – DULUTH

WA #04-14

HISTOLOGY AND HISTOPATHOLOGY GUIDELINES FOR PHASE 1B OF THE OECD FISH SCREENING ASSAY FOR EDC'S

TASK A: HISTOTECHNICAL GUIDANCE MODULE
TASK B: GONADAL HISTOPATHOLOGY GLOSSARY
AND DIAGNOSTIC CRITERIA MODULE
TASK C: GONADAL STAGING CRITERIA MODULE

Prepared by:
Experimental Pathology Laboratories, Inc.
P.O. Box 474
Herndon, VA 20172-0474
(703) 471-7060

Submitted to:
U.S. EPA Mid-Continent Ecology Division-Duluth
Duluth, MN 55804-2595
March 3, 2005



Phase 1A Review (cont.)

- Remedy: Develop guidance document for Phase 1B
- Collaborate effort of fish pathologists, sponsored by USEPA

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Phase 1A Review (cont.)

- Remedy: Develop guidance document for Phase 1B
- Collaborate effort of fish pathologists, sponsored by USEPA
- Resulting 103 page document:
 - Necropsy techniques and histologic processing SOP's for each of the three fish species
 - Illustrated glossary of diagnostic terms and criteria for reproductive histopathology
 - Description of method for gonad staging

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Phase 1B

- Total of 32 studies performed at 14 laboratories in 7 countries
-- ~80-100 fish per study



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- Total of 25 studies performed at 14 laboratories in 7 countries
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- Study Design: Negative control + 3 concentrations of test article in water, +/- positive control, 21-day exposure

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- Test Articles:
 - **Prochloraz**: aromatase inhibitor (**fadrozole** pos. control)
 - **Flutamide**: androgen antagonist
 - **4-tert-pentylphenol (4tPP)**: weak estrogen agonist (**estradiol** pos. control)

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 - **4-tert-pentylphenol (4tPP)**: weak estrogen agonist (**estradiol** pos. control)
- Each combination of species + test article performed by at least two, and usually three, different labs



Phase 1B

PATHOLOGIST	SPECIES	CHEMICALS	LABS	No. STUDIES
1	zbf	pro, 4tpp	G	2
2	jmd	pro, 4tpp	J	2
3	fhm	flu, 4tpp	B	2
4	zbf	pro, flu, 4tpp	E	3
5	jmd	flu, 4tpp	I	2
6	fhm	pro, 4tpp	C	2
7	zbf	flu, 4tpp	F	2
8	jmd	pro, 4tpp	D	2
9	zbf	flu	K	1
10	jmd, fhm	pro, flu, 4tpp	A, H, M, N	9

- Studies read by 10 pathologists
 - Most pathologists evaluated two chemicals, a single fish species, and the results from a single laboratory



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1	zbf	pro, 4tpp	G	2
2	jmd	pro, 4tpp	J	2
3	fhm	flu, 4tpp	B	2
4	zbf	pro, flu, 4tpp	E	3
5	jmd	flu, 4tpp	I	2
6	fhm	pro, 4tpp	C	2
7	zbf	flu, 4tpp	F	2
8	jmd	pro, 4tpp	D	2
9	zbf	flu	K	1
10	jmd, fhm	pro, flu, 4tpp	A, H, M, N	9

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Phase 1B – Overview of U.S. Studies

- All U.S. studies sponsored by USEPA



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- Species: FHM and JMD



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 - Species: FHM and JMD
 - Studies conducted at 4 laboratories:
 - USEPA → 4 studies
 - Wildlife International → 2 studies
 - ABC Laboratories → 2 studies
 - Springborn Smithers → 1 study
- 9 studies

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 - Wildlife International → 2 studies
 - ABC Laboratories → 2 studies
 - Springborn Smithers → 1 study
 - USEPA Non-OECD → 2 extra studies
11 studies

Phase 1B – Overview of U.S. Studies

- All U.S. studies sponsored by USEPA
- Species: FHM and JMD
- Studies conducted at 4 laboratories:
 - USEPA
 - Wildlife International
 - ABC Laboratories
 - Springborn Smithers
 - USEPA Non-OECD
- All histopathology evaluated by EPL



Phase 1B – Overview of U.S. Studies

	USEPA	WI	ABC	SS
Prochloraz	JMD FHM, FHM**		FHM	
Fadrozole	JMD* FHM*, JMD**		FHM*	
Flutamide	JMD FHM	JMD		FHM
4-tPP		JMD	FHM	
Estradiol		JMD*	FHM*	

*Used as positive control

**Non-OECD study



Phase 1B – Overview of U.S. Studies

	1) USEPA	2) WI	3) ABC	4) SS
Prochloraz	JMD FHM, FHM**		FHM	
Fadrozole	JMD* FHM*, JMD**		FHM*	
Flutamide	JMD FHM	JMD		FHM
4-tPP		JMD	FHM	
Estradiol		JMD*	FHM*	

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**Non-OECD study



Phase 1B – Overview of U.S. Studies

	USEPA	WI	ABC	SS
Prochloraz	JMD FHM, FHM**		FHM	
Fadrozole	JMD* FHM*, JMD**		FHM*	
Flutamide	JMD FHM	JMD		FHM
4-tPP		JMD	FHM	
Estradiol		JMD*	FHM*	

*Used as positive control

**Non-OECD study



Phase 1B – Overview of U.S. Studies

	USEPA	WI	ABC	SS
Prochloraz	JMD FHM, FHM**		FHM	
Fadrozole	JMD* FHM* , JMD**		FHM*	
Flutamide	JMD FHM	JMD		FHM
4-tPP		JMD	FHM	
Estradiol		JMD*	FHM*	

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**Non-OECD study



Phase 1B – Overview of U.S. Studies

	USEPA	WI	ABC	SS
Prochloraz	JMD FHM, FHM**		FHM	
Fadrozole	JMD* FHM*, JMD**		FHM*	
Flutamide	JMD FHM	JMD		FHM
4-tPP		JMD	FHM	
Estradiol		JMD*	FHM*	

*Used as positive control

**Non-OECD study



The background of the slide is a histopathology image showing cross-sections of biological tissue, likely from a rodent, stained with hematoxylin and eosin (H&E). The image shows various cellular structures, including what appears to be the renal cortex with glomeruli and tubules, and possibly the adrenal gland. The staining highlights the nuclei in purple and the cytoplasm/extracellular matrix in pink.

U.S. Phase 1B Studies: Histopathology Results by Chemical



Prochloraz -- Males

Exposure-Related Findings	FHM			JMD
	EPA1	EPA2*	ABC	EPA
Increased Interstitial Cells (Grade 1-2)	-	-	-	M, H
Increased Spermatozoa (Grade 1-2)	-	-	-	H
Increased Average Testicular Stage	-	M, H	-	H

*Non-OECD study

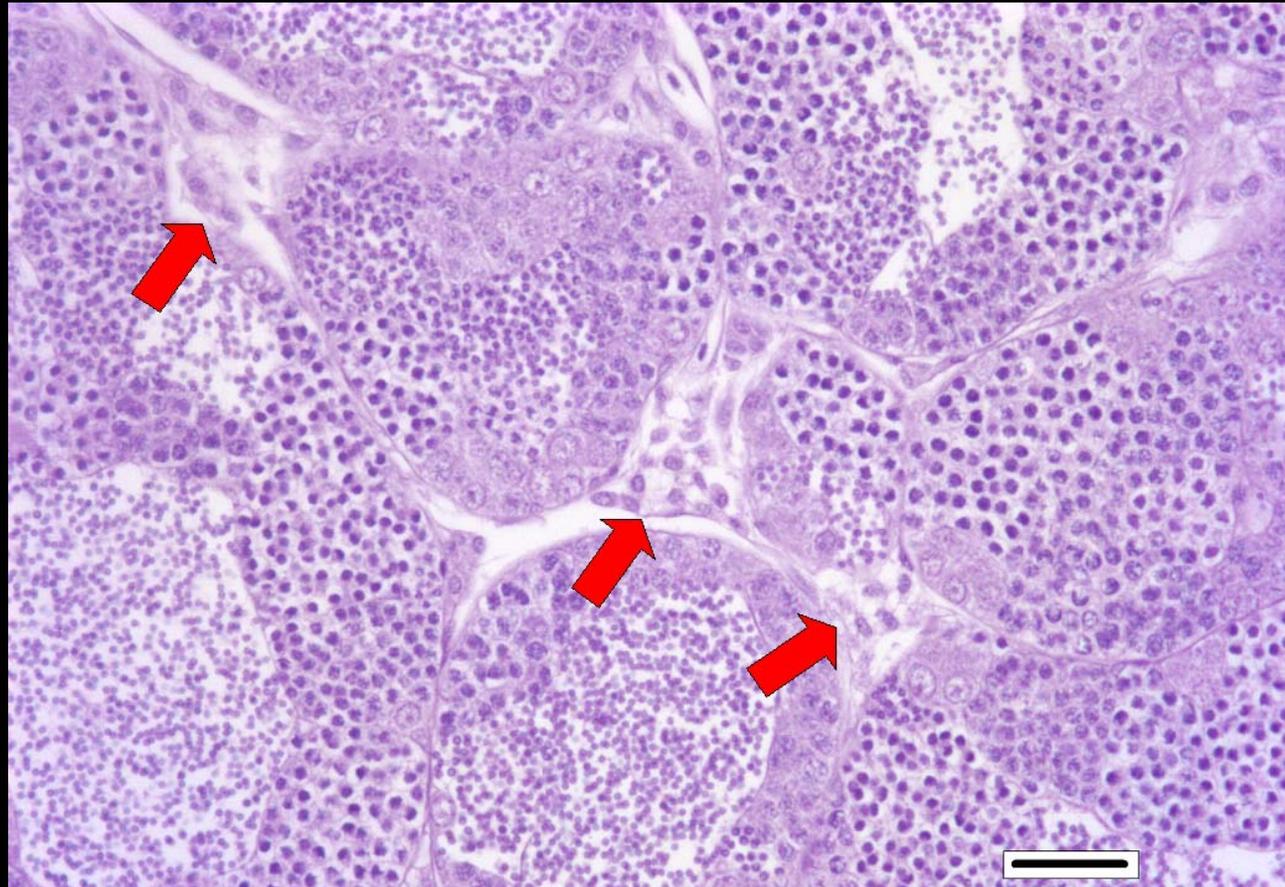
Fadrozole -- Males

Exposure-Related Findings	FHM		JMD	
	EPA	ABC	EPA1	EPA2
Increased Interstitial Cells (Grade 1-2)	P	P	-/+	H
Increased Spermatozoa (Grade 1-2)	P	-	P	H
Increased Average Testicular Stage	-	-	P	H



Control Testis FHM

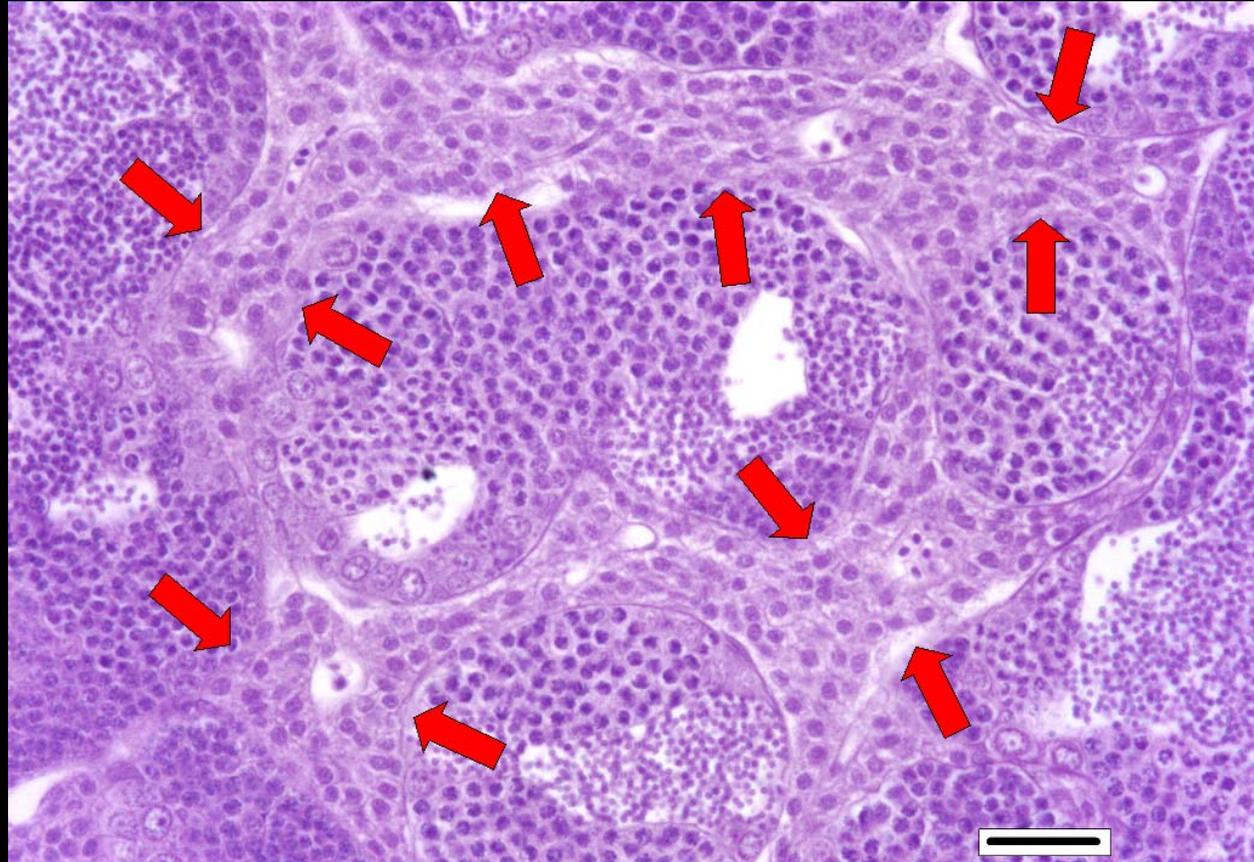
Arrows indicate small interstitial cell aggregates



Z10597.tif EPA2-4 Fathead Minnow Testis H&E 40x Fadrozole 0µg/L

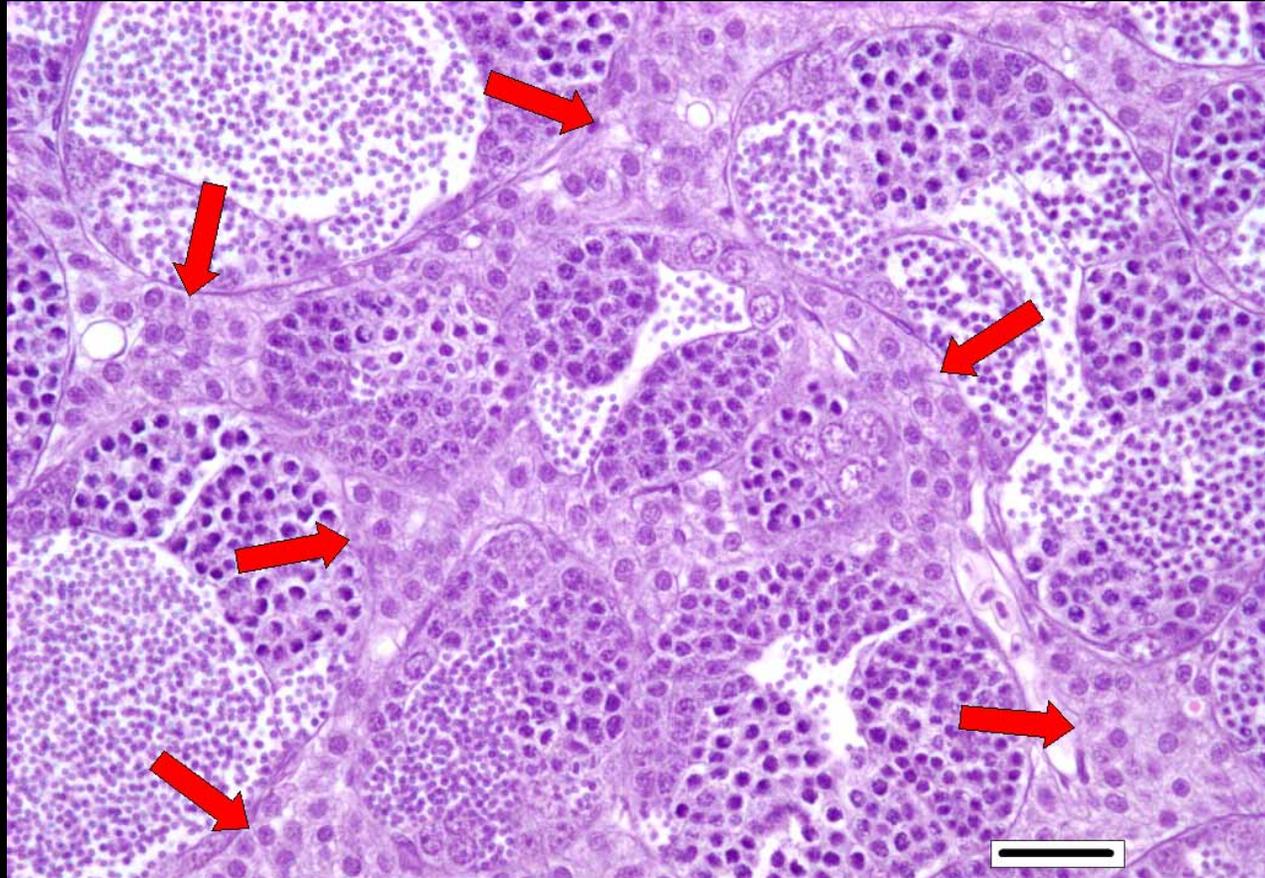
Fadrozole – Increased Interstitial Cells FHM (EPA study)

Severity
Grade 3



Z10596.tif EPA2-81 Fathead Minnow Testis H&E 40x Fadrozole 100µg/L

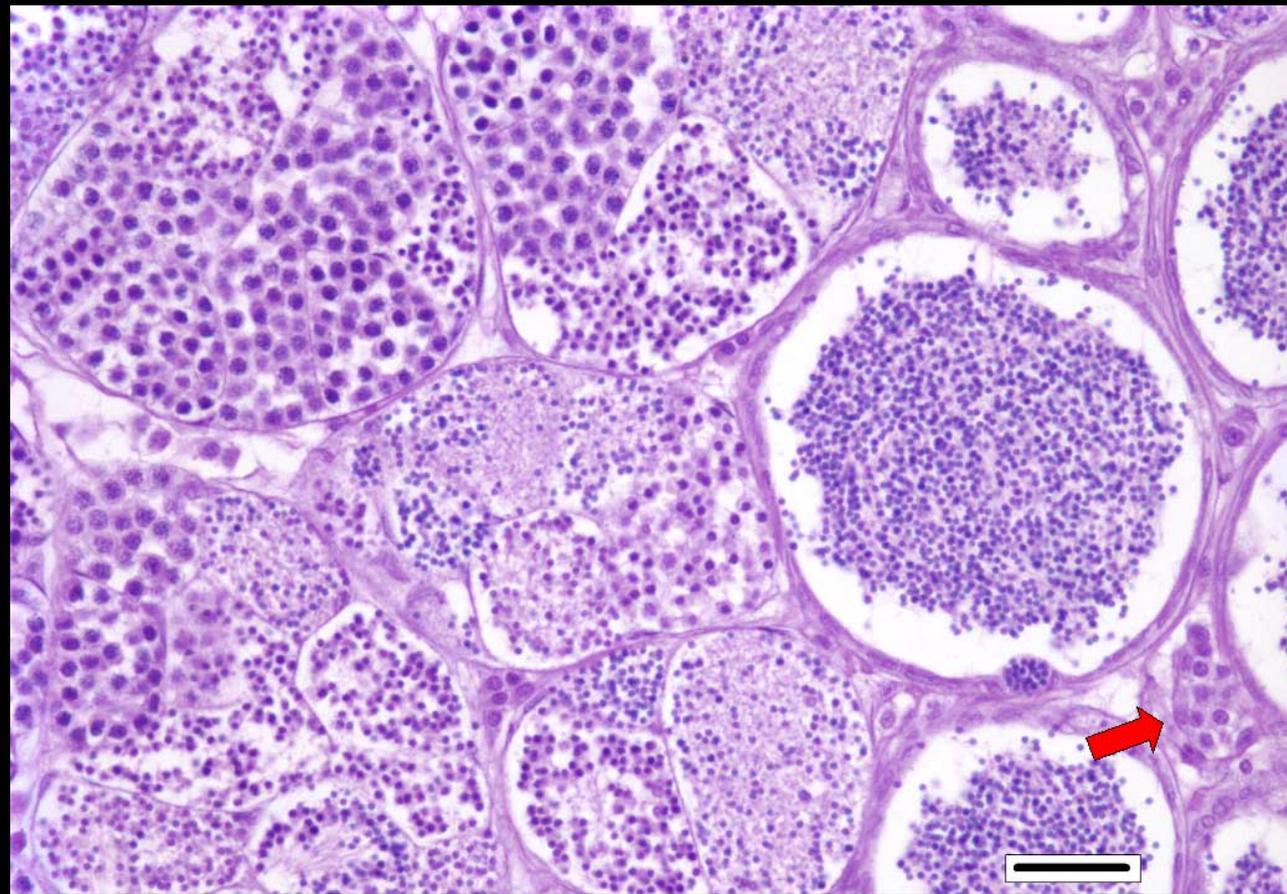
Fadrozole – Increased Interstitial Cells FHM (ABC Labs study)



Z10699.tif 01B1-2 Fathead Minnow Testis H&E 40x Fadrozole 100 ug/L

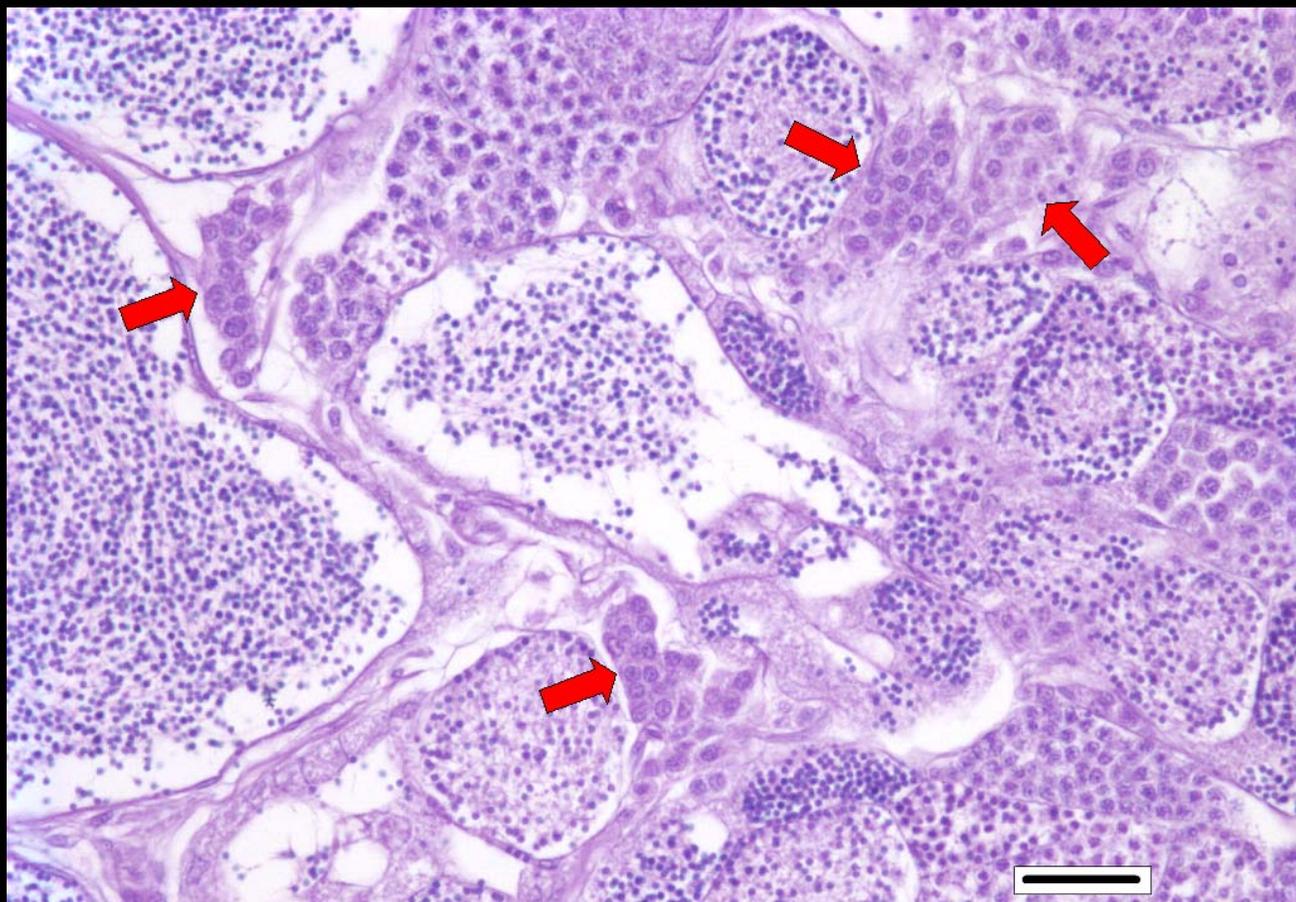
Control Testis JMD

Arrow indicates a small interstitial cell aggregate



Z10573.tif T1-A-6-3942 Medaka Testis H&E 40x Prochloraz 0ppm

Prochloraz – Increased Interstitial Cells JMD



Z10572.tif T4-A-8-4004 Medaka Testis H&E 40x Prochloraz 300ppm

Prochloraz -- Females

Exposure-Related Findings		FHM			JMD
		EPA1	EPA2*	ABC	EPA
Decreased Vitellogenesis	(Grade 2-4)	-	-	-	M, H
Increased Oocyte Atresia	(Grade 1-4)	H	-	H	M, H
Perifollicular Cell Hyperplasia	(Grade 1-3)	-	-	-	M, H
Perifollicular Cell Hypertrophy	(Grade 1-3)	-	-	-	M, H
Decreased Average Ovarian Stage		-	-	H	M, H

*Non-OECD study

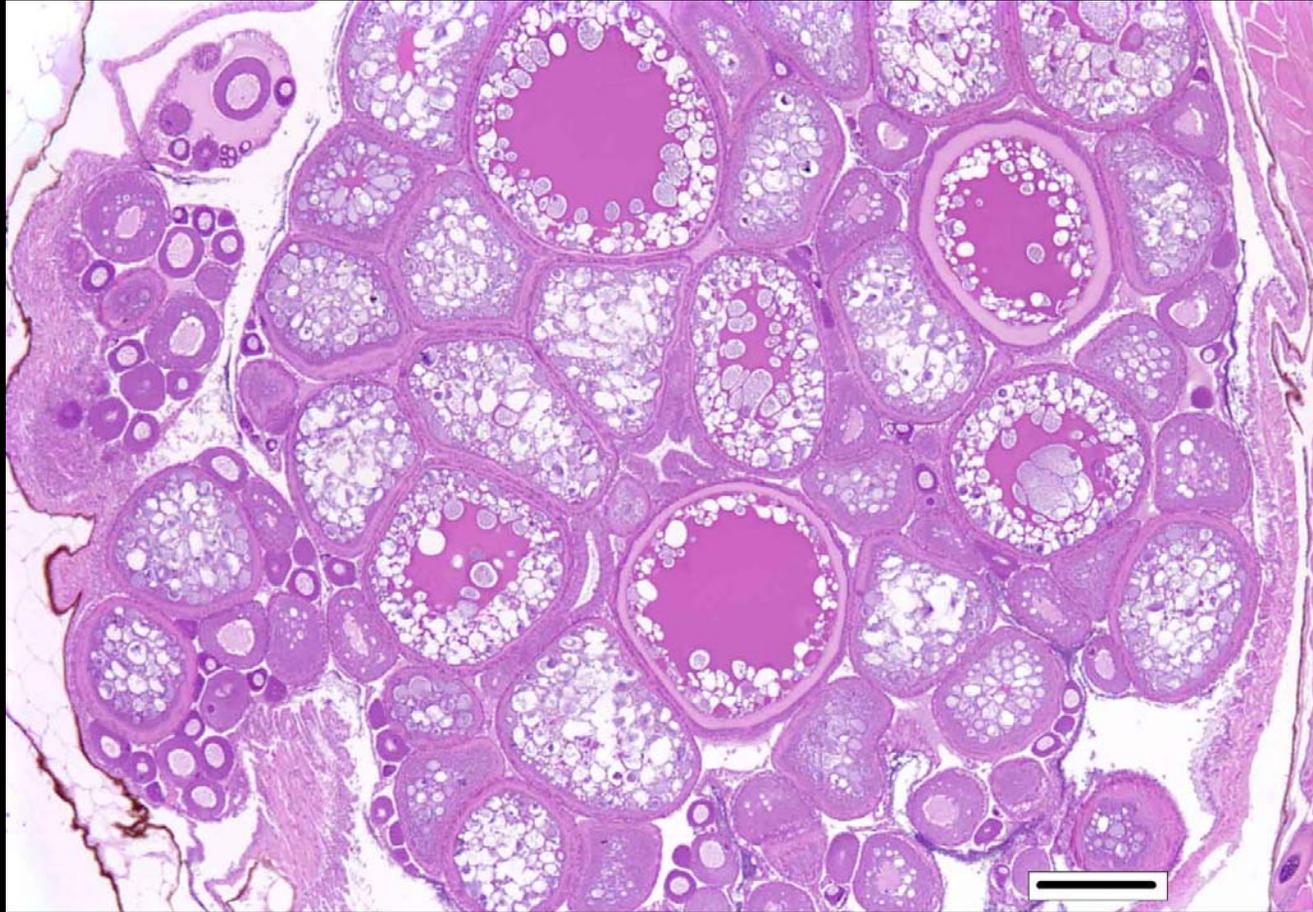
Fadrozole -- Females

Exposure-Related Findings		FHM		JMD	
		EPA	ABC	EPA1	EPA2*
Decreased Vitellogenesis		P	P	P	H
Increased Oocyte Atresia		P	P	P	H
Perifollicular Cell Hyperplasia		-	-	P	M, H
Perifollicular Cell Hypertrophy		-	-	P	L, M, H
Decreased Average Ovarian Stage		P	P	P	H

*Non-OECD study



Control Ovary JMD



Z10577.tif T1-B-2-3692 Medaka Ovary H&E 4x Prochloraz 0ppm

Fadrozole – Decreased Vitellogenesis JMD

Red arrow indicates dec. vitel.

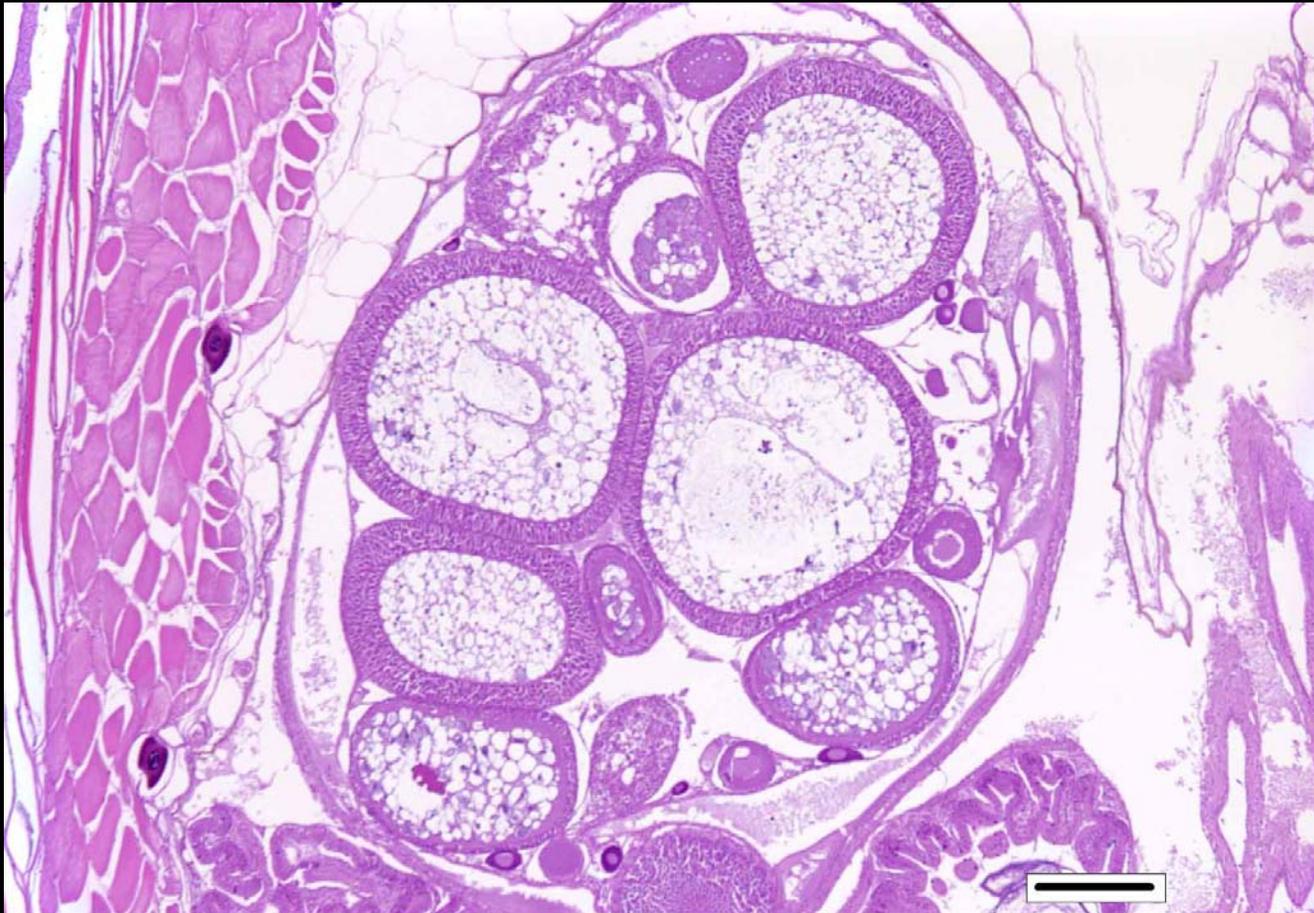
Open arrow indicates atresia

Small arrow indicates atresia of mature oocyte



Z10542.tif T4-A-6 Medaka Ovary H&E 4x Fadrozole 100ppm

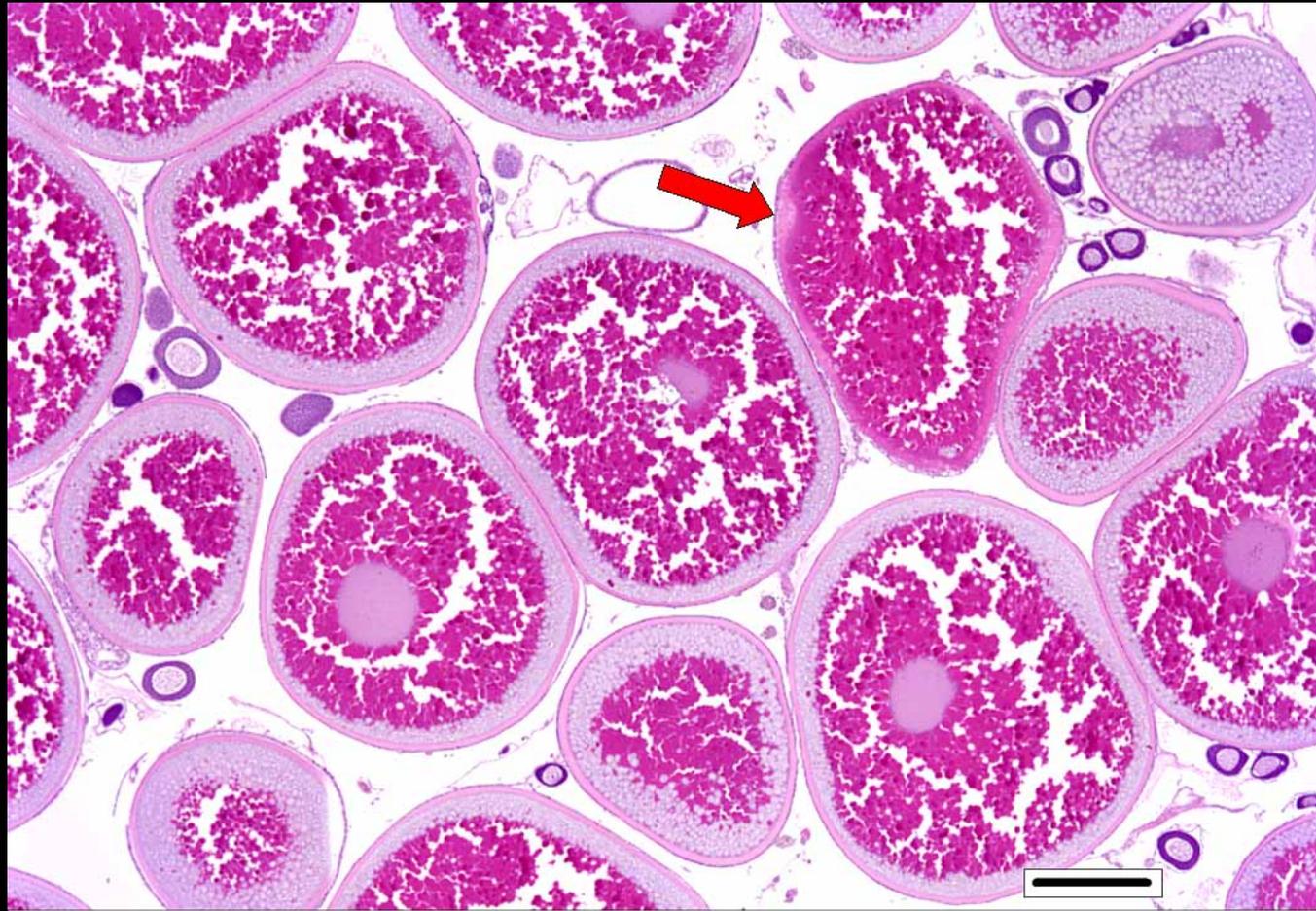
Prochloraz – Decreased Vitellogenesis JMD



Z10575.tif T4-B-1-3751 Medaka Ovary H&E 4x Prochloraz 300ppm

Control Ovary FHM

Arrow indicates atretic oocyte

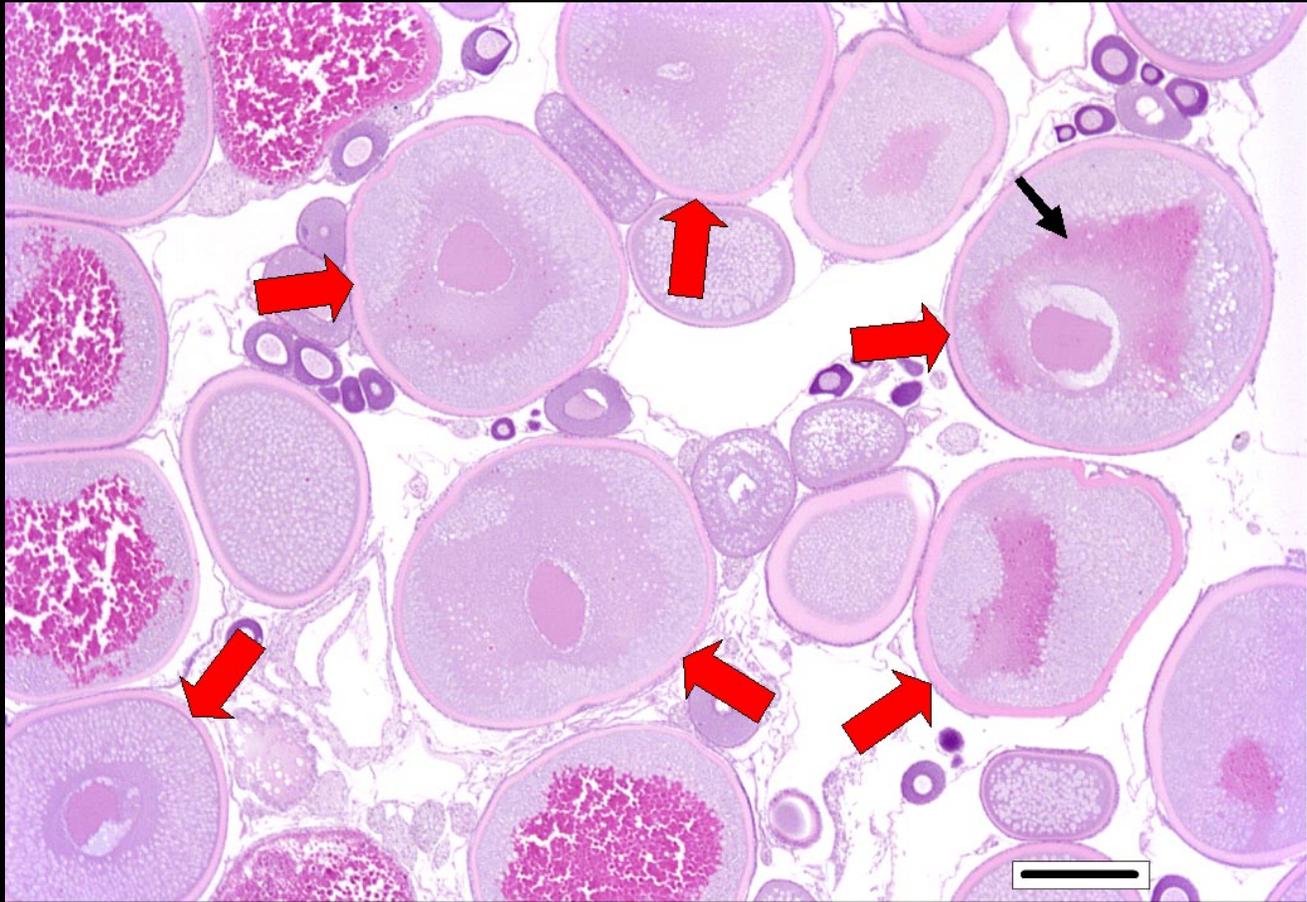


Z10607.tif EPA2-6 Fathead Minnow Ovary H&E 4x Prochloraz 0µg/L

Fadrozole – Decreased Vitellogenesis FHM (Fish from USEPA study)

Red arrows indicate oocytes with dec. vitel.

Black arrow indicates small amount of yolk material in one oocyte



Z10605.tif EPA2-97 Fathead Minnow Ovary H&E 4x Fadrozole 100µg/L

Fadrozole – Decreased Vitellogenesis FHM

(Fish from the ABC Labs study)

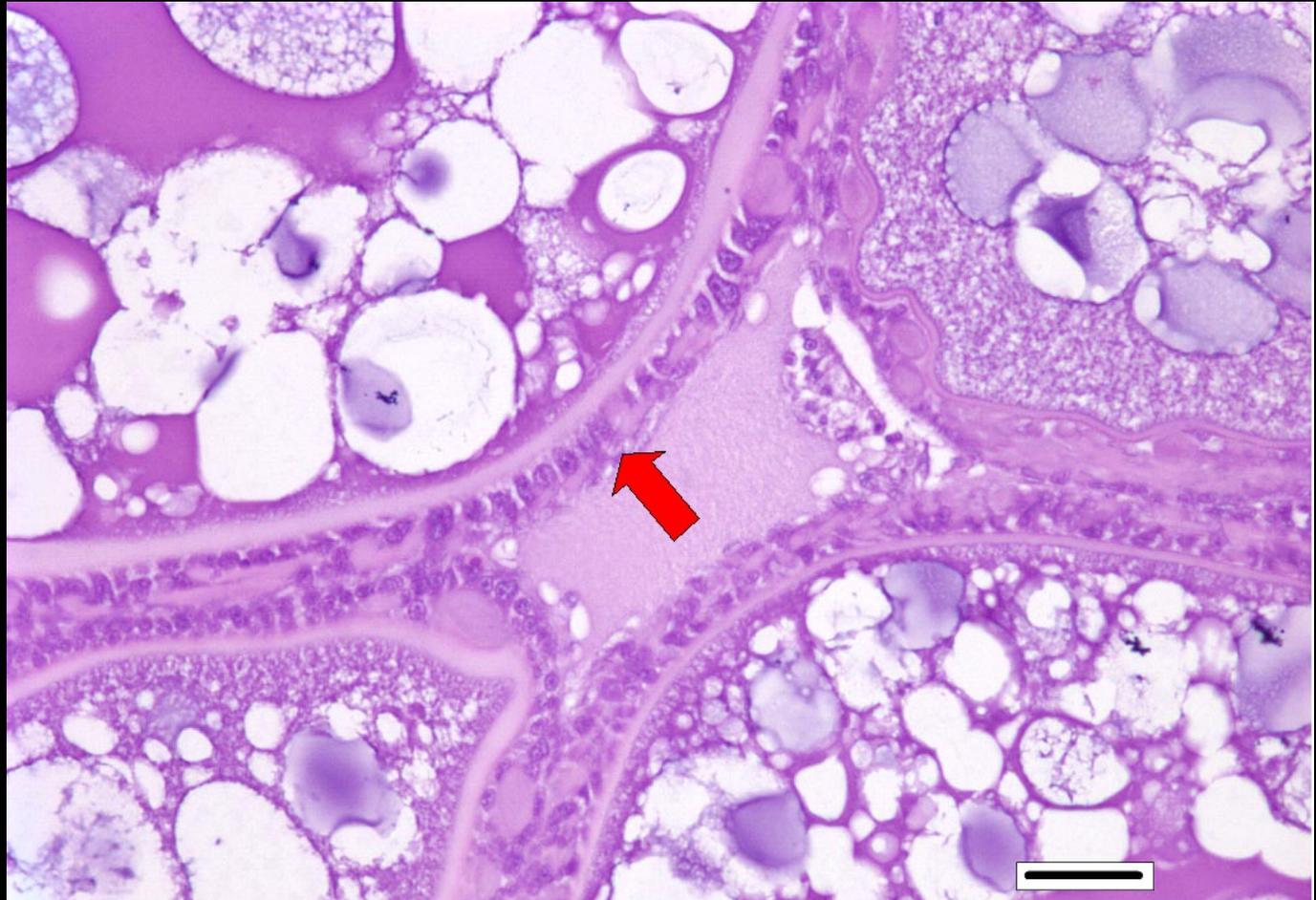
Arrow indicates area of oocyte that should contain yolk granules



Z10705.tif 18B2-2 Fathead Minnow Ovary H&E 10x Fadrozole 100 ug/L

Control Ovary JMD

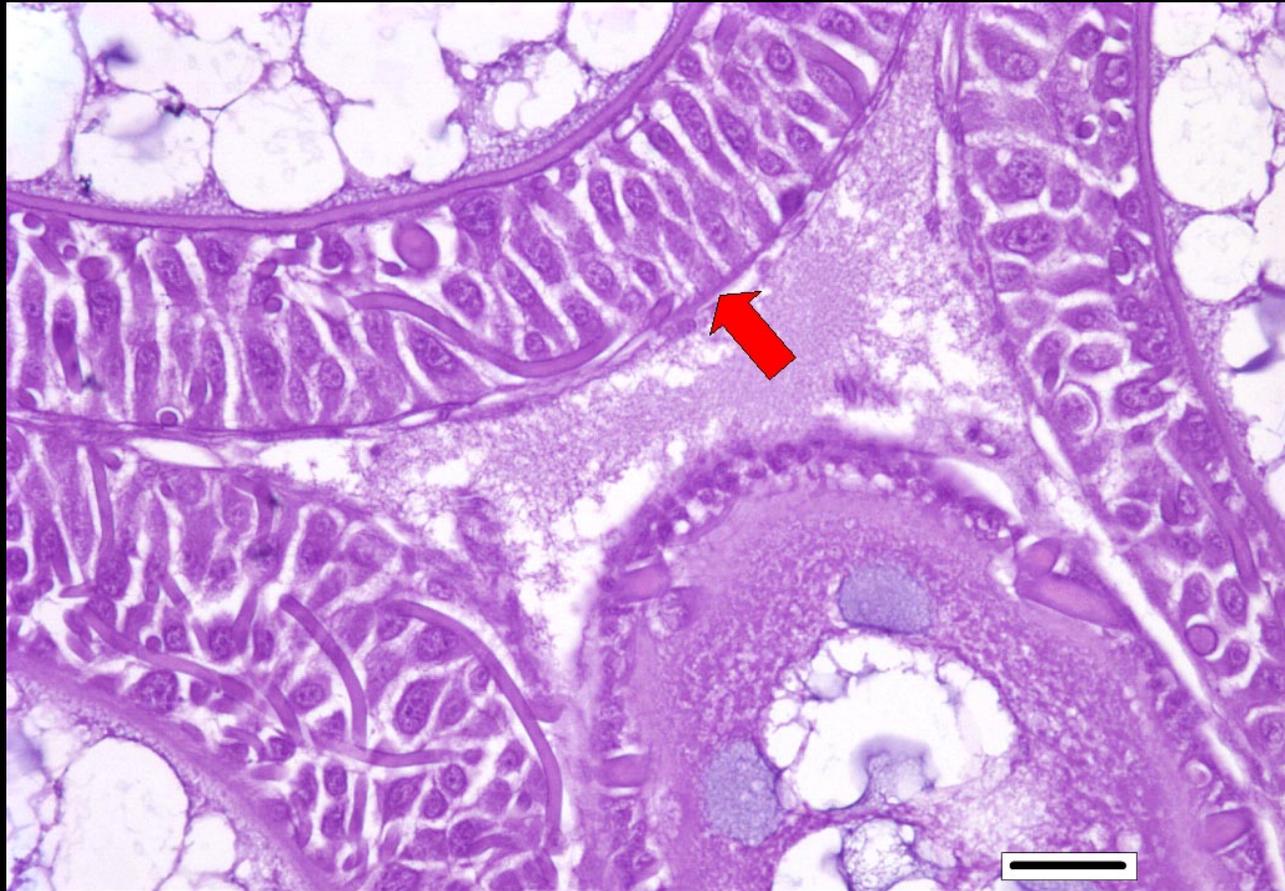
Arrow indicates perifollicular (granulosa & theca) cells



Z10578.tif T1-B-2-3692 Medaka Ovary H&E 40x Prochloraz 0ppm

Prochloraz – Perifollicular Cell Hypertrophy / Hyperplasia JMD

Severity
Grade 4



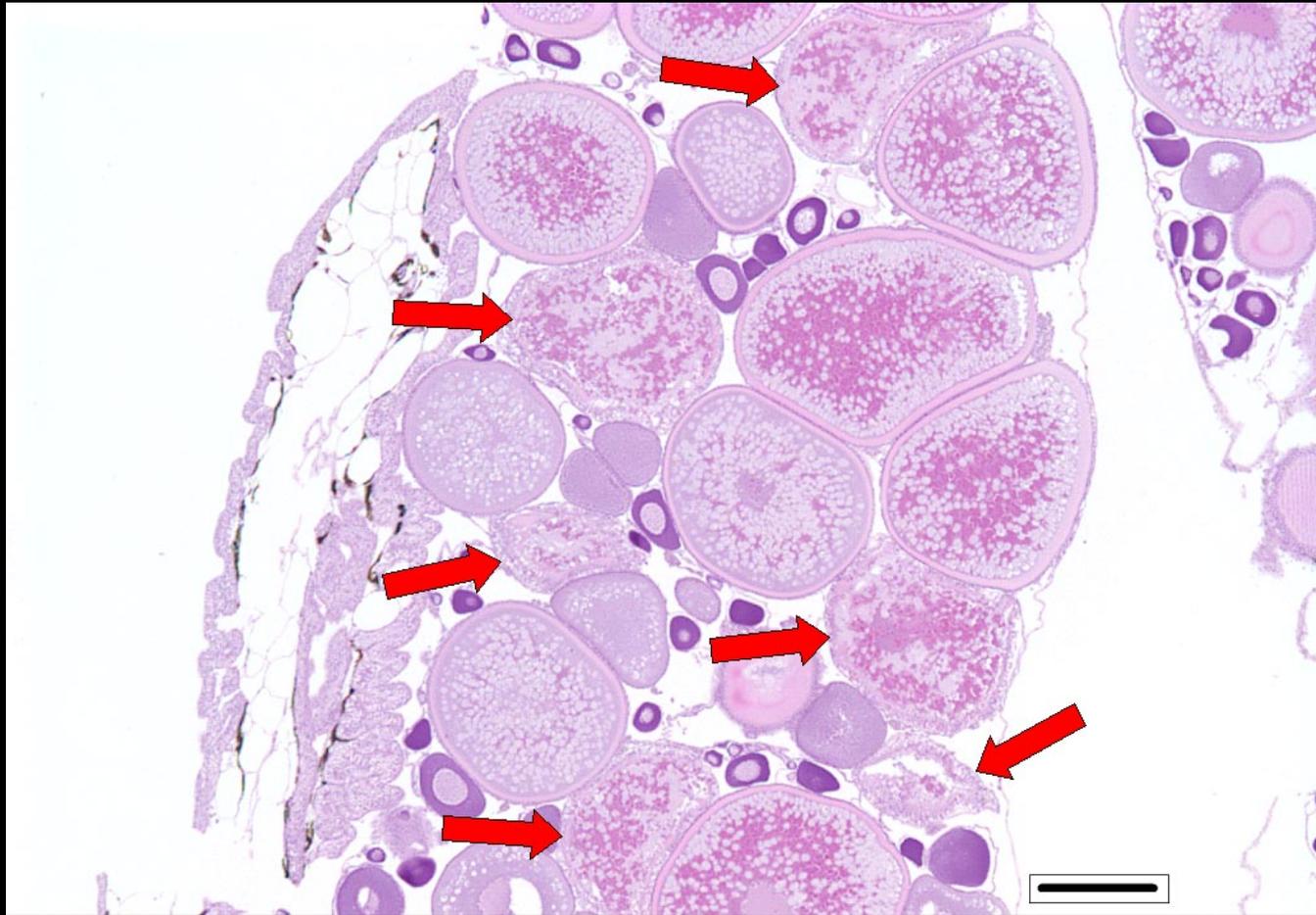
Z10576.tif T4-B-8-3751 Medaka Ovary H&E 40x Prochloraz 300ppm

Fadrozole – Increased Oocyte Atresia FHM



Z10704.tif 06B1-2 Fathead Minnow Ovary H&E 10x Fadrozole 100 ug/L

Prochloraz – Increased Oocyte Atresia FHM



Z10645.tif EPA2-68 Fathead Minnow Ovary H&E 4x Prochloraz 1000 ug/L

Flutamide -- Males

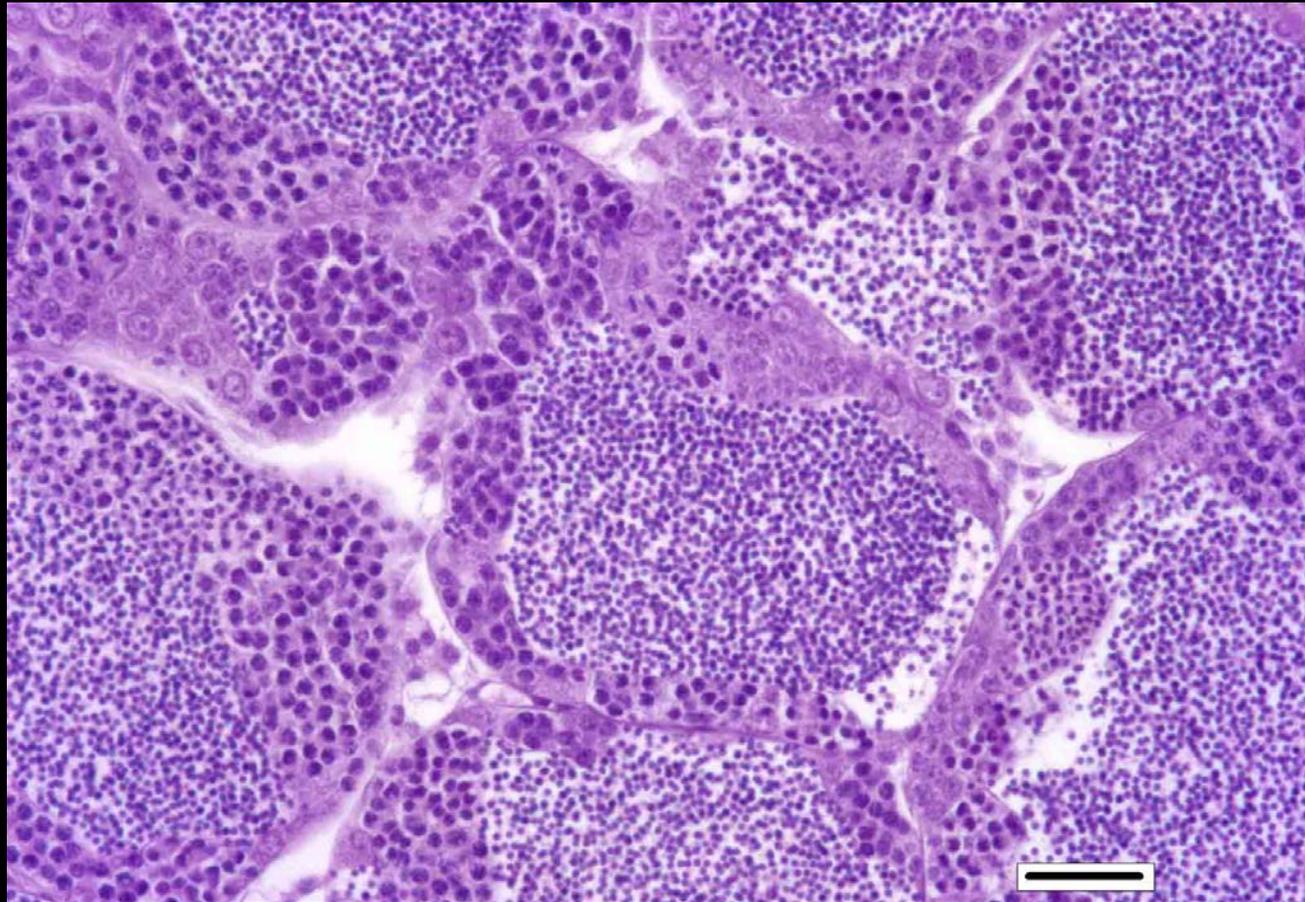
Exposure-Related Findings		FHM		JMD	
		EPA	SS	EPA	WI
Increased Spermatogonia	(Grade 1-3)	M, H	-	-	-
Decreased Spermatoocytes	(Grade 1-2)	H	-	-	-
Increased Average Testicular Stage		H	-	H	-

Flutamide -- Females

Exposure-Related Findings		FHM		JMD	
		EPA	SS	EPA	WI
Decreased Post-ovulatory Follicles	(not graded)	-	-	L, M, H	-
Increased Oocyte Atresia	(Grade 1-4)	M, H	-	-	-
Altered Average Ovarian Stage		M(i), H(d)	-	-	-



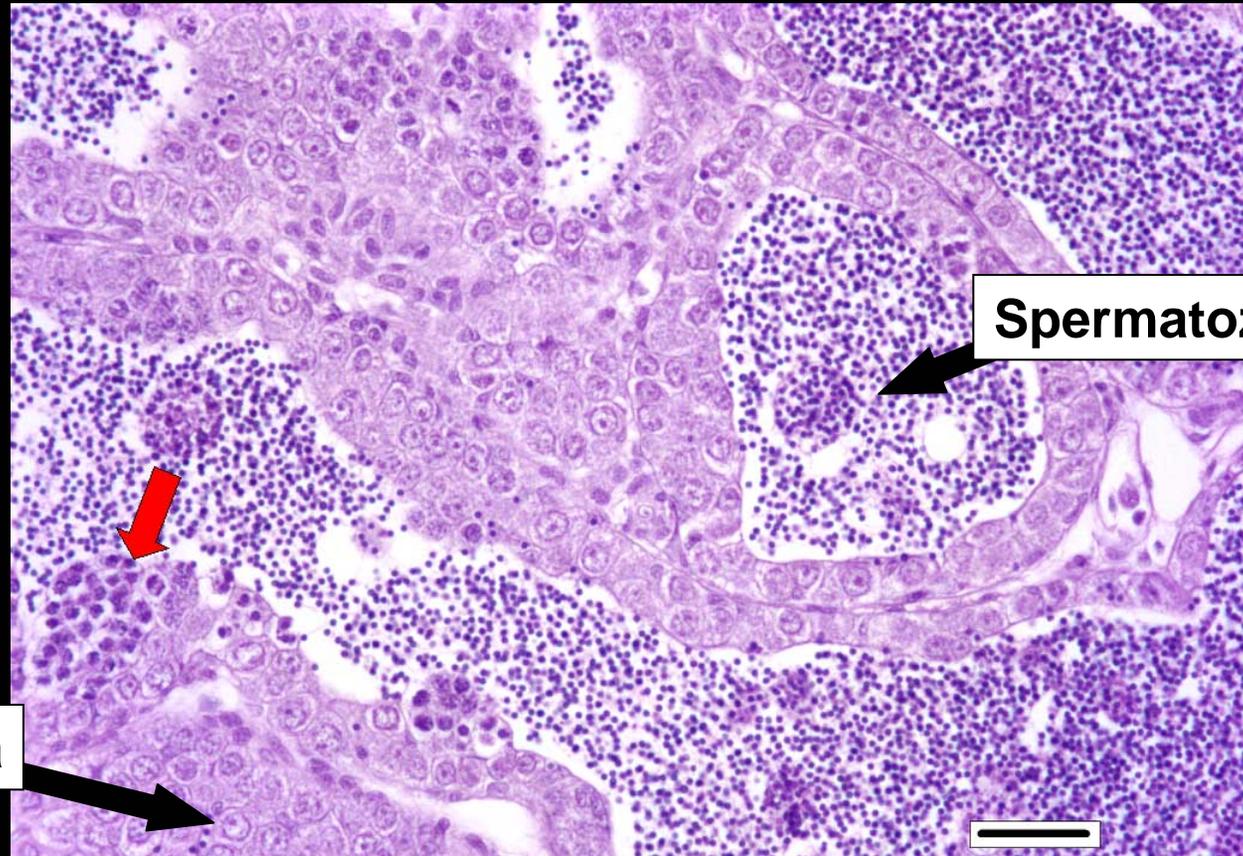
Control Testis FHM



Z10592.tif EPA1-1 Fathead Minnow Testis H&E 40x Flutamide 0 μ g/L

Flutamide – Increased Spermatogonia, Decreased Spermatozoa FHM

Red arrow
indicates
spermatocytes

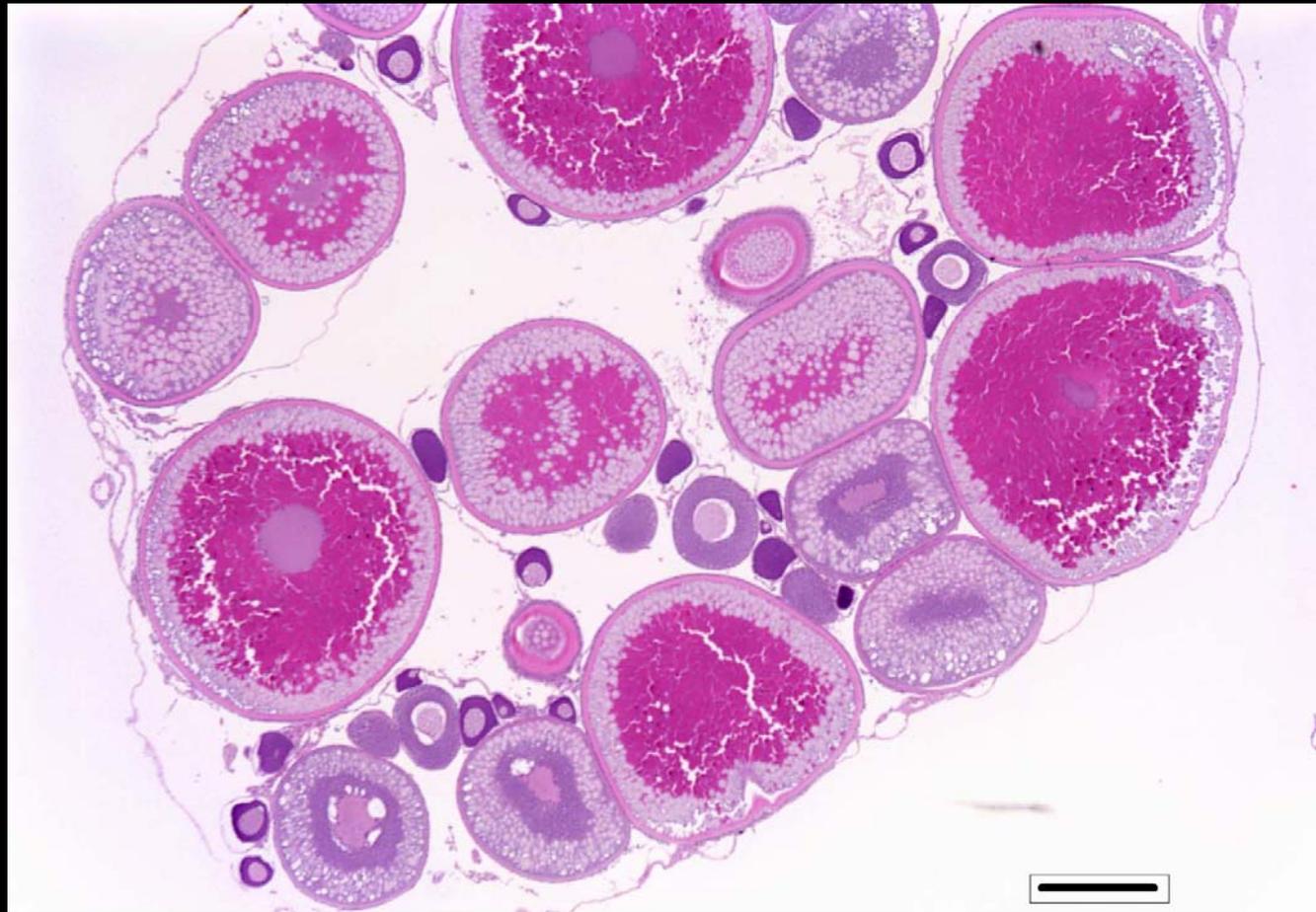


Spermatozoa

Spermatogonia

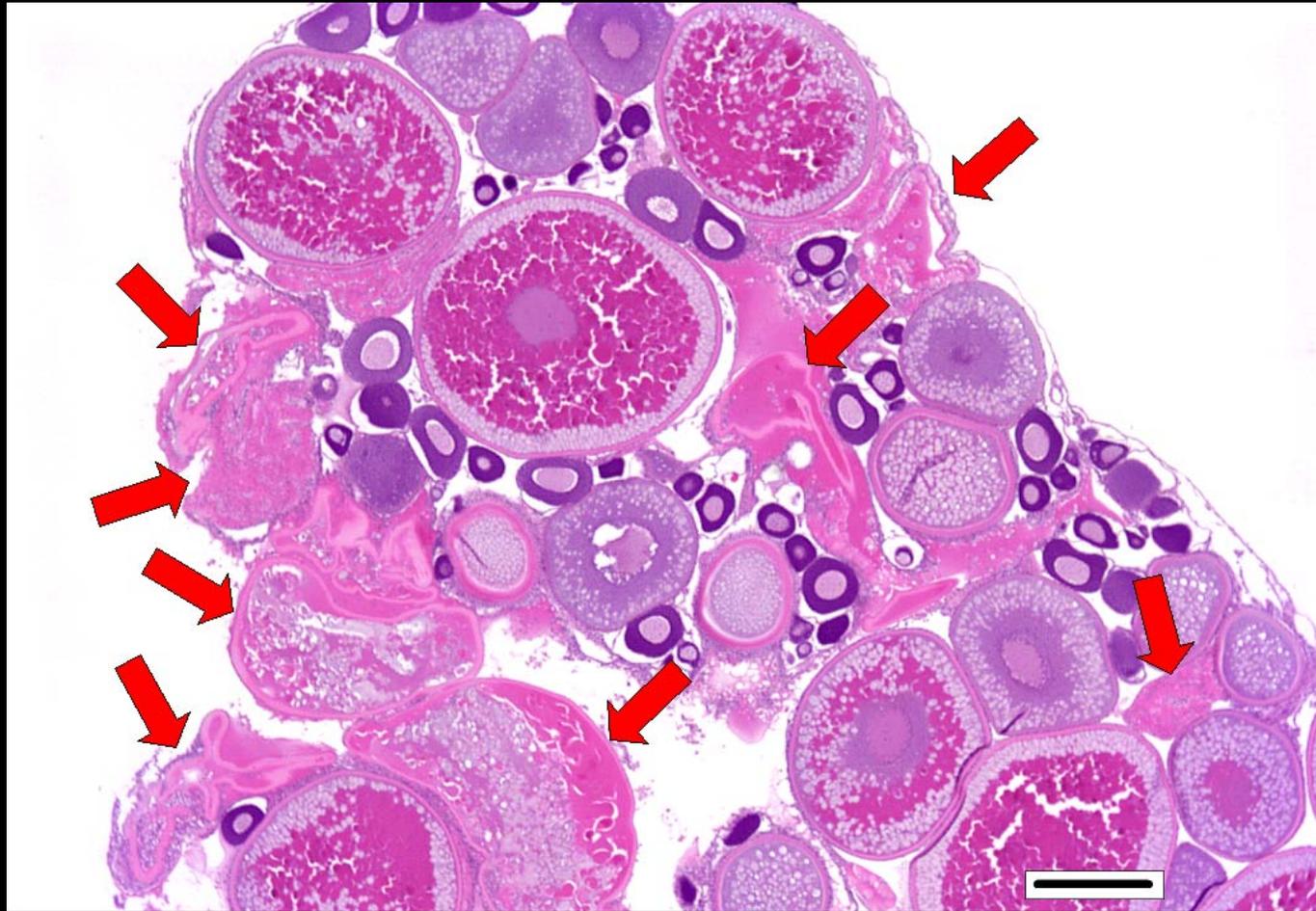
Z10590.tif EPA1-63 Fathead Minnow Testis H&E 40x Flutamide 1000µg/L

Control Ovary FHM



Z10595.tif EPA1-6 Fathead Minnow Ovary H&E 4x Flutamide 0 μ g/L

Flutamide – Oocyte atresia FHM



Z10594.tif EPA1-76 Fathead Minnow Ovary H&E 4x Flutamide 1000µg/L

4tPP -- Males

Exposure-Related Findings		FHM	JMD
		ABC	WI
Increased Testicular Degeneration	(Grade 1-3)	H*	H
Increased Spermatogonia	(Grade 1-3)	H*	-
Intravascular Proteinaceous Fluid	(Grade 1-2)	H*	-
Nephropathy	(Grade 1-3)	n/a	H

*No L or M dose group

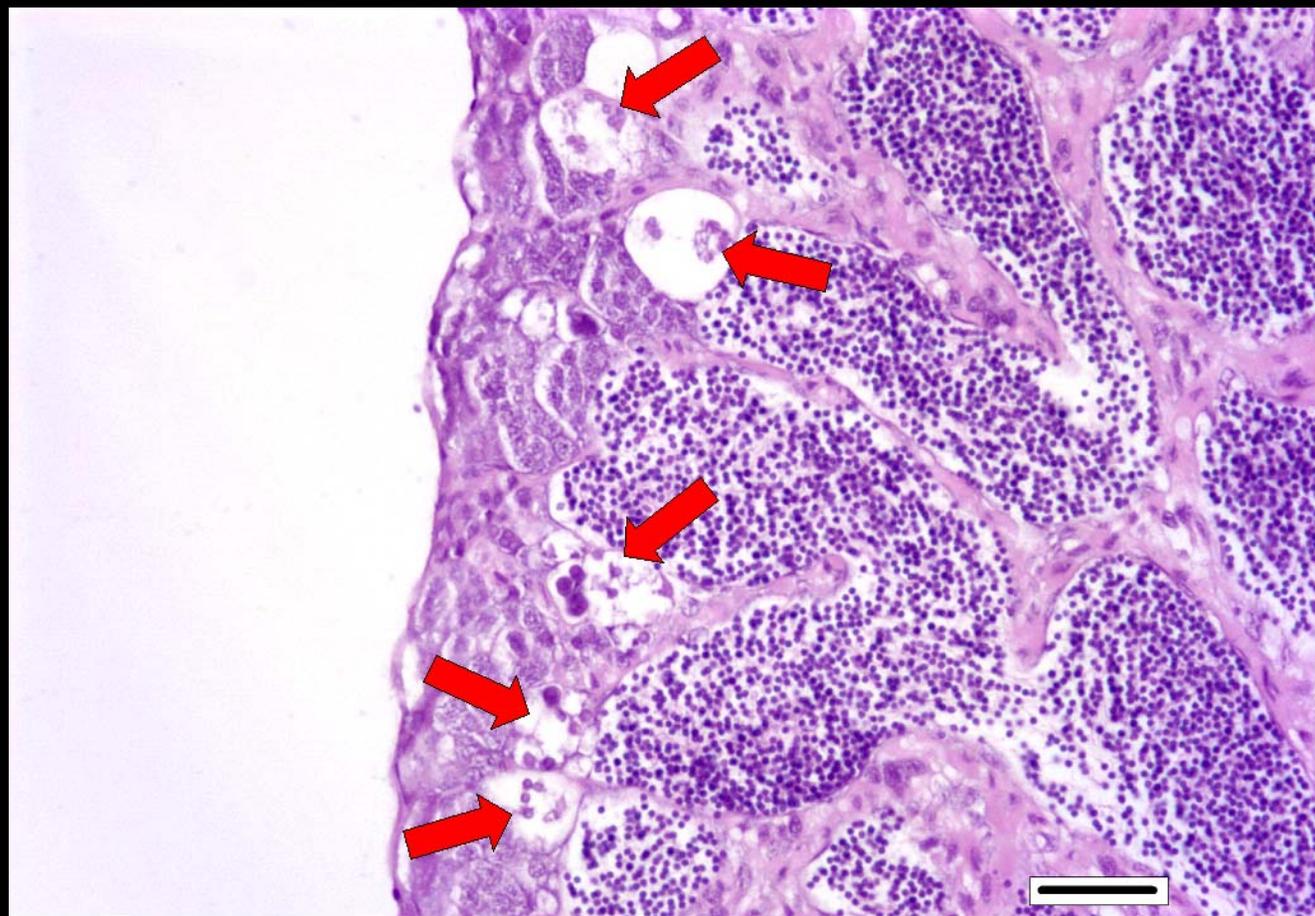
Estradiol (E2) -- Males

Exposure-Related Findings		FHM	JMD
		ABC	WI
Increased Testicular Degeneration		-	P
Increased Spermatogonia		P	-
Intravascular Proteinaceous Fluid		P	P
Nephropathy		n/a	P



E2 – Testicular Degeneration JMD

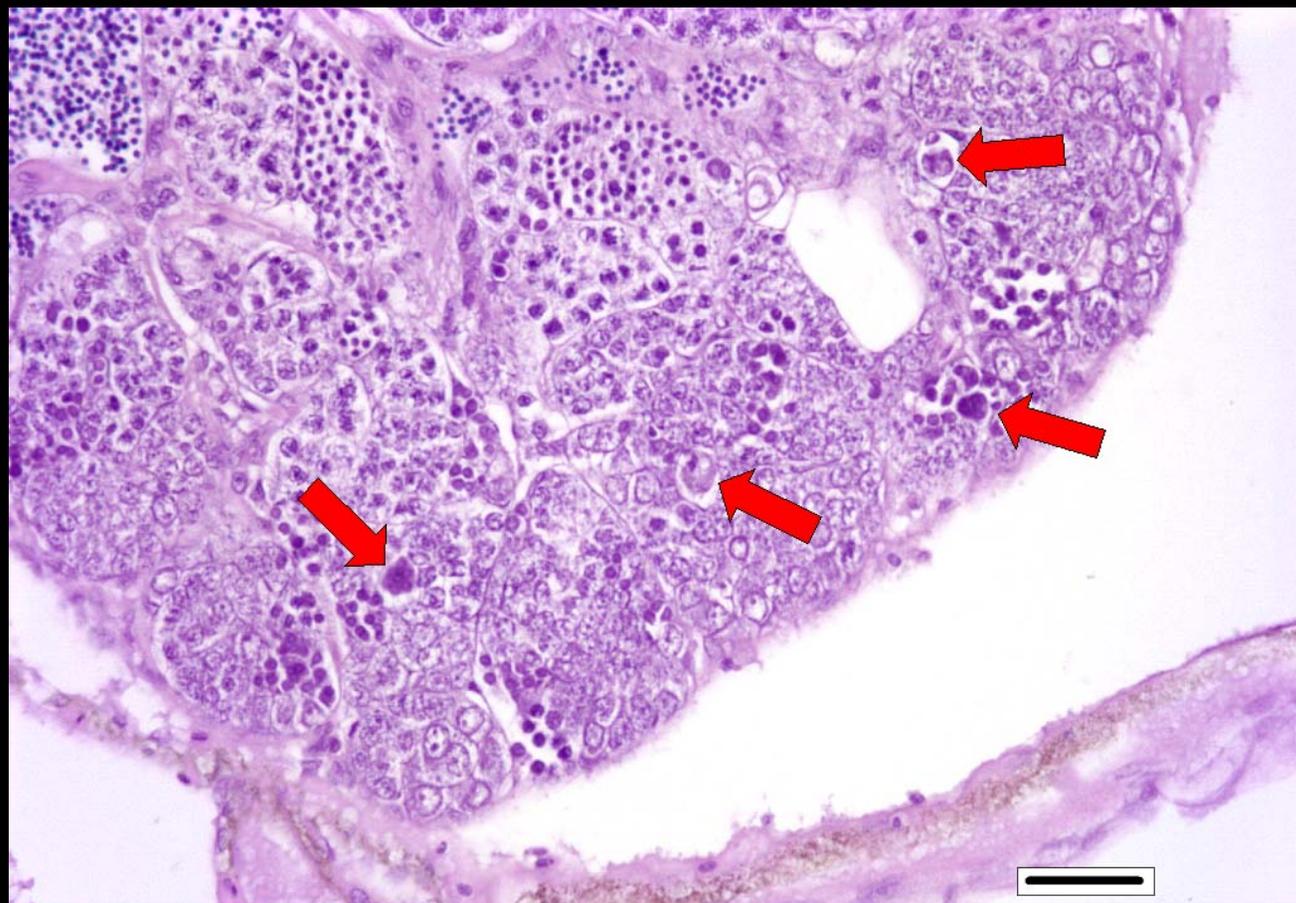
Arrows indicate degenerating germ cells



Z10625.tif 81B Medaka Testis H&E 40x E2 100ng/L

4tPP – Testicular Degeneration JMD

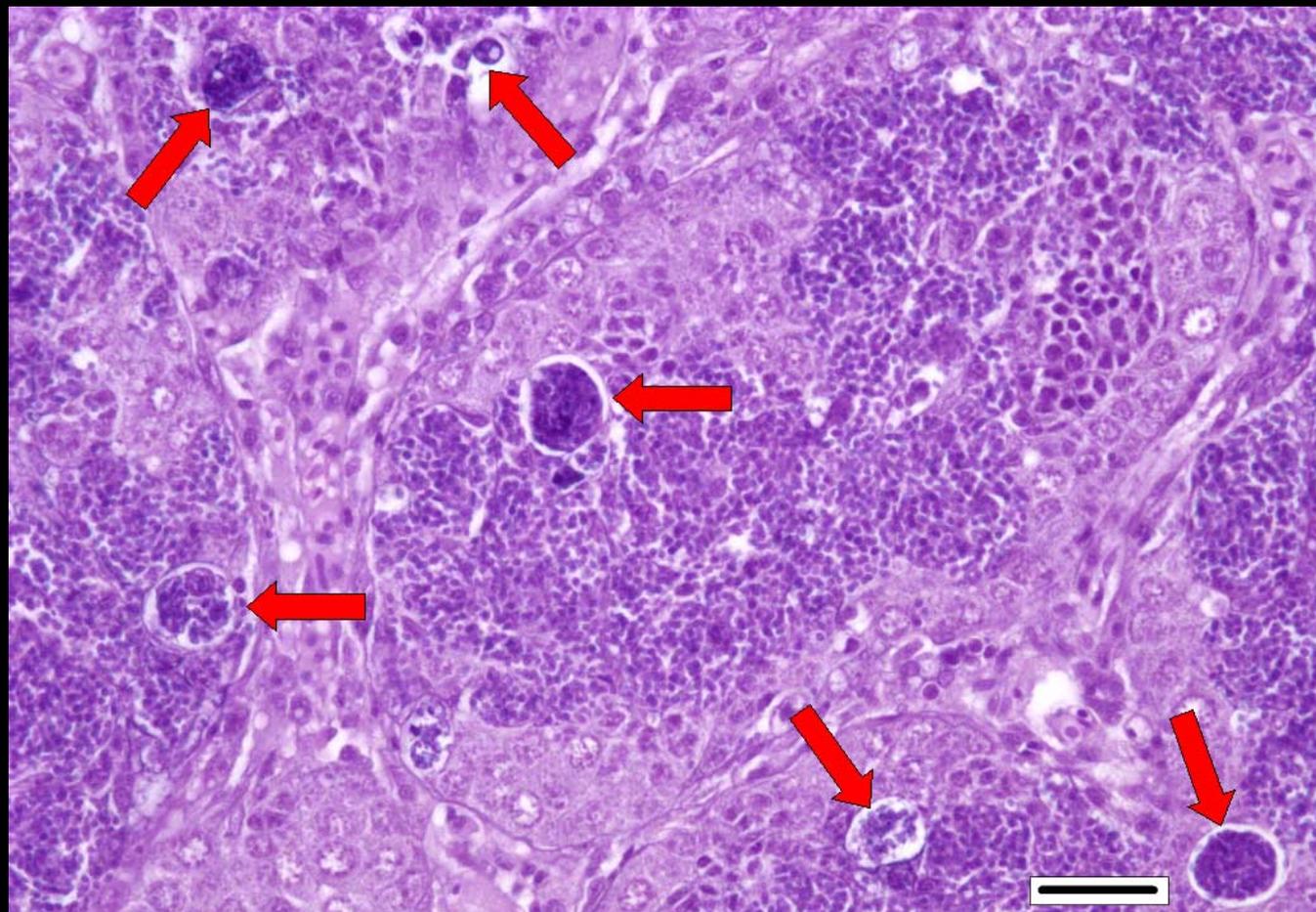
Arrows indicate degenerating germ cells



Z10624.tif 71B Medaka Testis H&E 40x 4-PP 1000µg/L

4tPP – Testicular Degeneration FHM

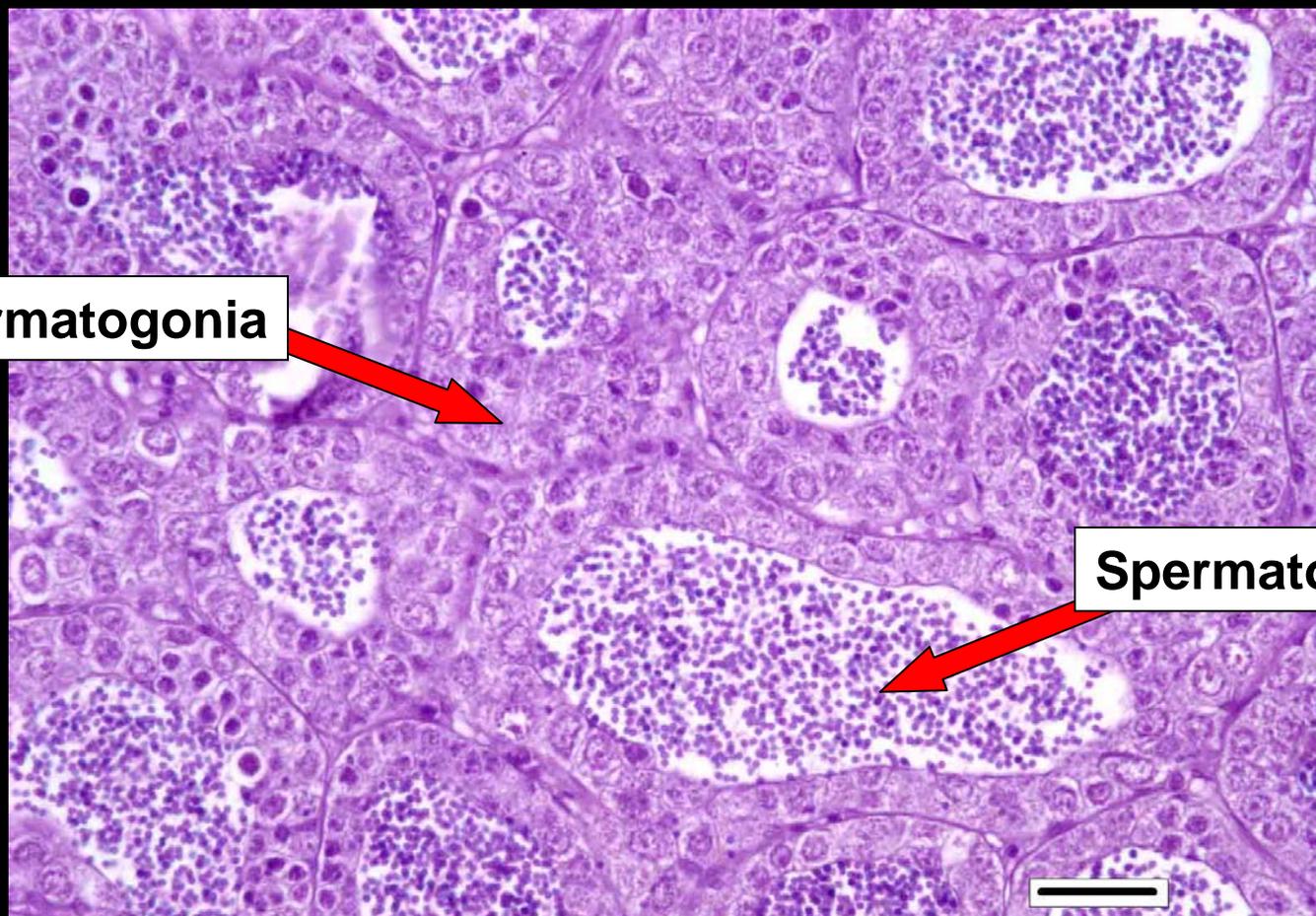
Arrows indicate degenerating germ cells



Z10690.tif 03E1-1 Fathead Minnow Testis H&E 40x 4tPP 1000 ug/L

E2 – Increased Spermatogonia FHM

Spermatogonia



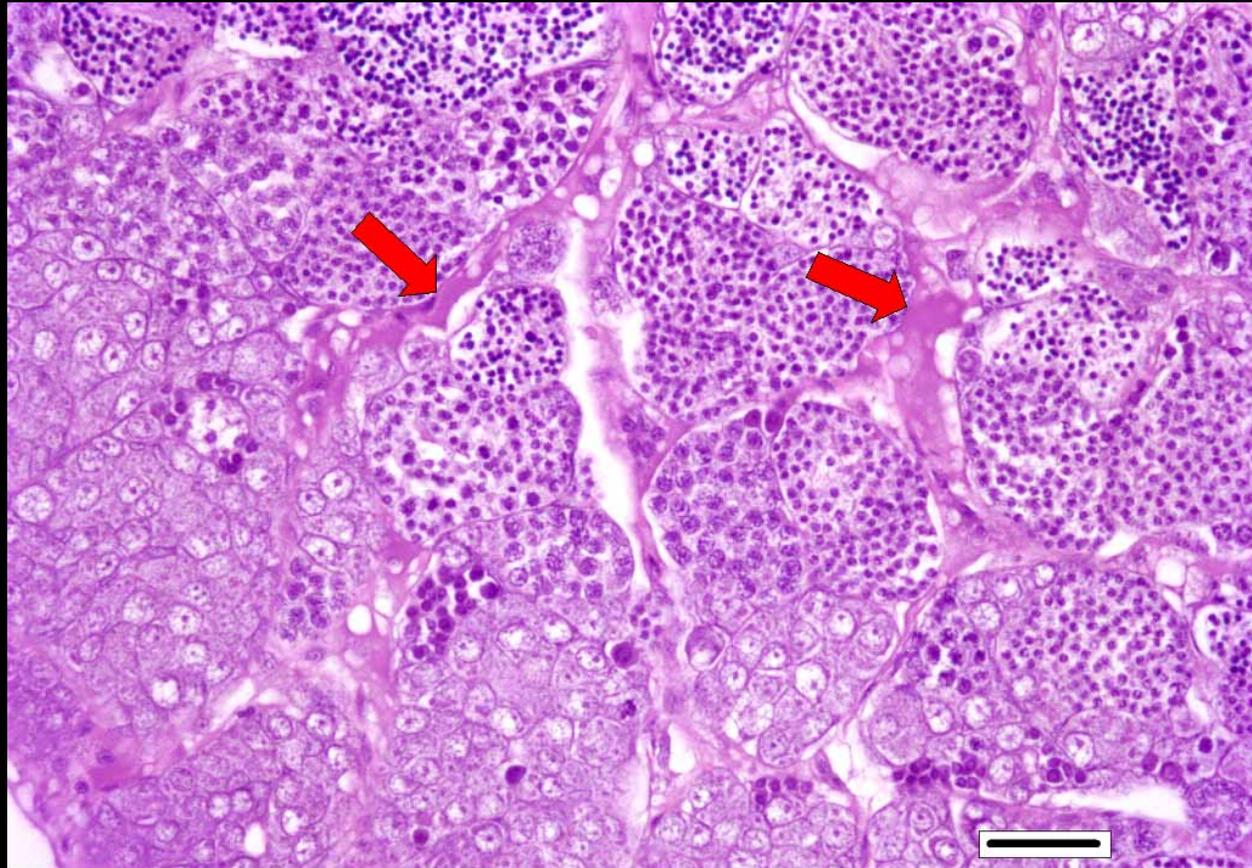
Spermatozoa

Z10691.tif 12B2-1 Fathead Minnow Testis H&E 40x E2 100 ng/L

E2 – Proteinaceous IV Fluid in Testis JMD

(increased spermatogonia, also)

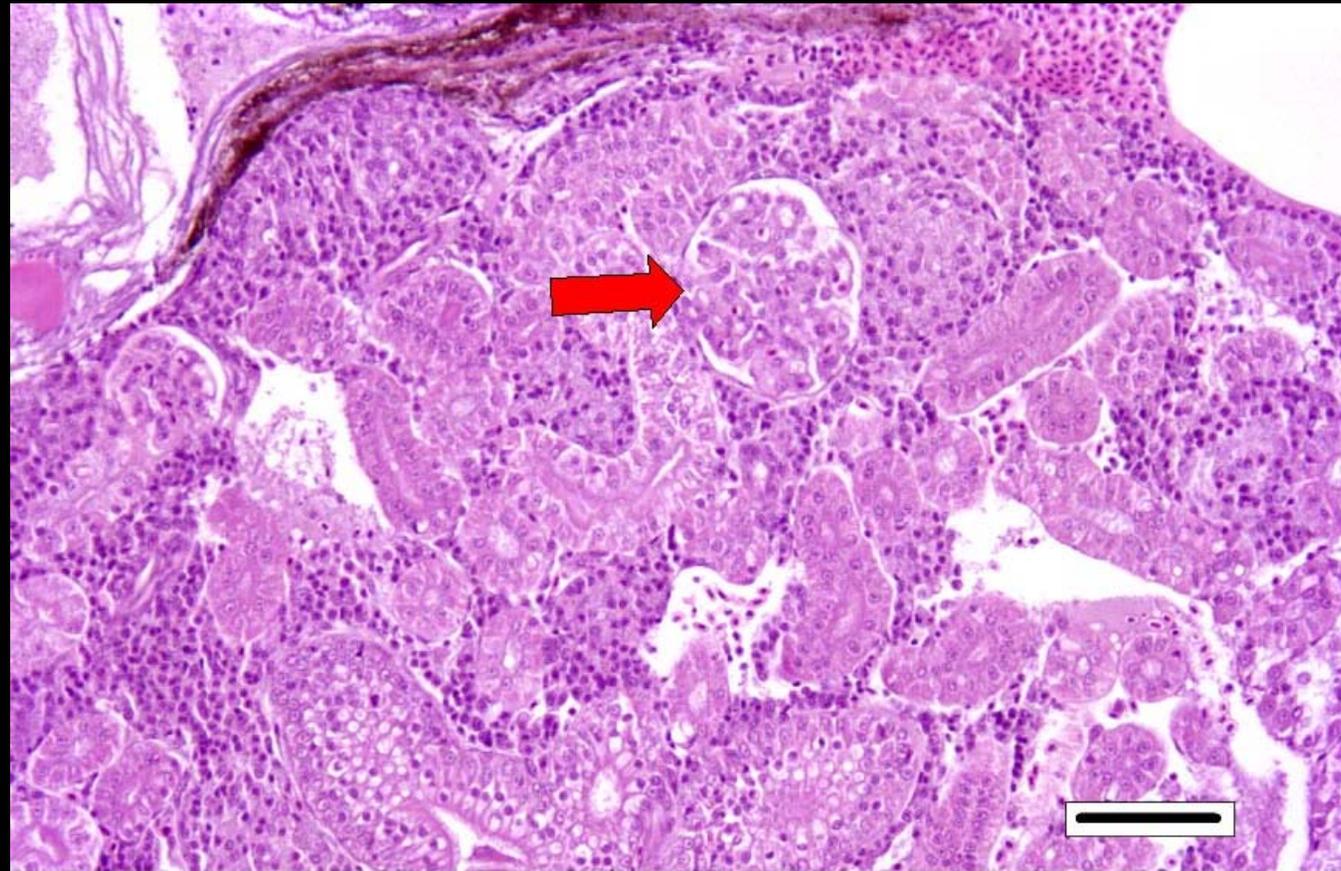
Arrows
indicate
fluid



Z10623.tif 92B Medaka Testis H&E 40x E2 100ng/L

Control Kidney JMD

Arrow indicates normal glomerulus

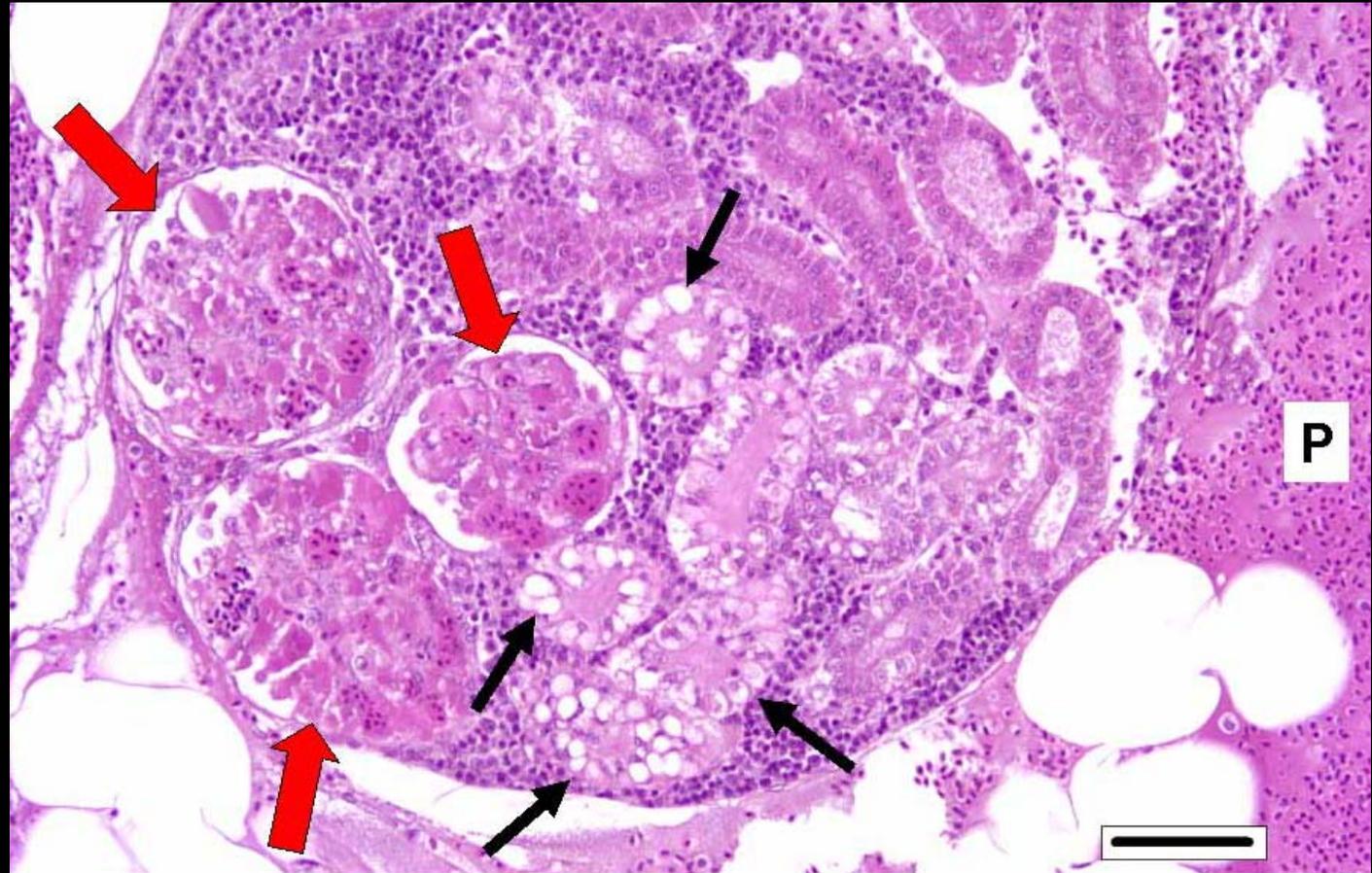


Z10619.tif 6B Medaka Kidney H&E 20x 4-PP 0µg/L

E2 – Nephropathy JMD (male)

Red arrows indicate massively enlarged glomeruli

Black arrows indicate vacuolar hypertrop. of tubule epithelium



Z10617.tif 92B Medaka Kidney H&E 20x E2 100ng/L

4tPP -- Females

Exposure-Related Findings		FHM	JMD
		ABC	WI
Interstitial Proteinaceous Fluid	(Grade 1-2)	-	M, H
Increased Oocyte Atresia	(Grade 1-2)	-	H
Decreased Ovarian Stage Score		-	H
Nephropathy	(Grade 1-2)	n/a	M, H

E2 -- Females

Exposure-Related Findings		FHM	JMD
		ABC	WI
Interstitial Proteinaceous Fluid		P	P
Intravascular Proteinaceous Fluid		P	-
Decreased Ovarian Stage Score		-	P
Nephropathy		n/a	P



4tPP -- Females

		FHM	JMD
		ABC	WI
Interstitial Proteinaceous Fluid	(Grade 1-2)	-	M, H
Increased Oocyte Atresia	(Grade 1-2)	-	H
Decreased Ovarian Stage Score		-	H
Nephropathy	(Grade 1-2)	n/a	M, H

E2 -- Females

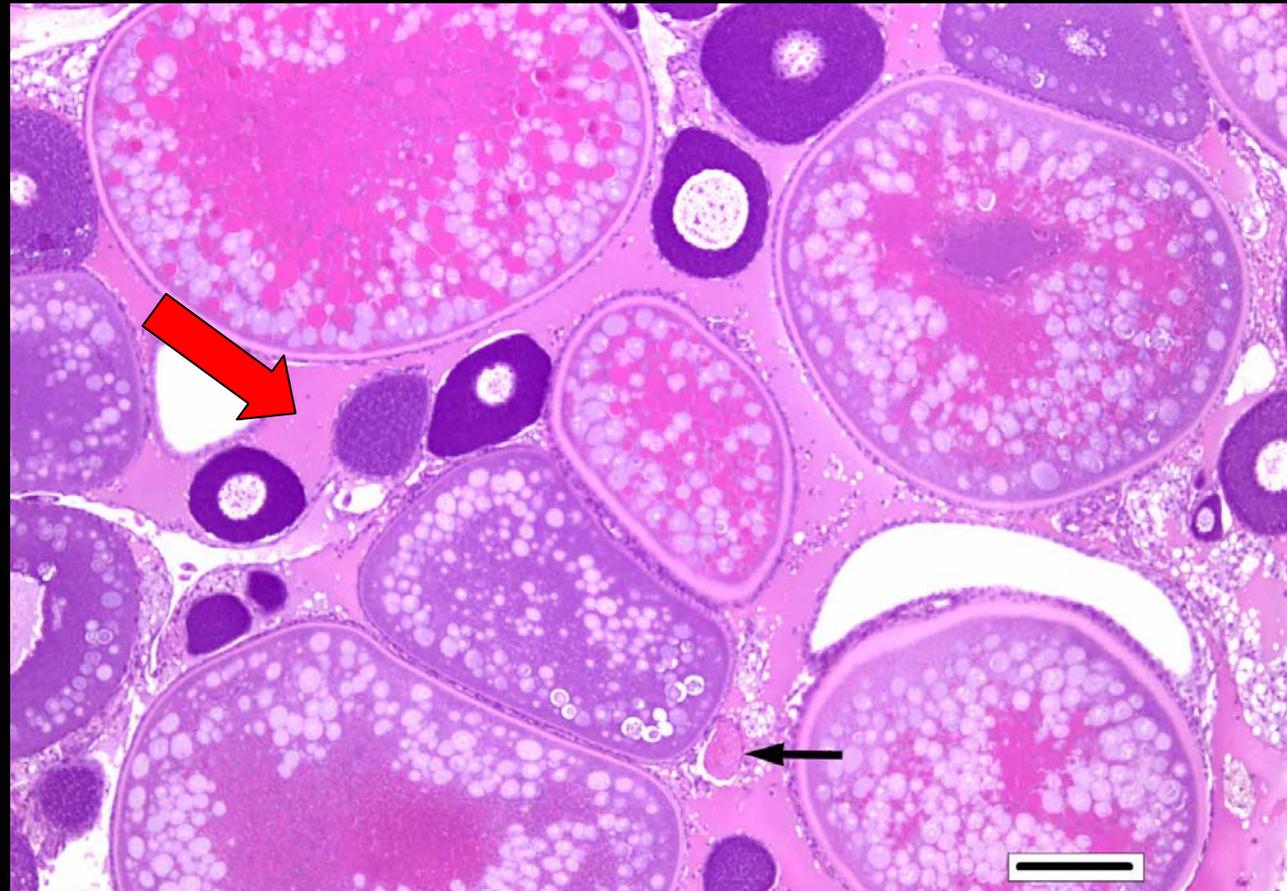
		FHM	JMD
		ABC	WI
Interstitial Proteinaceous Fluid		P	P
Intravascular Proteinaceous Fluid		P	-
Decreased Ovarian Stage Score		-	P
Nephropathy		n/a	P



E2 – Proteinaceous Interstitial and Intravascular Fluid in Ovary FHM

Red arrow indicates dark pink interstitial fluid

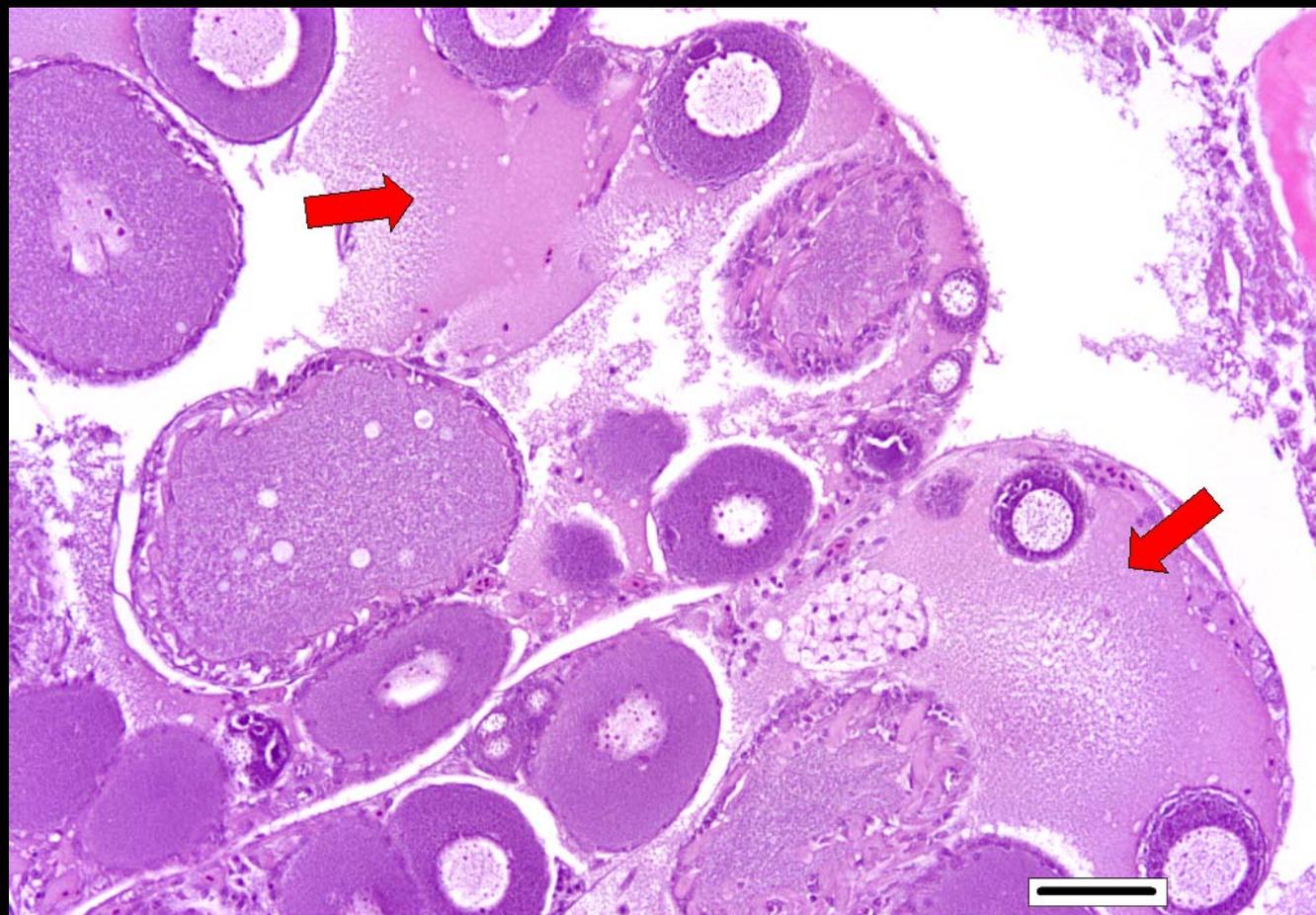
Small arrow indicates dark pink IV fluid



Z10696.tif 19B2-1 Fathead Minnow Ovary H&E 10x E2 100 ng/L

E2 – Proteinaceous Interstitial Fluid Ovary JMD

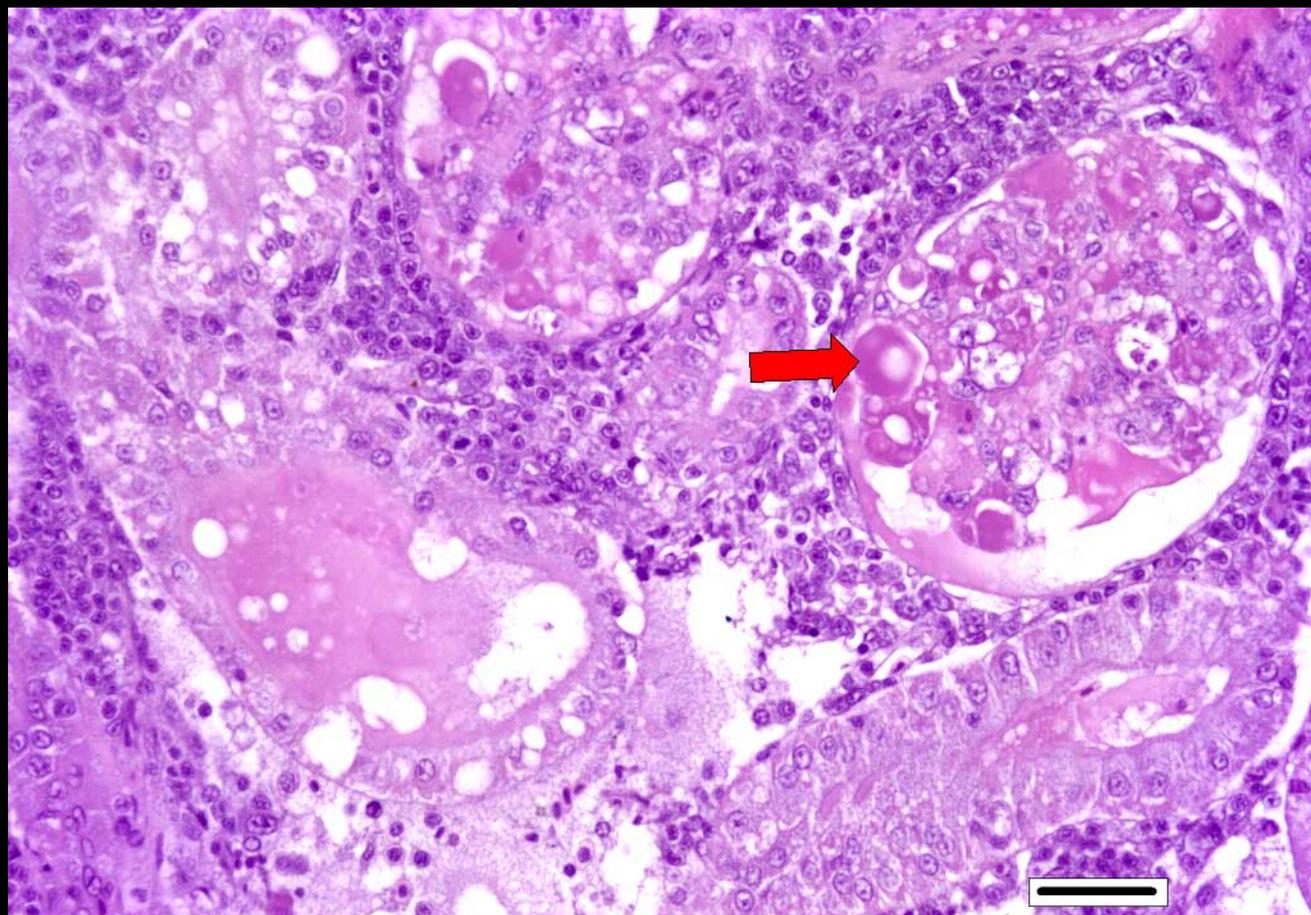
Arrows indicate increased interstitial fluid



Z10631.tif 84B Medaka Ovary H&E 20x E2 100ng/L

4tPP – Nephropathy JMD (female)

Arrow indicates hyaline deposits in glomerulus



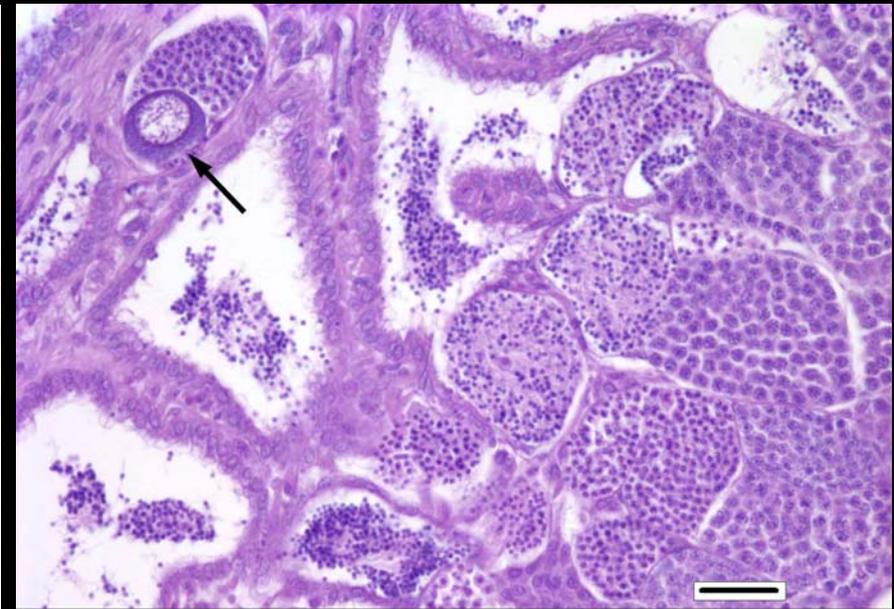
Z10620.tif 68B Medaka Kidney H&E 40x 4-PP 1000µg/L

By the way...

- **Testis-ova** were not factors in the U.S. studies
 - Only saw testis-ova in medaka from one of two laboratories, evenly distributed among control and compound-exposed fish
 - Suggests strain and/or husbandry factors involved?



Z10571.tif T1-A-8 Medaka Testis H&E 40x Flutamide 0ppm



Z10564.tif T1-A-3 Medaka Testis H&E 40x Fadrozole 0ppm

Testis-ova Data: Two Studies

Flutamide	Dose (ppm)	0			100			500			1000		
		Replicate	A	B	Total	A	B	Total	A	B	Total	A	B
	Number Examined	5	5	10	5	5	10	5	4	9	5	5	10
Testis-ova		1	0	1	1	0	1	0	0	0	1	0	1
	minimal	1	-	1	-	-	-	-	-	-	1	-	1
	mild	-	-	-	-	-	-	-	-	-	-	-	-
	moderate	-	-	-	1	-	1	-	-	-	-	-	-
	severe	-	-	-	-	-	-	-	-	-	-	-	-

Fadrozole	Dose (ppm)	0			3			10			100		
		Replicate	A	B	Total	A	B	Total	A	B	Total	A	B
	Number Testes Examined	4	5	9	5	5	10	5	5	10	5	5	10
Testis-ova		1	0	1	0	1	1	0	1	1	0	1	1
	minimal	1	-	1	-	1	1	-	-	-	-	-	-
	mild	-	-	-	-	-	-	-	1	1	-	1	1
	moderate	-	-	-	-	-	-	-	-	-	-	-	-
	severe	-	-	-	-	-	-	-	-	-	-	-	-

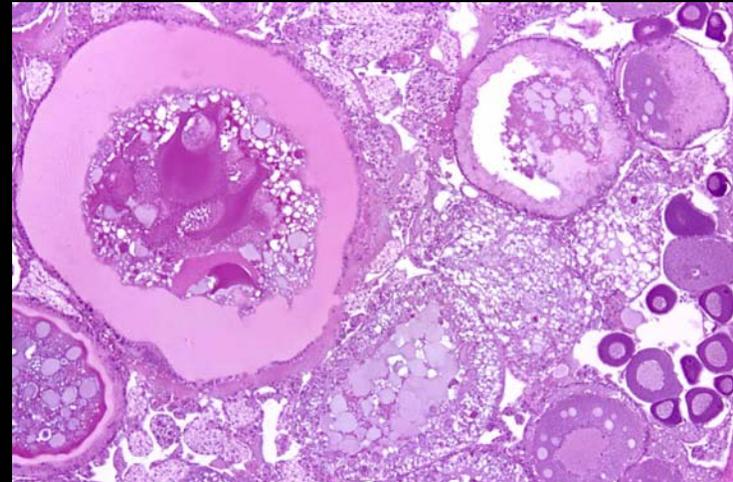


Second Meeting of the Fish Pathologists for the Validation of Gonadal Histopathology in the Fish Screening Assay – Phase 1B

University of Heidelberg, Germany, Nov. 22-23, 2004

Thomas Braunbeck, Christine
Ruehl-Fehlert, Narisato Hirai,
Rodney Johnson, Gerd Maack,
Leif Norrgren, Helmut Segner,
Masanori Seki, Leo van der Ven,
Klaus Weber, Jeffrey Wolf

Anne Gourmelon, Christiana
Grim, Les Touart



Goals of Heidelberg Meeting

1. To determine whether histopathology is a **useful** and **feasible** endpoint in a screening assay for endocrine-active chemical effects in fish

Goals of Heidelberg Meeting

useful

Is histopathology a sensitive and discriminating endpoint?



Goals of Heidelberg Meeting

feasible

Is histopathology an
efficient, economical
and reliable endpoint?



Goals of Heidelberg Meeting

1. To determine whether histopathology is a **useful and feasible** endpoint in a screening assay for endocrine-active chemical effects in fish
2. To further refine our use of histopathology as an endpoint
 - ➔ Revisions to the Guidance Document

Meeting Objectives

A. Evaluate **similarities** among laboratories / pathologists



Heidelberg Castle



Meeting Objectives

A. Evaluate similarities among laboratories / pathologists

1. Assess overall degree of similarity – is it satisfactory?



Heidelberg Castle



Meeting Objectives

A. Evaluate similarities among laboratories / pathologists

1. Assess overall degree of similarity – is it satisfactory?

Initial perception → probably not



Heidelberg Castle

Meeting Objectives

A. Evaluate similarities among laboratories / pathologists

1. Assess overall degree of similarity
– is it satisfactory?

Initial perception → probably not

Ultimately → perhaps!



Heidelberg Castle



Meeting Objectives

A. Evaluate similarities among laboratories / pathologists

1. Assess overall degree of similarity
– is it satisfactory?

2. Develop consensus findings for each chemical



Heidelberg Castle



Meeting Objectives

B. Evaluate **differences** among laboratories / pathologists



Heidelberg Castle



Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results
2. **Artificial** differences in results



Heidelberg Castle



Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results

→ From study to study, **the test subjects reacted differently** when exposed to the same chemical



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Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results
2. **Artificial** differences in results

→ From study to study, **the test subjects reacted similarly** when exposed to the same chemical



Heidelberg Castle

Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results
2. **Artificial** differences in results

- From study to study, the test subjects reacted similarly when exposed to the same chemical
- BUT, findings were **assessed differently** by different pathologists



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Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results –
Possible causes:



Heidelberg Castle

Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results –

Possible causes:

a) Nominal vs. actual dosages



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Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results –

Possible causes:

a) Nominal vs. actual dosages

b) Differences in fish

i. Source differences

-- genetics, early husbandry

ii. Age differences

-- nominal vs. actual

iii. Husbandry differences

-- protocol deviations

iv. Other

-- concurrent disease, e.g.



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Meeting Objectives

B. Evaluate differences among laboratories / pathologists

1. **Genuine** differences in results –

Possible causes:

- a) Nominal vs. actual dosages
- b) Differences in fish
 - i. Source differences
 - ii. Age differences
 - iii. Husbandry differences
 - iv. Other
- c) **Reproductive cycle variability** – intrinsic to assay
- d) **General biological variability** -- intrinsic to bioassays



Heidelberg Castle

Meeting Objectives

- B. Evaluate differences among laboratories / pathologists
- 2. **Artificial** differences in results

Possible causes:



Heidelberg Castle



Meeting Objectives

- B. Evaluate differences among laboratories / pathologists
- 2. **Artificial** differences in results

Possible causes:

- a) Differences in slide quality – hopefully not!



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Meeting Objectives

B. Evaluate differences among laboratories / pathologists

2. **Artificial** differences in results

Possible causes:

a) Differences in slide quality

b) Differences in diagnostic terminology

i. Mutual understanding of definitions is less than ideal

ii. Use of new terminology not present in Guidance Document



Heidelberg Castle

Meeting Objectives

B. Evaluate differences among laboratories / pathologists

2. **Artificial** differences in results

Possible causes:

- a) Differences in slide quality
- b) Differences in diagnostic terminology
 - i. Mutual understanding of definitions is less than ideal
 - ii. Use of new terminology not present in Guidance Document
- c) Differences in lesion observation
 - i. Pathologists may preferentially diagnose certain findings
 - ii. Chance differences in observation



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Meeting Objectives

B. Evaluate differences among laboratories / pathologists

2. **Artificial** differences in results

Possible causes:

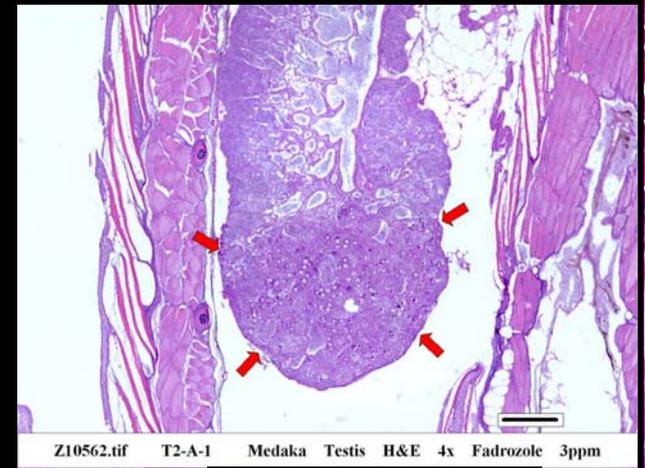
- a) Differences in slide quality
- b) Differences in diagnostic terminology
 - i. Mutual understanding of definitions is less than ideal
 - ii. Use of new terminology not present in Guidance Document
- c) Differences in lesion observation
 - i. Pathologists may preferentially diagnose certain findings
 - ii. Chance differences in observation
- d) **Differences in interpretation**
 - i. **Individual lesions** – lack of a true consensus
 - ii. **Overall results** – which are significant?



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Meeting Agenda

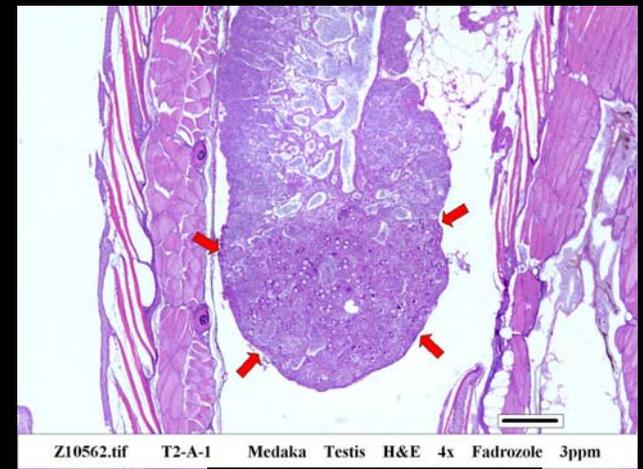
- Meeting objectives, election of chair, approval of agenda



Gonadal Tumor in
Medaka Testis

Meeting Agenda

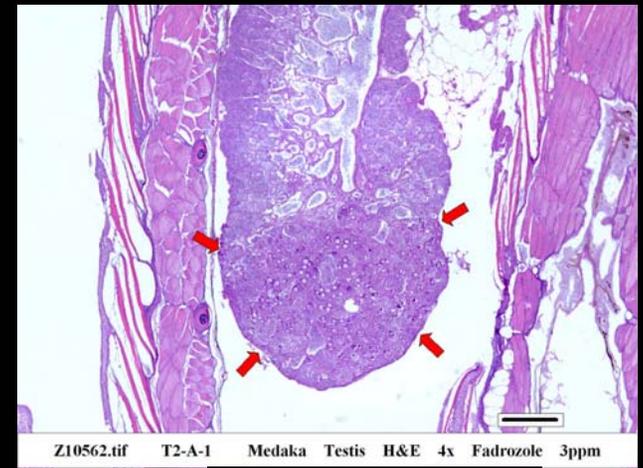
- Meeting objectives, election of chair, approval of agenda
- Pathologists' presentations of results
 - M. Seki: Overview of Phase 1B
 - N. Hirai
 - Th. Braunbeck /H. Segner
 - J.Wolf /R. Johnson
 - L. Norgren
 - Ch. Ruehl-Felhert
 - Gerd Maack
 - Klaus Weber



Gonadal Tumor in
Medaka Testis

Meeting Agenda

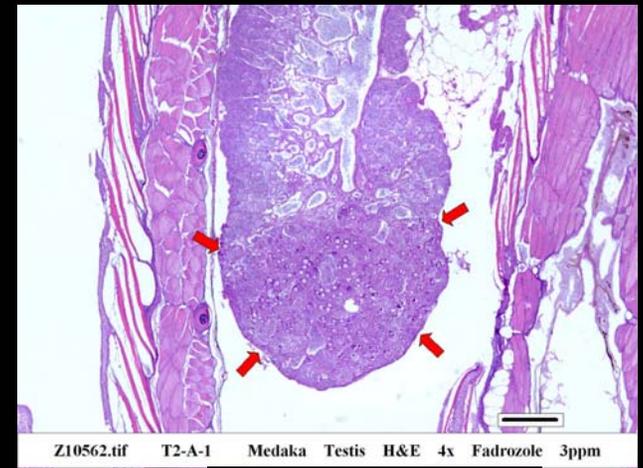
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 - L. Norgren
 - Ch. Ruehl-Felhert
 - Gerd Maack
 - Klaus Weber
- Review of glass slides (pertinent findings)



Gonadal Tumor in
Medaka Testis

Meeting Agenda

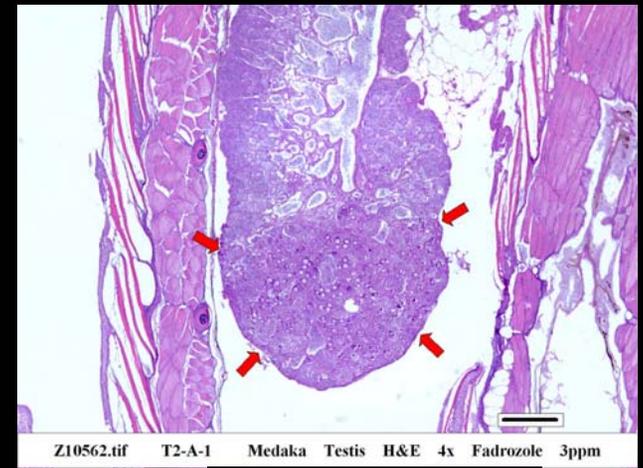
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 - Ch. Ruehl-Felhert
 - Gerd Maack
 - Klaus Weber
- Review of glass slides (pertinent findings)
- Discussion of previously unreported findings



Gonadal Tumor in
Medaka Testis

Meeting Agenda

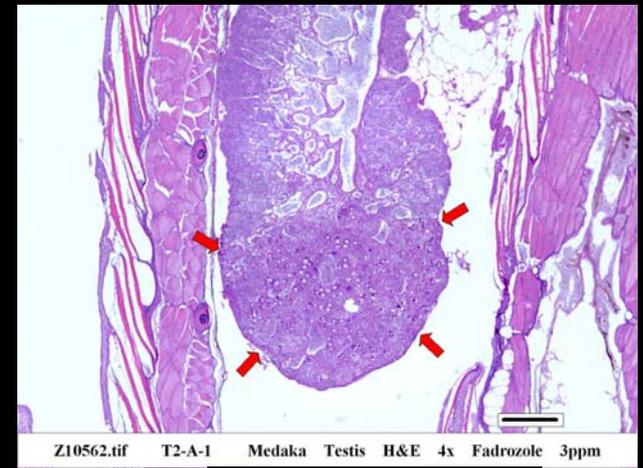
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 - L. Norgren
 - Ch. Ruehl-Felhert
 - Gerd Maack
 - Klaus Weber
- Review of glass slides (pertinent findings)
- Discussion of previously unreported findings
- Review of results from Excel spreadsheets



Gonadal Tumor in
Medaka Testis

Meeting Agenda

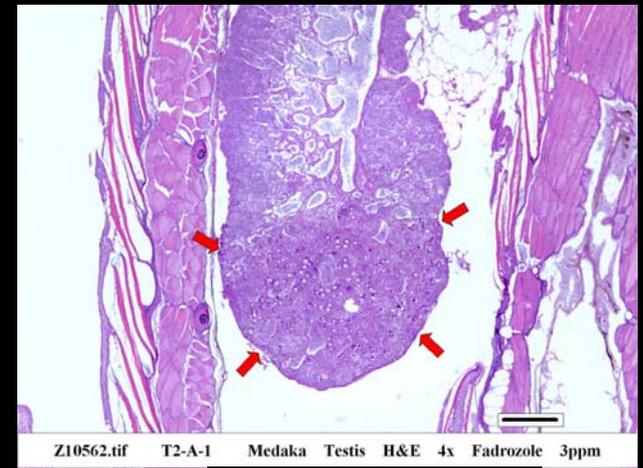
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 - Ch. Ruehl-Felhert
 - Gerd Maack
 - Klaus Weber
- Review of glass slides (pertinent findings)
- Discussion of previously unreported findings
- Review of results from Excel spreadsheets
- Review of Guidance Document questionnaire



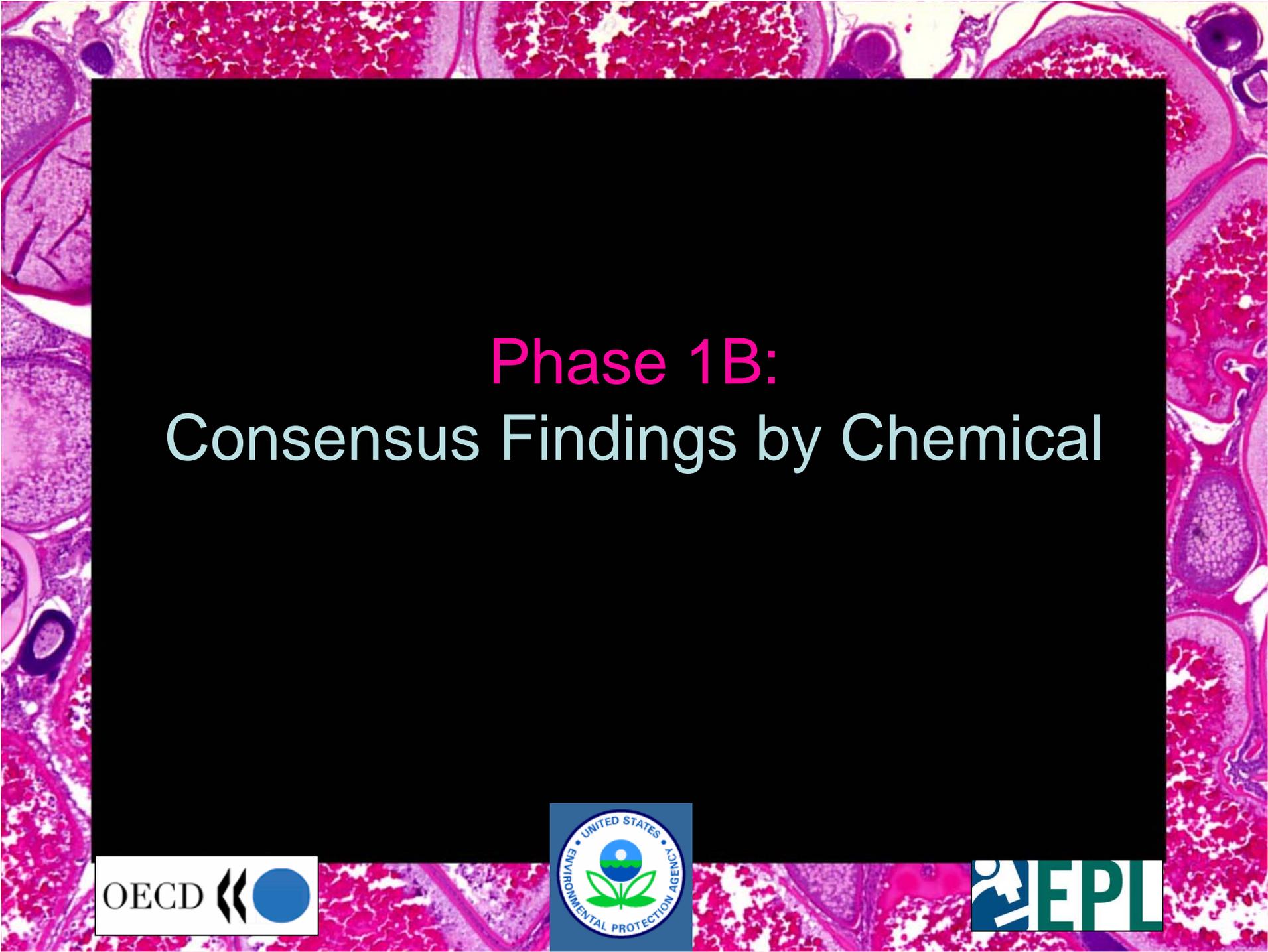
Gonadal Tumor in
Medaka Testis

Meeting Agenda

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 - M. Seki: Overview of Phase 1B
 - N. Hirai
 - Th. Braunbeck /H. Segner
 - J.Wolf /R. Johnson
 - L. Norgren
 - Ch. Ruehl-Felhert
 - Gerd Maack
 - Klaus Weber
- Review of glass slides (pertinent findings)
- Discussion of previously unreported findings
- Review of results from Excel spreadsheets
- Review of Guidance Document questionnaire
- Consensus on recommendations for the VMG-eco



Gonadal Tumor in
Medaka Testis



Phase 1B: Consensus Findings by Chemical



Phase 1B – Consensus Findings by Chemical

Prochloraz (Aromatase inhibitor)					
Species	Sex	Spawning	VTG	Secondary sex characteristics	Histopathology
JMD	M		↓	-	↑ Spermatozoa ↑ Interstitial fibrosis
	F	↓	↓**		↑ Oocyte atresia
FHM	M		-	-	↑ Spermatozoa
	F	equivocal	↓**		↑ Oocyte atresia
ZBF	M		↓		-
	F	↓	↓**		↑ Oocyte atresia

↓ decrease, ↑ increase

** significant level: 1%



Phase 1B – Consensus Findings by Chemical

Prochloraz (Aromatase inhibitor)					
Species	Sex	Spawning	VTG	Secondary sex characteristics	Histopathology
JMD	M		↓	-	↑ Spermatozoa ↑ Interstitial fibrosis
	F	↓	↓**		↑ Oocyte atresia
FHM	M		-	-	↑ Spermatozoa
	F	equivocal	↓**		↑ Oocyte atresia
ZBF	M		↓		-
	F	↓	↓**		↑ Oocyte atresia

↓ decrease, ↑ increase

** significant level: 1%

Phase 1B – Consensus Findings by Chemical

Flutamide (Anti-androgen)					
Species	Sex	Spawning	VTG	Secondary Sex Characteristics	Histopathology
JMD	M		equivocal	-	-
	F	equivocal	-		-
FHM	M		-	-	↑ Spermatogonia
	F	↓	-		↑ Oocyte atresia
ZBF	M		-		↑ Interstitial cells, ↑ Spermatogonia
	F	equivocal	-		-

↓ decrease, ↑ increase



Phase 1B – Consensus Findings by Chemical

Flutamids (Anti-androgen)					
Species	Sex	Spawning	VTG	Secondary Sex Characteristics	Histopathology
JMD	M		equivocal	-	-
	F	equivocal	-		-
FHM	M		-	-	↑ Spermatogonia
	F	↓	-		↑ Oocyte atresia
ZBF	M		-		↑ Interstitial cells, ↑ Spermatogonia
	F	equivocal	-		-

↓ decrease, ↑ increase

Phase 1B – Consensus Findings by Chemical

4- <i>t</i> -Pentylphenol (Weak estrogen agonist)					
Species	Sex	Spawning	VTG	Secondary Sex Characteristics	Histopathology
JMD	M		↑ **	↓	↑ Testicular deg.
	F	-	↑ **		↑ Oocyte atresia
FHM	M		↑ **	↓ **	↑ Spermatogonia
	F	↓	↑ **		↑ Oocyte atresia
ZBF	M		↑ **		↑ Spermatogonia
	F	-	↑		-

↓ decrease, ↑ increase

** significant level: 1%

Shaded cells: endpoint not measured



Phase 1B – Consensus Findings by Chemical

4- <i>t</i> -Pentylphenol (Weak estrogen agonist)					
Species	Sex	Spawning	VTG	Secondary Sex Characteristics	Histopathology
JMD	M		↑**	↓	↑ Testicular deg.
	F	-	↑**		↑ Oocyte atresia
FHM	M		↑**	↓**	↑ Spermatogonia
	F	↓	↑**		↑ Oocyte atresia
ZBF	M		↑**		↑ Spermatogonia
	F	-	↑		-

↓ decrease, ↑ increase

** significant level: 1%

Shaded cells: endpoint not measured

Phase 1B: What are the key histopathological diagnoses?

PRIMARY DIAGNOSES		
	Males	Females
1	Increased proportion of spermatogonia	Increased oocyte atresia
2	Presence of testis-ova	Perifollicular cell hyperplasia / hypertrophy
3	Increased testicular degeneration	Decreased vitellogenesis ¹
4	Interstitial (Leydig) cell hyperplasia / hypertrophy	Gonadal staging (based on improved staging criteria)

¹ "Decreased yolk formation" in future

Phase 1B: Consistency Among Laboratories / Pathologists

Prochloraz (Aromatase inhibitor)									
Sex	Finding	JMD			FHM			ZBF	
		Lab1	Lab 2	Lab3	Lab1	Lab2	Lab3	Lab1	Lab2
Male	Testis-ova (substantially increased to controls)	-	-	-	-	-	-	-	-
	Increased proportion of spermatogonia	-	-	-	-	-	-	-	-
	Increased interstitial cells	-	-	+	-	-	-	-	-
	Increased testicular degeneration	-	-	-	-	-	-	-	-
Female	Increased oocyte atresia	+	+	+	+	+	+	+	+
	Perifollicular Cell Hypertrophy / Hyperplasia	ne*	ne*	+	-	-	-	ne	ne
	Decreased vitellogenesis	ne*	ne*	+	-	-	-	ne	ne
	Altered gonadal stage	+	+	+	-	+	-	+	-

- Endpoint not responsive; + Consistent trend (up or down); ne: not evaluated

* Endpoint not evaluated, but peer-reviewed slides showed that perifollicular cell hyperplasia / hypertrophy and decreased vitellogenesis (or decreased yolk formation) were present

Phase 1B: Consistency Among Laboratories / Pathologists

Prochloraz (Aromatase inhibitor)									
Sex	Finding	JMD			FHM			ZBF	
		Lab1	Lab 2	Lab3	Lab1	Lab2	Lab3	Lab1	Lab2
Male	Testis-ova (substantially increased to controls)	-	-	-	-	-	-	-	-
	Increased proportion of spermatogonia	-	-	-	-	-	-	-	-
	Increased interstitial cells	-	-	+	-	-	-	-	-
	Increased testicular degeneration	-	-	-	-	-	-	-	-
Female	Increased oocyte atresia	+	+	+	+	+	+	+	+
	Perifollicular Cell Hypertrophy / Hyperplasia	ne*	ne*	+	-	-	-	ne	ne
	Decreased vitellogenesis	ne*	ne*	+	-	-	-	ne	ne
	Altered gonadal stage	+	+	+	-	+	-	+	-

- Endpoint not responsive; + Consistent trend (up or down); ne: not evaluated

* Endpoint not evaluated, but peer-reviewed slides showed that perifollicular cell hyperplasia / hypertrophy and decreased vitellogenesis (or decreased yolk formation) were present

Phase 1B: Consistency Among Laboratories / Pathologists

Prochloraz (Aromatase inhibitor)									
Sex	Finding	JMD			FHM			ZBF	
		Lab1	Lab 2	Lab3	Lab1	Lab2	Lab3	Lab1	Lab2
Male	Testis-ova (substantially increased to controls)	-	-	-	-	-	-	-	-
	Increased proportion of spermatogonia	-	-	-	-	-	-	-	-
	Increased interstitial cells	-	-	+	-	-	-	-	-
	Increased testicular degeneration	-	-	-	-	-	-	-	-
Female	Increased oocyte atresia	+	+	+	+	+	+	+	+
	Perifollicular Cell Hypertrophy / Hyperplasia	ne*	ne*	+	-	-	-	ne	ne
	Decreased vitellogenesis	ne*	ne*	+	-	-	-	ne	ne
	Altered gonadal stage	+	+	+	-	+	-	+	-

- Endpoint not responsive; + Consistent trend (up or down); ne: not evaluated

* Endpoint not evaluated, but peer-reviewed slides showed that perifollicular cell hyperplasia / hypertrophy and decreased vitellogenesis (or decreased yolk formation) were present

Phase 1B: Consistency Among Laboratories / Pathologists

Flutamide (Antiandrogen)									
Sex	Finding	JMD			FHM			ZBF	
		Lab1	Lab2	Lab3	Lab1	Lab 2	Lab3	Lab1	Lab 2
Male	Testis–ova (substantially increased over controls)	-	-	-	-	-	-	-	-
	Increased proportion of spermatogonia	-	-	-	-	+	-	+	+
	Increased interstitial cells	-	-	-	-	-	-	+	+
	Increased testicular degeneration	-	-	-	-	-	-	-	-
Female	Increased oocyte atresia	+	-	-	-	+	-	-	-
	Perifollicular cell hypertrophy / hyperplasia	-	-	-	-	-	-	-	-
	Decreased vitellogenesis	-	-	-	-	-	-	-	-
	Altered gonadal stage	-	-	-	+	-	-	-	-

- endpoint not responsive; + consistent trend (up or down)



Phase 1B: Consistency Among Laboratories / Pathologists

Flutamide (Antiandrogen)									
Sex	Finding	JMD			FHM			ZBF	
		Lab1	Lab2	Lab3	Lab1	Lab 2	Lab3	Lab1	Lab 2
Male	Testis–ova (substantially increased over controls)	-	-	-	-	-	-	-	-
	Increased proportion of spermatogonia	-	-	-	-	+	-	+	+
	Increased interstitial cells	-	-	-	-	-	-	+	+
	Increased testicular degeneration	-	-	-	-	-	-	-	-
Female	Increased oocyte atresia	+	-	-	-	+	-	-	-
	Perifollicular cell hypertrophy / hyperplasia	-	-	-	-	-	-	-	-
	Decreased vitellogenesis	-	-	-	-	-	-	-	-
	Altered gonadal stage	-	-	-	+	-	-	-	-

- endpoint not responsive; + consistent trend (up or down)



Phase 1B: Consistency Among Laboratories / Pathologists

4-t-Pentylphenol (Weak estrogen agonist)											
Sex	Finding	JMD				FHM			ZBF		
		Lab1	Lab2	Lab3	Lab4	Lab1	Lab2	Lab3	Lab1	Lab2	Lab3
Male	Testis-ova (substantially increased over controls)	-	-	-	-	-	-	-	+	-	-
	Increased proportion of spermatogonia	-	-	-	-	+	+	+	-	+	+
	Increased interstitial cells	-	-	-	-	-	-	-	-	-	-
	Increased testicular degeneration	+	+	-	+	-	-	-	-	-	-
Female	Increased oocyte atresia	-	+	-	+	-	-	-	-	-	-
	Perifollicular cell hypertrophy / hyperplasia	-	-	-	-	-	-	-	-	-	-
	Decreased vitellogenesis	-	-	-	-	-	-	-	-	-	-
	Altered gonadal stage	-	+	-	-	+	-	-	-	-	-

- endpoint not responsive; + consistent trend (up or down)



Phase 1B: Consistency Among Laboratories / Pathologists

4-t-Pentylphenol (Weak estrogen agonist)											
Sex	Finding	JMD				FHM			ZBF		
		Lab1	Lab2	Lab3	Lab4	Lab1	Lab2	Lab3	Lab1	Lab2	Lab3
Male	Testis-ova (substantially increased over controls)	-	-	-	-	-	-	-	+	-	-
	Increased proportion of spermatogonia	-	-	-	-	+	+	+	-	+	+
	Increased interstitial cells	-	-	-	-	-	-	-	-	-	-
	Increased testicular degeneration	+	+	-	+	-	-	-	-	-	-
Female	Increased oocyte atresia	-	+	-	+	-	-	-	-	-	-
	Perifollicular cell hypertrophy / hyperplasia	-	-	-	-	-	-	-	-	-	-
	Decreased vitellogenesis	-	-	-	-	-	-	-	-	-	-
	Altered gonadal stage	-	+	-	-	+	-	-	-	-	-

- endpoint not responsive; + consistent trend (up or down)

Phase 1B: Relative Sensitivity of Histopathology

4-t-Pentyl-phenol	Growth	Spawning	Vitellogenin	Histology	Secondary sex characteristics
JMD	–	–	100	100	1000 *
FHM	–	320 / 1000 **	320	320	320 / 1000 **
ZF	–	1000 *	100 / 320 **	100	

Prochloraz	Growth	Spawning	Vitellogenin	Histology	Secondary sex characteristics
JMD	–	100	(20*)100 **	100	–
FHM	–	–	100 / 300 **	100	300 *
ZF	–	300 *	100	20	

Flutamide	Growth	Spawning	Vitellogenin	Histology	Secondary sex characteristics
JMD	–	1000 *	100 *	100	–
FHM	–	500 / 1000 **	500 *	320	1000 *
ZF	–	–	–	500	

– no effect at concentrations tested; * one laboratory only; ** differences between laboratories



Phase 1B: Relative Sensitivity of Histopathology

4-t-Pentyl-phenol	Growth	Spawning	Vitellogenin	Histology	Secondary sex characteristics
JMD	–	–	100	100	1000 *
FHM	–	320 / 1000 **	320	320	320 / 1000 **
ZF	–	1000 *	100 / 320 **	100	

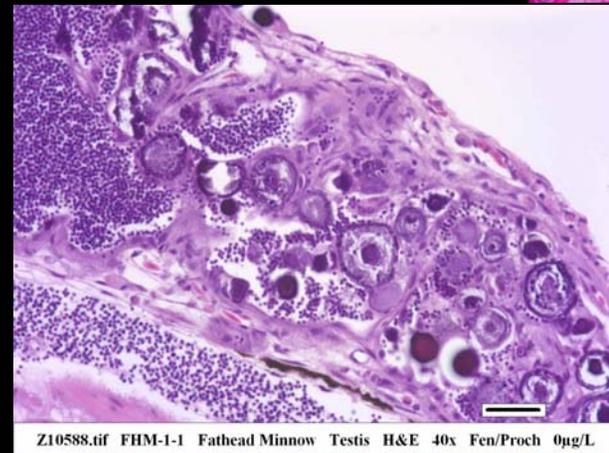
Prochloraz	Growth	Spawning	Vitellogenin	Histology	Secondary sex characteristics
JMD	–	100	(20*)100 **	100	–
FHM	–	–	100 / 300 **	100	300 *
ZF	–	300 *	100	20	

Flutamide	Growth	Spawning	Vitellogenin	Histology	Secondary sex characteristics
JMD	–	1000 *	100 *	100	–
FHM	–	500 / 1000 **	500 *	320	1000 *
ZF	–	–	–	500	

– no effect at concentrations tested; * one laboratory only; ** differences between laboratories

Phase 1B: What were the sources of inconsistency?

- **Genuine Differences** – possibly due to:
 - Exposure levels differed from nominal
 - Reproductively immature fish
 - Fish substantially older than stipulated
 - High mortality in controls, cause undetermined (disease?)



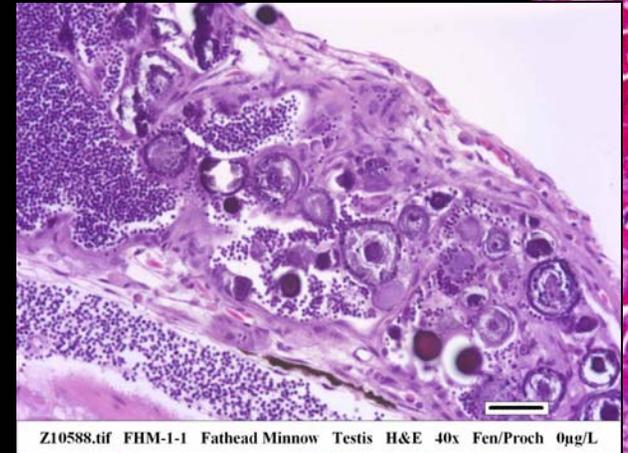
Mineralization in FHM Testis

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- **Artificial Differences**

- At least one major misdiagnosis
- Extra-gonadal changes in JMD not universally observed
- Previously unrecognized findings →
 - Different diagnostic terms used by pathologists, or
 - Diagnosis not made by all pathologists
- Not all pathologists attended the Paris meeting
- Guidance Document good, but incomplete



Mineralization in FHM Testis

**How can we make the histopathology
endpoint more accurate, efficient,
and cost effective?**



How can we make the histopathology endpoint more accurate, efficient, and cost effective?

- Streamline list of anticipated diagnoses
- Evaluate only gonads
- Evaluate slides non-blinded
- Simplify data recording worksheet
- Eliminate pathology narrative report
- Improvements to Guidance Document
- Training workshops for pathologists

Conclusions

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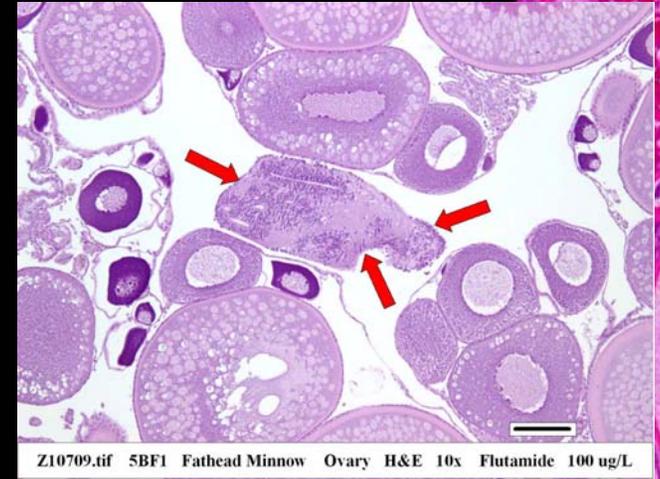
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 - It may be possible to make histopathology more cost-effective

Recommendations and Other Considerations

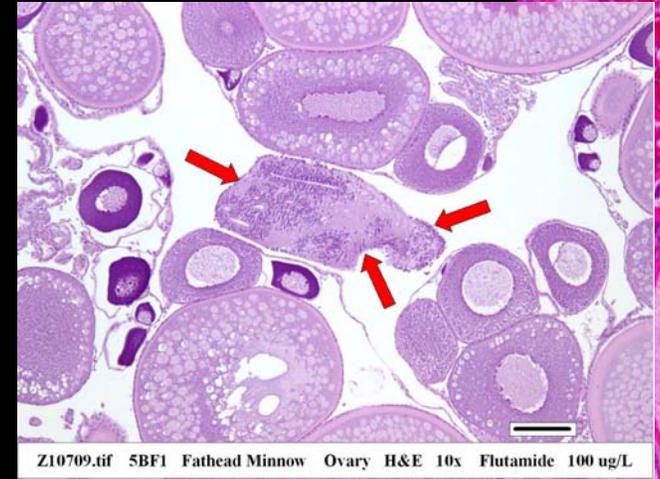
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Brain Tissue in FHM Ovary

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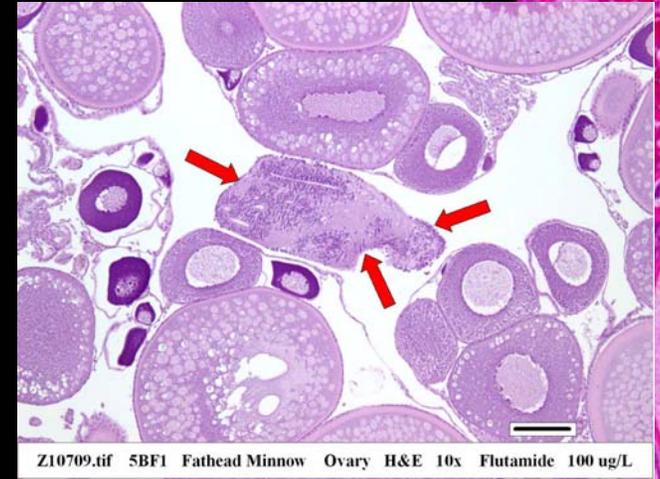
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 - FHM in appropriate group size



Brain Tissue in FHM Ovary

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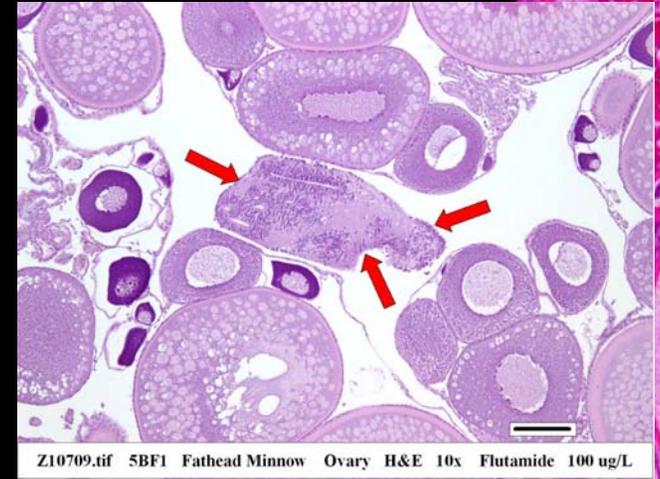
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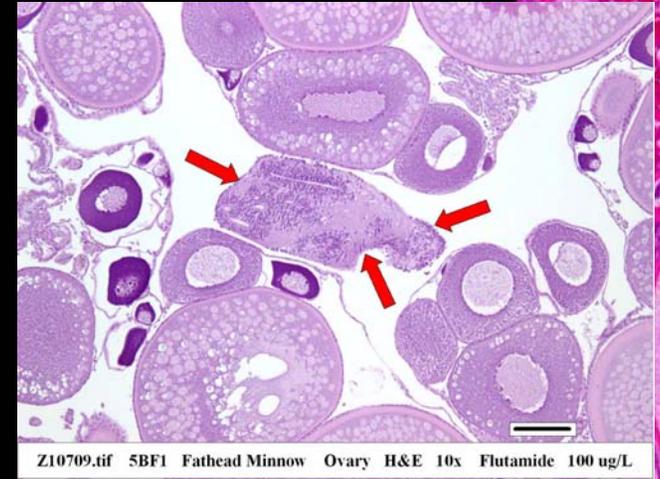
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- Further validate test system with negative controls?
- Consider re-reading Phase 1B slides?



Brain Tissue in FHM Ovary