

**Apoptosis in Ovarian Cells of Nile  
Tilapia (*Oreochromis niloticus*)  
Chronically Exposed to  
Pyrethroid**

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# Introduction

- ✿ *Palay-isdaan*, a sustainable measure to increase tilapia and rice yield is practiced in some parts of the country. The environmental impact of toxic pesticide accumulation on pond sediments had adverse effects on the life cycle of the fish. (Cagauan, 2001 & Fisher, 2002)
- ✿ Pyrethroids are the safest insecticides used worldwide but are highly toxic to most fish. (WHO, 1986)
- ✿ The Fertilizer and Pesticide Authority (FPA) has no records of monitoring activities regarding pesticide use in the country. (Balmedilla, 2003)

- Hormones and pesticides act as endocrine-disruptors in fish. (Janz & Van der Kraak, 1997, Wood & Van der Kraak, 2002, Weber et al, 2002)
- Endocrine-disrupting chemicals are correlated with early or precocious sexual maturity in fish. (Colbourn as cited by Gillete, 1997)
- Increased fecundity and fertility of Nile Tilapia at a smaller size due to environmental harshness. (Khallaf et al, 2000)

- ✿ Gonadosomatic Index (GSI) is the percentage ratio of the gonad weight and body weight used to determine fecundity among fish. (Janz et al, 1997)
- ✿ Spontaneous apoptosis (programmed cell death without inflammation) is necessary to maintain homeostasis in reproductive cells. (King, 2000)
- ✿ Induction of apoptosis by endocrine-disruptors in the environment (ex. pyrethroid) could alter the reproductive potential of Nile Tilapia.

# This study aims :

- ✿ to determine the effect of the time of exposure and the different concentrations of pyrethroid to the GSI of Nile Tilapia.
- ✿ to correlate the Gonadosomatic Index of Nile Tilapia with the body weight and gonad weight.
- ✿ to determine the histological signs of apoptosis in the ovarian cells of Nile Tilapia.

# Methodology

**Acclimation**

**Range-Finding Test**

**Sublethal Exposure Test**

**Control**

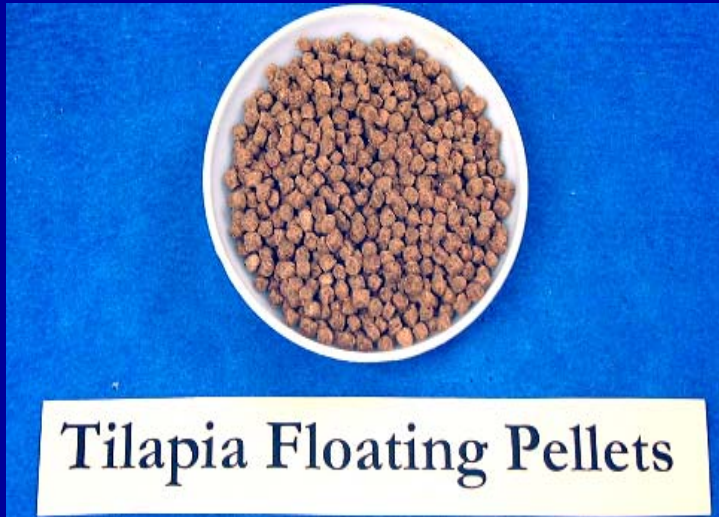
**25%**

**50%**

**75%**

# Housing





**Feeds : 30-35% crude protein**

**Amount: 30% @ AM  
and 10% @PM of  
total fish BW.**

**Time: 3x a day 8 AM,  
11 AM, 4 PM**

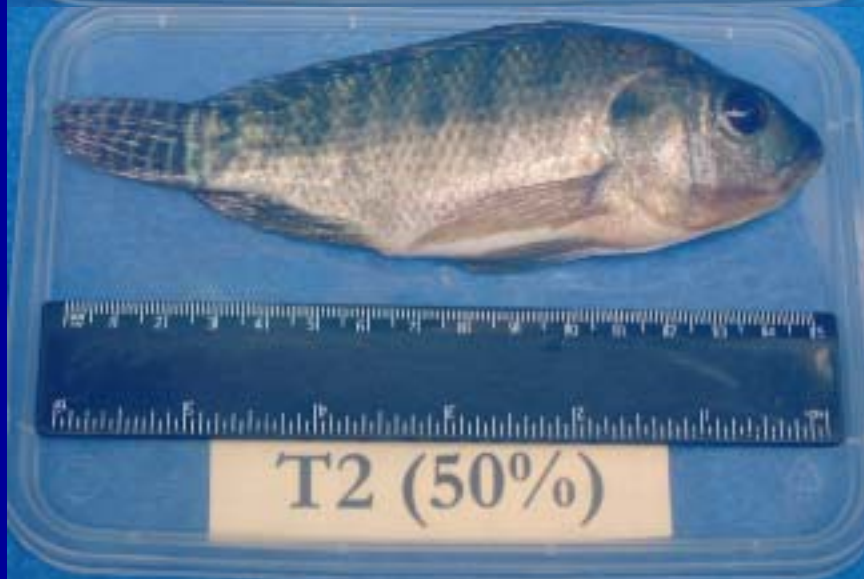
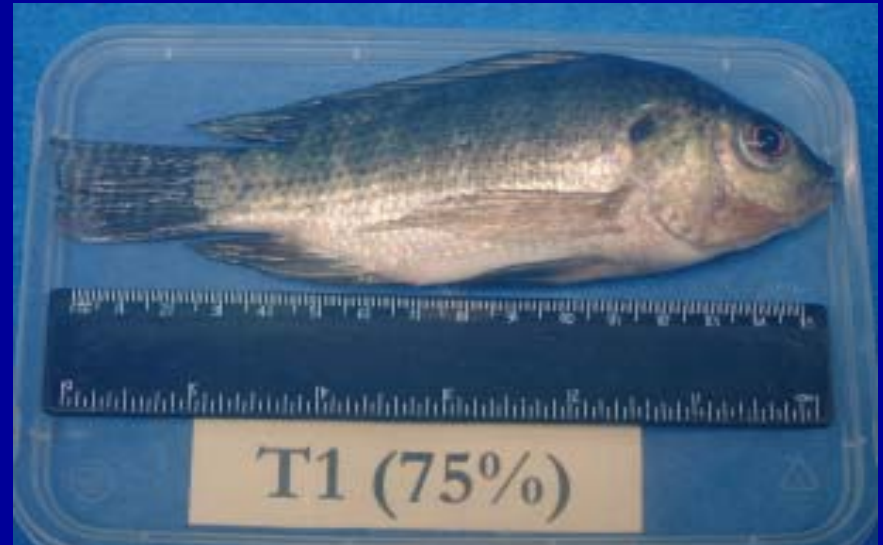


**Pyrethroid: 2mg tablet, 25mg/L  
deltamethrin as an active  
ingredient**

**Dilution: 3 tabs/L = 0.10 mg/L**



# Nile Tilapia (BFAR 2000) Samples



# Laboratory Analyses Tools





# Data Collection

- *In situ* analysis of apoptotic signs in the ovarian cells (+ present, - absent)
- GSI (body weight, gonad weight)
- Physico-chemical parameters were monitored
  - Temperature (28-32 °C)
  - Dissolved oxygen (3-6 mg/L)
  - pH (7-8)
  - Conductivity ( $\mu\text{S}/\text{cm}$  (0.65) = TDS mg/L)  
243-300 mg/L
  - Water hardness as  $\text{CaCO}_3$  (hard – very hard)

# Statistical Analysis

- PROC ANOVA – applying of treatments on 2 variables
- Two -factorial design
  - *The doses of pyrethroid were used as factor A with 4 levels (0%, 25%, 50% and 75%)*
  - *Weeks of treatment was factor B with 4 levels (1, 2, 3 & 4)*
- Student Newman Keul Test (SNK]- to detect treatment mean significance

# RESULTS

## A. Gonadosomatic Index

- GSI was not influenced ( $P > 0.14$ ) by the interaction of the different concentrations and exposure time to the pyrethroid.
- The different sublethal concentrations of the pyrethroid significantly ( $P < 0.03$ ) decreased the GSI.
- The GSI was highly influenced ( $P < 0.006$ ) by the time of exposure.

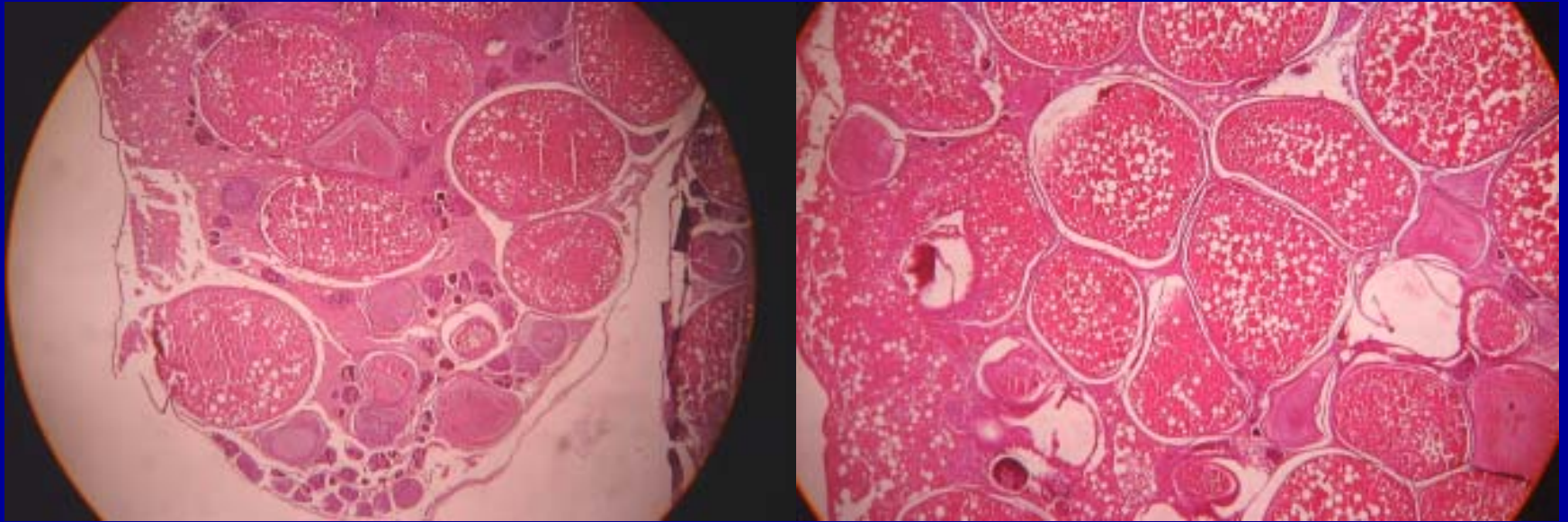
## B. Correlations

- GSI and BW showed a negative correlation ( $r = -0.124$ )
- A significant ( $P < 0.05$ ) relationship exists ( $r = 0.896$ ) between GSI and GW.
- A low correlation ( $r = 0.291$ ) exists between GW and BW



# C. Histological Analysis

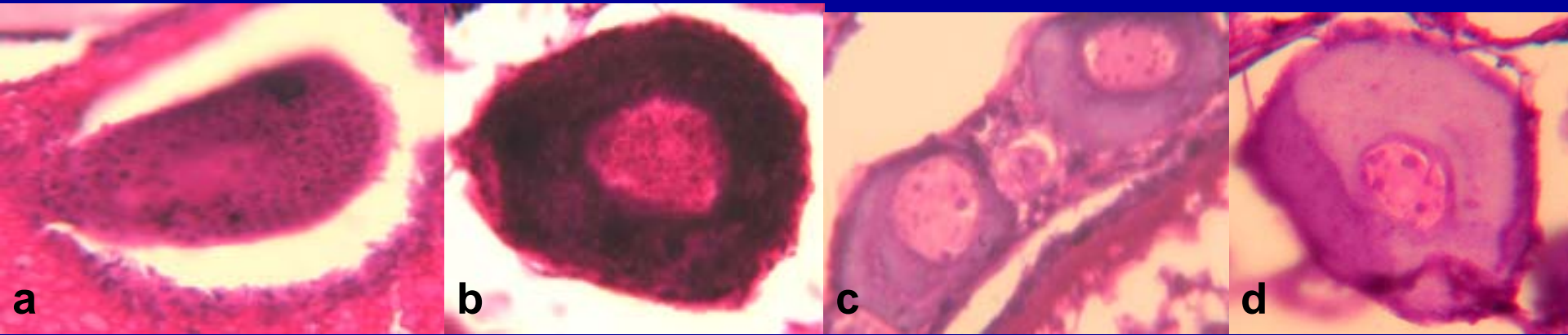
## Asynchronous ovary of Nile Tilapia



Oocytes at different stages of development dominated by yolk granular stages, few cortical-alveolar stages and previtellogenic oocytes

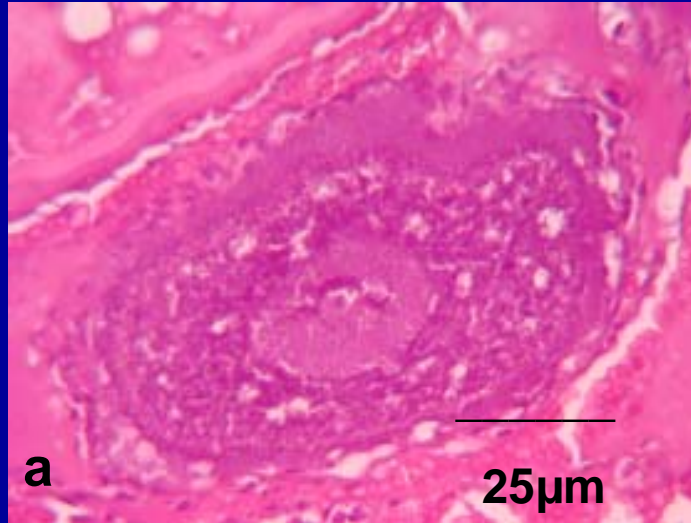


# Proliferating Oogonia



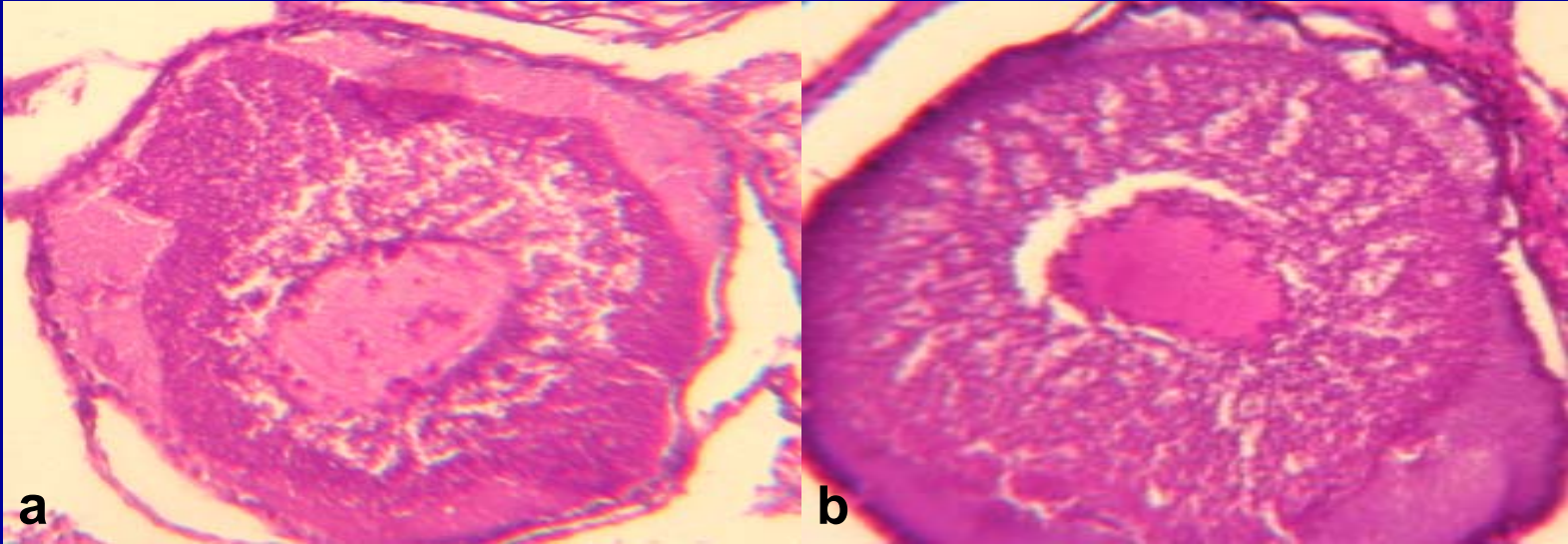
- (a) Slightly ovoid oogonium with granular materials occupying the entire cytoplasm,
- (b) oogonium with distinct nuclear area,
- (c) oogonia next to yolk granular oocyte with enlarged nuclear area and several primer nucleoli and
- (d) slightly spherical oogonium with reduced cytoplasmic area and four primer nucleoli. (H&E, HPO 3x)

# Previtellogenic Oocytes



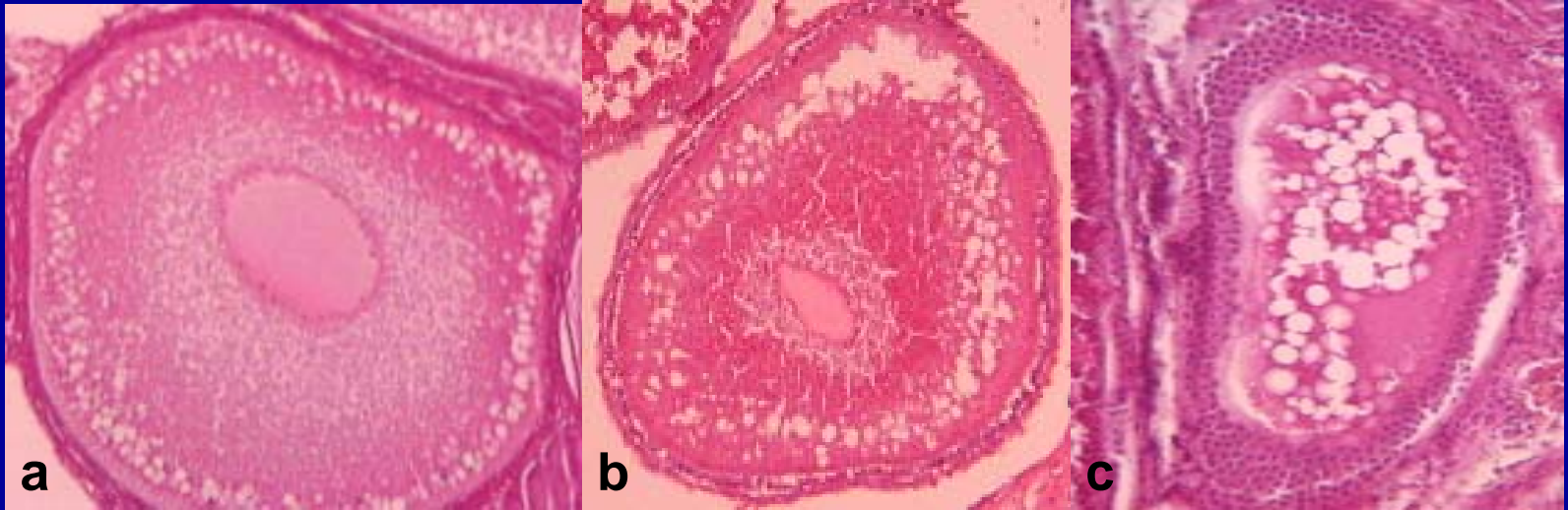
- (a) Oocyte at chromatin nucleolar stage with large nucleus,
- (b) oocyte at chromatin-nucleolar stage with prime nucleoli and distinct chromatids.

# Previtellogenic Oocytes



- a. Oocyte at perinucleolar stage with several perinucleoli and surrounding theca folliculi cells
- b. Perinucleolar stage with nucleoli along the periphery of the nucleoplasm surrounded by the developing zona radiata.

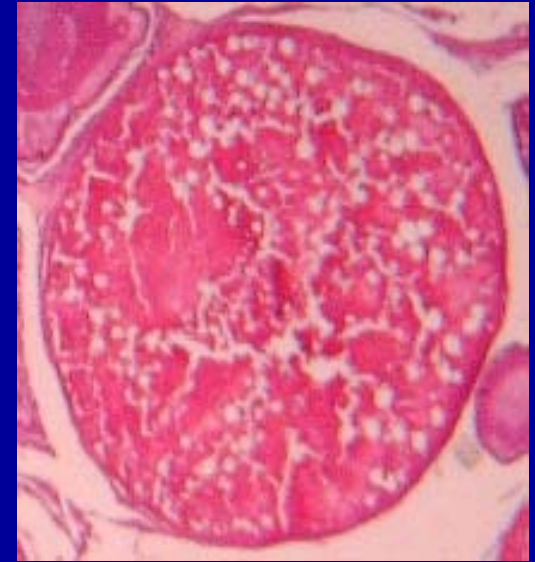
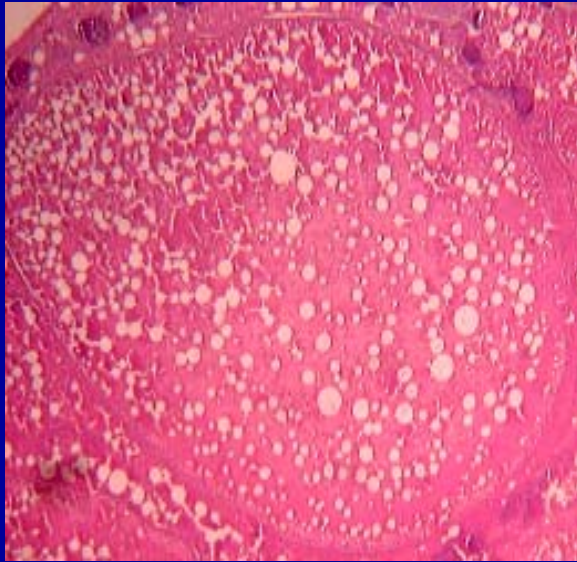
# Vitellogenic Oocytes



- (a) cortical alveolar oocyte with distinct ring of cortical alveoli anterior to the zona radiata and prominent nucleus with several nucleoli,
- (b) cortical alveolar oocyte with smaller nucleus
- (c) irregularly-shaped cortical alveoli along the center of the ooplasm.



# Vitellogenic Oocytes



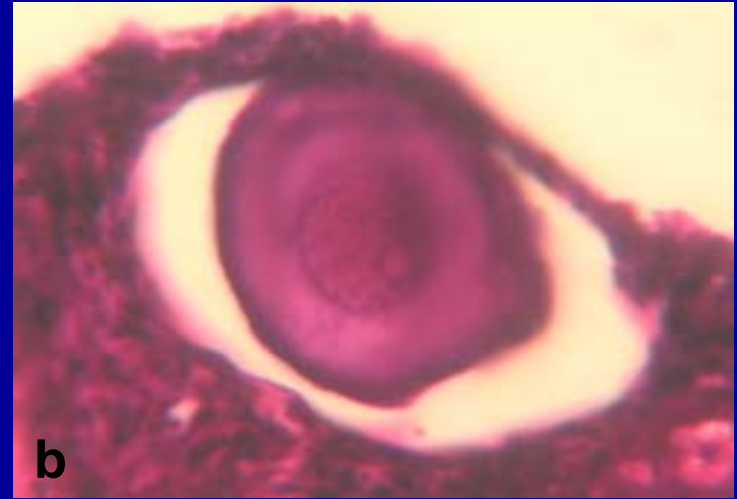
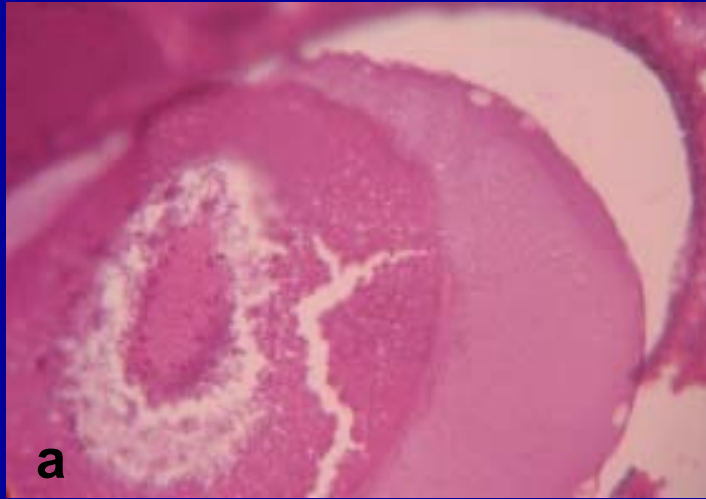
- Oocytes at yolk granular stage with nuclear area almost entirely covered with yolk granules (HPO)

**Table 2.** Histological apoptotic signs in the ovarian cells of *O.niloticus* exposed to different sublethal concentrations of pyrethroid observed on a weekly interval.

Apoptotic Signs	Level of Pyrethroid Treatment Week	CONTROL				25%				50%				75%				
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
Blebbing membrane		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
chromatin Aggregation		-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Condensed nucleus/cytoplasm		-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Plasma membrane shrinkage		-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Theca & granulosa disruption		-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Vesicle-formation		-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-

# Histological Features of Apoptosis

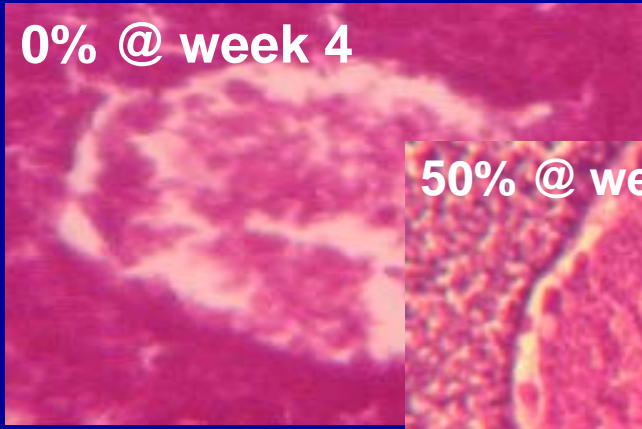
## Blebbing Membrane



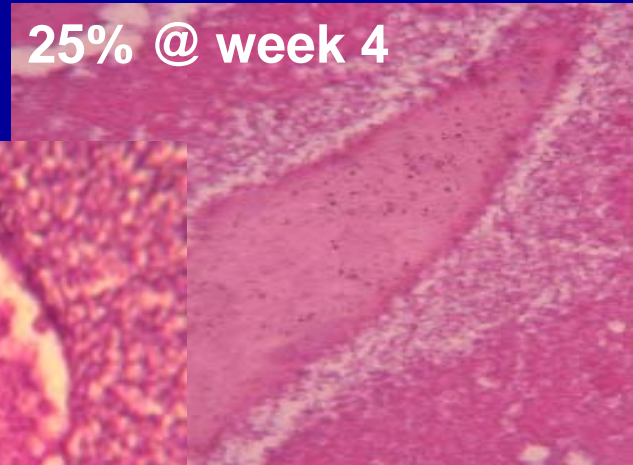
Perinucleolar oocyte (a) and oogonia (b) exposed to 75% sublethal concentration of pyrethroid (a) LPO 5.3x

# Chromatin Aggregation

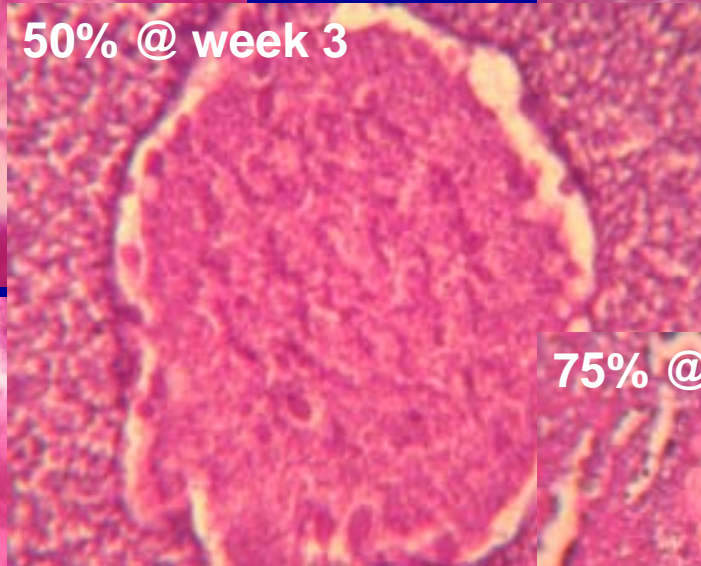
0% @ week 4



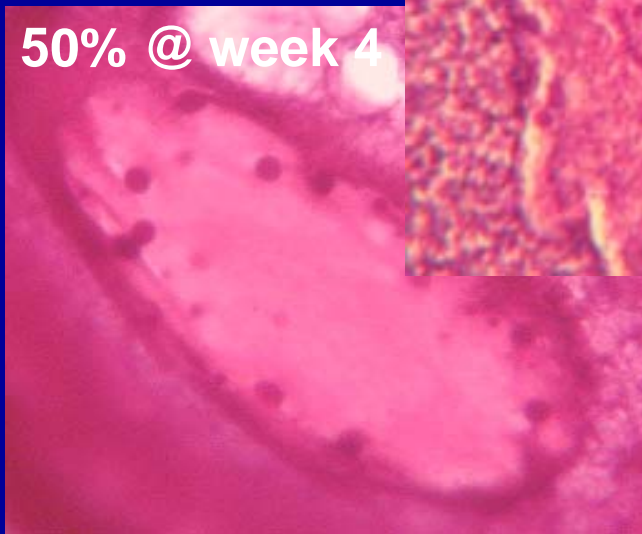
25% @ week 4



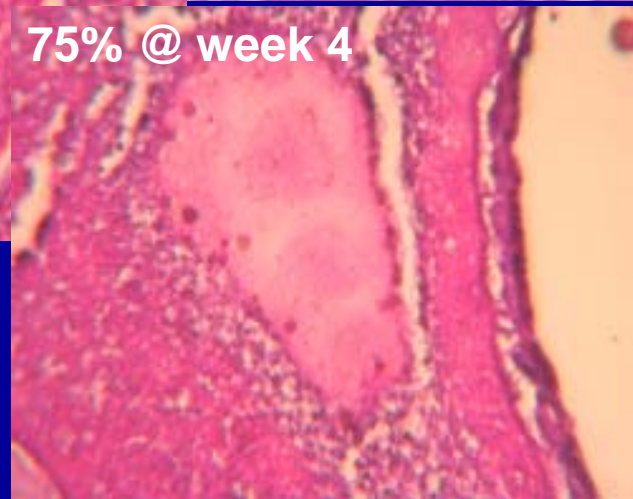
50% @ week 3



50% @ week 4

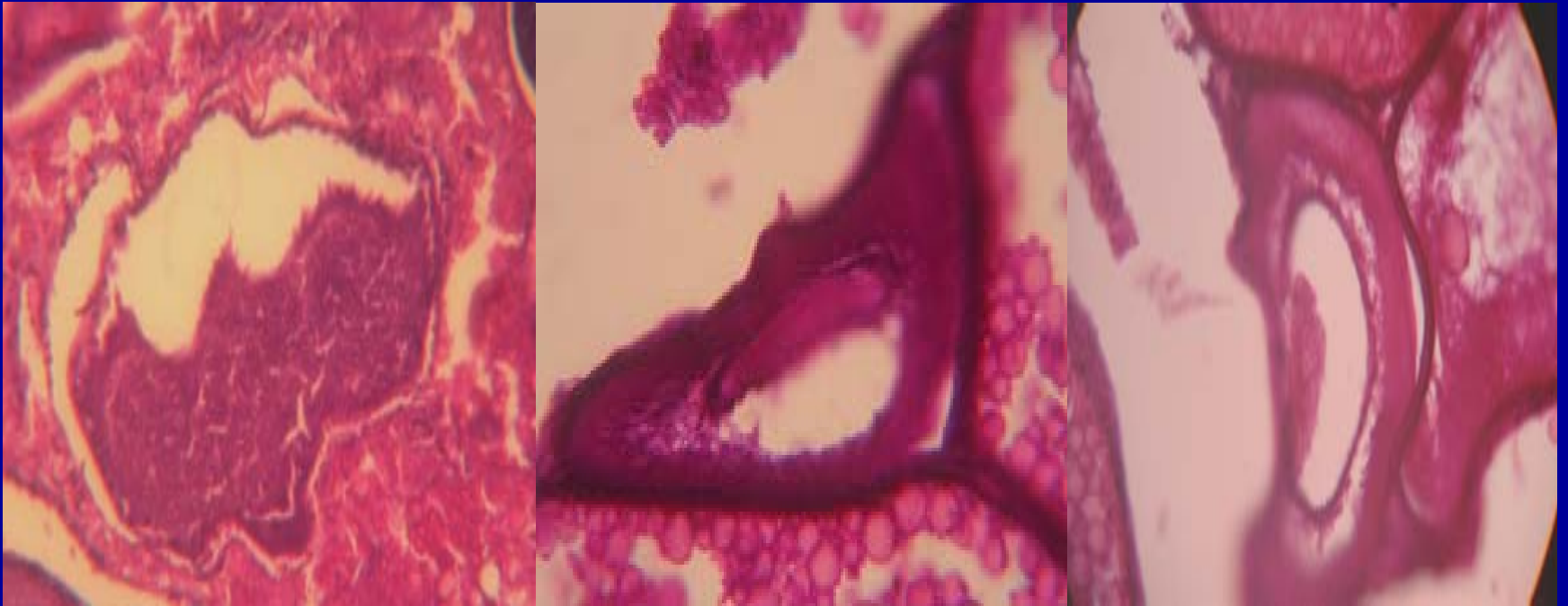


75% @ week 4



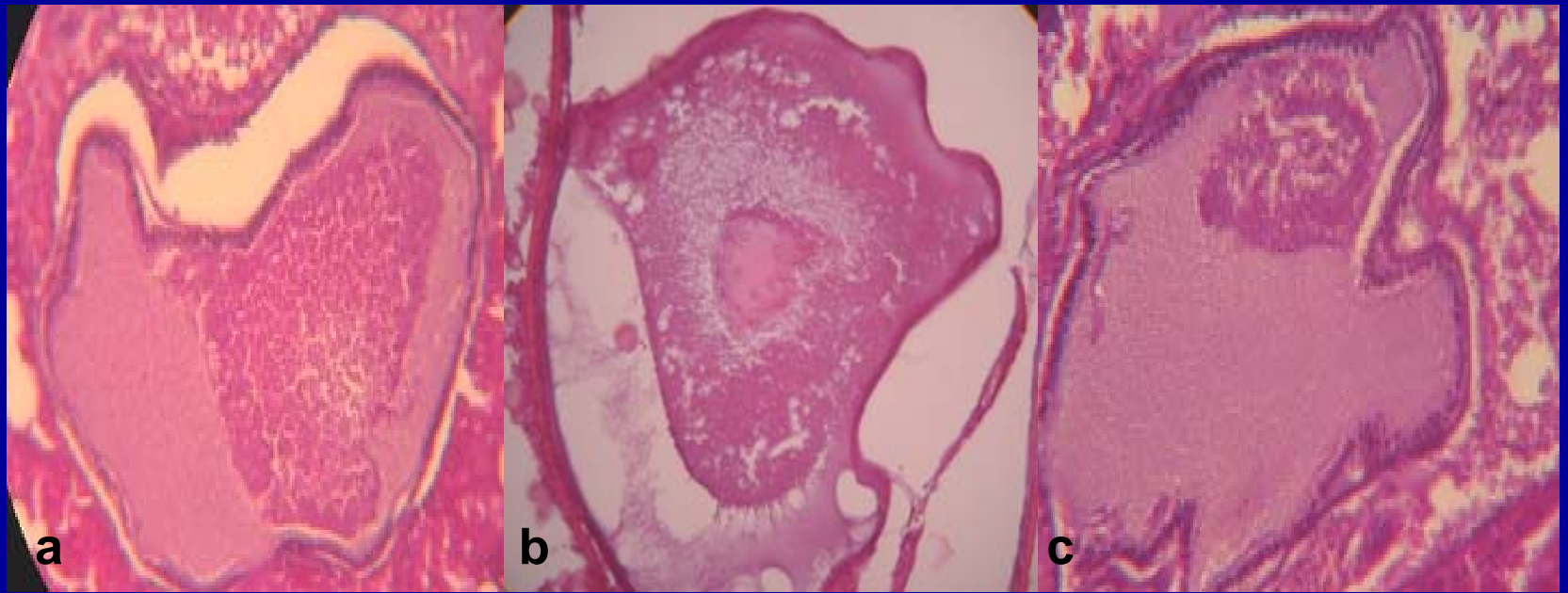


# Nuclear Condensation



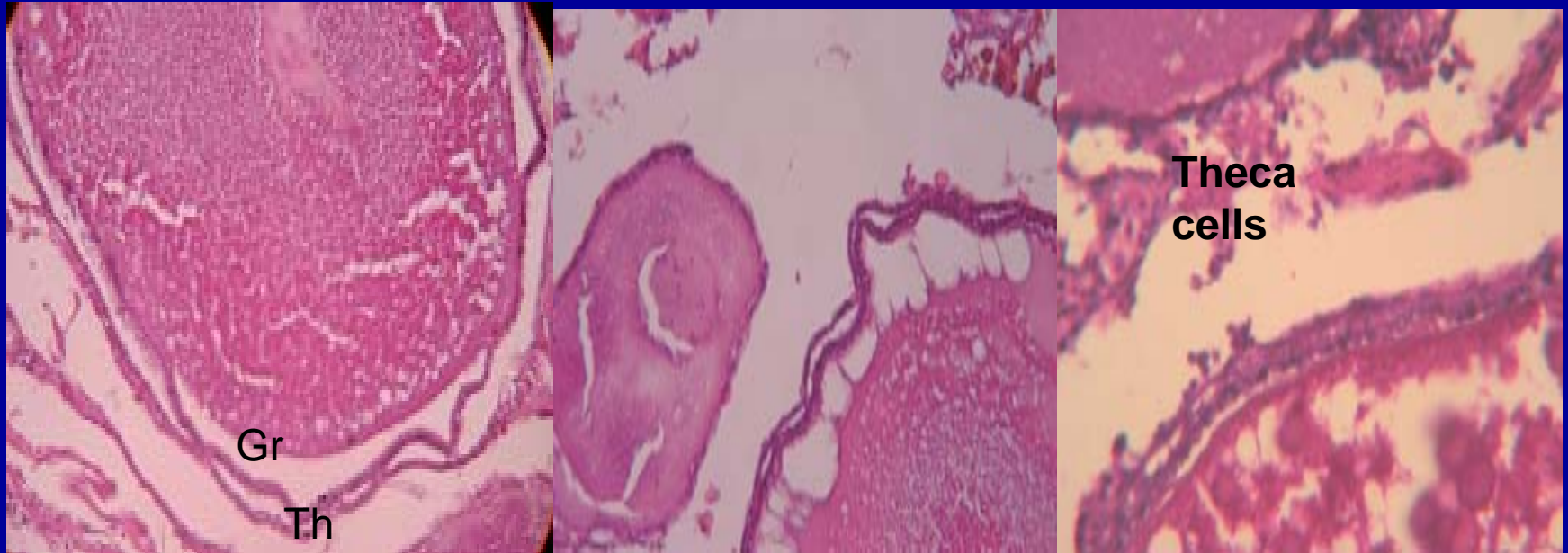
Nuclear condensations of the oocytes at week 4

# Cytoplasmic condensation



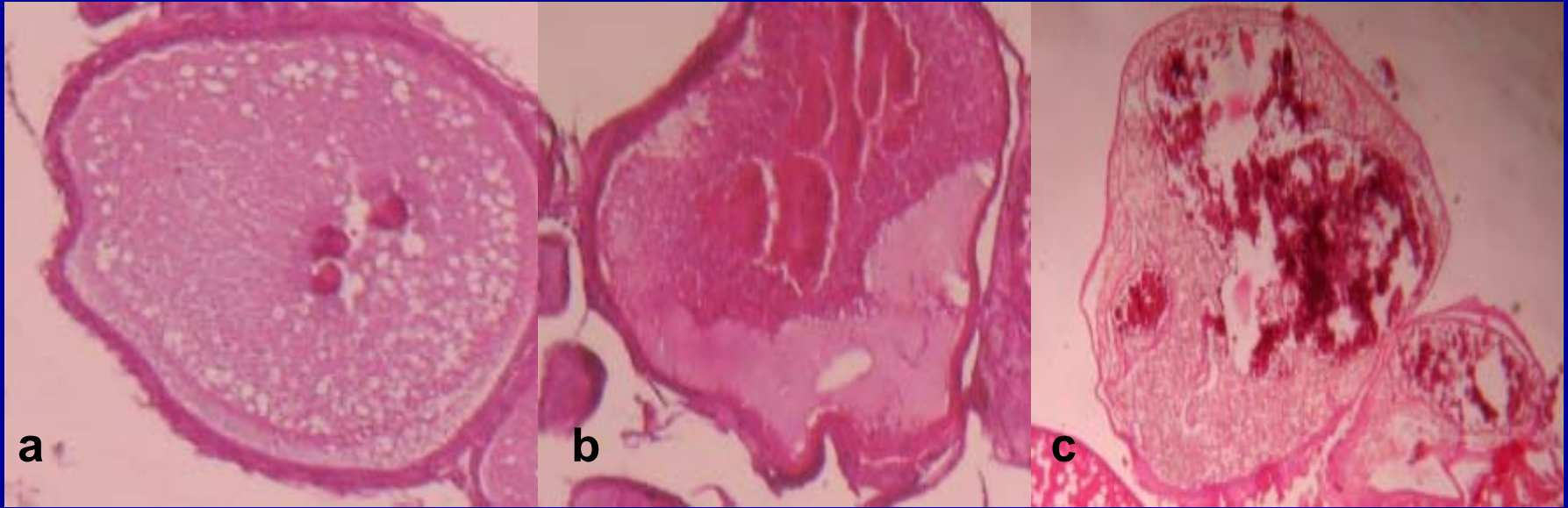
Cytoplasmic condensations in the oocytes exposed to 75% sublethal concentration of pyrethroid at week 4.

# Theca / Granulosa breakdown



- Theca and granulosa layer disruption at the basement membrane exposed to 75% sublethal concentration of pyrethroid.

# Vesicle formation



Formation of membrane-bound vesicles in oocytes exposed to 25% @ wk 3 (a), 50% @ wk 3 (b) and 75% @ wk 1 (c) sublethal concentration of pyrethroid. (LPO/ H & E 4.3x).

# Conclusions

- GSI was influenced by the exposure to pyrethroid.
- GSI is highly correlated with gonad weight but not body weight.
- Prolonged exposure to endocrine disruptor (pyrethroid) resulted into early apoptotic signs on the ovarian cells.

# Recommendation

Assessment on the use of pesticides or fertilizers as endocrine disruptors in fish ponds and the possibility of early sexual maturation in tilapia brought about by the induction of apoptosis.



# Acknowledgements

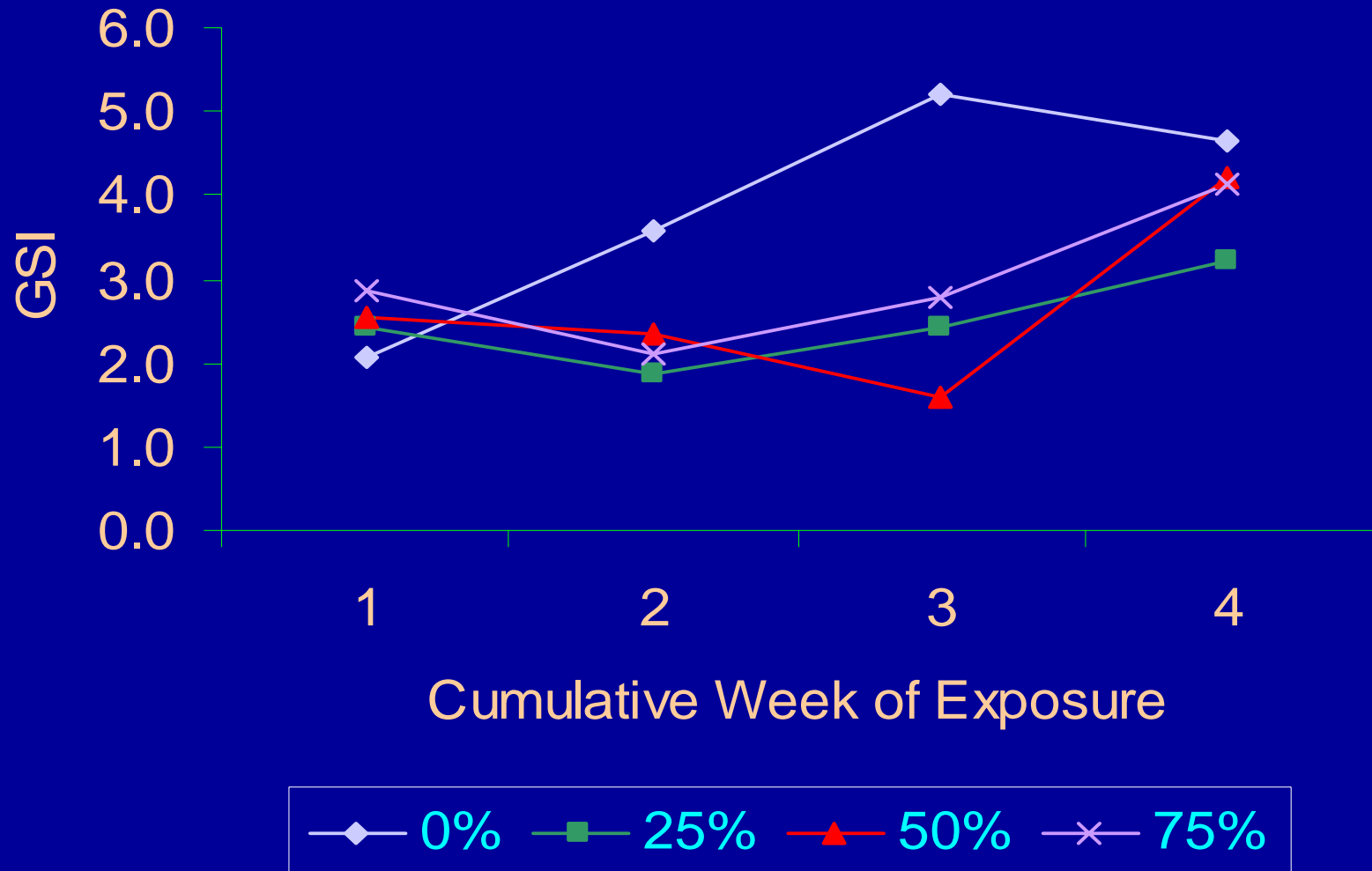


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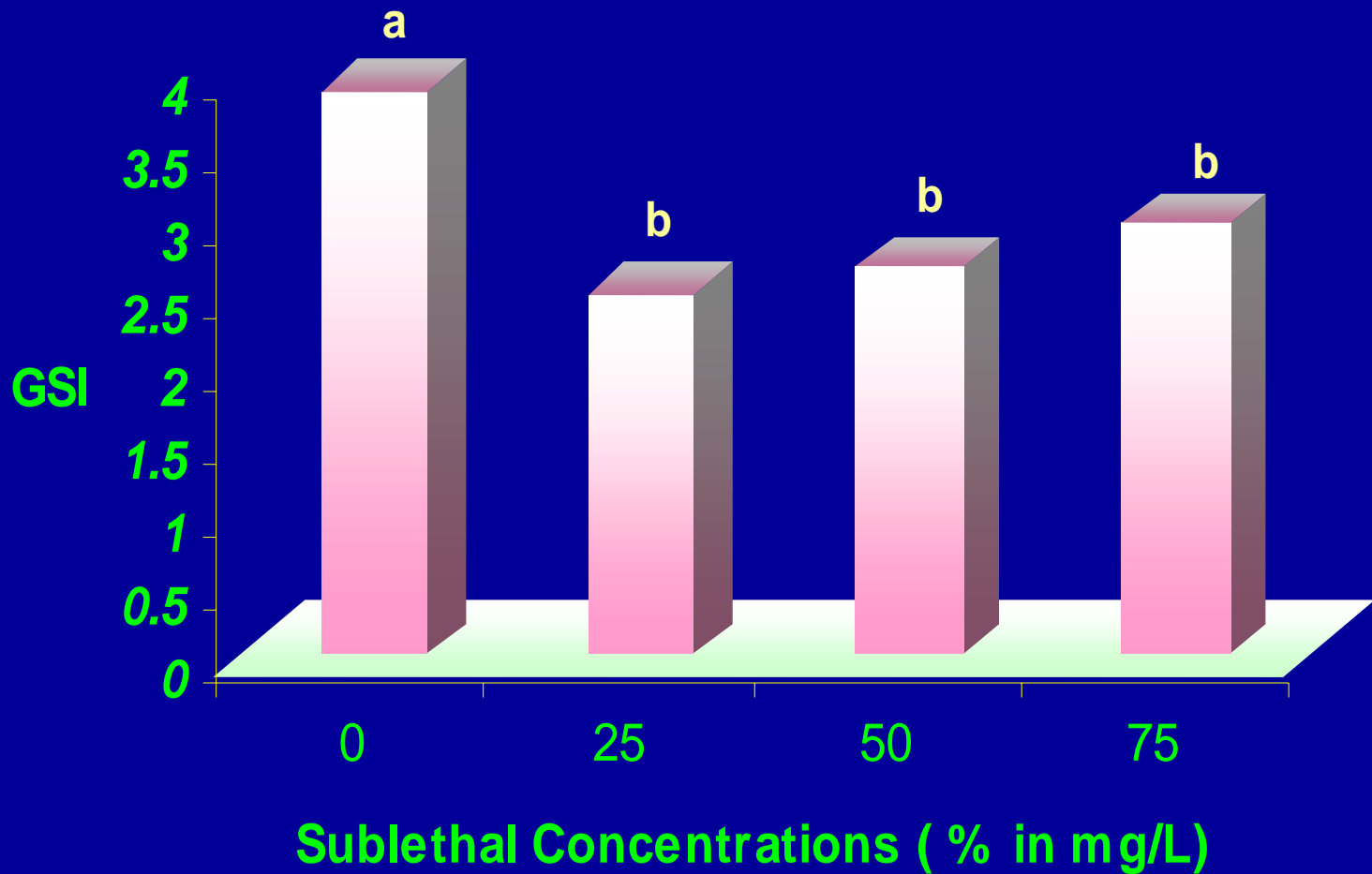


**Thank you very much...**



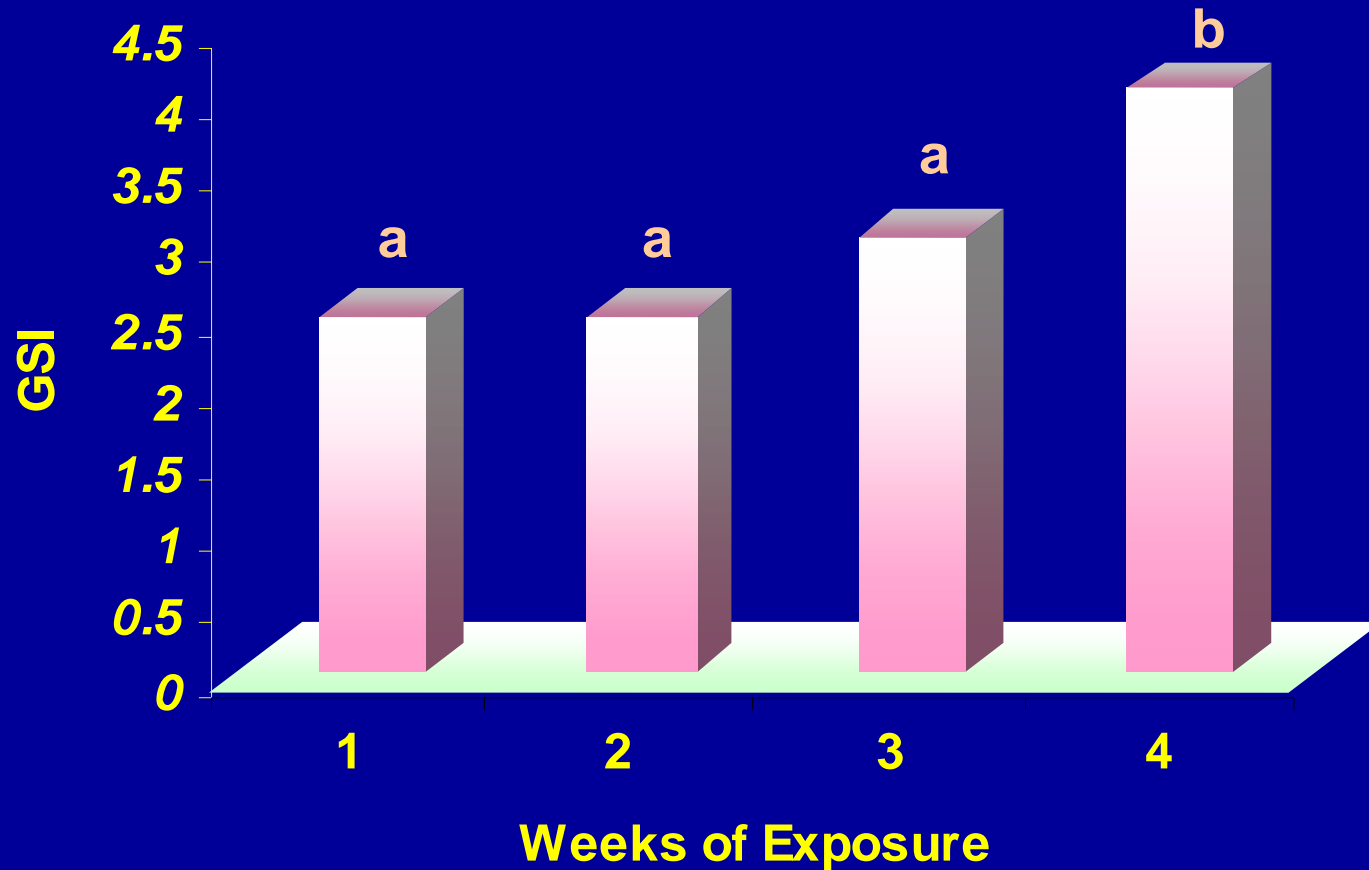
“GSI was not influenced ( $P > 0.14$ ) by the interaction of the different concentrations and exposure time to the pyrethroid.”





“ Compared with the control group which has higher GSI, treated groups regardless of concentrations showed reduction in GSI.”

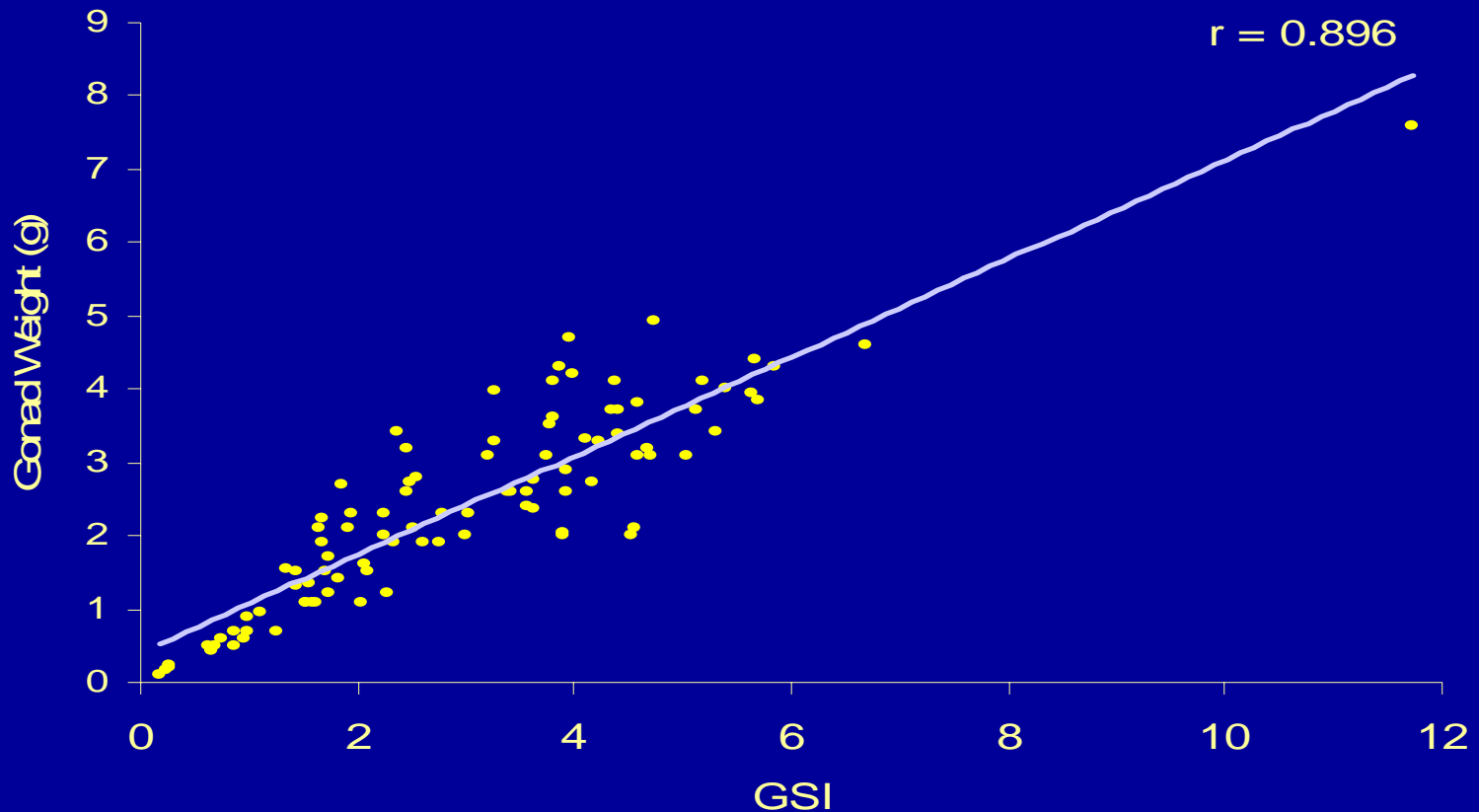




“ The GSI @ 4th week was significantly different with the first 3 weeks. As the week of exposure progresses, the GSI increases“



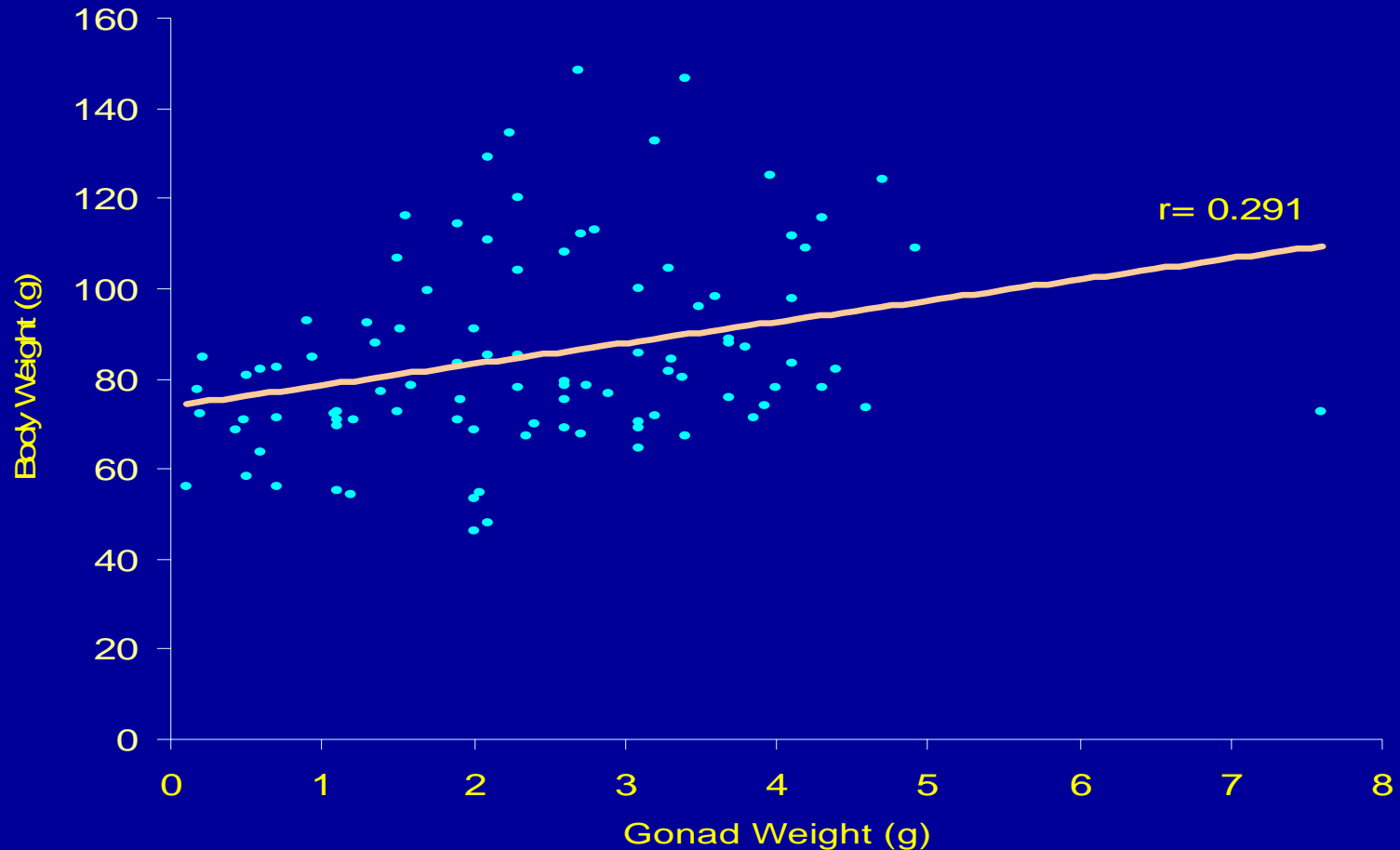
# Correlation Between GSI and GW



“Gonad weight regardless of the body weight can be used as an indicator of fecundity to measure the reproductive fitness of *O. niloticus*.”



# Correlation Between GW and BW



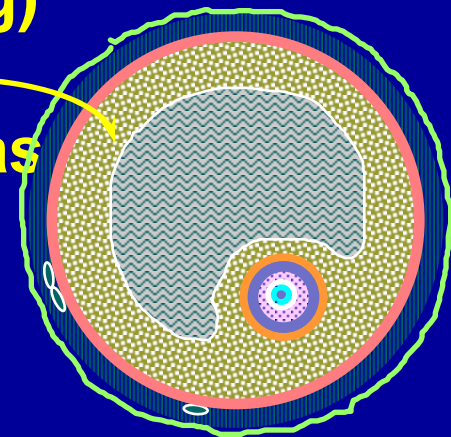
“ Body weight is not an indicator of gonad weight. *O. niloticus* can be more fecund even at lower body weight.”



# APOPTOSIS

- ◆ In development, apoptosis removes unwanted cells, **more examples: after matrix synthesis in the growth plate, in old bone awaiting resorption,**
- ◆ Some *defensive* cells die in the course of protecting the body: **lymphocytes can kill each other, thus *limiting* immune reactions, & avoiding autoimmunity**
- ◆ Apoptosis is part of running down unused or unwanted female reproductive tissue as it cycles, e.g., uterine endometrium, corpus luteum, breast secretory epithelium

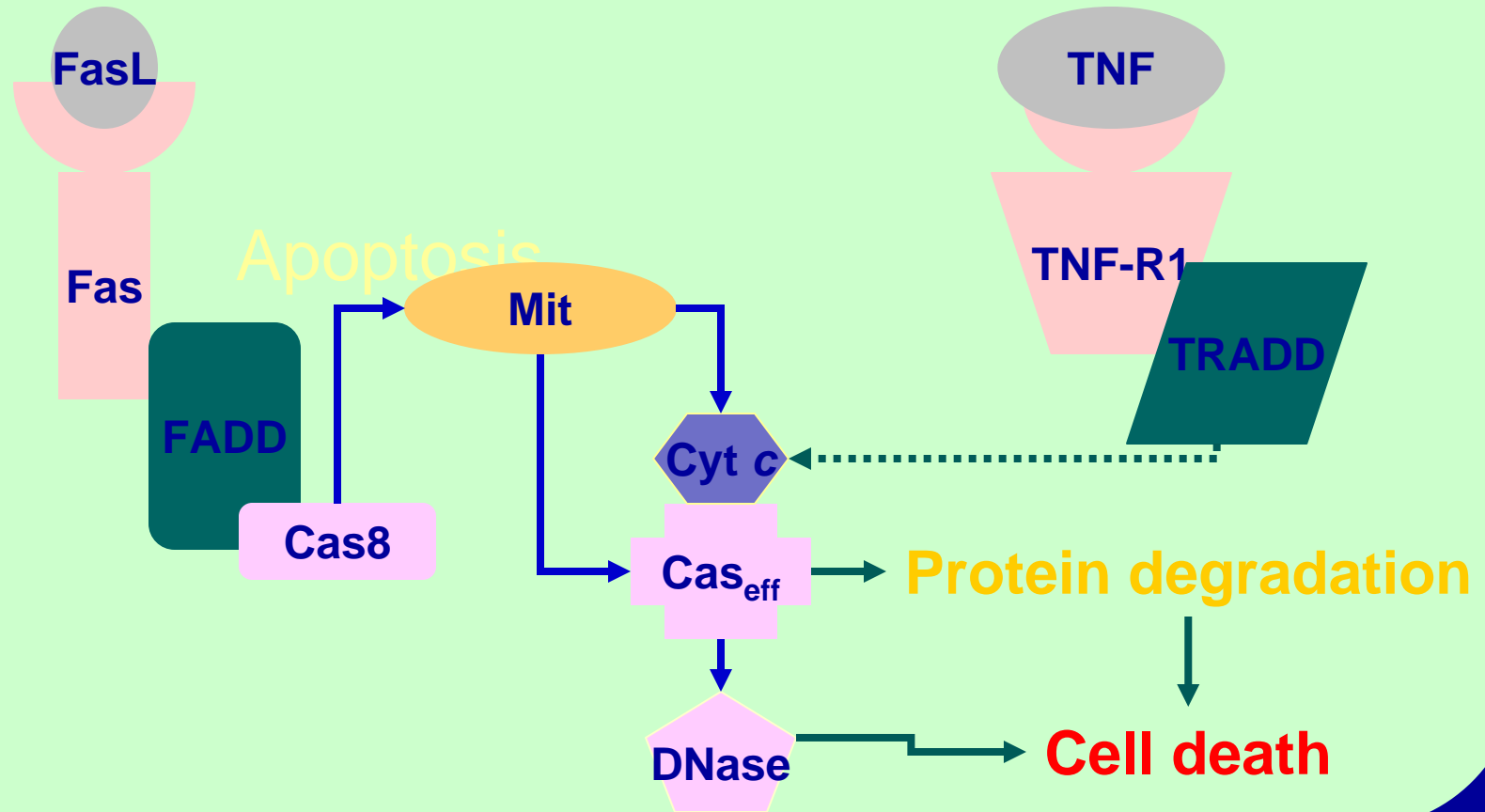
**In mammalian ovarian late *atretic* (degenerating) follicles, granulosa cells display hallmarks of apoptosis, e.g., fragmented nuclei, shrinkage, as they shed into the lumen**





# APOPTOSIS: Contexts | Where used

- ◆ In *development*, apoptosis removes unwanted cells; for example in separating the digits of the hand, culling excess neurons, eliminating inappropriately sensitive lymphocytes
- ◆ In mature tissues that undergo *renewal*, e.g., blood cells, some epithelia, the outdated cells deliberately destroy themselves, if they can
- ◆ Some *defensive* cells die in the course of protecting the body, e.g. neutrophils, or in developing maximum affinity for attacking an invader, e.g. B lymphocytes
- ◆ *Infected* cells may be removed by apoptosis
- ◆ Most *tumor* cells grow; some become apoptotic
- ◆ Apoptosis is used in running down unused or unwanted *female reproductive* tissue as it cycles, e.g., uterine endometrium, corpus luteum, breast gland epithelium



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