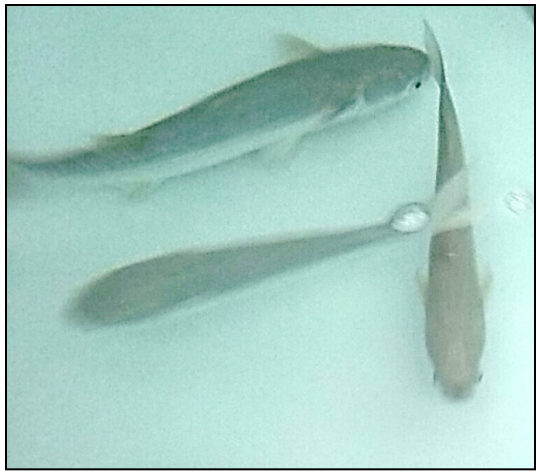
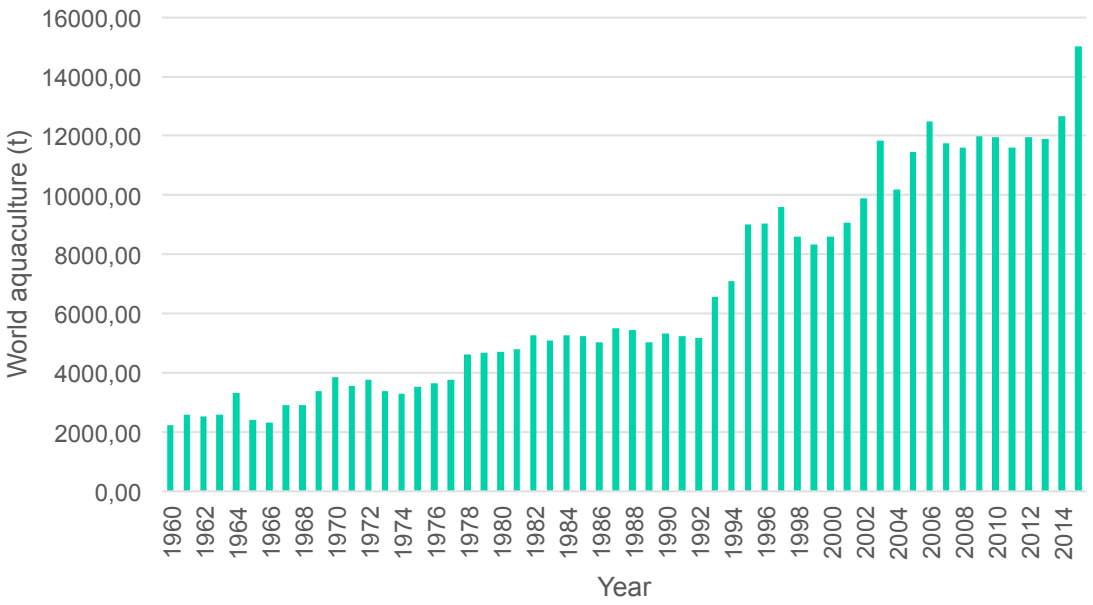
# INTRODUCTION


- Fingerling supplies come almost exclusively from the wild.
- **Reproductive dysfunction in captive breeders.**



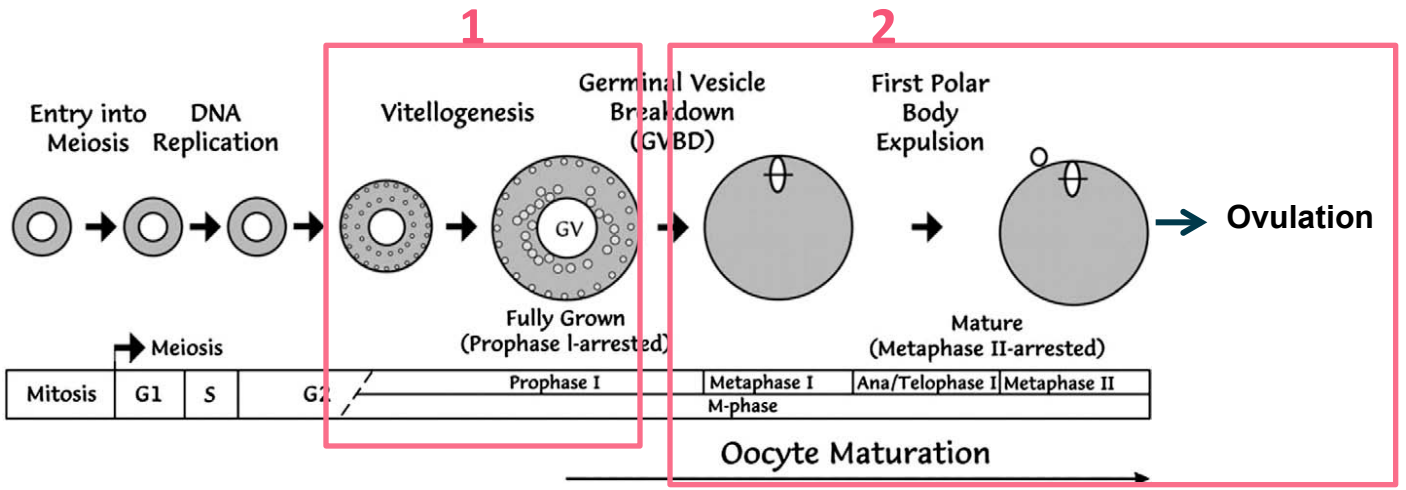



*Yaron and Levavi-Sivan, 2011.*
*FAO, 2015.*

# INTRODUCTION

♂ No running milt.

♀ Have dysfunctions at (1) early stages of vitellogenesis and (2) final oocyte maturation and ovulation.

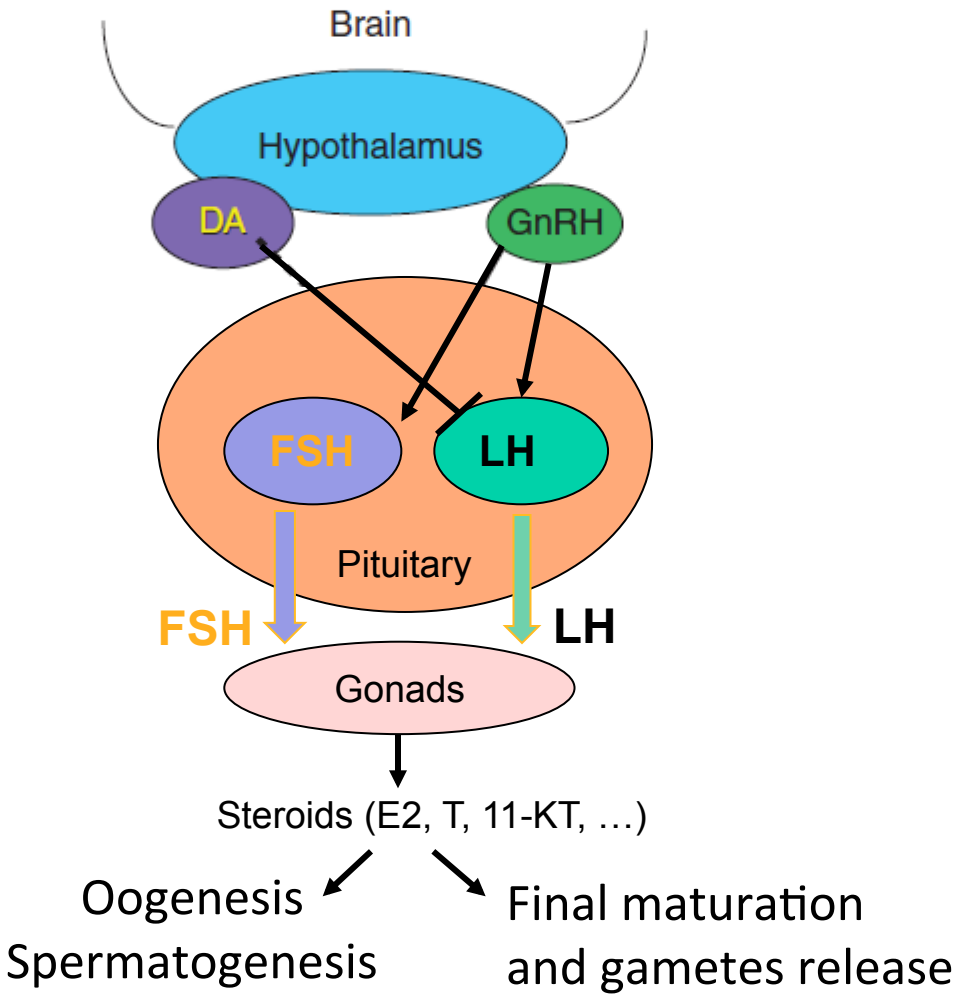



**vitellogenesis: yolk accumulates in the oocyte which increases in diameter*

**oocyte absorbs water and is released into the ovarian lumen*

*Yaron and Levavi-Sivan, 2011.*

# INTRODUCTION




*Modified from Yaron and Levavi-Sivan, 2011.*

# OBJECTIVE

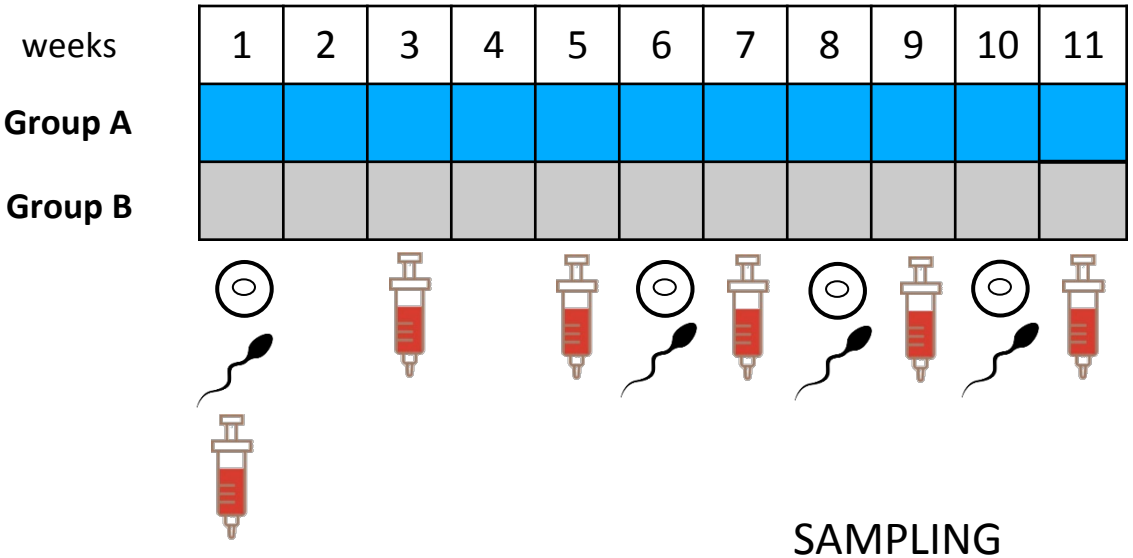
Induce vitellogenesis and spermatogenesis and synchronize gonadal development in male and female grey mullet.

# METHODS

- Treatments with mainly grey mullet recombinant follicle-stimulating hormone (mugil-rFSH).
  - ✓ **Grey mullet FSHβ and α subunit sequenced.**
  - ✓ **Single-chain rFSH produced in mammalian CHO (Chinese Hamster Ovary) cells.** Polypeptides are more stable in fish plasma and enhance their biological activity (Chauvigné et al., 2017).

# Induction protocol

- 2 groups of 12 immature grey mullet (8♀ and aprox. 4♂, mean weight 990 ± 212g)
- Weekly intramuscular injections of:



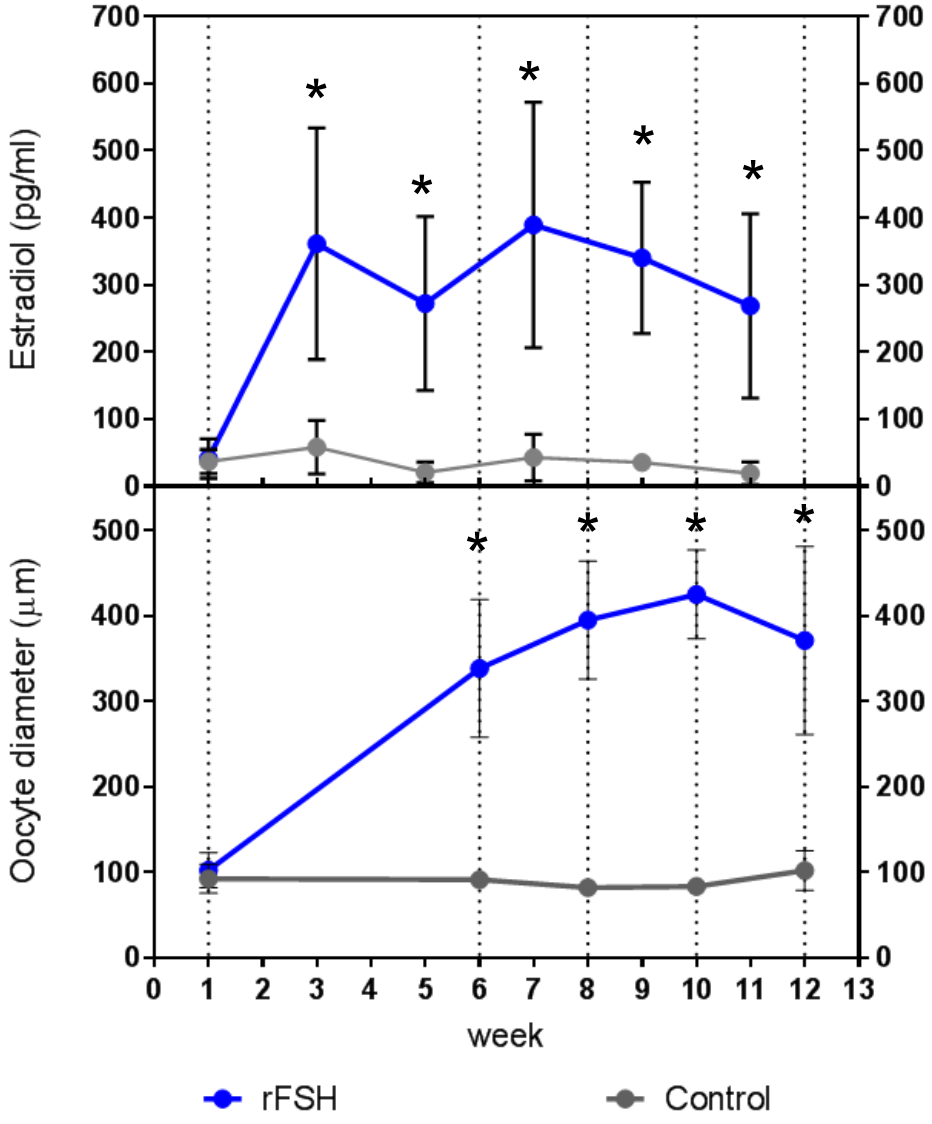

Grey mullet-rFSH (15 µg/kg)

CHO cells medium (control fish)

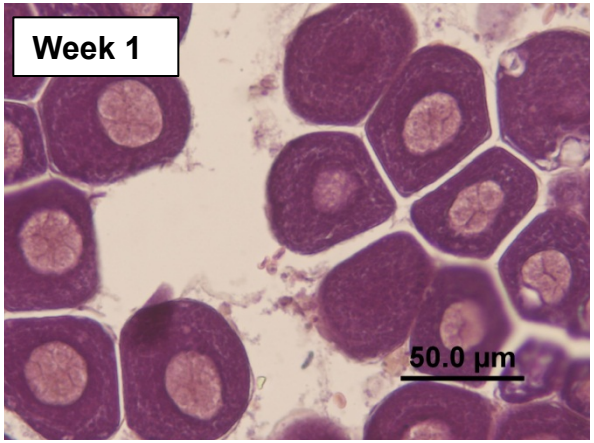
SAMPLING

- Blood samples (steroid hormones analysis).
- Ovarian biopsies (oocyte diameter).
- Presence of milt.
  (Sperm concentration and motility).

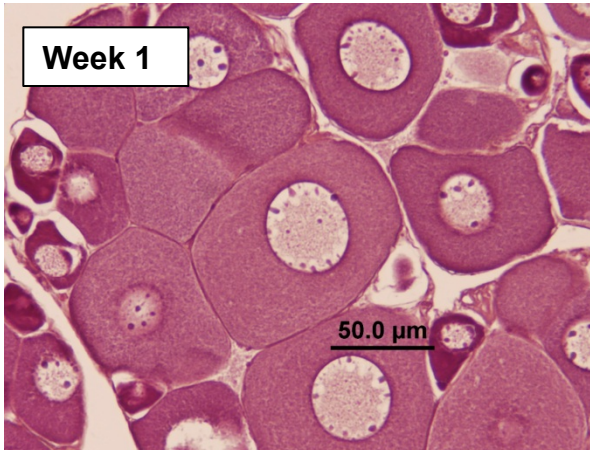
# RESULTS ♀



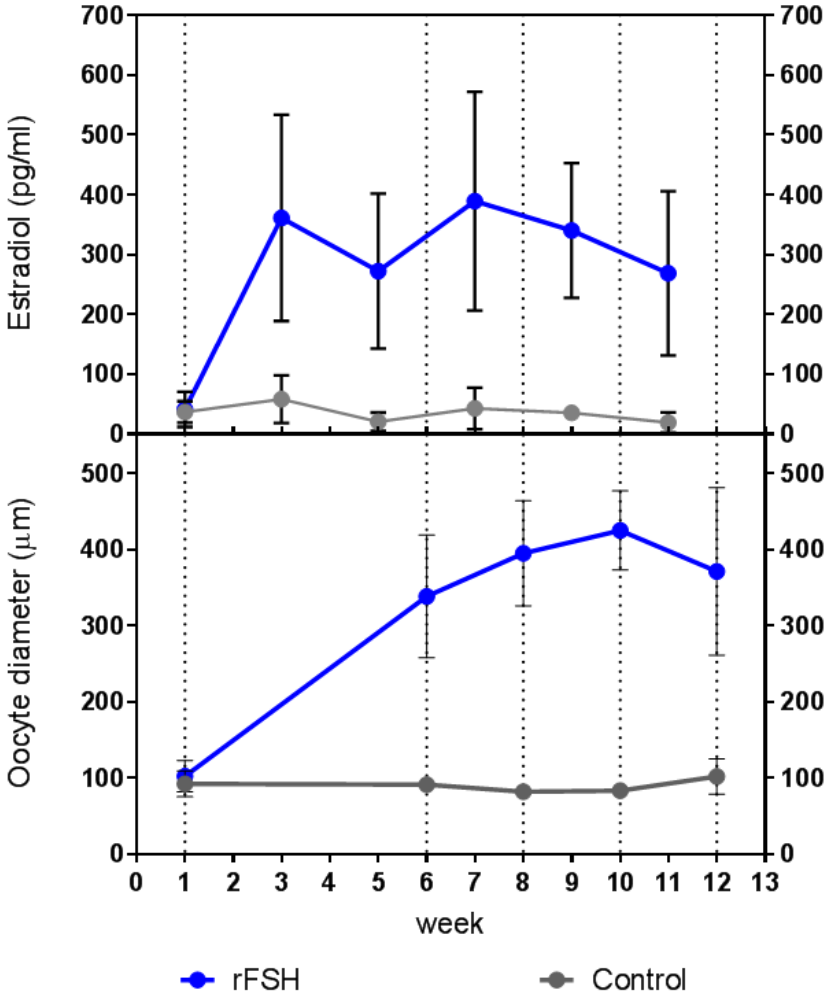
# RESULTS ♀



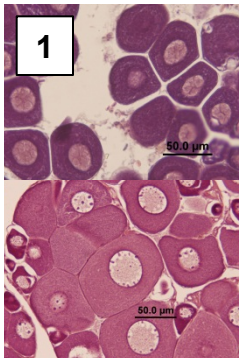

One nucleolus primary growth oocytes (PGon)

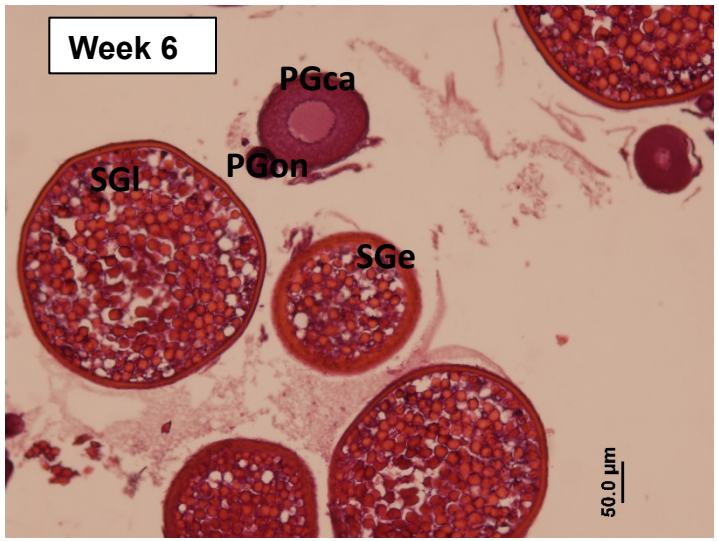


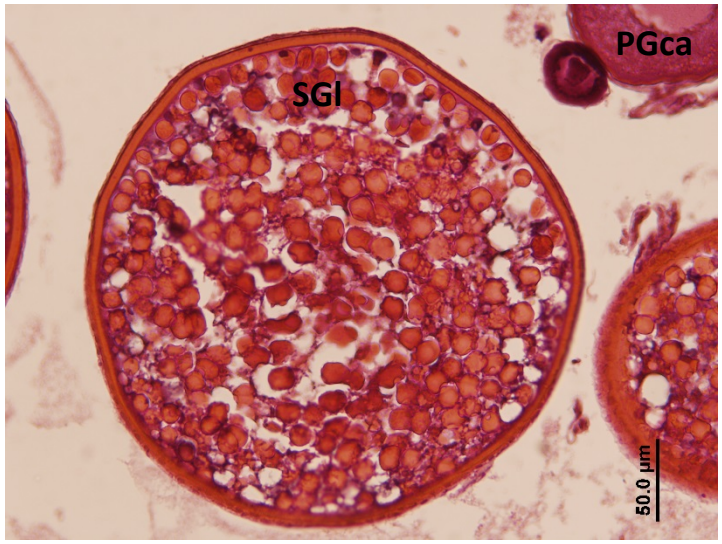

Perinuclear primary growth oocytes (PGpn)

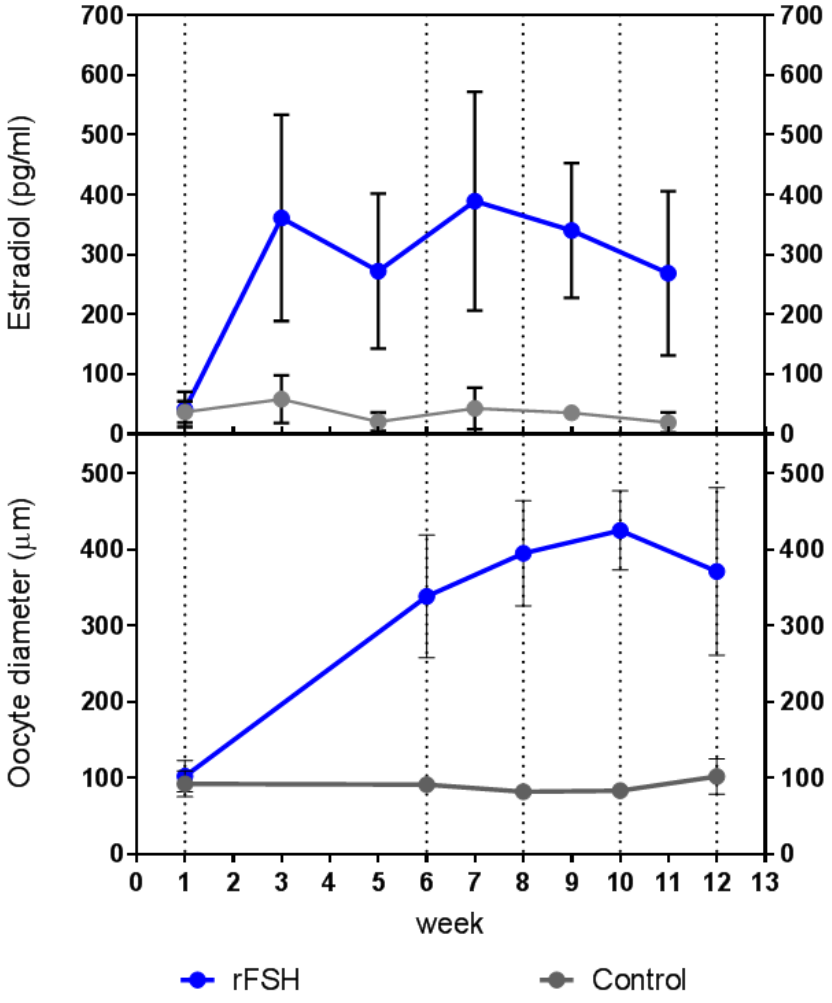


# RESULTS ♀



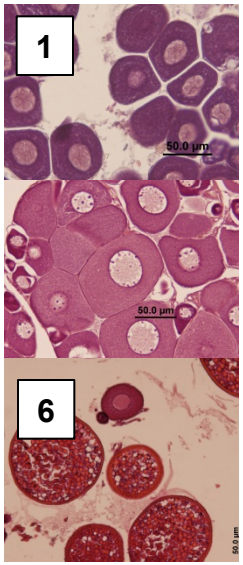



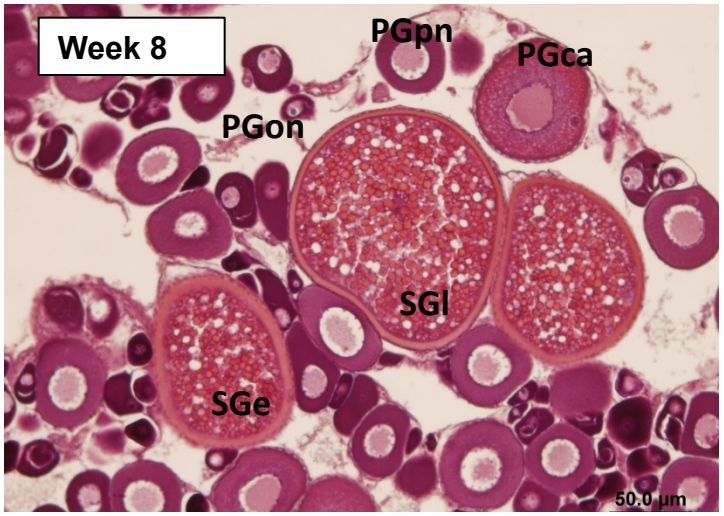



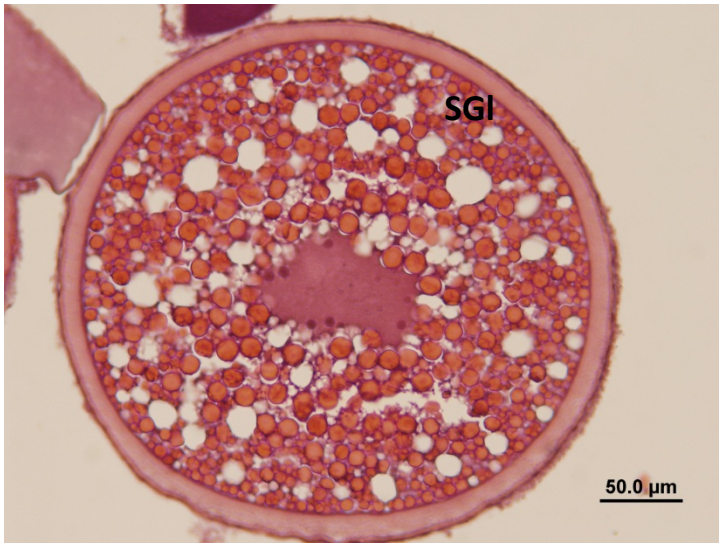



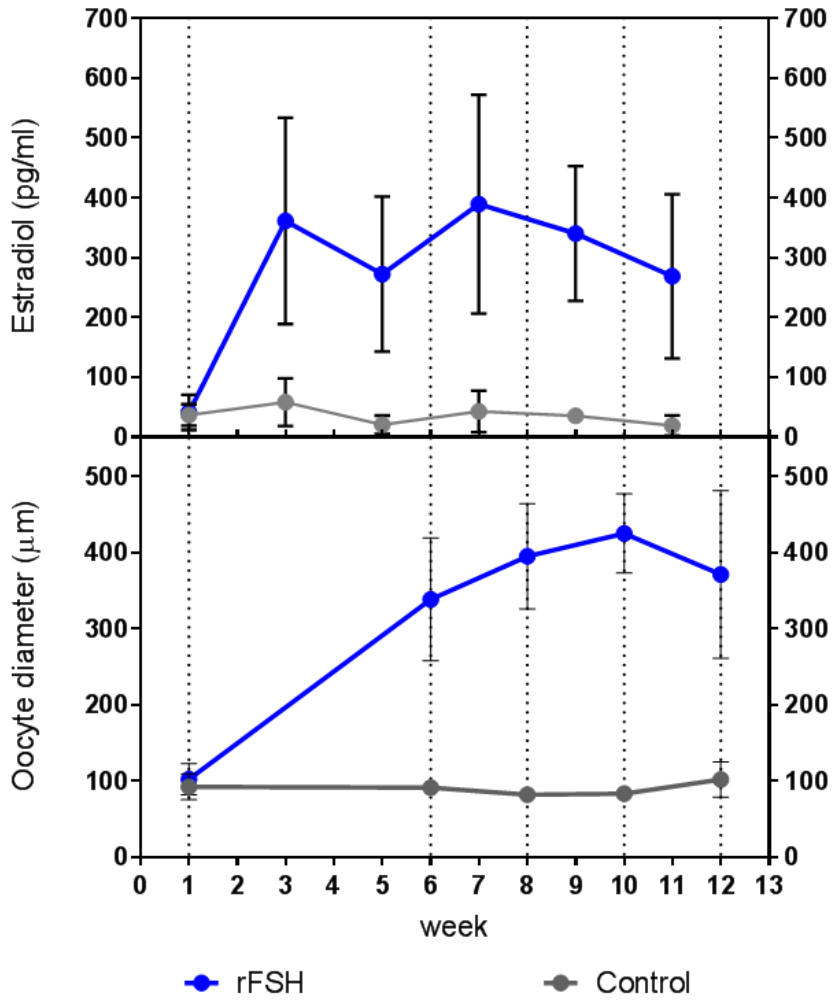

- PGon
- Cortical alveoli primary growth oocyte (PGca)
- Early secondary growth oocyte (SGe)
- **Late secondary growth oocyte** (SGl)

# RESULTS ♀



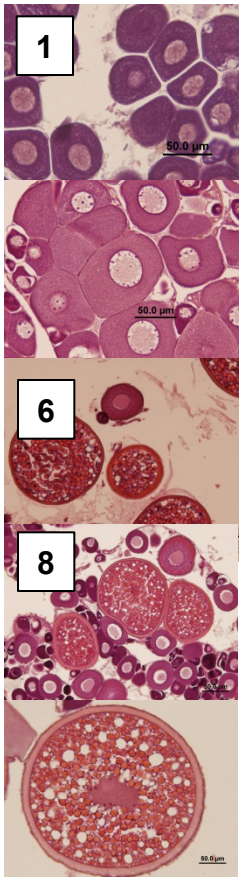



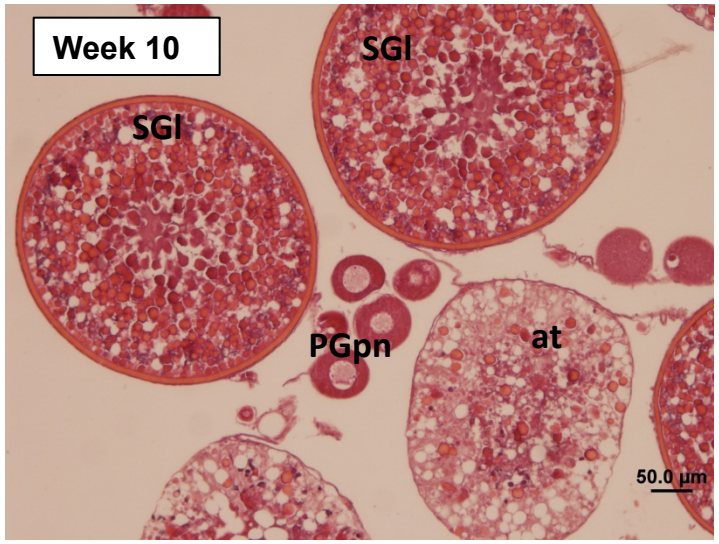



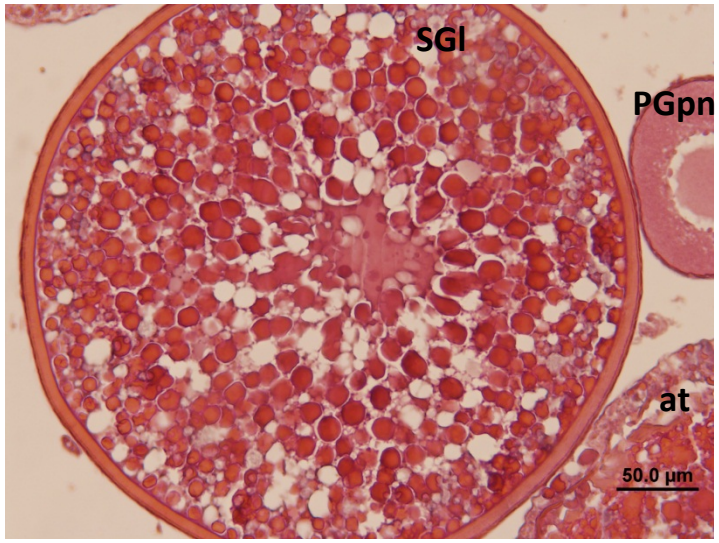



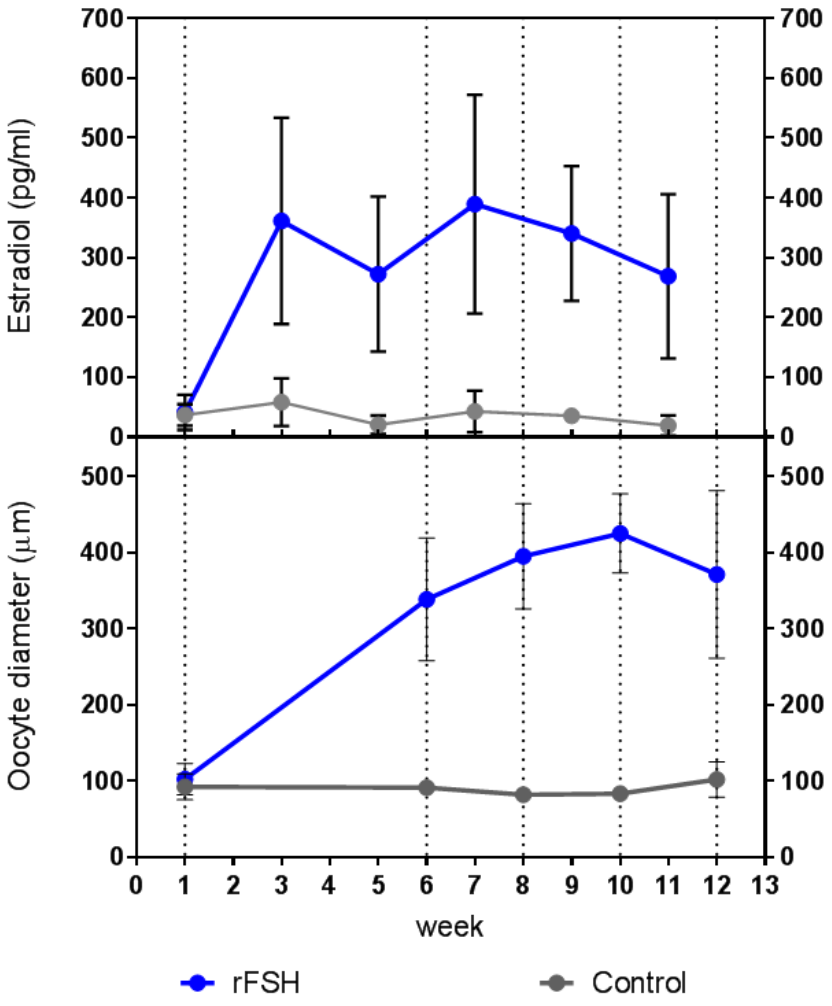

- PGon and PGpn
- Cortical alveoli primary growth oocyte (PGca)
- Early secondary growth oocyte (SGe)
- **Late secondary growth oocyte** (SGl)

# RESULTS ♀



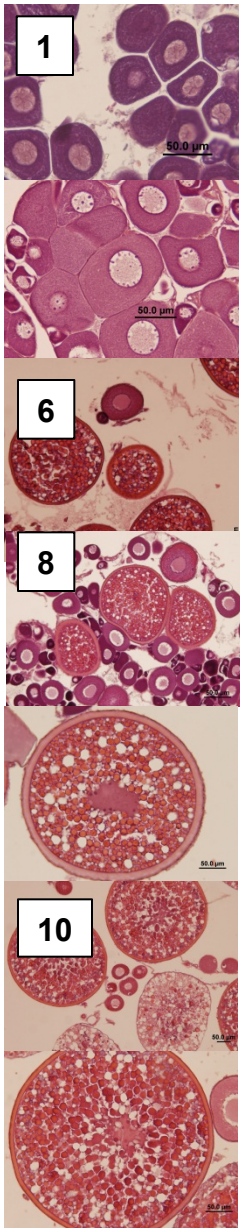



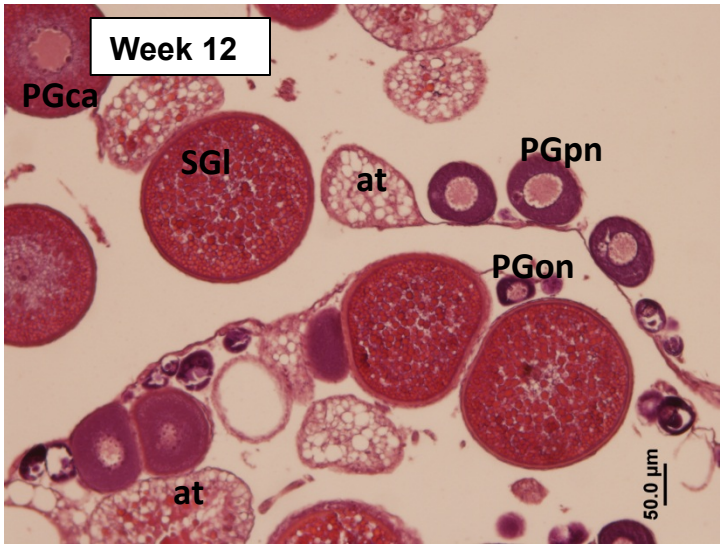



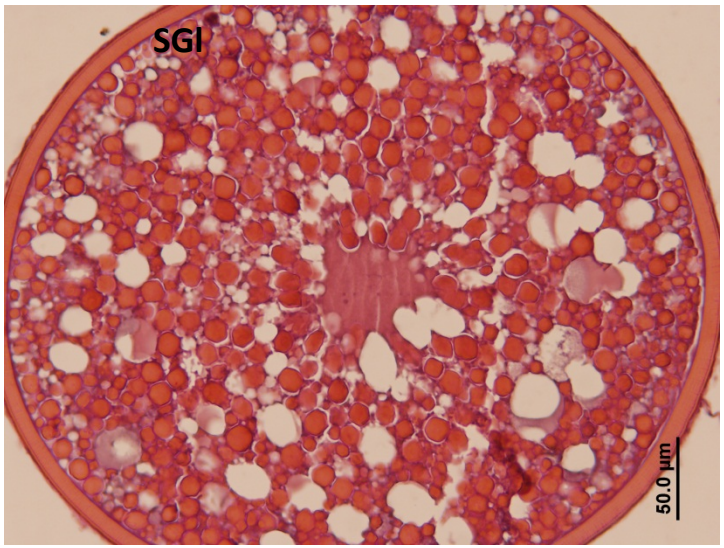




- PGpn and PGca.
- **Late secondary growth oocyte** (SGl).
- **First atretic oocytes** (at).

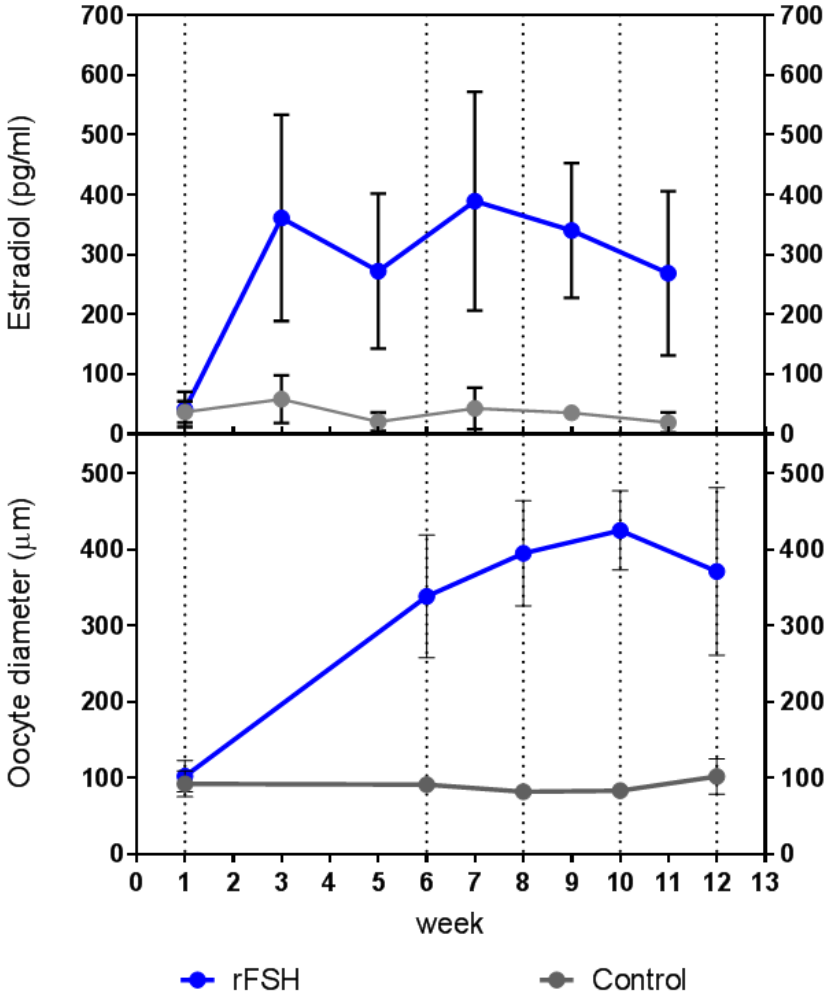
# RESULTS ♀



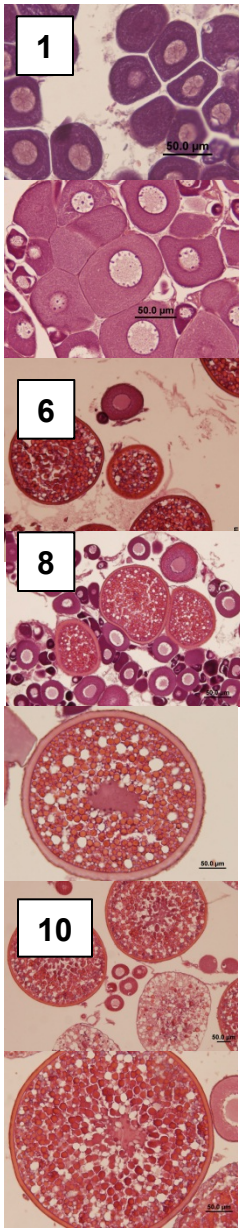



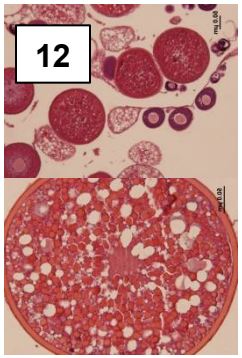

- PGon, PGpn and PGca.
- **Late secondary growth oocyte** (SGl).
- **Atretic oocytes** (at).




- Developed up to 400-450 µm.

# RESULTS ♀



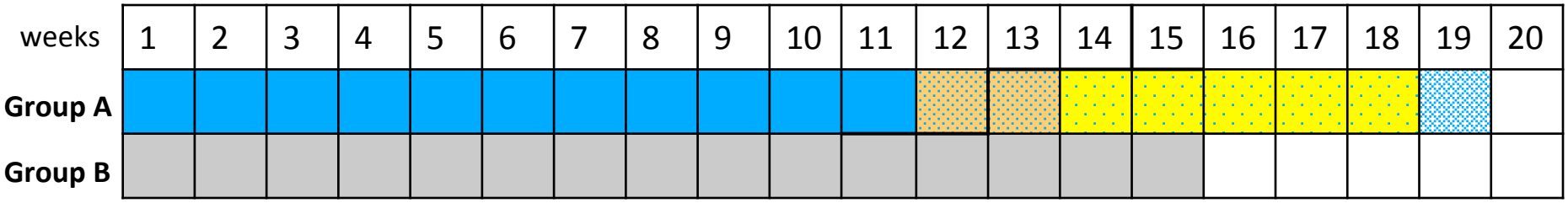



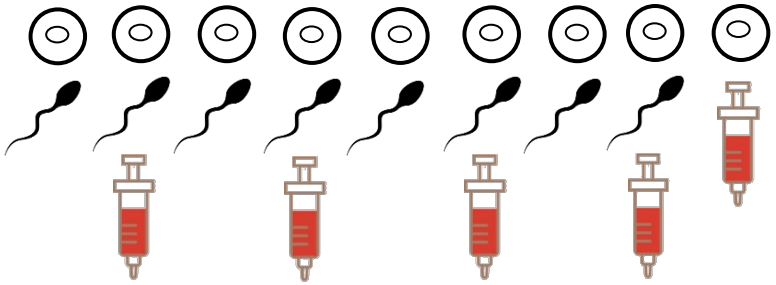

rFSH induced gametogenesis.

(?) The fact that atretic oocytes were found after 9 weeks of treatment, means that **additional hormonal stimulus seems to be required** prior to week 10 to complete vitellogenesis and start maturation.

# Induction protocol

- Combined treatment of different hormones with rFSH. Dose adjustments were performed according to the ovarian response.



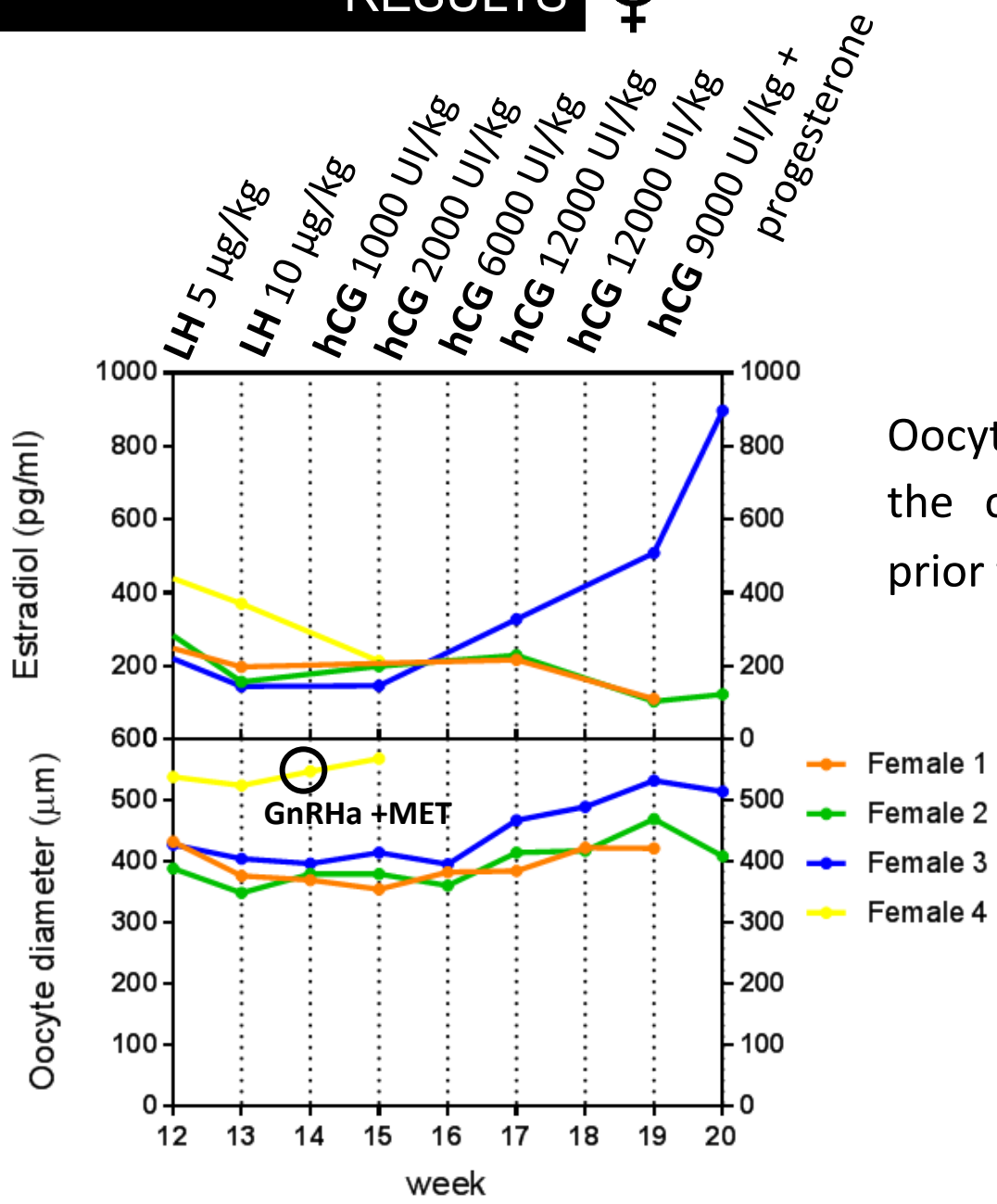
If oocytes >350 µm…

rFSH + ***Solea senegalensis* rLH** 5 and 10 µg/kg ♀♂

rFSH + **hCG** 1000, 2000, 6000, 12000, 12000 IU/kg ♀♂

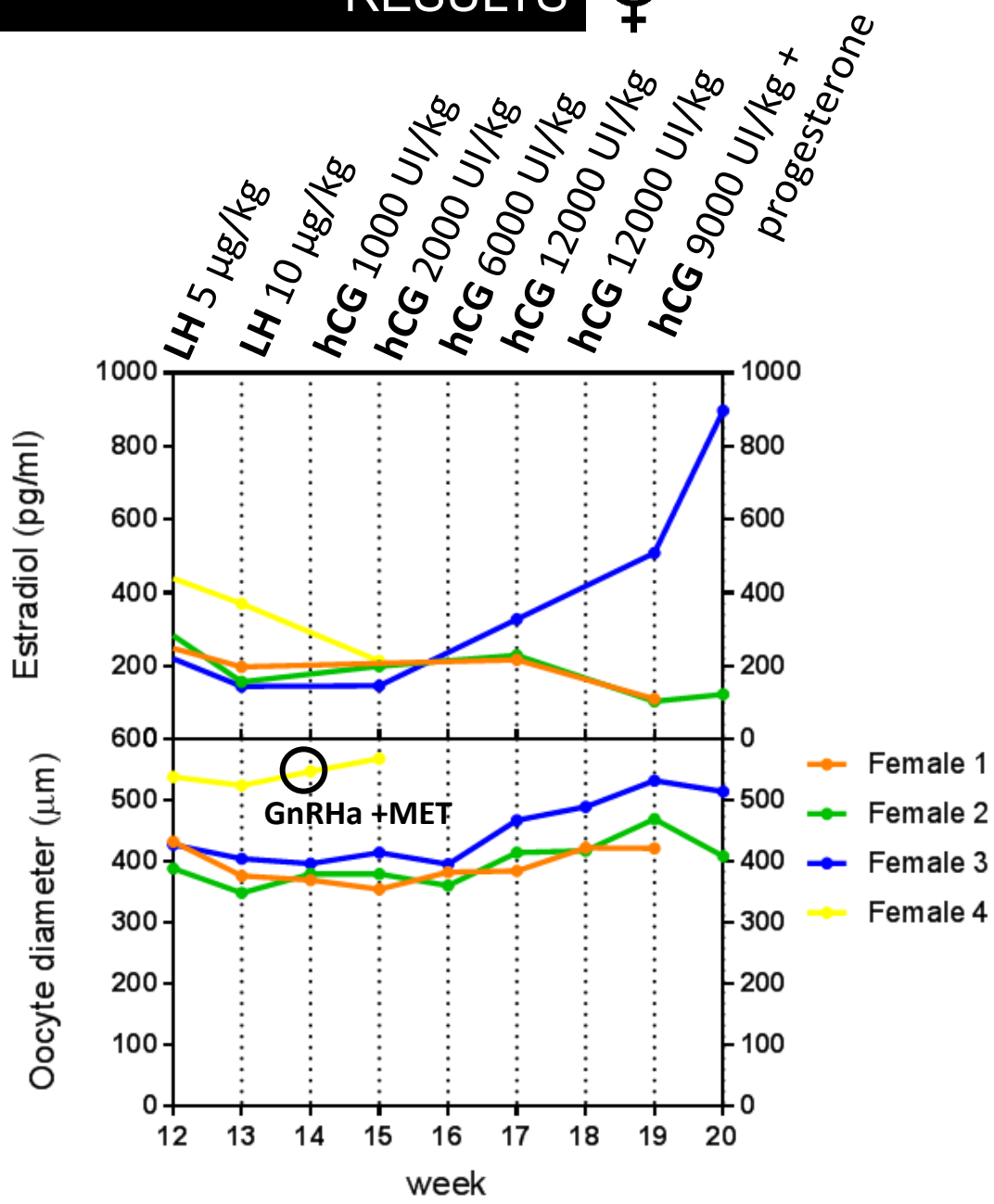
rFSH + **hCG** (9000 IU/kg) **+ Progesterone** ♀

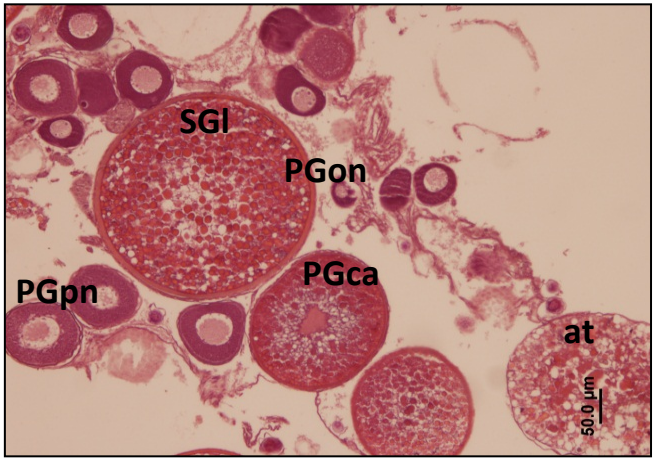
# RESULTS ♀

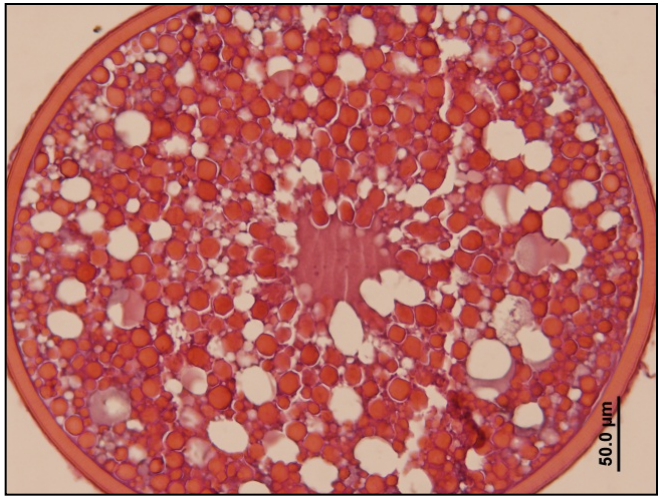



Oocyte diameter did not increase to the completion of oocyte growth prior to maturation.

# RESULTS ♀

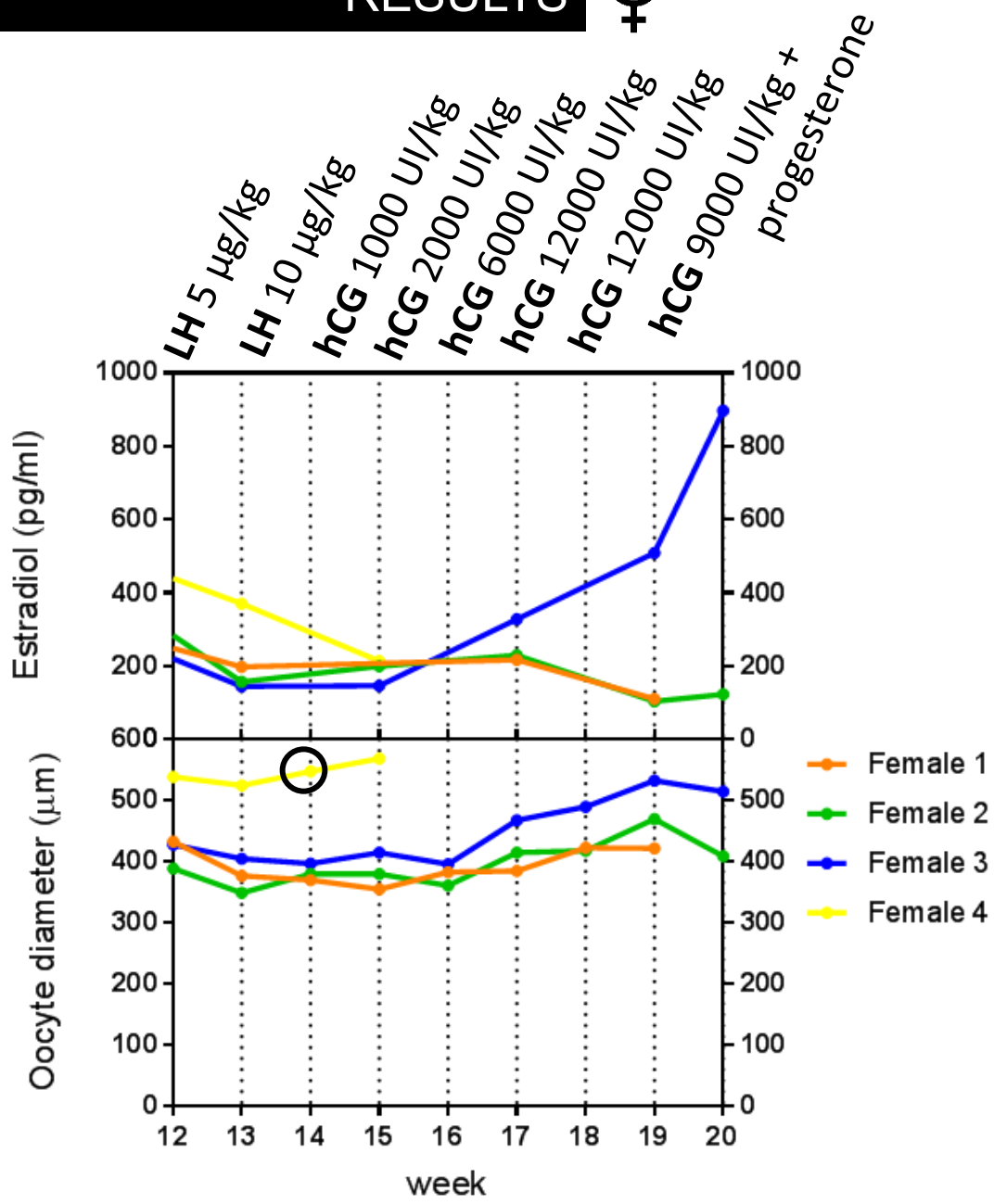


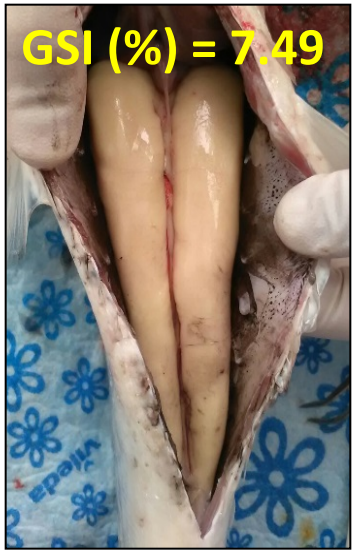



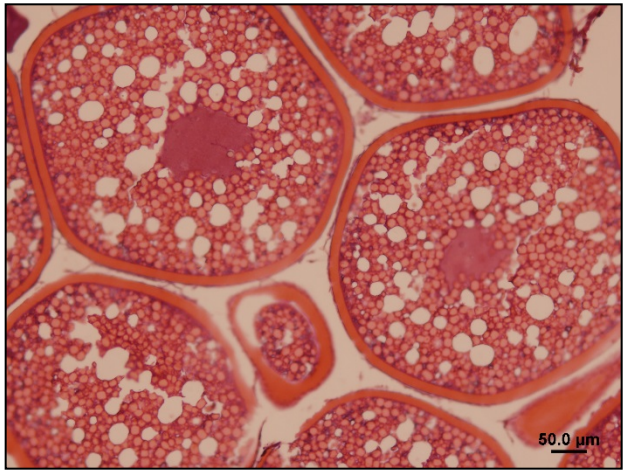

- Developed up to 450-500 µm.
- PGon, PGpn, PGca.
- **Late secondary growth oocyte** (SGl).
- **Atretic oocytes** (at).

# RESULTS ♀

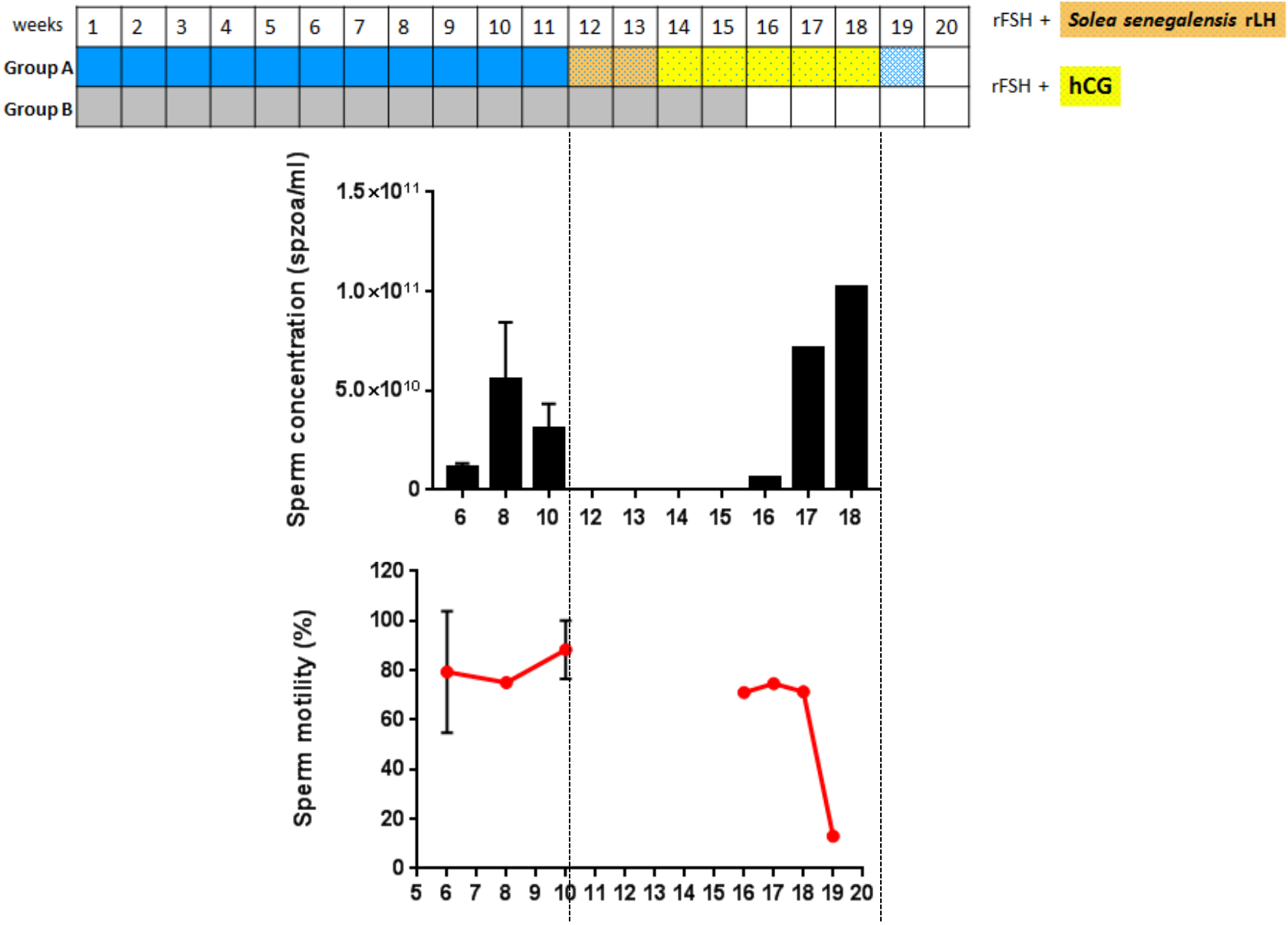



570 µm
GnRHa + metoclopramide

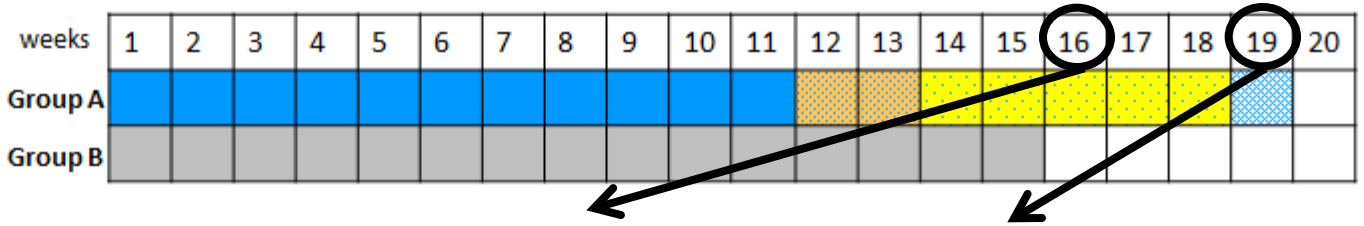


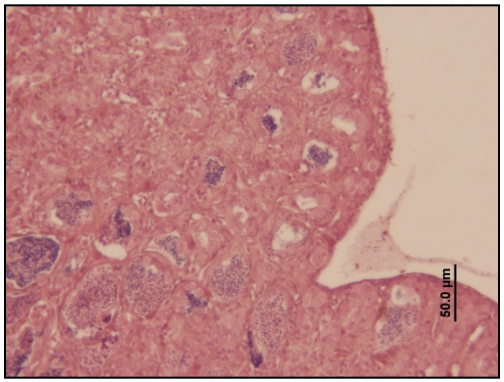
# RESULTS ♂

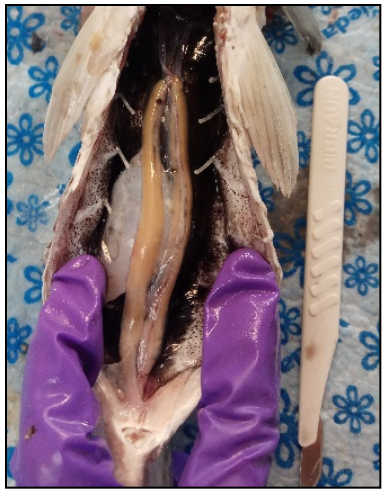

weeks
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Group A
Group B
rFSH + Solea senegalensis rLH
rFSH + hCG
Sperm concentration (spzoa/ml)
1.5×10^11
1.0×10^11
5.0×10^10
0
6 8 10 12 13 14 15 16 17 18
Sperm motility (%)
120
100
80
60
40
20
0
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

# RESULTS ♂

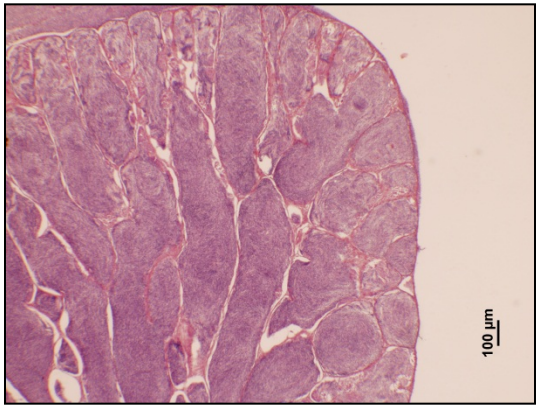


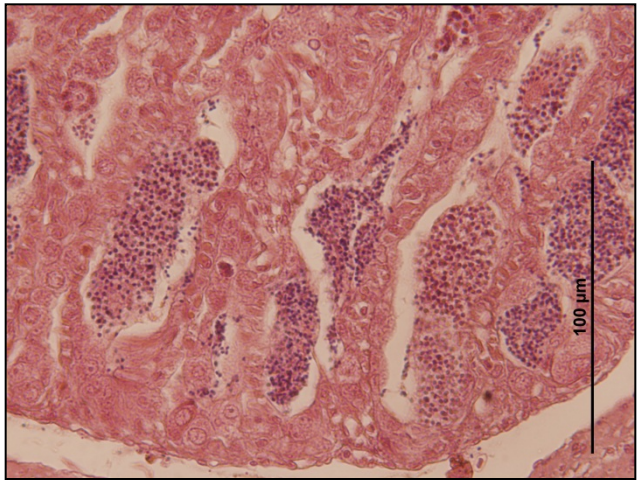

**GSI (%) = 0.17**

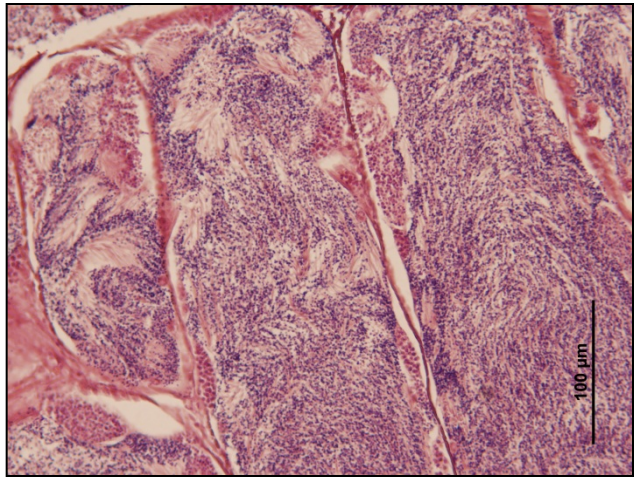


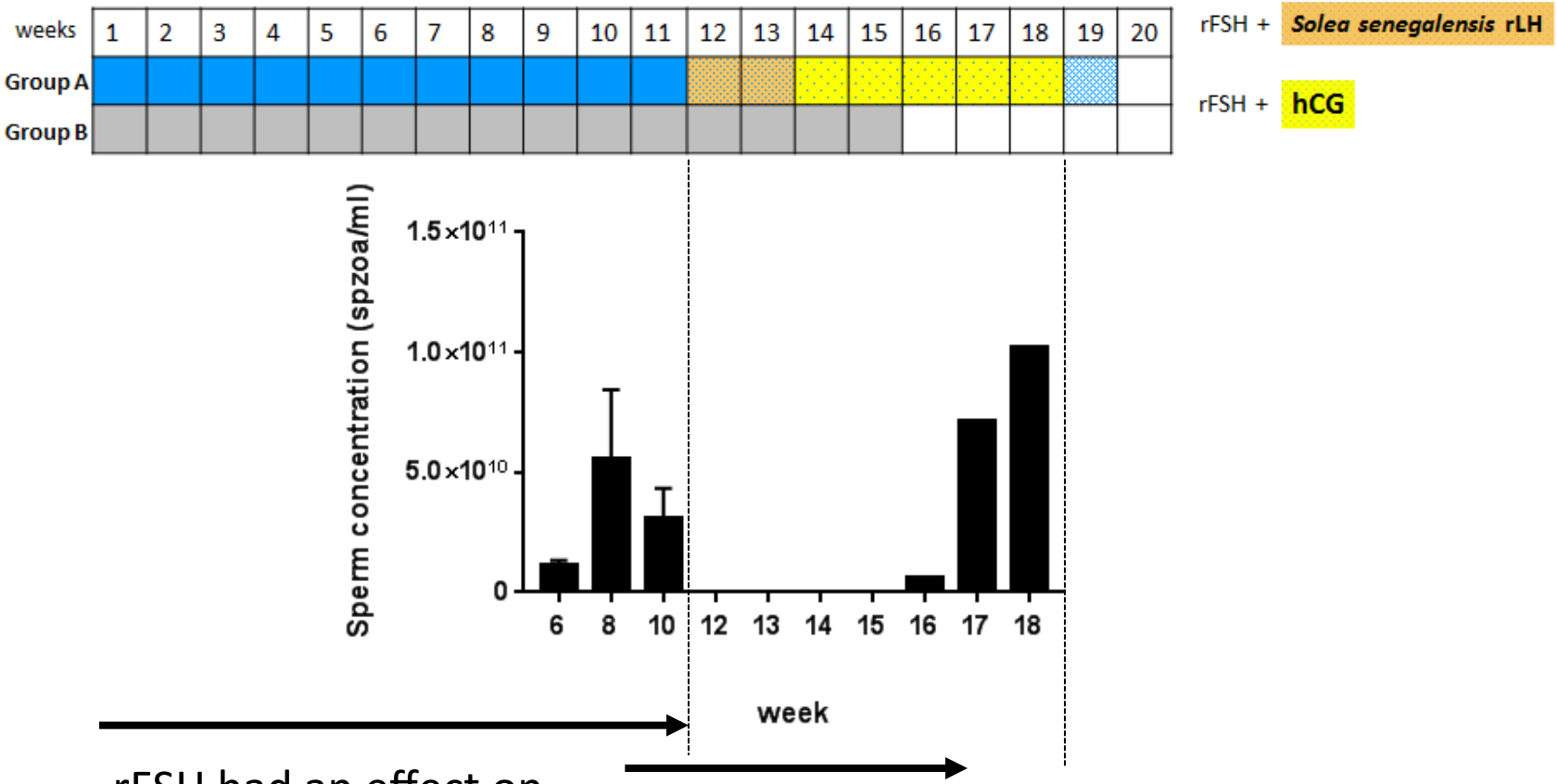
**GSI (%) = 1.81**



# RESULTS ♂




rFSH had an effect on spermatogenesis

- Additional hormonal stimulus is required to increase the seminal fluid volume and reduce sperm density ensuring high motility.
- It is difficult to conclude due to the low number of individuals tested.

# CONCLUSIONS

- rFSH produced in CHO cells can induce vitellogenesis from primary oocyte growth stages to mid and late stages of vitellogenesis.

- Additional hormonal stimulus is required prior to week 9 of r-FSH treatment to complete vitellogenesis and start maduration processes. The treatments combined with *Solea senegalensis*-LH, hCG and GnRHa plus Metoc did not increase oocyte diameter.

- rFSH produced in CHO cells can induce spermatogenesis.

- Also, additional hormonal stimulus is required from week 6 to 10 to increase the seminal fluid volume.

- Gonadal development appeared not to be synchronized. Males produced sperm earlier than females were in late-vitellogenesis. Administration of rFSH may be needed later in the case of males in order to ensure gonadal synchronization.


IRTA
RECERCA I TECNOLOGIA
AGROALIMENTÀRIES
DIVERSIFY
Thank you for your attention