



Induction of gonadal maturation in teleosts by recombinant gonadotropins

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ENVIRONMENTAL STIMULI

CENTRAL NERVOUS SYSTEM

HYPOTHALAMUS

GnRH

HYPOPHYSIS

FSH
LH

Testes

- Spermatogenesis
- Spermiogenesis

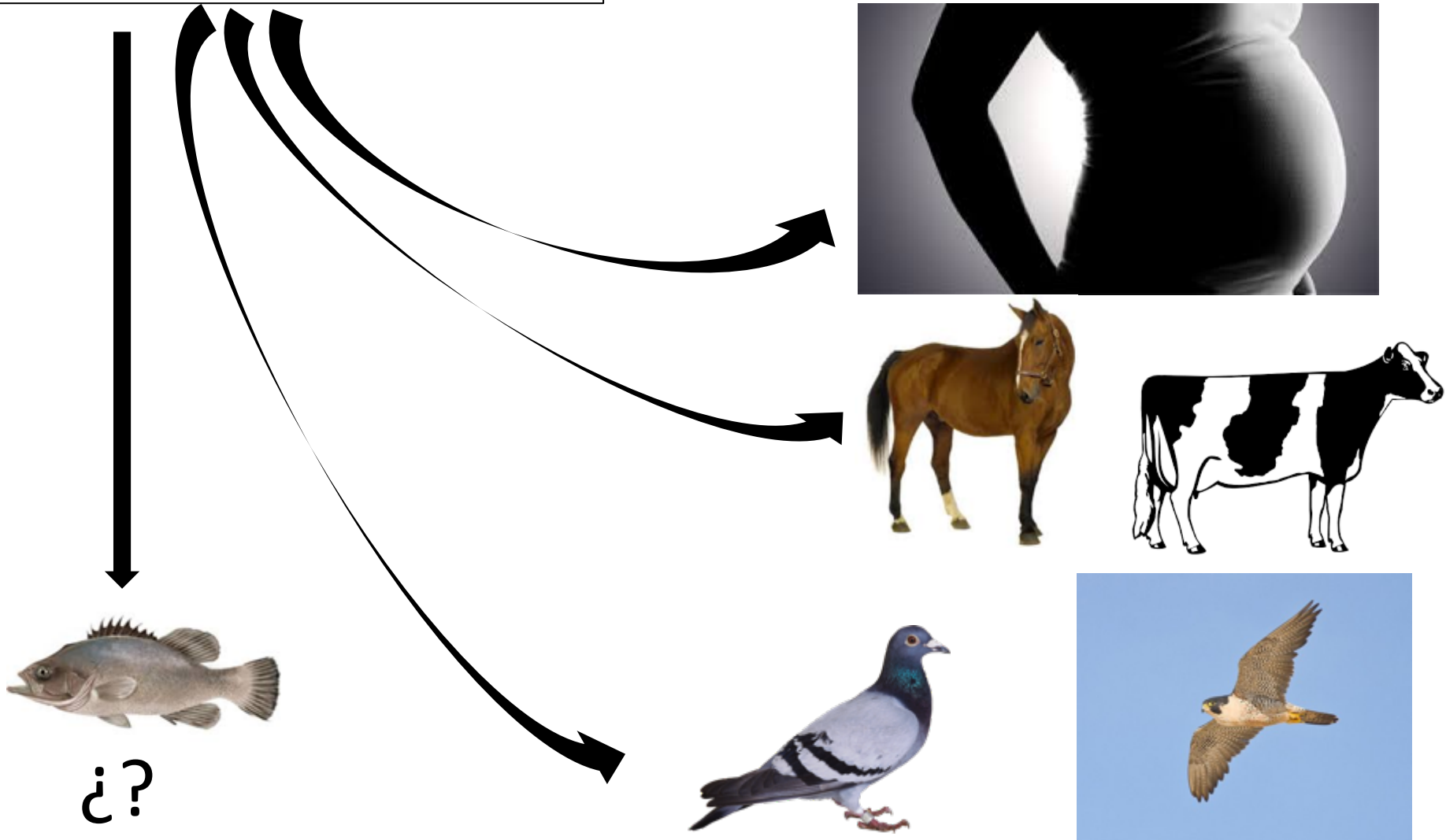
Ovaries

- Vitellogenesis
- Final maturation
- Ovulation

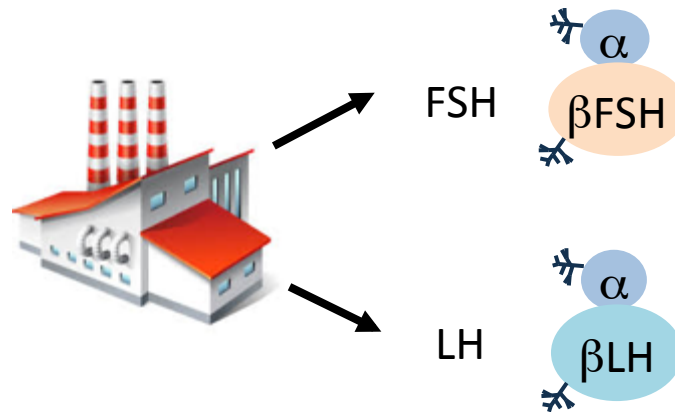


Full induction of reproductive cycles can be done

Recombinant Gonadotropins



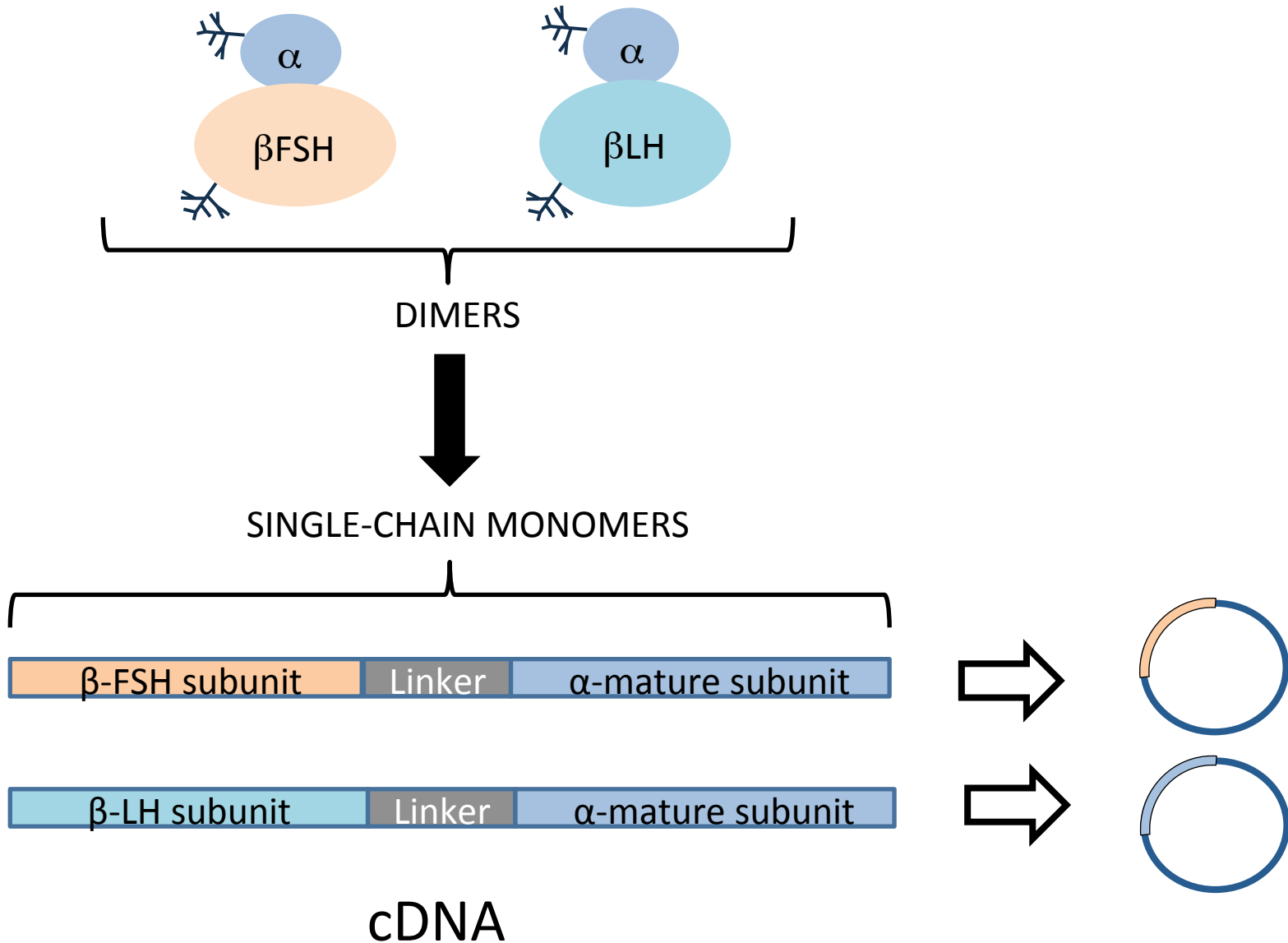
Production of recFSH and recLH



Difficulties:

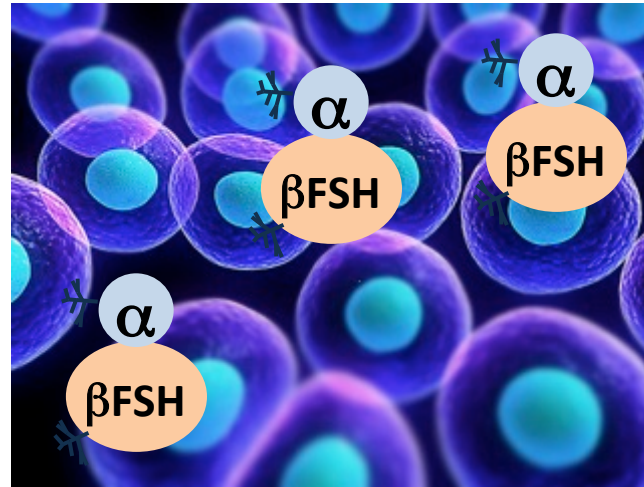
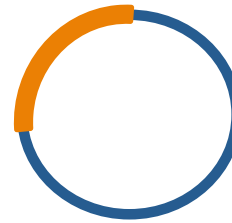
- Dimers
- Highly glycosylated proteins
- Small yields

Production of recFSH and recLH



Production of recFSH and rec LH

- Detection (developed antibodies)
- Purification
- Concentration
- Optimization of expression levels
- Bioassay



CHO CELLS

CHINESE HAMSTER OVARY CELLS

Why CHO cells?

Proper glycosilation is essential for activation of glycoprotein hormone receptors

Mammalian glycosylation pattern (more sialic acid capping)



Improved bioactivity

Pharmacokinetics: **Longer half-life**

CHO glycosylations are thought to be less immunogenic in vertebrates than glycosylations from other expression systems.

Disavandtage: Lesser protein yields than other expression systems.

But....

Glycoproteins used in human therapeutics are expressed in CHO cells (rhFSH and rHLH)

Dose?

Normal values



FSH : 2 ng/ml
LH : 1-10 ng/ml



FSH: 4-12 ng/ml
LH : 1-5 ng/ml



FSH: 1-3 ng/ml
LH : 2-3 ng/ml



FSH : 2-4 ng/ml
LH : 4-18 ng/ml



FSH : 5-15 ng/ml
LH : 2-14 ng/ml

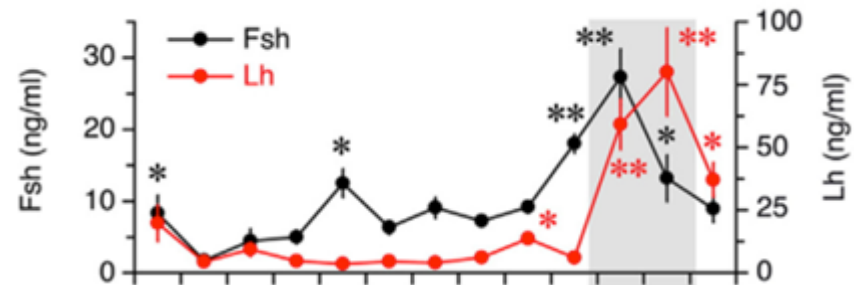


FSH : 18-40 ng/ml
LH : 1-2.7 ng/ml

Rocha et al. (2009)
Moles et al. (2012)



FSH: 80 ng/ml
LH : 85 ng/ml



Chauvigné F. et al. (2016)

Dose?

Human daily dose of rhFSH = 75 IU (standard dose) = 5,5 μg

0,1 $\mu\text{g}/\text{kg}$



FSH: 0,1 μg \longrightarrow 1 $\mu\text{g}/\text{kg}$



4,3 $\mu\text{g}/\text{kg}$

FSH: 0,15 μg \longrightarrow 0,6 $\mu\text{g}/\text{kg}$

FSH: 0,5 μg \longrightarrow 0,5 $\mu\text{g}/\text{kg}$



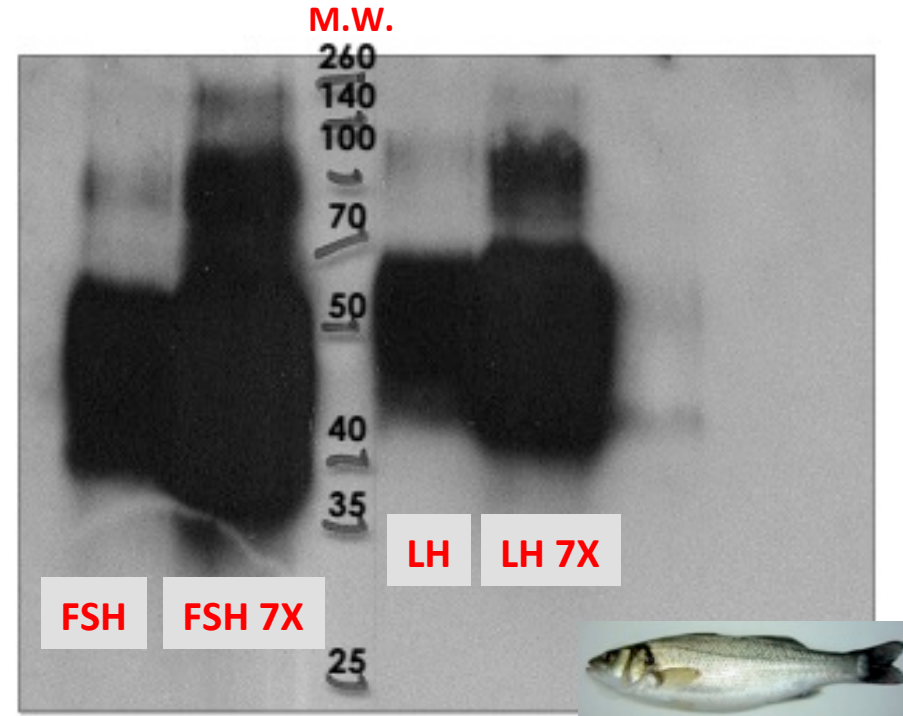
17 $\mu\text{g}/\text{kg}$ (weekly)

2,4 $\mu\text{g}/\text{kg}$ (daily equivalent)

Production of teleost recFSH and rec LH by RARA AVIS

Teleost recFSH and rec LH produced

- Sea bass
- Sole
- European eel



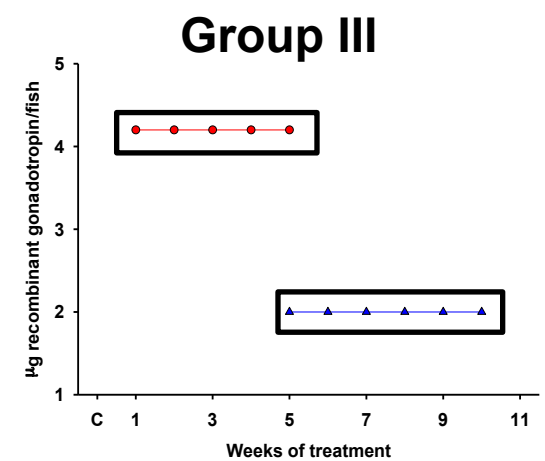
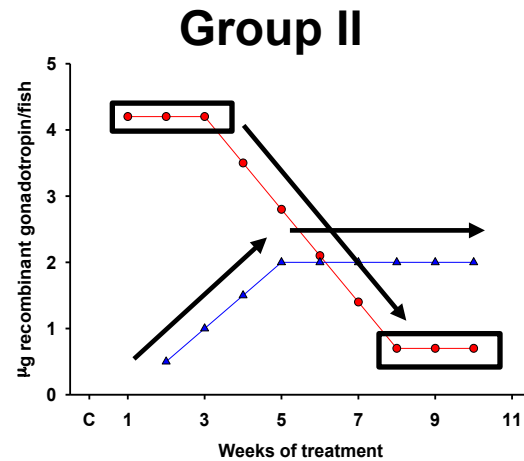
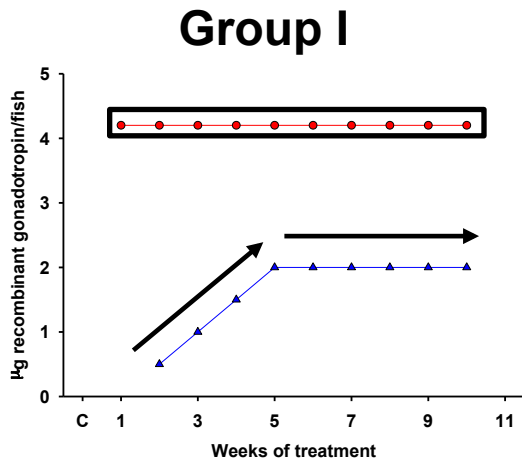
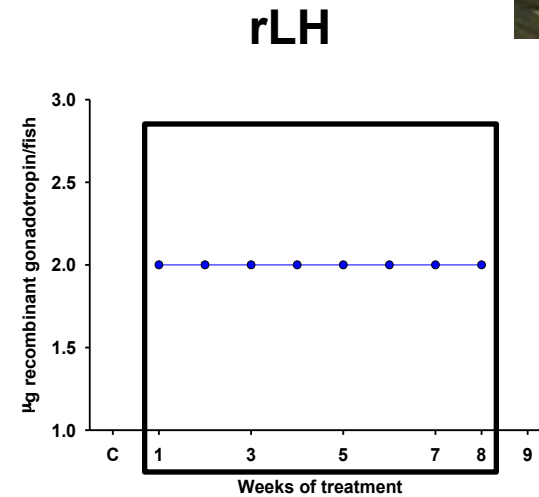
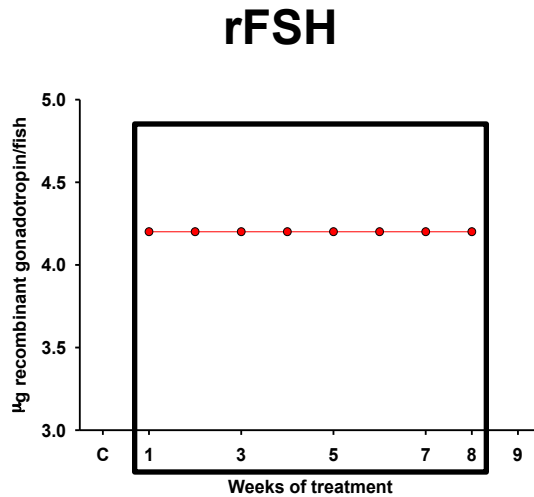
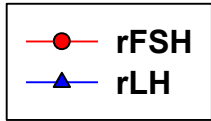
-Anti-alpha *Dicentrarchus* , Dil:1: 1000
-Anti-Rabbit-HRP, Dil : 1: 5000

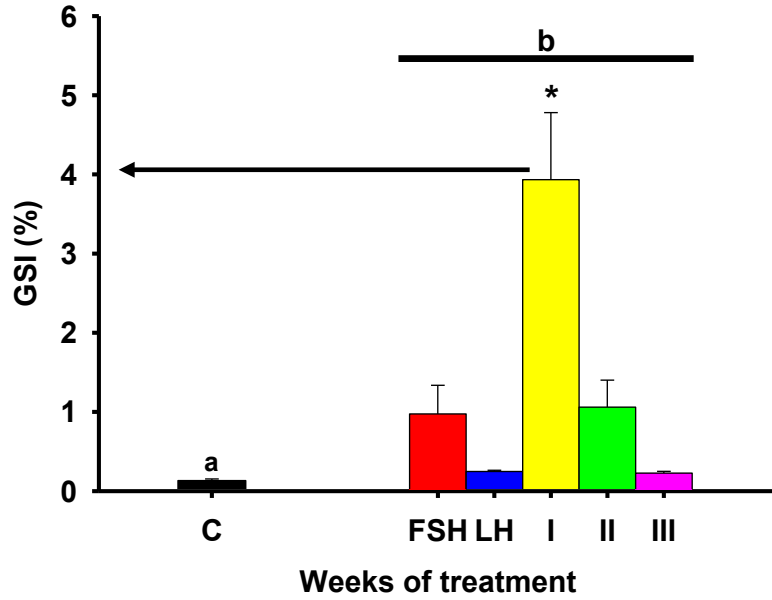
M.W.:Molecular Weight Marker.

Yield:

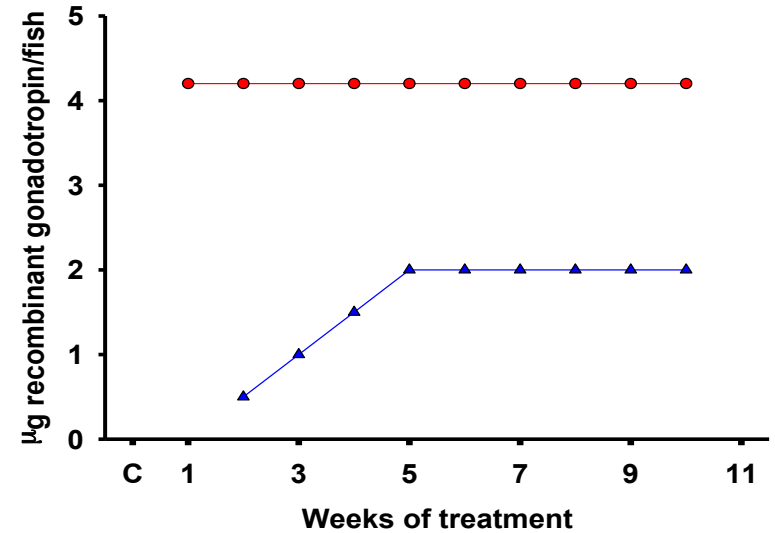
-FSH : 1,4 -2,2 mg/l
-LH : 1,8-2mg/l

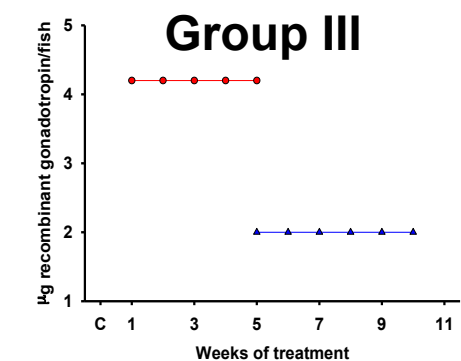
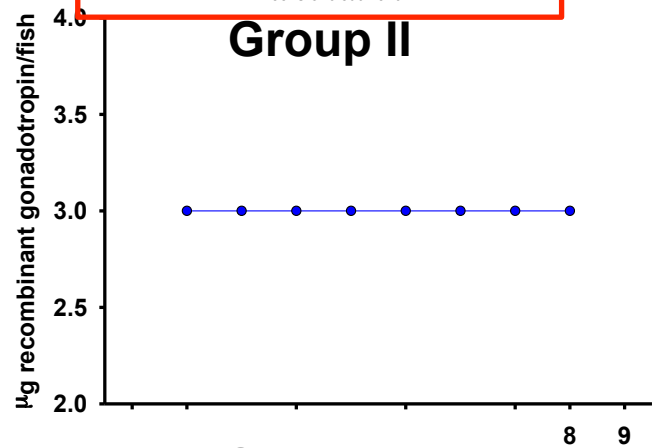
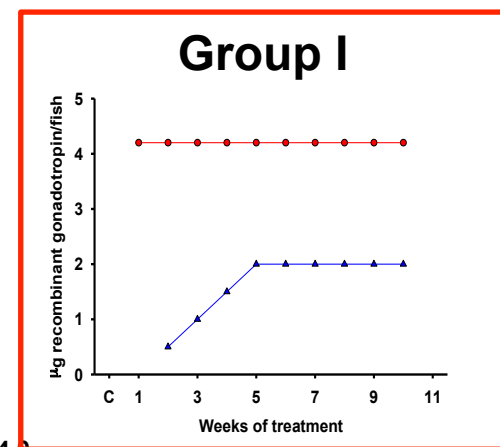
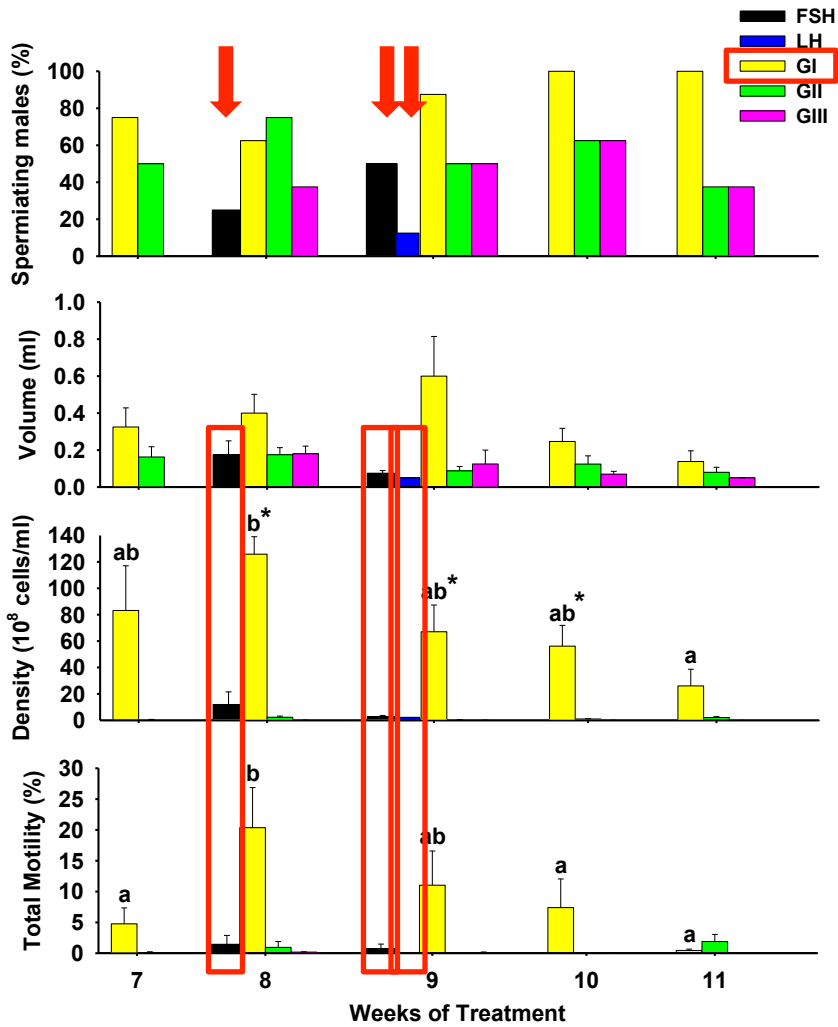
Male eels. Experimental Design

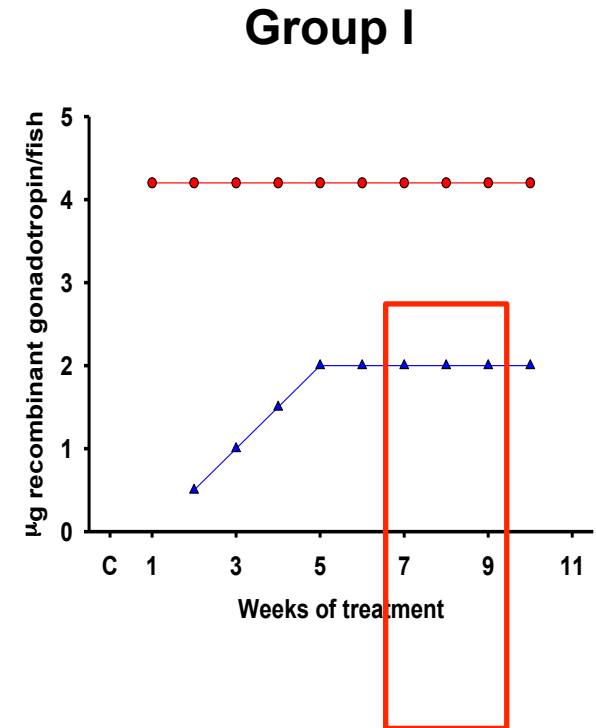
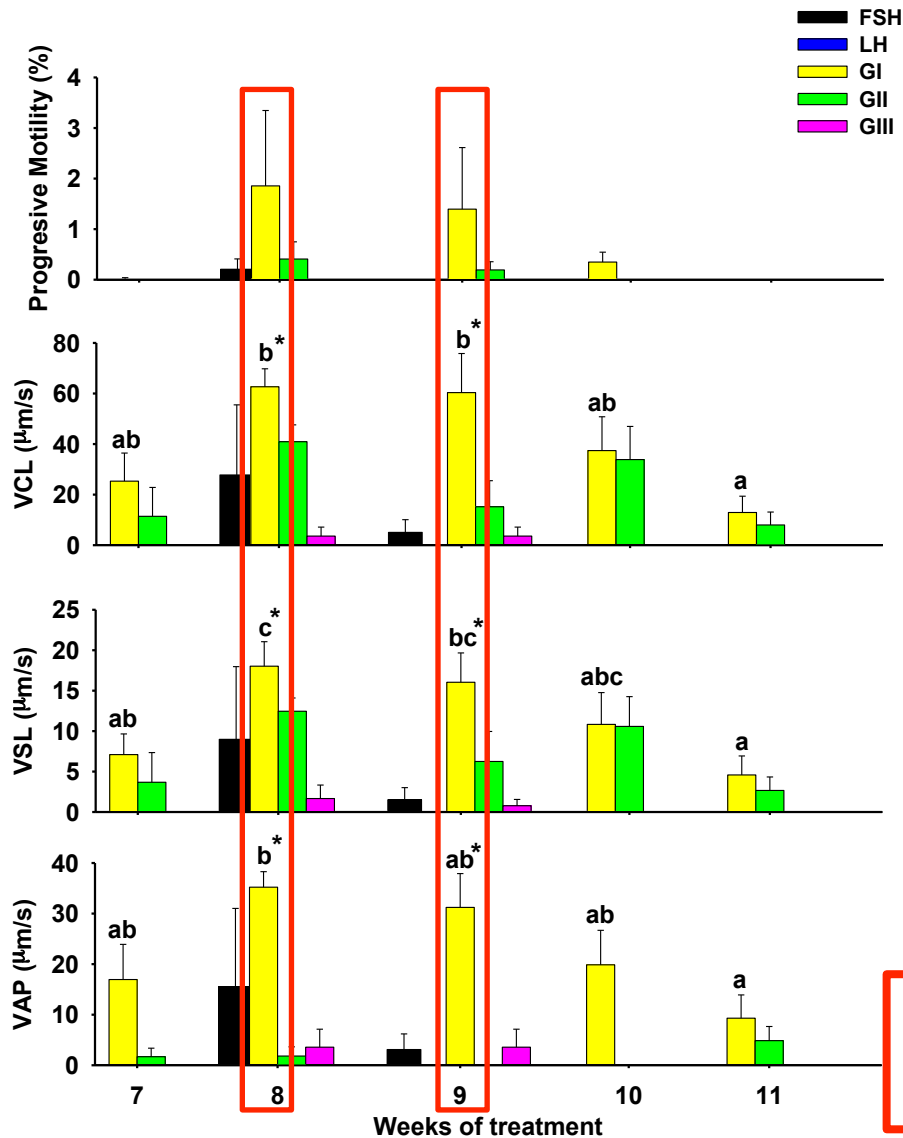




Group I



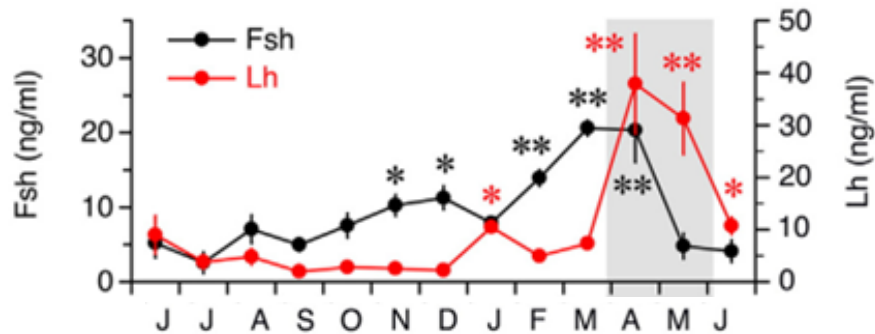




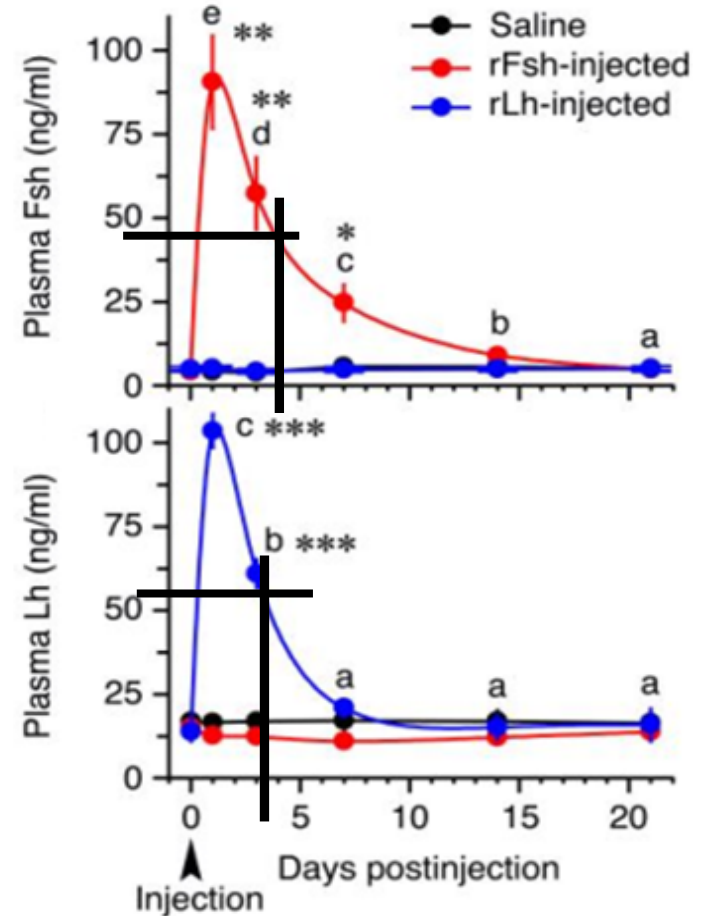
Actually, we are getting better results with **higher doses**

Half - life

Recombinant Sole FSH and LH
Dose = 17 $\mu\text{g}/\text{kg}$ (IM)



Native Sole FSH and LH (Males)

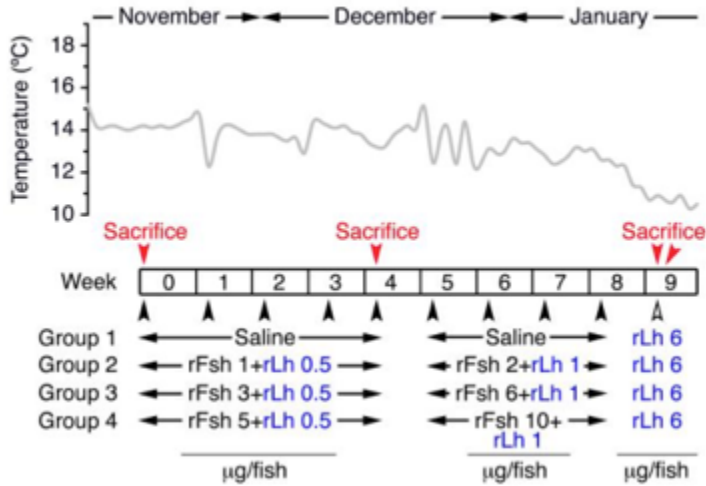


EXPERIMENT 1

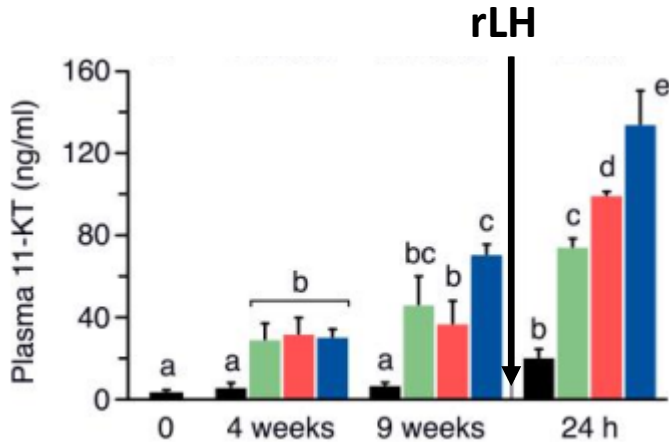
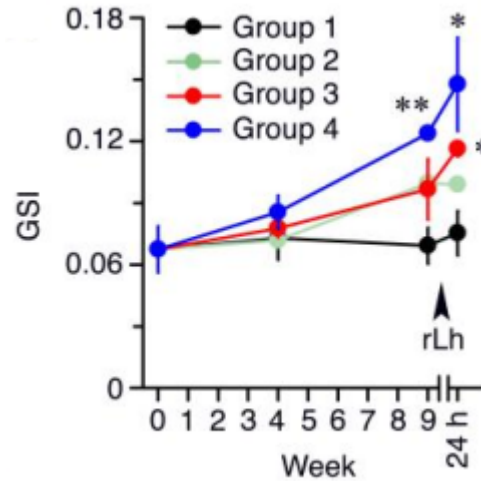


SOLE MALES

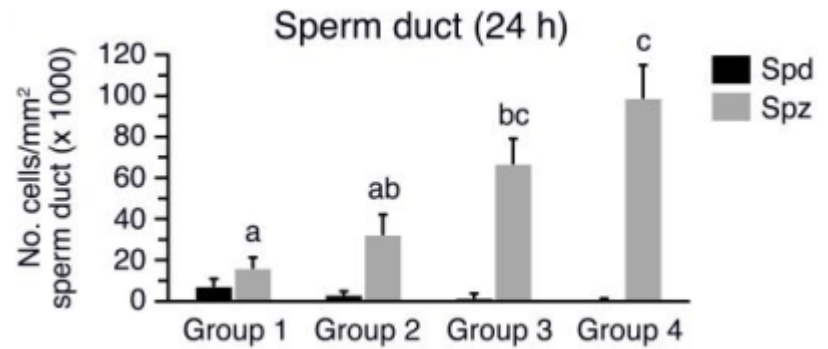
Experiment 1



EXPERIMENTAL DESIGN



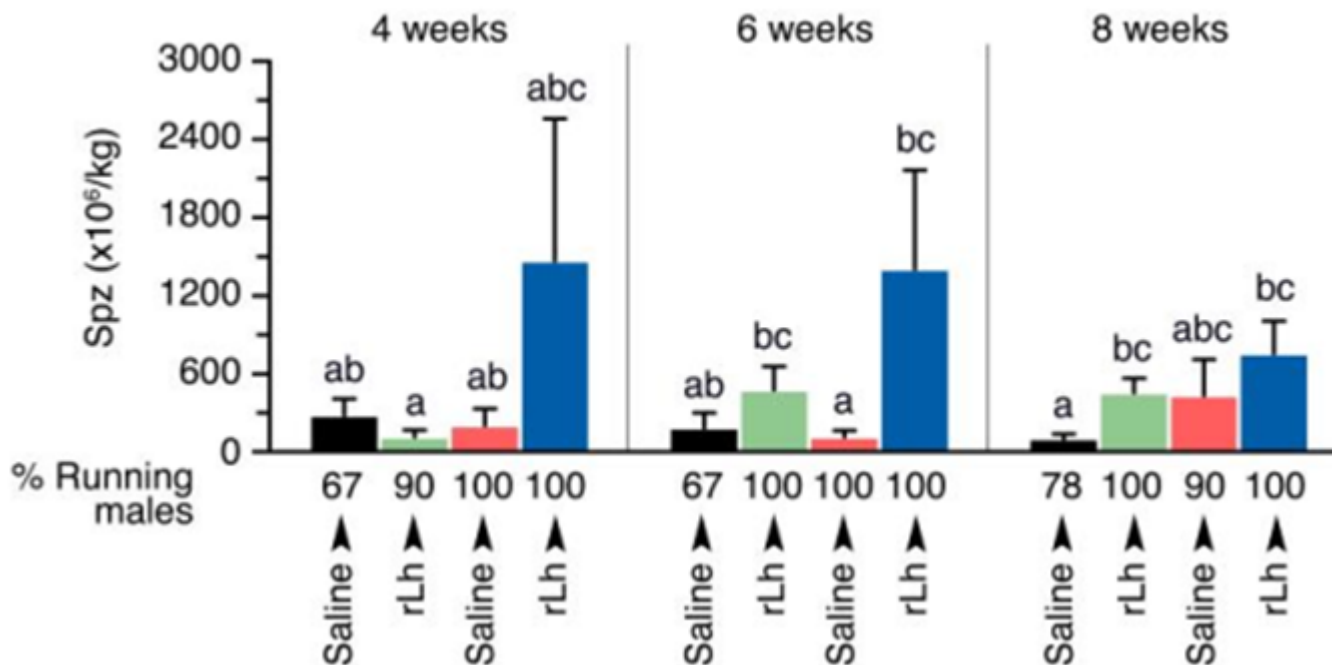
STEROIDOGENESIS



HISTOLOGY

EXPERIMENT 2

SPERM PRODUCTION



- Saline
- 6 µg LH biweekly
- 6 µg FSH weekly
- 6 µg FSH weekly + 6µg biweekly



WEIGHT: 354 ± 7 g



RESTARTING VITELLOGENESIS

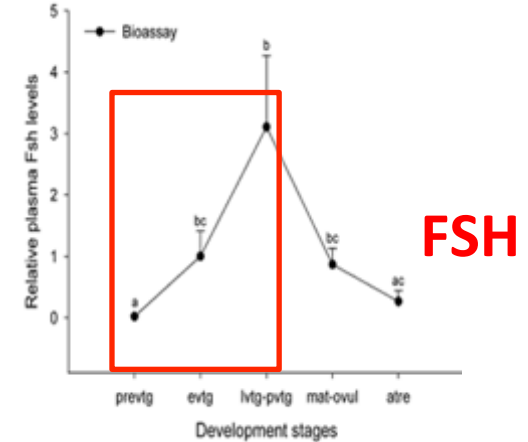
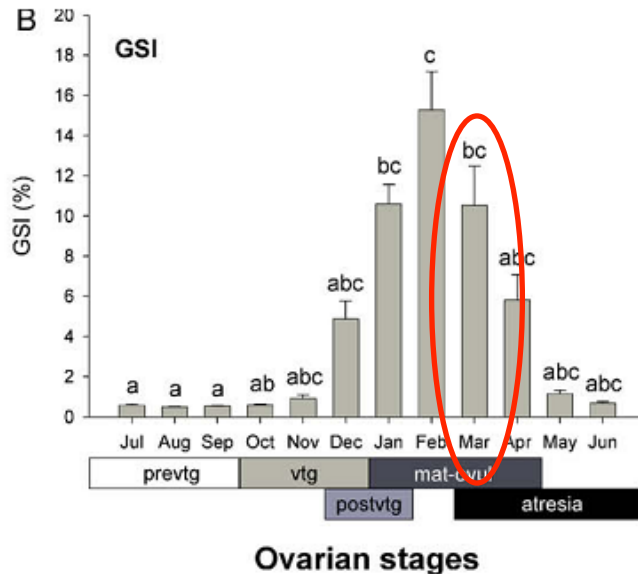
Dicentrarchus labrax



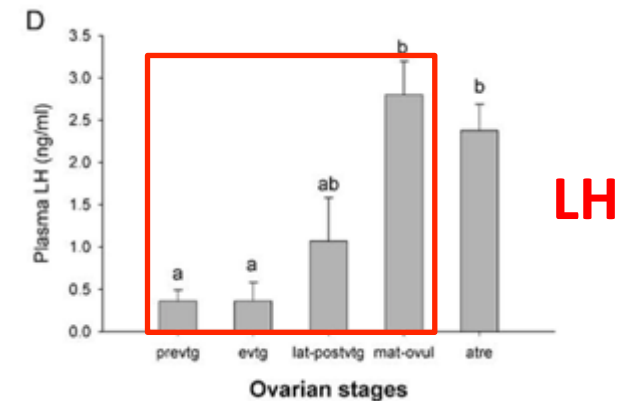
Weight: 204,2 ± 17,1 g

22 months

Natural conditions of photoperiod and T.



Molés G. et al. 2011





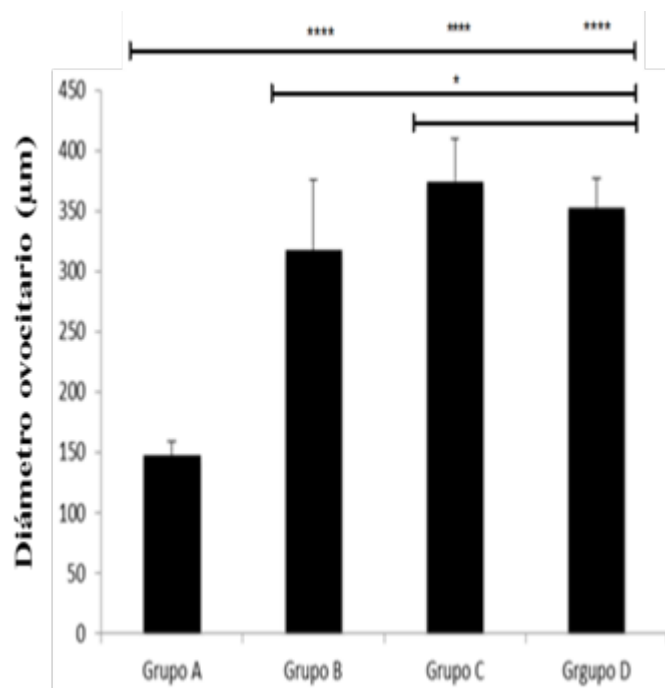
Group A: Saline

Group B: 1 µg rsbFSH (weekly) x 8 weeks

Group C: 2 µg rsbFSH (weekly) x 8 weeks

Group D: 4 µg rsbFSH (biweekly) x 8 weeks

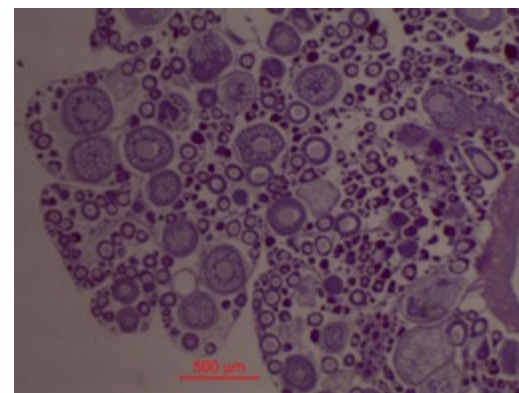
} + 1 µg rsbLH weekly (last 4 weeks)



OOCYTE DIAMETER

(100 greater oocytes/slide)

	Group A	Group B	Group C	Group D
Mean	146,8	317,1	374,3	352,2
SD	12,5	58,6	35,7	25,2
	100%	216%	255%	240%

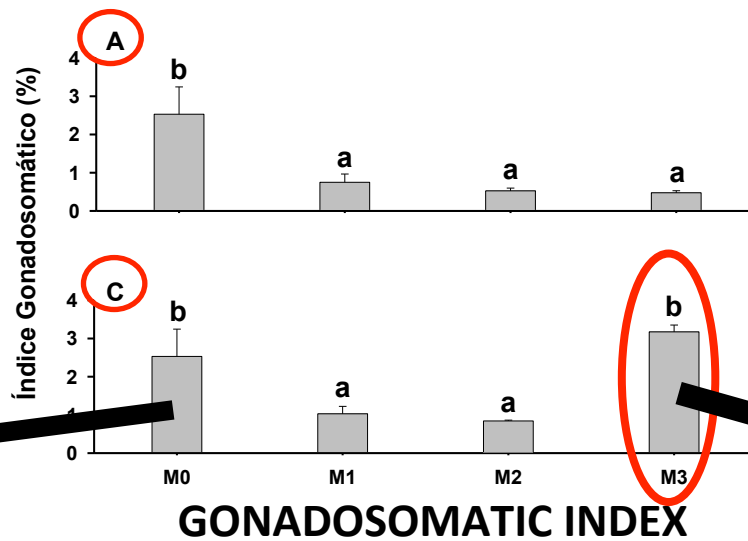
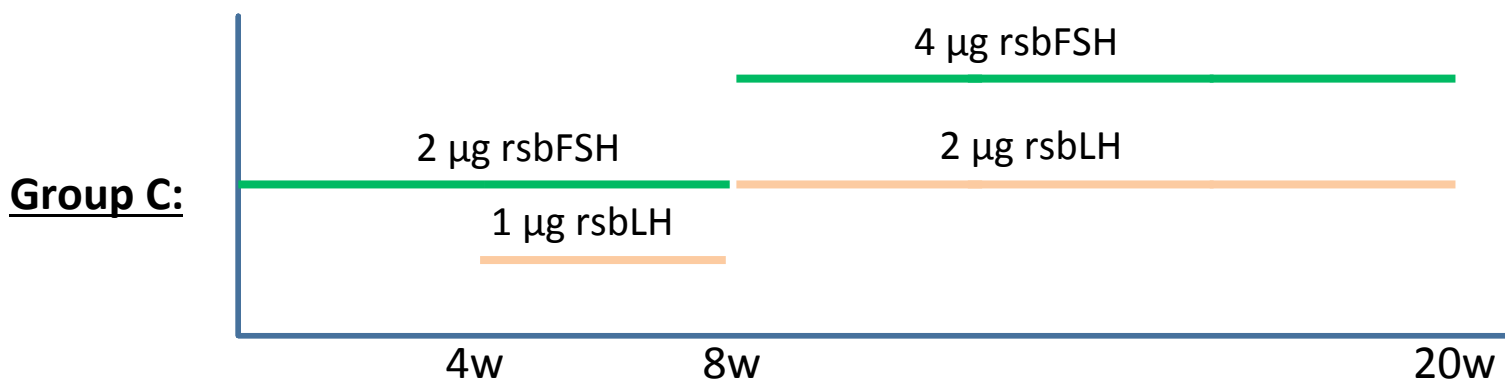




Experimental extension

Individuals of Groups **A** (n=10) and **C** (n=10) were treated for 12 more weeks.

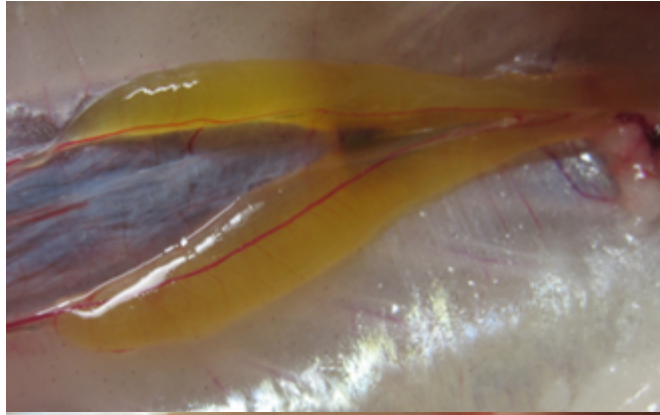
Group A: Saline



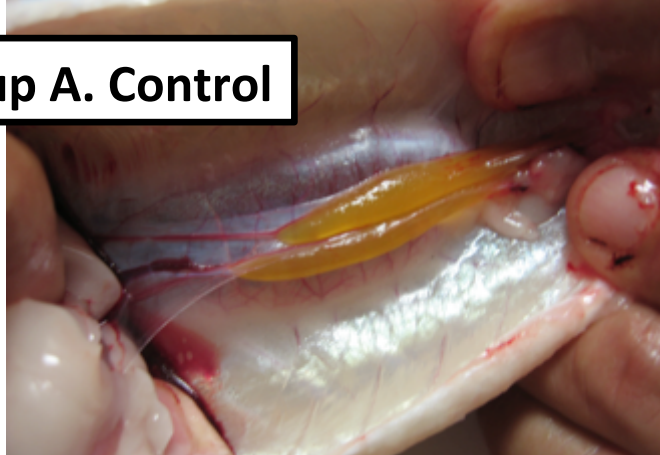
February

July 31 2015

End of the experiment. Macroscopy



Group A. Control

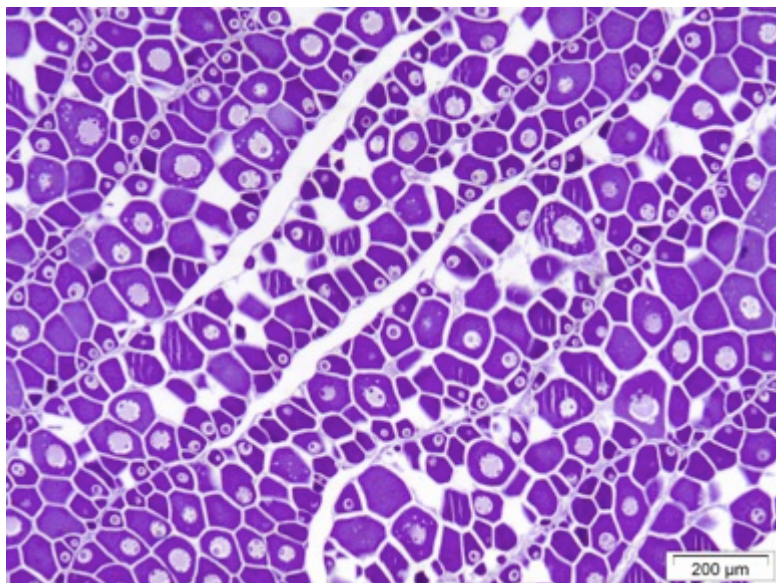


Group C

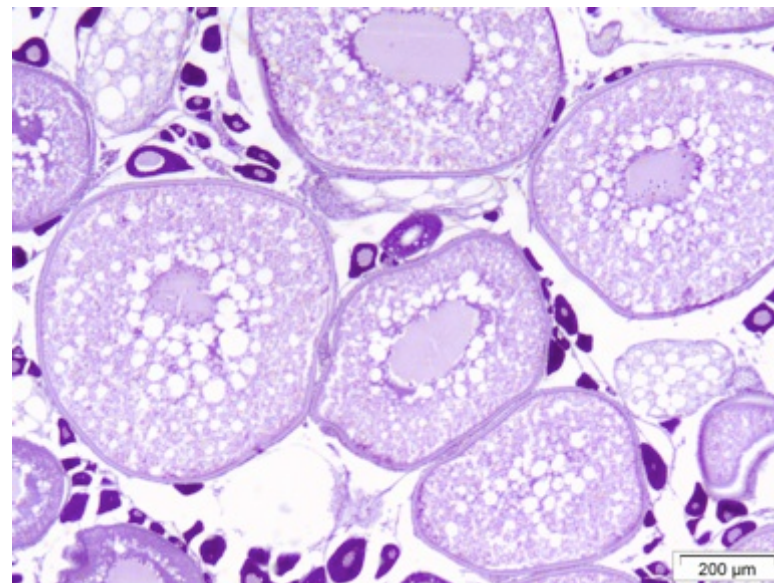


End of the experiment. Microscopy

Group A. Control



Group C



Measurement of 25 bigger oocyte diameters (μm) /slide.

	Group A	Group C
Mean	137,1	709,1
SD	11,5	55,5



Wreckfish : First attempt

Sea bass recombinant FSH and LH

+

2 GnRH IMPLANTS



Eggs laid and fertilized in July 2016

Conclusions

- Single-chain homologous recombinant gonadotropins have been expressed in CHO cells for :



- *Anguilla anguilla*
- *Solea senegalensis*
- *Dicentrarchus labrax*



- The single-chain recombinant gonadotropins have biological activity ***in vivo*** at the doses tested.
- The effective doses are in the range of doses used on birds and mammals
- The half-life of the hormones allows weekly (at least) treatments
- These recombinant gonadotropins are able to induce full gametogenesis from immature gonads and possibly, from prepubertal individuals. Also, they could be used to induce «out of season» cycles
- These recombinant gonadotropins could be useful in aquaculture to induce reproductive cycles and for research purposes



Thanks for your attention