

# Broodstock and Breeding



*Dicentrarchus labrax* and *Sparus aurata* egg diameters ~ 1mm

# Broodstock management

- Broodstock collection
- Breeding programs
- Spawning control
  - Natural - Photoperiod/temperature control
  - Induced - hormones
- Broodstock nutrition
- Water quality and environment
- Hygiene aspects
- Disease aspects
- Egg quality

# Broodstock management

## Prophylaxis



Fresh water / Formalin baths, Oxytetracycline

## Nutrition 2% body weight per day

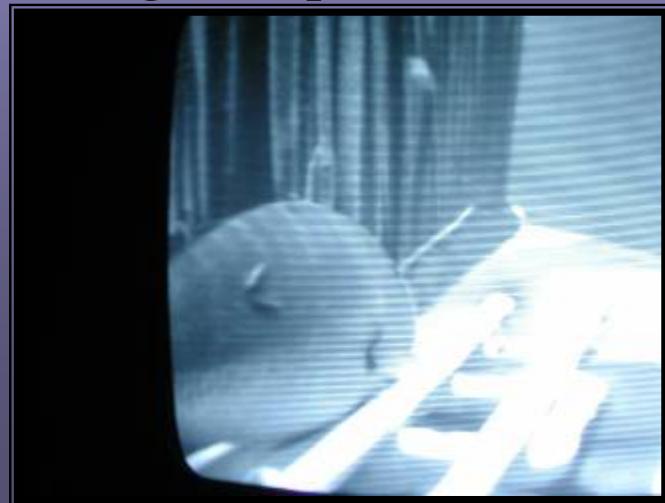


## Substrate / Monitoring



Aquamat®, Underwater Video, Artificial Cleaning Station

## Long-term parasite control

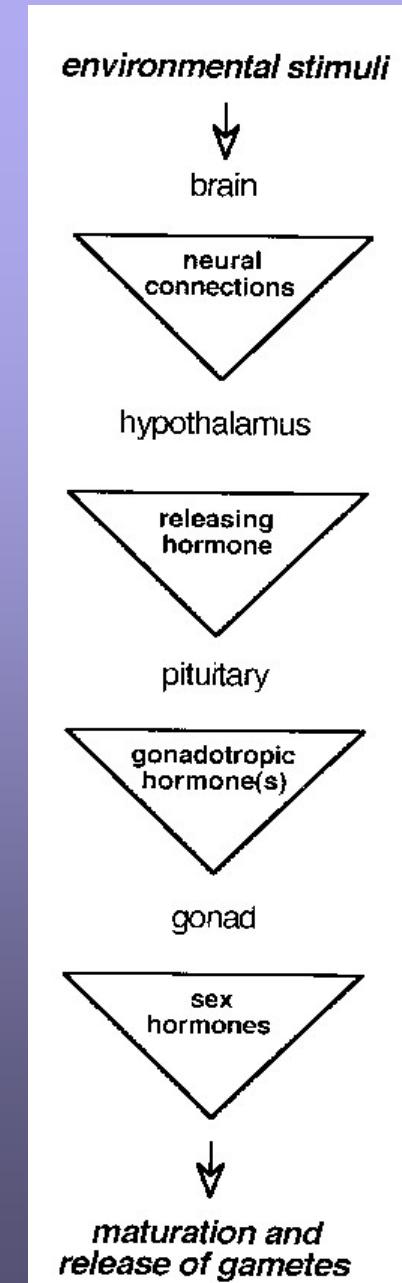


# Breeding Strategies

- Maximize survival of eggs and fry
  - natural habitat
- Ultimate Factors
  - food supply
  - absence of predators
  - water quality

# Breeding Strategies

- Proximate Factors
  - environmental cues
    - Temperature
    - Day length
    - Rainfall
    - Salinity
    - Lunar cycle
  - anticipate future spawning



# 3 strategies for production of eggs and spawning

- Synchronous spawning
  - One crop of eggs and sperm are produced and spawned at one time after which the fish dies e.g. Salmon
- Group synchronous
  - Groups of eggs are produced and spawned at one time but several cycles of development of spawning can occur (annual or other interval e.g. common aquaculture species)
- Asynchronous spawning
  - Continual development and spawning of oocytes

# Reproductive biology

## - freshwater fish

# Reproduction stages

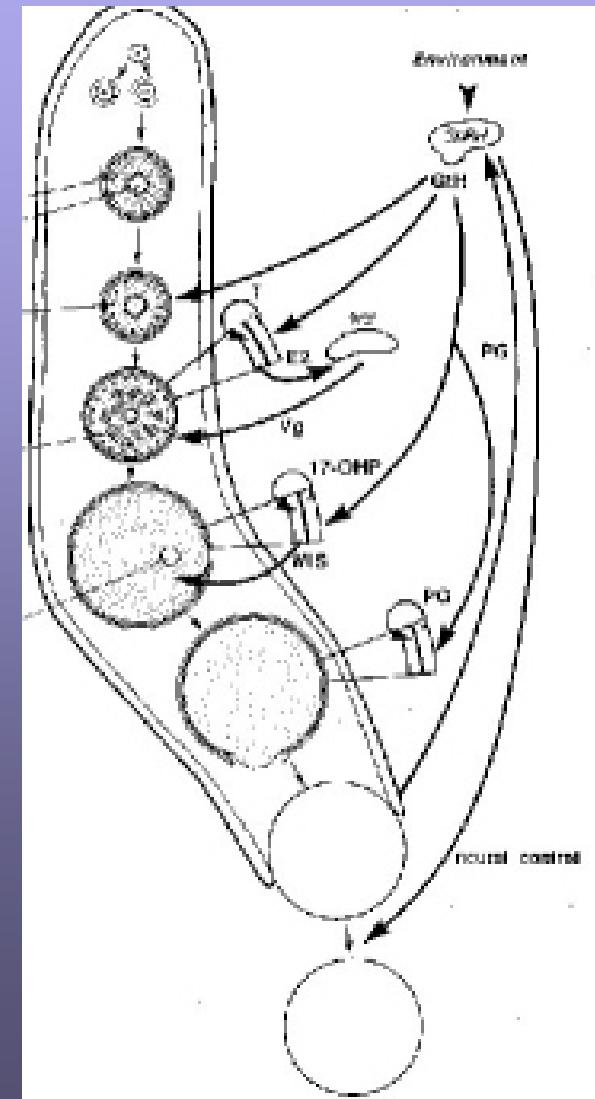
- Proliferation of oogonial cells in the ovary of the female fish that will develop into oocytes (eggs) & initiation of meiosis (reduction of chromosome numbers in half)
- **Stage I** – Development of basic cellular structures
- **Stage II** – Vitellogenesis – synthesis and uptake of egg yolk proteins which provide nutrients for the developing embryo
- **Stage III** – Maturation caused by the steroid hormone progesterone
- **Stage IV** – Spawning - hydration and fertilization
- **Stage V** - Recovery

# Stage I – Development of basic cellular structures

- Enlargement of the nucleus, appearance of multiple nucleoli and subcellular organelles including cortical aveoli (important in fertilization events)
- Endogenous vitellogenesis – protein synthesis
- Two cell layers ( theca & granulosa cells) develop and surround the oocyte to form a follicle
- Theca and granulosa cells are responsible for production of reproductive steroid hormones

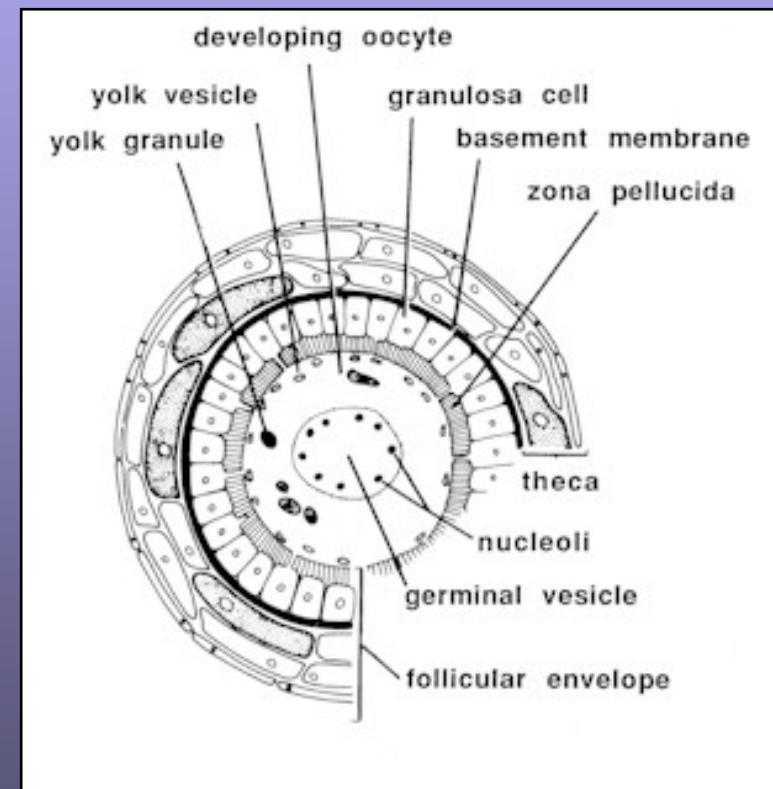
# Stage I: Oocyte Development

- Oogenesis
  - Oogonia give rise to oocytes
  - Oogonia undergo meiosis
  - Surrounded by epithelial cells (follicle)
  - Meiosis stops and enters a long state of cytoplasmic growth
- Gonadotropin independent and body size dependent



# Stage I: Yolk Vesicle Formation

- Response to Proximate Factors
- Surges in gonadotropin
  - Previtellogenic oocyte
  - Appearance of yolk vesicles
  - “endogenous vitellogenesis”
  - GtH triggers yolk vesicle formation

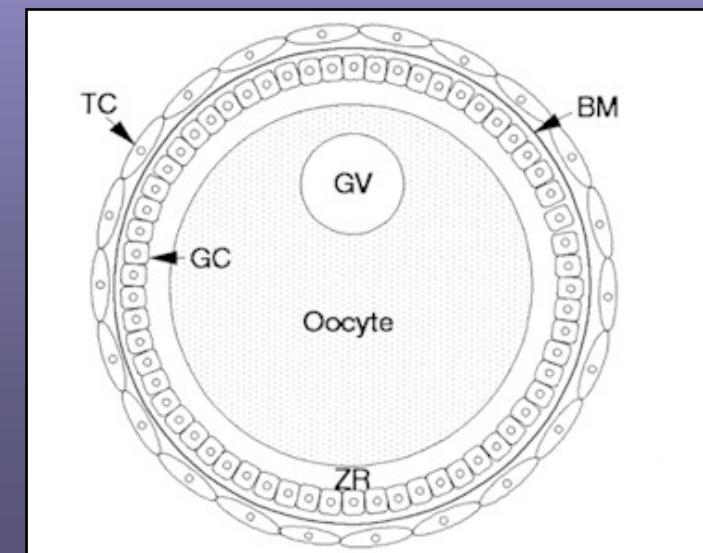


# Stage II – Vitellogenesis

- Interaction of the pituitary gland in the brain, the follicle cells, the liver and the eggs to produce hormones (gonadotrophins)
- Gonadotrophin stimulates the theca and granulosa cells to produce estrogen which in turn stimulates the liver to produce vitellogenin the precursor to egg yolk proteins
- Vitellogenesis is the longest stage of oocyte development and requires nutrient input either directly from feed or body stores of muscle and fat
- If insufficient amounts of egg proteins are deposited in the oocytes then fry development from these eggs will not complete development and have high mortalities as eggs or sac-fry

# Stage II: Vitellogenesis

- Vitellogenin (Vg)
  - Phospholipid produced in liver
  - Transported to oocyte via blood
  - Precursor to egg yolk protein
- High nutritional demand
- Longest phase



# Stage II: Vitellogenesis

- Egg yolk production
  - Theca cells
    - Testosterone (T)
    - Induced by GtH
  - Granulosa cells
    - Estradiol ( $E_2$ )
  - $E_2$  stimulates liver to produce Vg
- End of Vitellogenesis
  - GtH II produced in higher levels than GtH I
  - Follicle produces maturation inducing substance (MIS)



# Stage III – Maturation

- Final stage of oocyte development and lasts 24-72 hrs
- Nucleus of the egg migrates from the centre of the egg to the periphery and meiosis resumes again to pause before completion
- The membrane surrounding the nucleus disappears – geminal vesicle breakdown
- Uptake of water occurs in some species at this point
- When maturation is complete the oocytes are ovulated from the follicle due to influence of prostaglandin's
- Depending on the species
  - Some oocytes retained in the ovary
  - Eggs released into the peritoneal cavity until spawning

# Stage III: Oocyte Maturation

- Maturation occurs in less than 24 hrs
  - Germinal vesicle migration
  - Germinal vesicle breakdown
  - Resumption of meiosis
  - Water uptake



## Stage IV – Spawning - hydration and fertilization

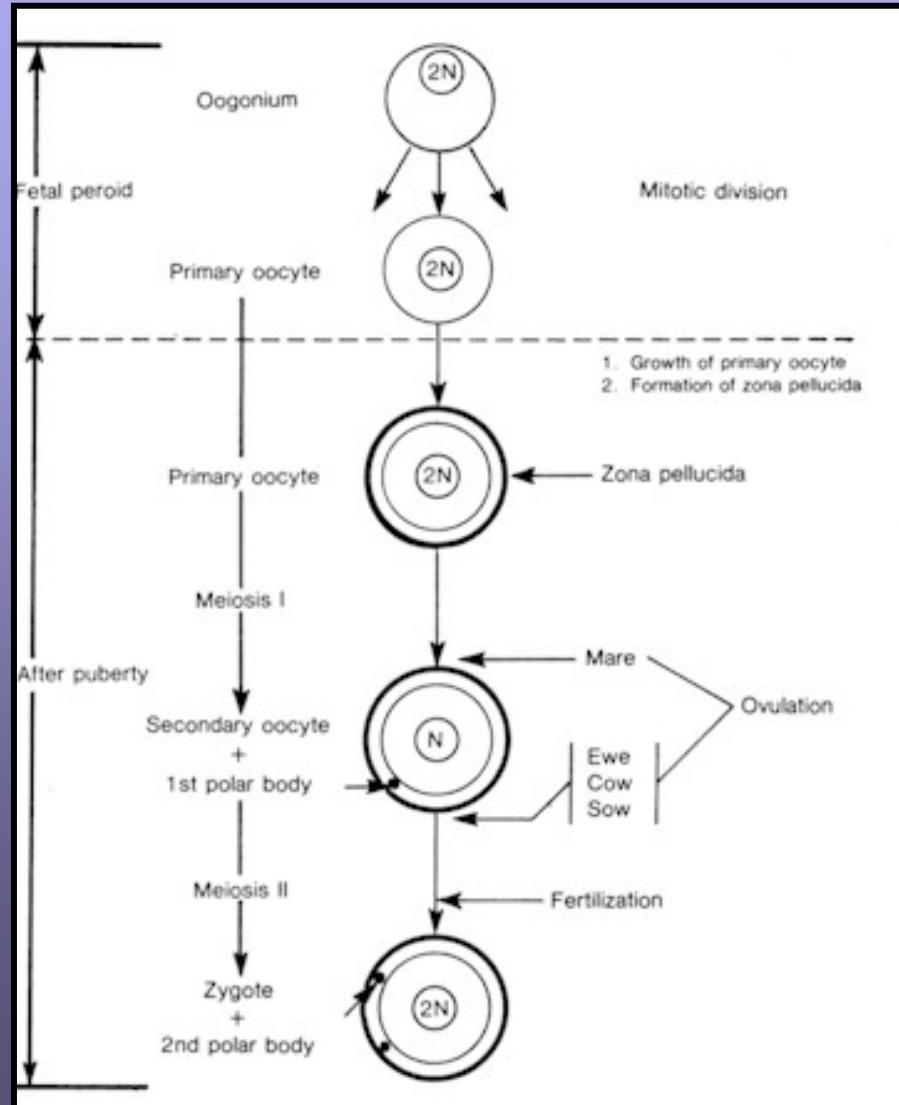
- Occurs shortly after maturation of the eggs or the eggs will become overripe
- Spawning behaviour depends on the species
- Spawning can be induced by injection of gonadotrophin hormones(stage III) – carp pituitary extract or human chorionic gonadotrophin
- At fertilization activation of the oocytes involves the release of the contents of the cortical alveoli into the space between the egg membrane and the chorion which forms the egg shell. There is a hole in the chorion called the micropyle which allows the sperm to pass through and fertilize the egg.
- On activation and the release of the cortical alveoli the chorion lifts off the egg and the micropyle begins to close

# Stage IV: Spawning

- Optimum environment
  - Mating behaviors
- Ovulation controlled by F-prostaglandin (PG)
  - PG release stimulated by MIS
  - GtH II is required for this process
- Common stage for hormone therapy

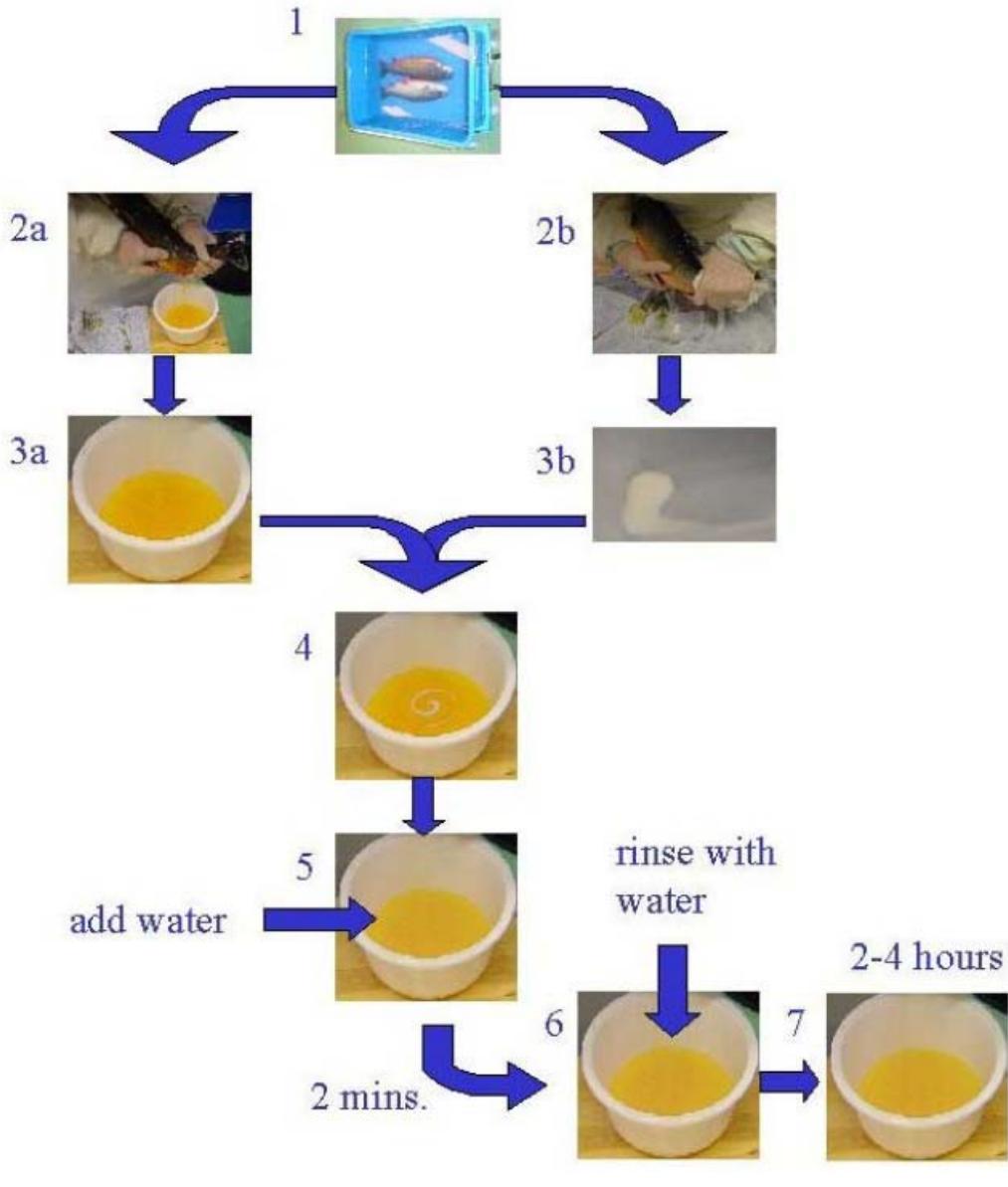
# Stage V Recovery

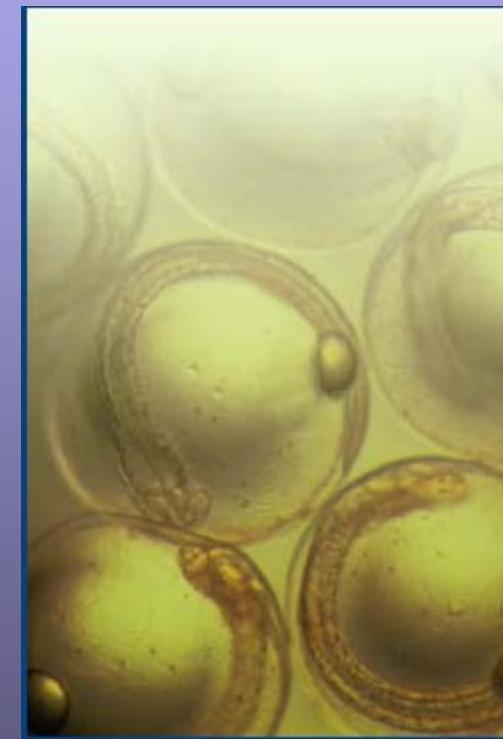
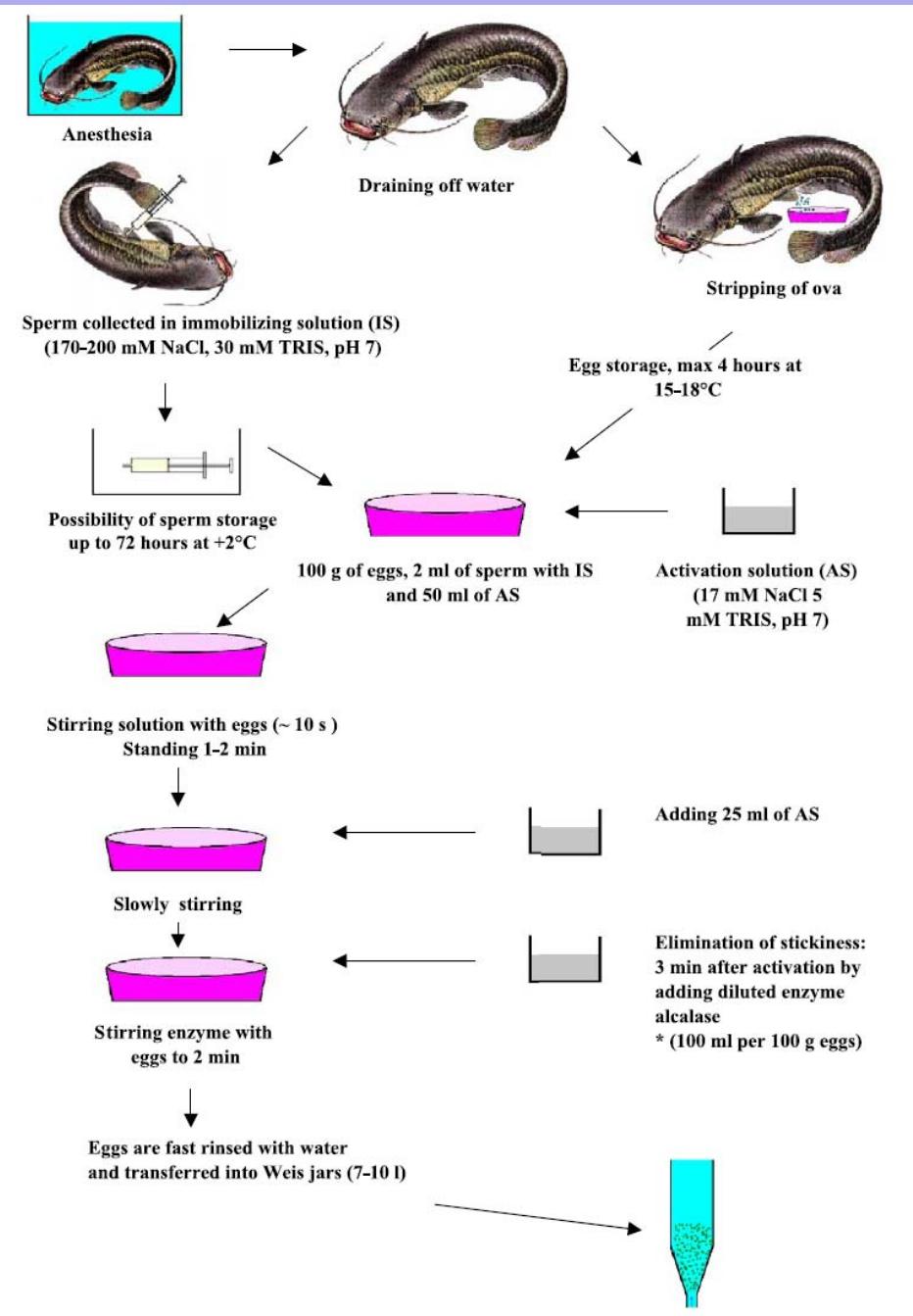
- Restore body condition
- New oocyte development



# Overview of the stripping process

Cover the head Towel to have  
to prevent good grip  
stress





# Spawning

- Spawning behaviour
- Egg release
- Pheromone release
- Release of milt
  - Fertilization

## Methods

- Simulate natural environment
- Dry stripping
- Hormone induction

# Spawning

- Simulate natural environment
  - Nesting basins
  - Floating plants
  - Floating nests
  - Fiber mats



# Spawning

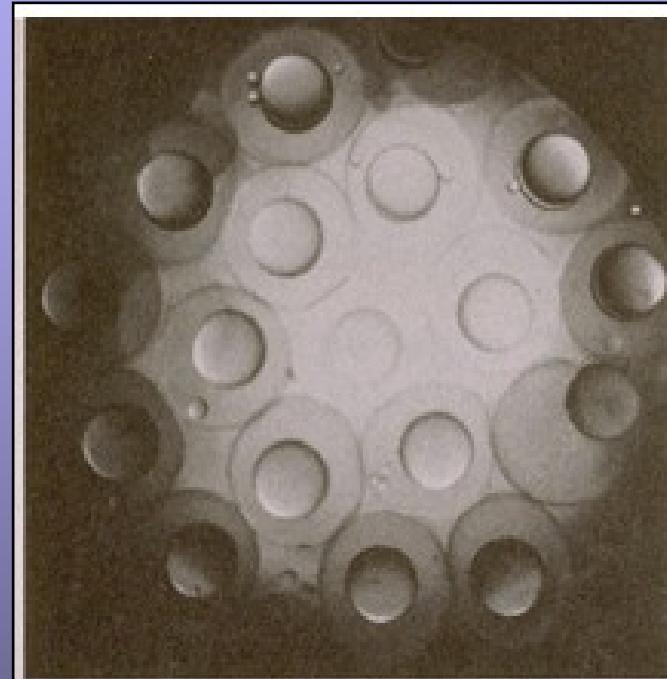
- Dry Stripping
  - Strip eggs and milt
  - Mix together
  - Add water
    - Activate eggs and milt  
closure of micropyle
  - Separation of sticky eggs  
eg Silting

**MS222 is toxic to eggs and milt!**



# Spawning

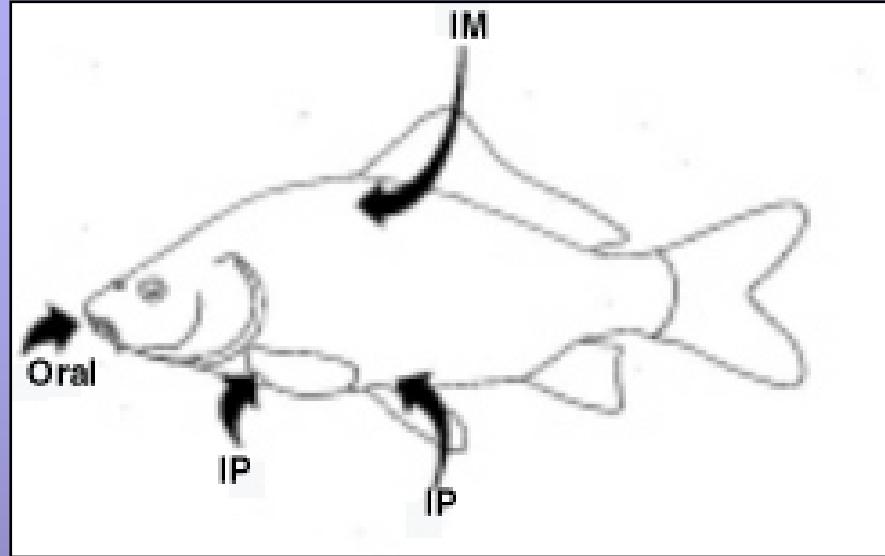
- Induced Spawning
  - Stage 3 Oocyte maturation
  - Egg Sampling
    - Egg size
    - Yolk clarity
    - Oil globules



Ripe eggs at ovulation

# Spawning

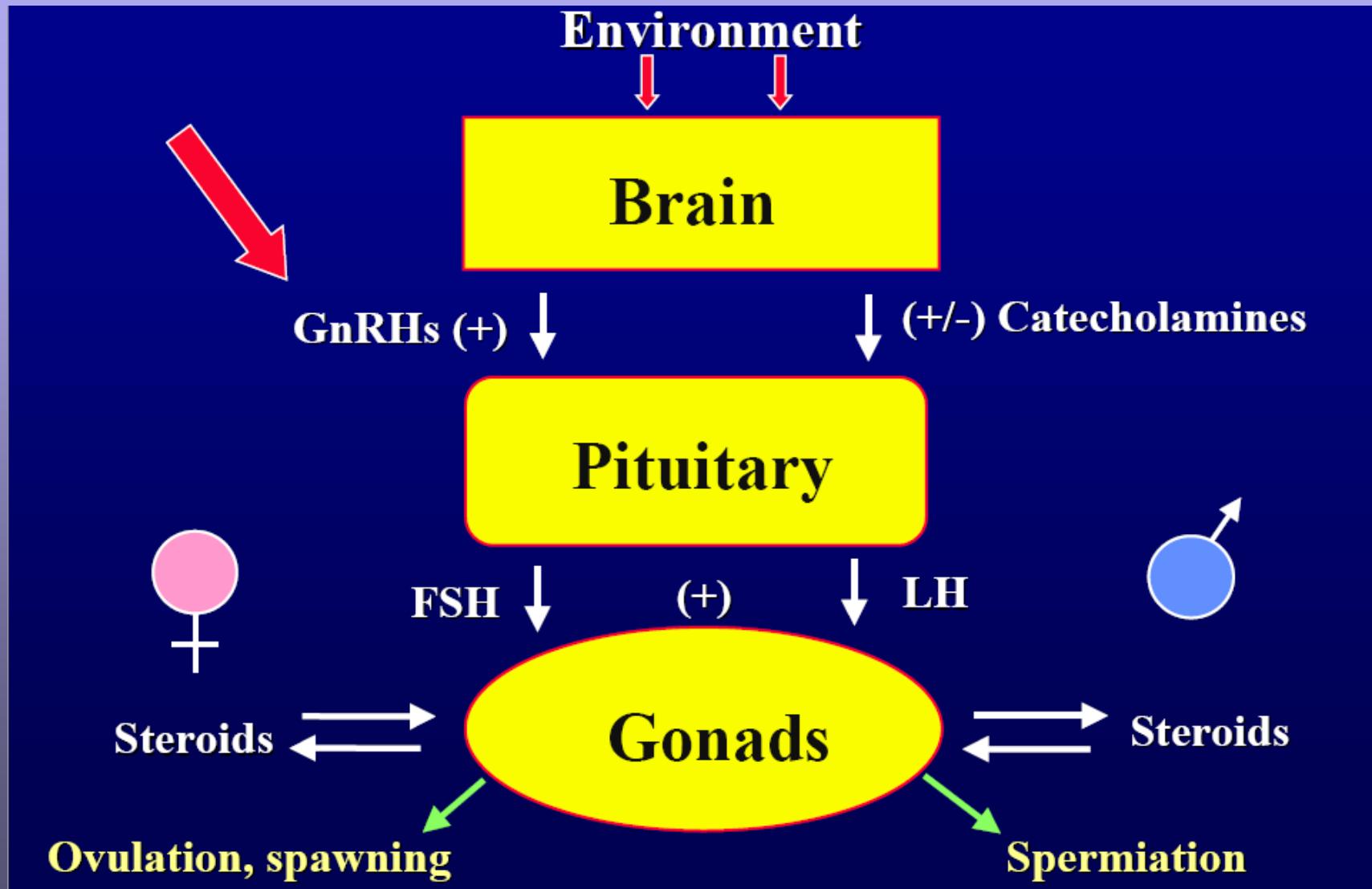
- Oral
- Hormone Injection
  - Intra muscular
  - Intra peritoneal
    - Carp Pituitary Extract
    - Human Chorionic Gonadotropin (HCG)
    - GnRHa
- Single or multiple injection
  - Stage 3 complete in 12 - 48 h
- Slow release capsules
- Natural Environment or Dry Stripping



# Induced spawning

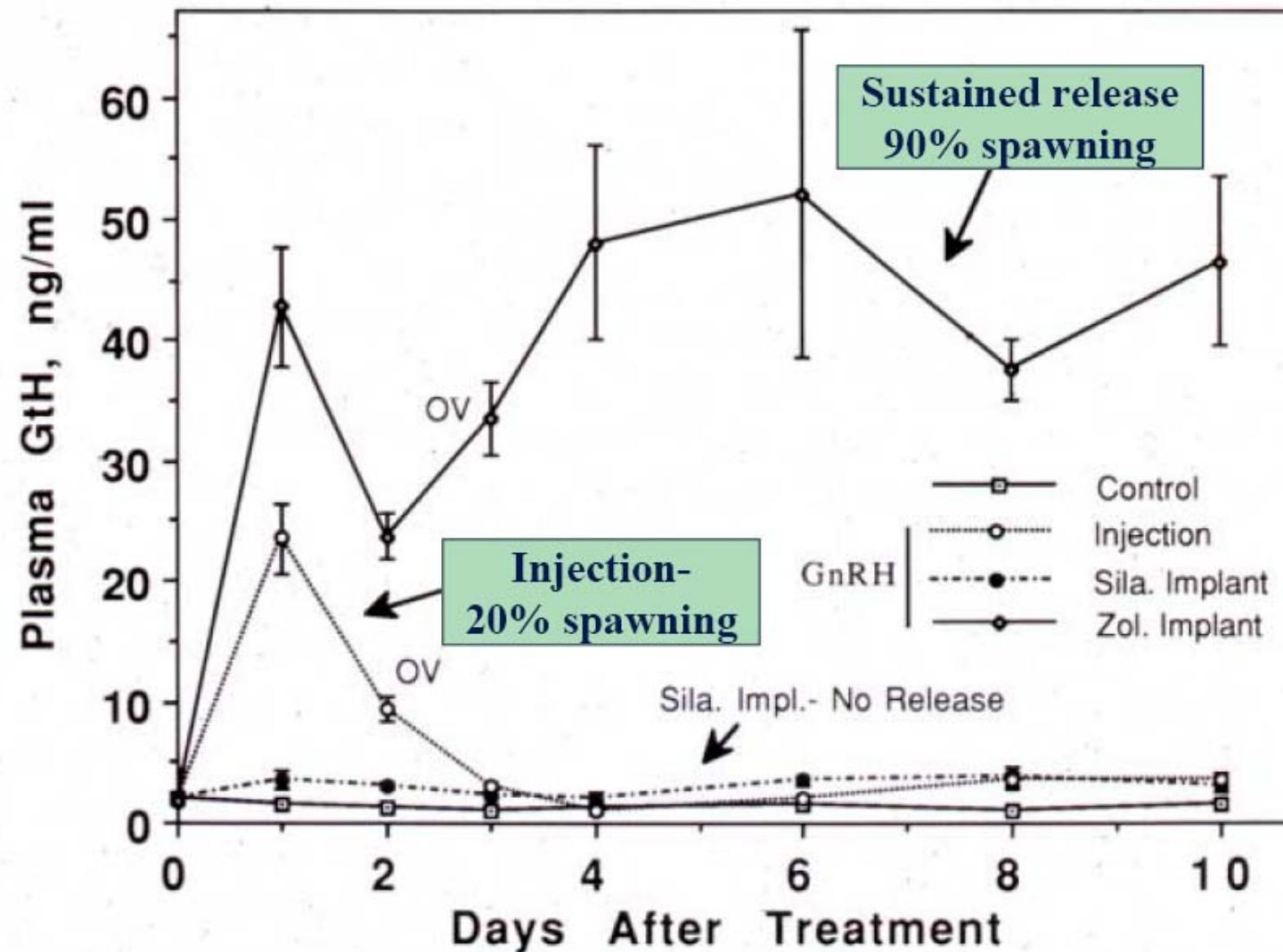
- Induced spawning
- Natural spawning

# Regulation of spawning



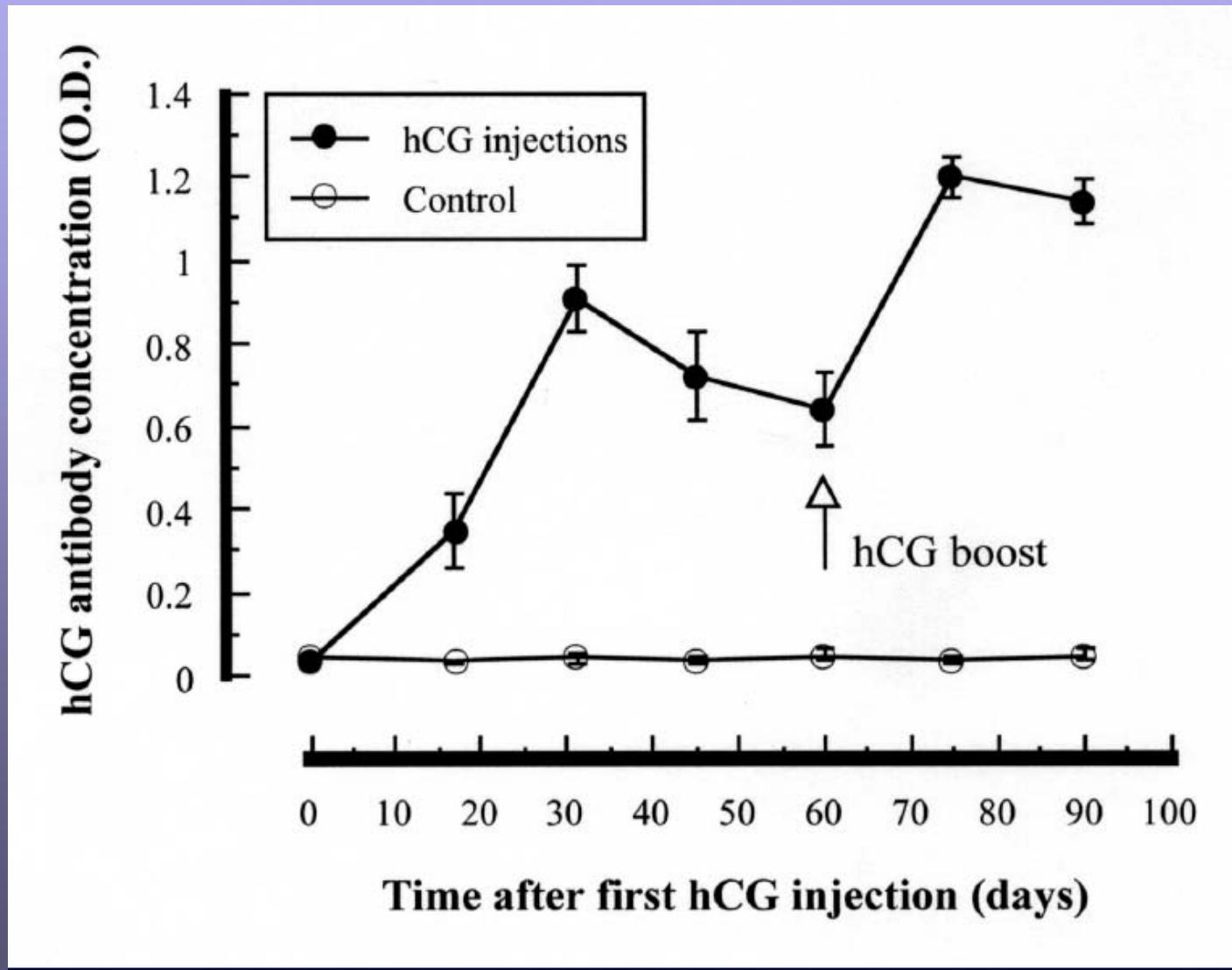
# Methods of inducing spawning

## Modes of GnRHa administration in gilthead seabream



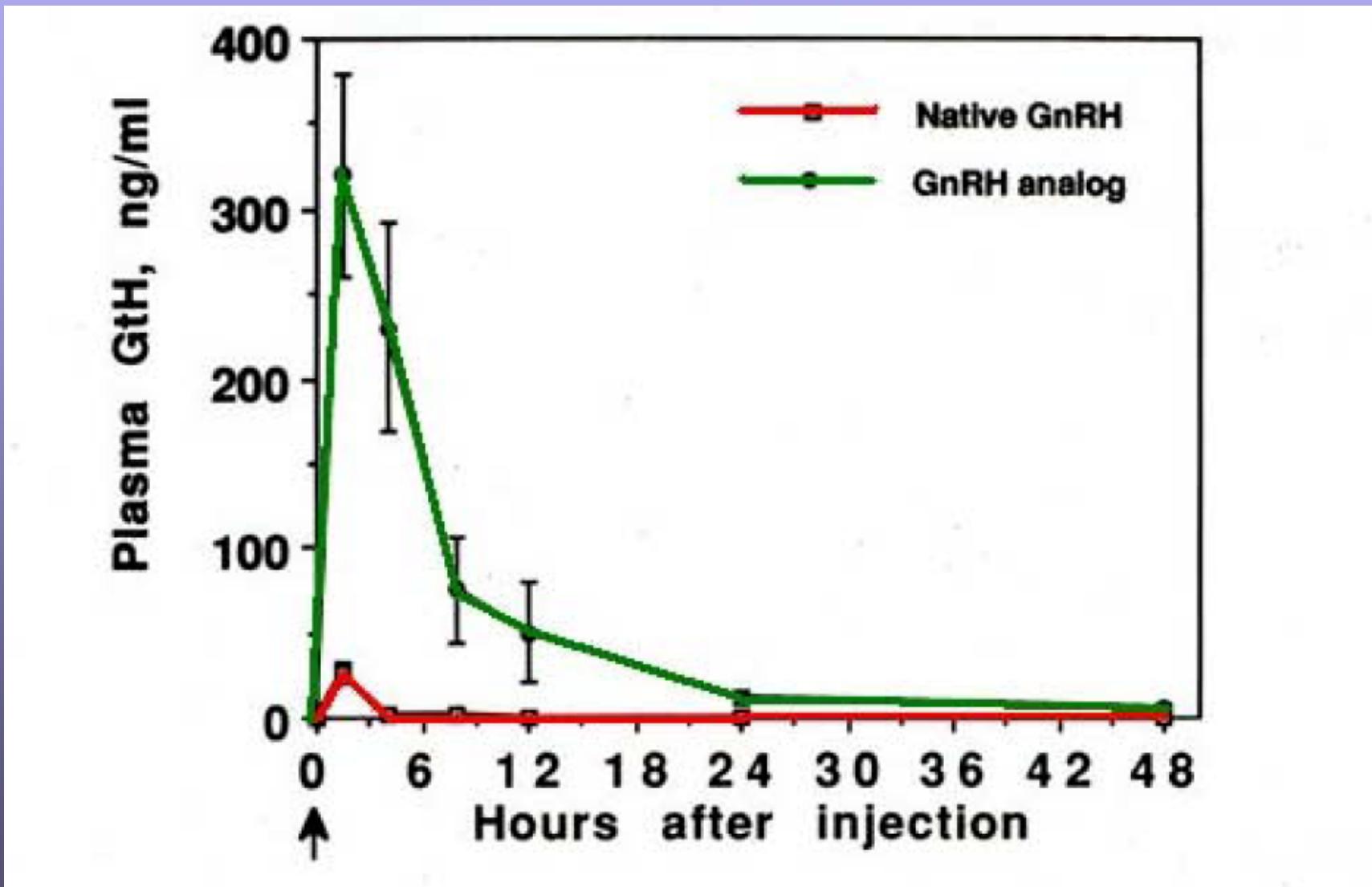
From Broodstock management and selective breeding Yonathan Zohar

# Development of antibodies



From Broodstock management and selective breeding Yonathan Zohar

# Use of analogues



From Broodstock management and selective breeding Yonathan Zohar

# Injecting implants or microspheres



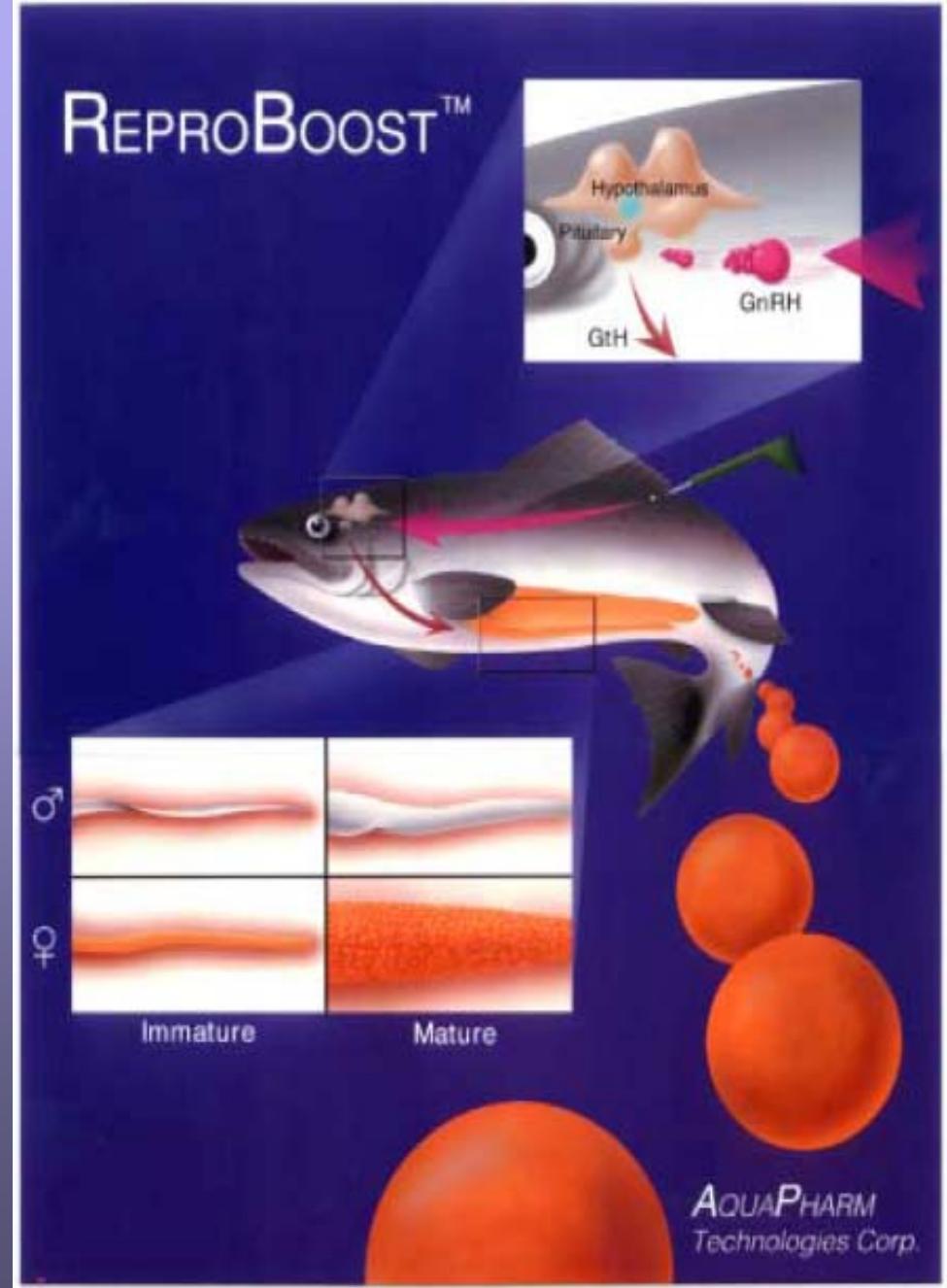
From Broodstock management and selective breeding Yonathan Zohar

# Induced sperm production using GnRH implants



From Broodstock management and selective breeding Yonathan Zohar

# Commercially available



From Broodstock management and selective breeding Yonathan Zohar