Bioteknologi pada pengembangbiakan ikan

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Topics

- Introduction
- Reproduksi: GnRH tool
- Induksi kematangan gonad ikan melalui kontrol lingkungan;
- Induksi kematangan gonad ikan melalui hormonal
- Kontrol Fertilitas
- Poliploidisasi
- Kontrol proporsi sex pada keturunan
- Transplantasi Germ cell

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Introduction

Biotechnology → To enhance aquaculture production holds great potential → meet demand & improve aquaculture practices

What need to be improved?

- ✓ fast growing disease resistant varieties,
- ✓ the development of cheap and effective vaccines,
- ✓ disease diagnostic methods,
- ✓ cell lines
- ✓ probiotics

Biotechnology

- ✓ superior breeds,
- ✓ fish health management,
- ✓ population genetics-biodiversity
- ✓ and use of molecular markers

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Introduction

Production is accelerated by:

1. improved breeds,
2. breeding programmes,
3. hybridisation,
4. chromosome manipulation
5. and transgenesis

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Reproduksi View

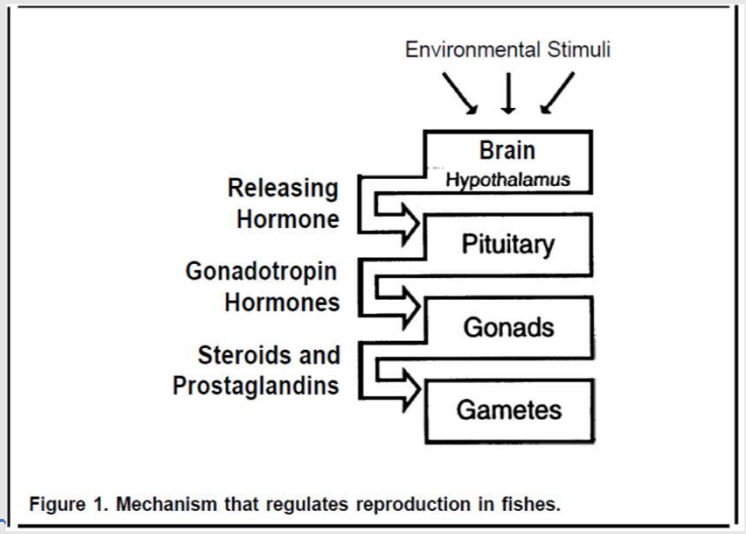

Figure 1. Mechanism that regulates reproduction in fishes.

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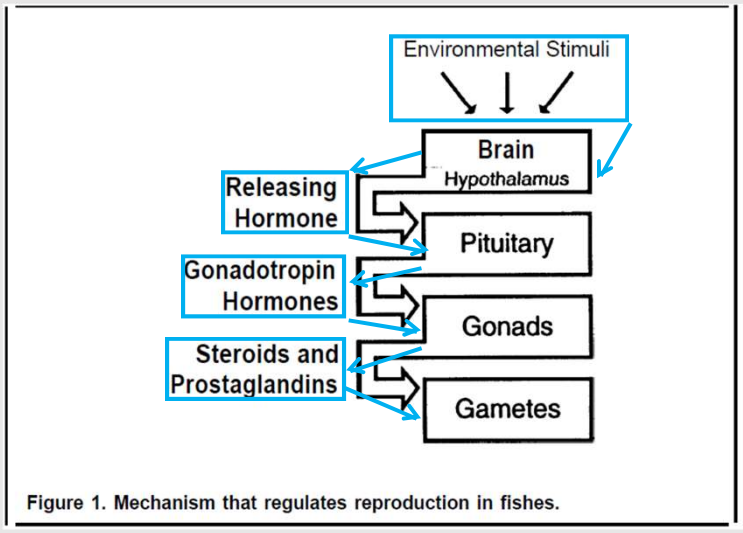

Figure 1. Mechanism that regulates reproduction in fishes.

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Reproduksi GnRH: tool

Gonadotropin-releasing hormones

- Presence of gonadotropin-releasing hormones (GnRH) in brain
  - Dorsalis pars medialis, meso-adenohypophysis, & pituitary stalk
- GnRH activity found in crude hypothalamic extracts from many fish (cyprinids, salmonids)
  - Hypothalamic GnRH extracts promote release of gonadotropins when injected into other fish
  - Partially characterized GnRH from extracts of common carp
    - Small molecule - molecular weight of <5000
    - Fish GnRH not same as mammalian LH-RH
      - Does not cross react with anti-mammalian-LH-RH

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Synthetic Gonadotropin-releasing Hormone in Fish

- Mammalian LH-RHa cheap, easily-made synthetic, & biologically active in many fish
  - Large doses mammalian LH-RH produces release of gonadotropins in common carp, brown trout, & goldfish
  - Nonapeptide analogue of LH-RH more potent & longer acting gonadotropin release than LH-RH itself
    - Multiple LH-RHa injections over several days induces ovulation
  - Male response to mammalian LH-RHa poor at onset of spermatogenesis
    - Response increases at spermatid stage & continues during rest of spermatogenesis & spermiation
  - Pituitary response of LH-RHa low in early-stage maturity fwmales
    - Becomes increased at vitelline maturation (vitellogenesis)
  - LH-RHa only useful as late-maturation induced spawning hormone

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Synthetic Gonadotropin-releasing Hormone in Fish

- GnRH from fish – amino acid sequence can be slightly different for each species
  - Salmon sGnRHa
    - Cheap, easily-made synthetic, active in many fish species, effective for maturation in early stage fish
- Preferred method of induced spawning

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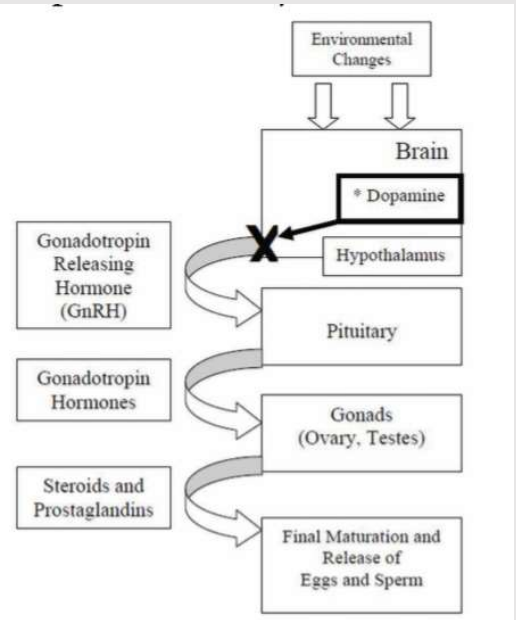
Induksi kematangan gonad ikan melalui kontrol lingkungan



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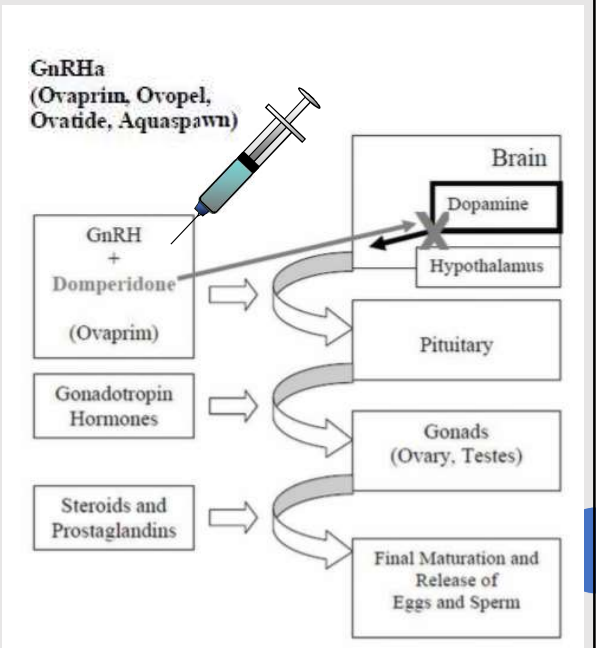
Induksi kematangan gonad ikan melalui hormonal



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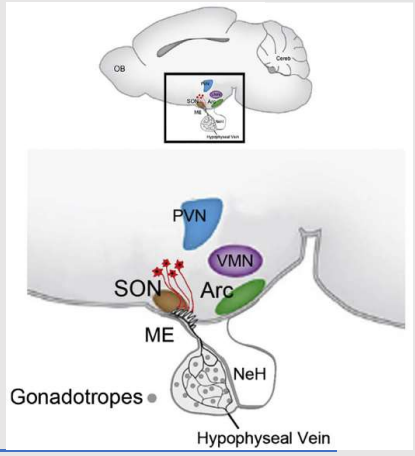
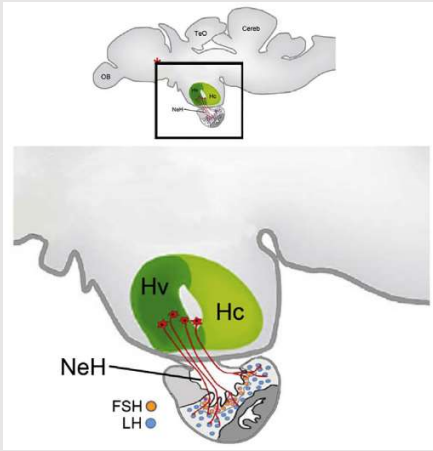
Structure of the vertebrate hypothalamus and description of gnrh/GnRH containing cells in the mammals and in the fish brain

In mammals

In fish





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Central regulation of reproduction in fish

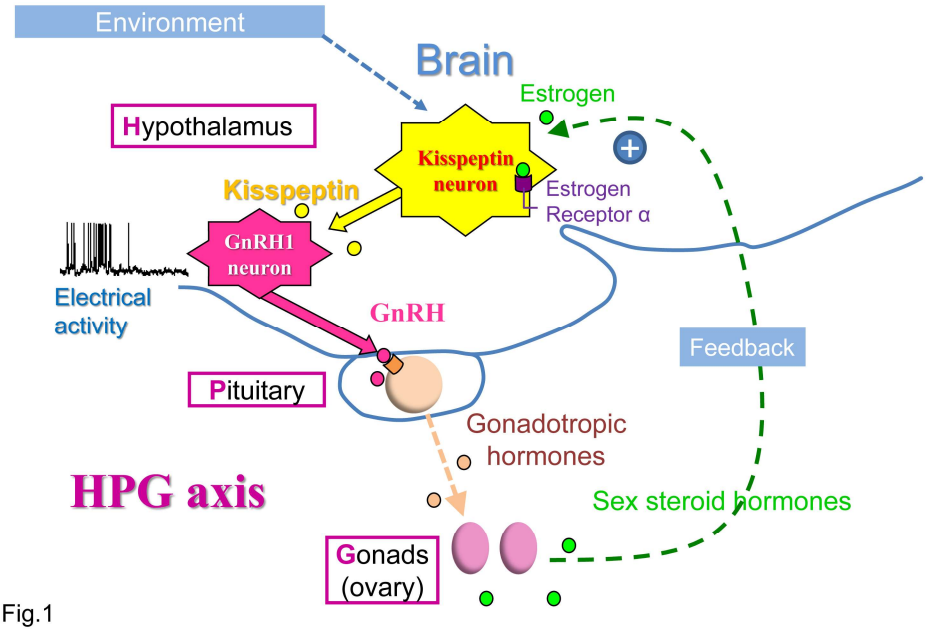

Fig.1

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Kontrol Fertilitas (Fertility control)

It is postulated that physiological and morphological changes related to maturation imply devoting a great deal of metabolic energy to these activities.

Technologies to produce reproductively sterile fish are becoming increasingly important to resolve the current and projected spread of genetic contamination caused by aquaculture escapees and the invasion of non-native species

The most common and practical methods currently used to induce sterility in the aquaculture industry are **chromosome manipulations** to cause triploidization or interspecies hybridization

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Poliploidisasi (Induced Polyploidy)

Induced polyploidy is artificial production of individuals with an increased number of haploid chromosome sets.

Triploid fish are genetically sterile, i.e. they are not capable of producing viable progeny

Genetic sterility and reduction in gonad development of triploid fish provides two possible benefits for their production in aquaculture and fisheries.

Triploid fish may be produced: • To prevent fish reproduction in ponds, reservoirs, or natural waters. • To obtain a better growth rate as compared with normal diploids

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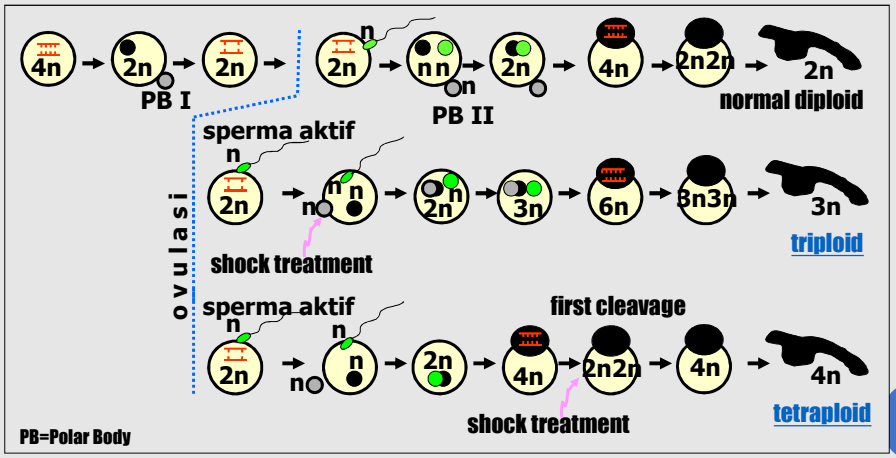
POLYPLOIDY TECHNIQUE



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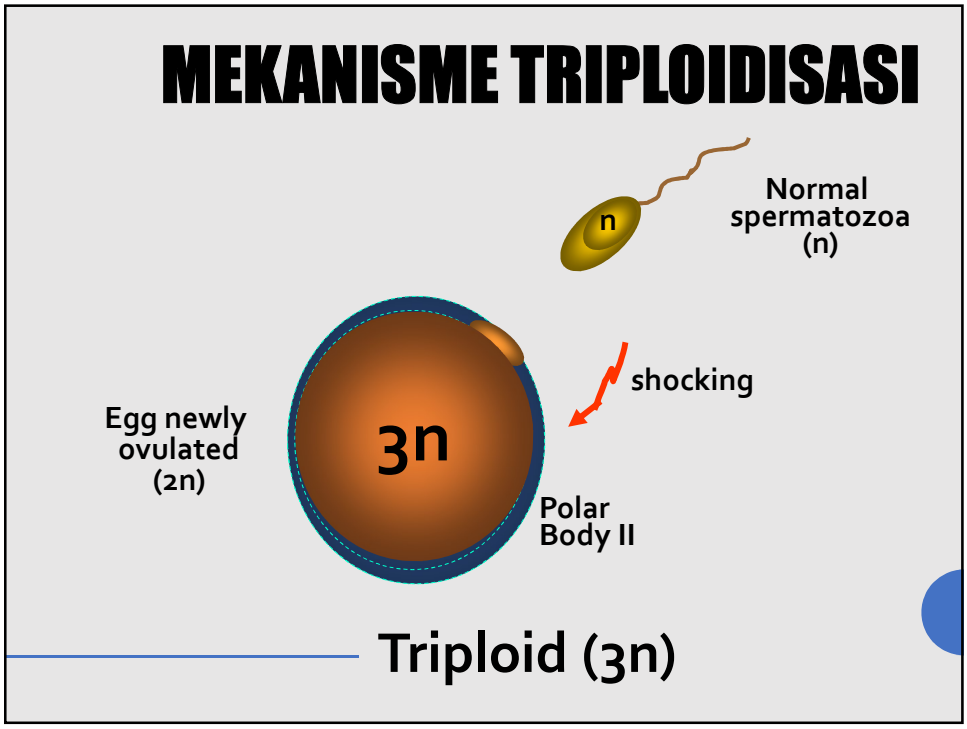
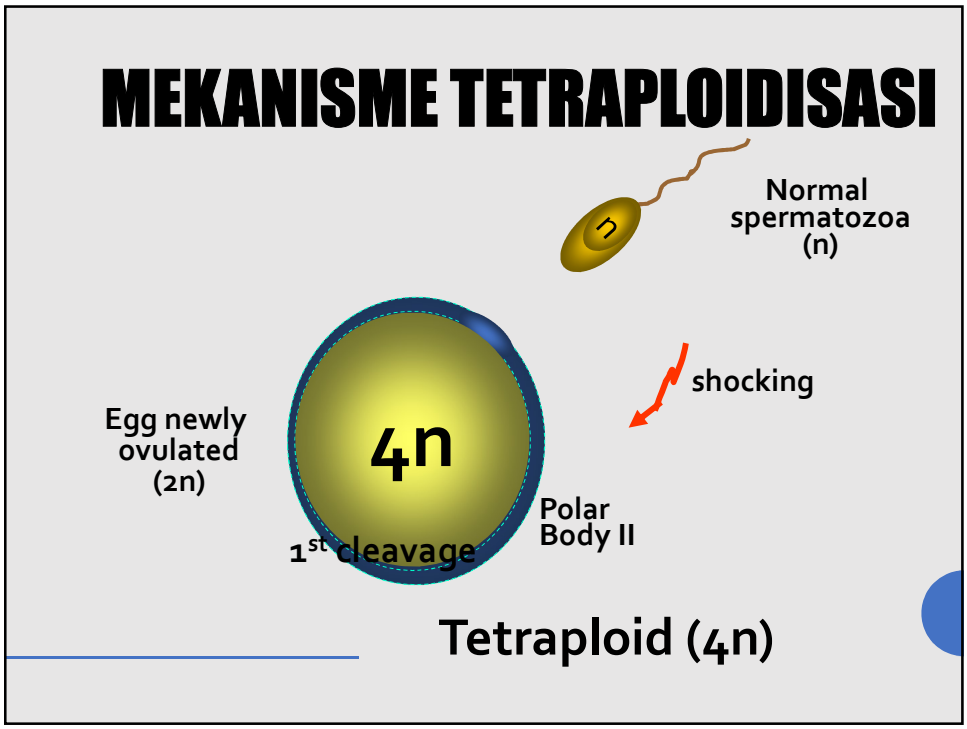

MEKANISME TRIPLOIDISASI
n
Normal spermatozoa (n)
shocking
Egg newly ovulated (2n)
3n
Polar Body II
Triploid (3n)

17


MEKANISME TETRAPLOIDISASI
n
Normal spermatozoa (n)
shocking
Egg newly ovulated (2n)
4n
1st cleavage
Polar Body II
Tetraploid (4n)

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Kontrol Proporsi Sex Pada Keturunan (Sex Reversal)

The phenomenon whereby organisms developing at sex-specific conditions such as temperatures or karyotypes hatch the opposite sex

The main purpose is to produce a monosex populations

Factors related to sex reversal are:

- ✓ **Chemical inducer**
- ✓ **Labil Period**
- ✓ **Methods administration**
- ✓ **Dosage**

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Chemical inducers used for sex reversal in fishes

Classification of inducer chemicals
I Androgens
1 Aromatizable (i) Naturals (a) Testosterone (T) (b) 11-Ketotestosterone (KT) (c) Androstenedione (AST)
(ii) Synthetics (a) 17α-methyltestosterone (MT) (b) Mibolerone (c) Fluxymestrone (d) 17α ethyltestosterone (ET)
2 Non-aromatizable (i) Steroids (a) 1,4,6-androstatrien 3, 17-dione (ATD) (b) 17α methyldihydrotestosterone (MDHT) (c) 4 hydroxy-4-androstene- 3, 17 dione (4OH) (d) 4 androsten-4-ol-3, 17 dione (formestone) 3 Non-steroids (a) Fadrozole (Fz) (*aromatase* inhibitor) (b) Letrozole (c) Tamoxifen (Tx) (estrogen antagonist) (d) Flutamide
4 Medicinal herb (a) *Tribulus terrestris*

Classification of inducer chemicals
II Estrogens
1 Naturals (a) Estrone (E_1) (b) 17β-Estradiol (E_2) (c) Estriol (E_3)
2 Synthetics (a) Diethylstilbestrol (DES) (b) 17α-Ethynylestradiol (EE_2) (c) Estradiol benzoate (EB)
3 Estrogen receptor agonist (a) 4-Nonyphenol (NP) (b) Spironolactone aldosterone antagonist

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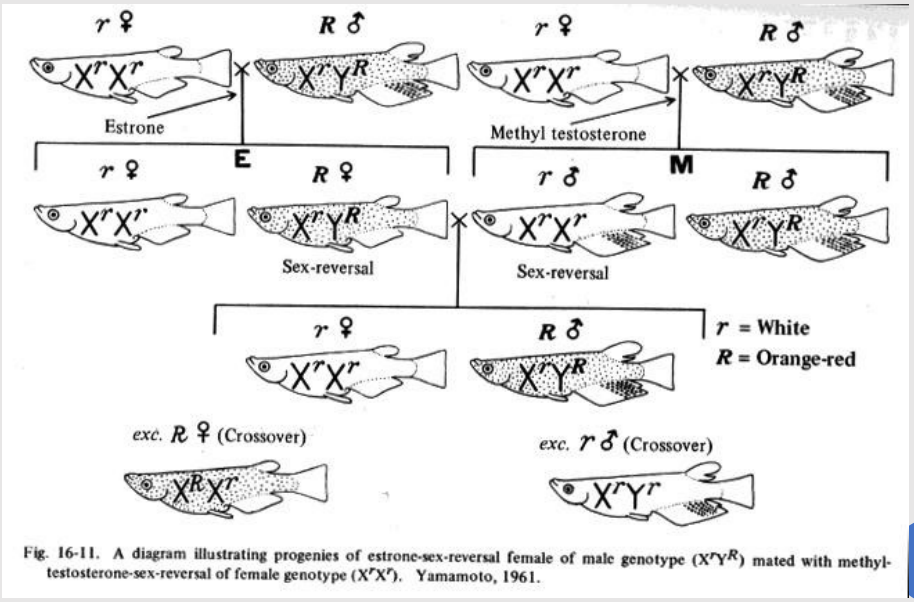

Fig. 16-11. A diagram illustrating progenies of estrone-sex-reversal female of male genotype (X^rY^R) mated with methyl-testosterone-sex-reversal of female genotype (X^rX^r). Yamamoto, 1961.

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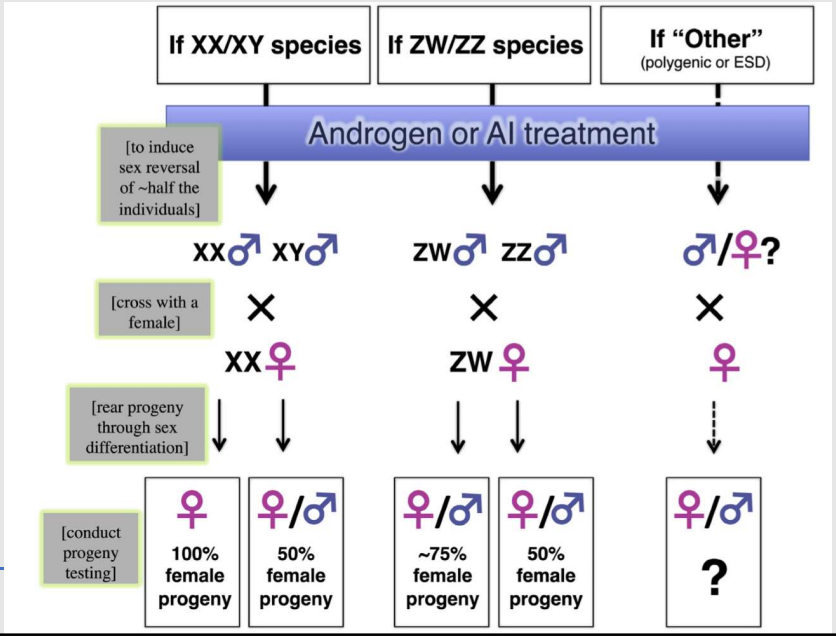
sex reversal techniques and progeny testing: to identify the underlying s
determination system of cold-blooded vertebrate species.



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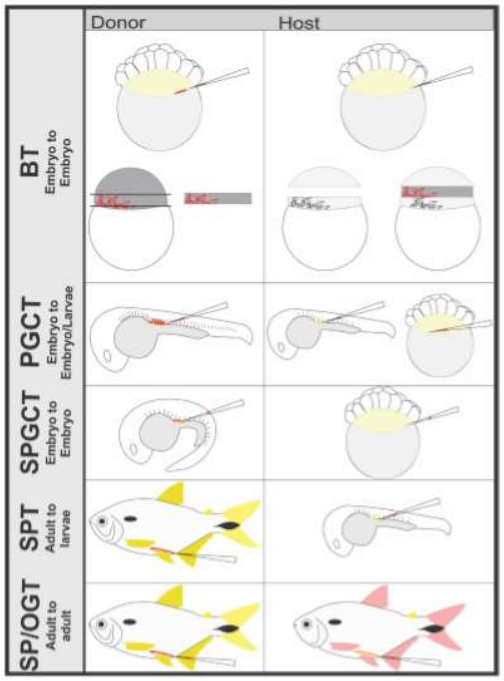
Germ cell transplantation

Germ cell transplantation in fish involves the production of heterologous gametes by production of germline chimera (Okutsu et al. 2007).



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Approaches used in germ cell transplantation in fish. Main advantages and disadvantages

Approach	Advantages	Disadvantages
Blastomeres transplantation between embryos	• Does not require great skills to be performed • Many PGC precursors can be easily sucked into a micropipette at once	• Requires a great supply of donor embryos • Domain of reproduction of both donor and host species • Synchrony of donor and host species reproduction • Months to years until target gamete and, consequently, production of the target offspring • Low rate of PGC migration to host genital region • Low percentage of donor-derived offspring
PGC transplantation from larvae to larvae and embryo	• Higher rate of PGC migration to host genital region • More efficient to reach to offspring production	• Requires a great supply of donor embryos • Domain of the reproduction of both donor and host species • Synchrony of donor and host species reproduction time • Months to years to start target gamete and, consequently, offspring production • Sophisticated equipment is needed • Skills needed
Single PGC transplantation between embryos	• High efficiency to produce offspring • Do not require a great supply of donor embryo • One cell is enough to resume gametogenesis	• Domain of the reproduction of both donor and host species • Synchrony of donor and host species reproduction time • Months to years to start target gamete and, consequently, offspring production • Sophisticated equipment is needed • Skills needed

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Approaches used in germ cell transplantation in fish. Main advantages and disadvantages

Spermatogonial transplantation to embryo and larvae	• Many spermatogonia are present at male testes • Do not need to synchronize donor and host reproduction time • Donor cells can be harvested from a wild specimen, reducing necessity of reproduction in laboratory	• Months to years to start target gamete and consequently offspring production • Sophisticated equipment is needed • Skills needed
Spermatogonial transplantation between adults	• Do not need sophisticated equipment • Do not need great skills • Donor cells can be harvested from a wild specimen, rejecting the necessity of reproduction in laboratory • Target gametes and offspring can be produced in few weeks or months • Donor germ cells can be injected any time with no possibility of rejection • Do not need to synchronize donor and host reproduction time	• Sterile host is necessary • A considerable number of donor cells is needed • Low efficiency • Loss of maternal genetic material, such as mitochondrial DNA and germplasm
Oogonia transplantation between adults	• Do not need sophisticated equipment • Do not need great skills • Donor cells can be harvested from a wild specimen, reducing necessity of reproduction in laboratory • Target gametes and offspring can be produced in few weeks or months • Donor germ cells can be injected any time with no possibility of rejection • Do not need to synchronize donor and host reproduction time • Transference of maternal genetic material to offspring	• Sterile host is necessary • A considerable number of donor cells is needed • Low efficiency

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Thank You

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