Biotechnologies for monosex and spawning induction

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Objectives to meet desired outcomes for industry

Techniques to produce all-female monosex populations
- Using recombinant insulin-like androgenic gland hormone \( (rPm\text{-IAG}) \)
- Using dispersed hypertrophied Androgenic gland \( (hAG) \) cells

Provide an alternative to eyestalk ablation (ESX)
- Induce ovarian maturation using hormonal treatments
Why Monosex?

*Penaeus monodon* females:
- Faster growth rate
- Grow larger
- Superior FCR
- Result in a uniform product
- Higher survival (reduced aggression)

Campos-Ramos et al., 1994
Monosex – how? Sexual differentiation in decapods

Male sexual differentiation in decapods is mediated by the Androgenic Gland (AG)

Mareddy et al., 2011

Wen-Ming Ma et al., 2010

(FAO, 2018)
IAG characterised in all commercial important decapod families
Monosex – how?

All male

- Silencing IAG using RNAi (dsRNA)

**Neo-female**: genetic male, functional female

Neo-female (ZZ) x male (ZZ) = 100%♂ (ZZ) embryos

All Female

- Injection of hypertrophied AG cells

**Neo-male**: genetic female, functional male

Neo-male (ZW) x female (ZW) = 25%♂ ZZ, 50%♀ ZW, 25%♀ WW

Female (WW) x male (ZZ) = 100%♀ (ZW) embryos

Is IAG manipulation possible in *P. monodon*?

Ventura et al., 2012

Levy et al., 2016
Recombinant IAG in *P. monodon*

Produced in yeast

- Obtained sequence NCBI
- Designed DNA expression construct
- Transfected into yeast (*P. pastoris*)
- Screened yeast transformants
- Induced rPm-IAG expression

**Pm-IAG**

MNQLASR\*TVGLMAMQLLLVQMLMLSLTSASSECVN
VTGIPVFDQADIGDTMCSCHKTFTPARPHSNIMPSL
TVSRSAIADLDWQGTRGQTPIIPLPPQXPRSTAN
NPMDYFELDMQELHLSPEAAHALVKTSGF
WDECOHVSQRITCAEEILEC

**Pm-IAG**

10.135 kDa

**pPIC9K**

9.3 kb
Injection trials

- Injected into thousands of post larvae (PL9-30) (Prior to the expression of IAG)
- Checked phenotype (PL75)
- Confirmed Genotype by PCR using sex specific markers

UNSUCCESSFUL...
Further knowledge needed!

• Application of RNA-Seq analysis

• BLASTX - Isolation and Identification of AG specific factors
AG specific factors identified:

**Single insulin binding domain (SIBD) proteins**
- essential for insulin-like peptide binding
- may be needed to infer the effect of IAG

**Crustacean hyperglycemic hormone (CHH)**
- Another AG – derived hormone!

Additional AG factors needed to induce sex change in *P. monodon*?
Injection of AG cells

• Dissected AG, dispersed cells

• Performed an *in vivo* injection trials
  In 3000 animals at PL10, 15 and 30

Checked the phenotypes and genotypes

Again UNSUCCESSFUL
Could their be a different mechanism at play?

Positive evidence for upstream sex determination mechanism is the key to successful sex reversal
Ovarian maturation – what is it?

Ovarian maturation/vitellogenesis is a four stage process → Spawning

• Accumulation of lipids (vitellogen) and other energy sources

Stage 1
Previtellogenic

Stage 2
Developing

Stage 3
Vitellogenic

Stage 4
Ripe ovary

Uawisetwathana et al., 2011
In vivo injection trial

Aim - Stimulate ovarian maturation

- **Postive Control- ESX (a)**
- **Group 1 – dsRNA of GIH (b)** (Functional)
- **Group 2 – 5-HT and spiperone (DA inhibitor) (c)**
- **Group 3 – Mixed dsRNA of GIH, MF, 17αHP, RPCH and lGnRH-I (d)**
- **NC – saline (e)**

Conclusions

Spawning events

- ESX (n = 26)
- **dsPm-GIH (n = 2)**
- 5HT + DA inhibitor (n = 2)
- NC (n = 0)
Integrated omics approach
Combination of Transcriptomic and Metabolomic analysis

- Mature ♀ previtellogenic and vitellogenic samples

Hemolymph (blood)
Antennal gland
Eyestalk
Brain + Thoracic ganglia

Previtellogenic  Developing  Vitellogenic  Ripe ovary

- Hemolymph (blood)
- Antennal gland
- Eyestalk
- Brain + Thoracic ganglia
Omics analysis

Identified key dopaminergic factors - DA transporter, DA receptors and all enzymes involved in DA biosynthesis and several DA related metabolites

Highlights

↑ Dopaminergic factors increase in stage 2

Is dopamine stimulatory?

Conclusions

No golden bullet, we don’t fully understand how complex the process is
Final conclusions

**Monosex**
- IAG/AG manipulation in *P. monodon* is ineffective,
- Factors upstream IAG and the AG such as DMRTs, could be key candidates to allow sex reversal in penaeids

**Future research - monosex**
Investigate the role multiple of the Dmrt5s in sex determination.

**Ovarian maturation**
- the dopaminergic system is significantly altered throughout the early stages of vitellogenesis
- dopamine may be stimulatory in the early stages of vitellogenesis and therefore essential for promoting successful spawning

**Future research – ovarian maturation**
Identification of new pharmacological approaches for the development of an alternative to eyestalk ablation
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Questions?