Assessment of ovarian maturation in *Chasmichthys dolichognathus* after exposure to single polycyclic aromatic hydrocarbons, benzo[a]pyrene

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Background
Oocyte maturation of fishes

**Vitellogenesis**
- Oocyte
  - Estradiol-17β (E2)

**Final maturation**
- 17α20βOHP (maturation inducing steroid)

**Long period**
- GSI increases gradually
- Increase in oocyte diameter

**Short period**
- Maximum in GSI
- Occurrence of **GVBD** and ovulation
- Induction of **final maturation**
- Ready to spawn

**Prerequisite process for stock recruitment**
General steroidogenic pathway of teleost
Endocrine Disrupting Chemicals and Fisheries

EDCs are natural or anthropogenic compounds that mimic endogenous hormones. Also known as hormone mimics, hormonally active agents, xenoestrogens.

The diagram illustrates the impact of EDCs on the life cycle of a fish, from Adult to Egg, with stages of Recruitment, Juvenile, Hatching, Spawning, and Reproduction.
Mechanism of endocrine disrupting chemicals

- Bind to hormone receptors; and/or interfere with normal hormone response

- Inhibition of oocyte maturation
- Alteration in reproduction

Reproductive success or not
Oil spills

Release of crude oil or refined petroleum products into the ocean and coastal waters

Spills take months or even years to clean up

Oil spills in Korea (2007)
Polycyclic Aromatic Hydrocarbons (PAHs)

- Component of crude oil
- Byproducts of incomplete combustion
- Bioaccumulation in lipid-rich tissues

Benzo[a]pyrene

- A representative PAHs that has strong toxicity
- A five-ring PAH that is mutagenic and highly carcinogenic
The aim of this study

To evaluate the effects of benzo[a]pyrene on oocyte maturation *in vitro*
**Ovary**

- **Oogonium**
- **Oocyte**

**Vitellogenesis**

**Final maturation**

- **Germinal vesicle break down (GVBD)**
  - Morphological index of maturation

**Steroid hormone production**

- Maturation inducing steroid (MIS, $17\alpha20\beta\text{OHP}$)
- Estrogen and testosterone
- Ratio of each steroid ($E2/T$ or $E2/17\alpha20\beta\text{OHP}$)
Gobiid fish: Ideal organism in EDCs study

1. Short life cycle
2. Small size
3. Easy to handle
4. Strong tolerance
5. World wide distribution

Longchin goby, *Chasmichthys dolichognathus*
Materials and methods
Experimental protocol

Dissection → Separation of oocytes → In vitro Incubation → Extraction of steroids in the media

Oocytes of **0.9-1.0 mm** and **0.8-0.9 mm** in diameters

Exposure to 1, 10, 100, 1,000, and 10,000 nM of **B[a]P**

Steroids levels - **RIA**

- Observation of GVBD
Results and discussion
Effects of B[a]P on *in vitro* steroid production (Φ=0.8-0.9 mm)

Bars represent the mean and SEM (n=3). Asterisks indicate significant differences from controls (P<0.05).

Lower concentrations of B[a]P
- Increase in 17α20βOHP production
- Related to final maturation
Effects of B[a]P on *in vitro* steroid production (Φ=0.9-1.0 mm)

Bars represent the mean and SEM (n=3). Asterisks indicate significant differences from controls (P<0.05).

Increase in E2 production
Increase in 17α20βOHP production
Effects of B[a]P on *in vitro* steroid production (E2/T and E2/17α20βOHP)

High E2/T and E2/17α20βOHP – Estrogenic activity or inhibition of maturation

Φ = 0.8 - 0.9 mm

Φ = 0.9 - 1.0 mm
Effects of B[a]P on *in vitro* GVBD

Bars represent the mean and SEM (n=3). Asterisks indicate significant differences from controls (P<0.05).

Higher concentrations of B[a]P – Inhibition in GVBD
Lower concentrations of B[a]P – Stimulation in GVBD
Conclusion and future study

Exposure to lower concentrations
Stimulation in maturation
Estrogenic effects

Exposure to higher concentrations
Inhibition in maturation

Investigation of long-term effects
Mechanism of endocrine disruption on oocyte maturation by PAHs, B[a]P

In vivo experiment

Molecular biological methods

But, I’m not sure. Study more in detail!
Thank you.
Don’t spill your oil.