

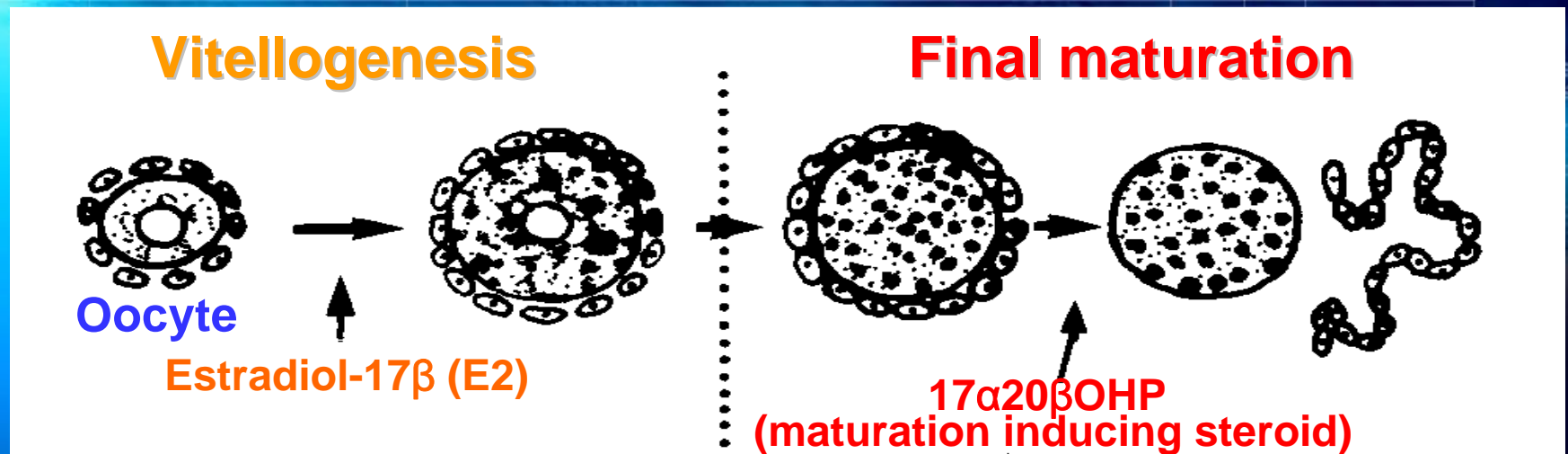
Assessment of ovarian maturation in *Chasmichthys dolichognathus* after exposure to single polycyclic aromatic hydrocarbons, benzo[a]pyrene



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Background

Oocyte maturation of fishes



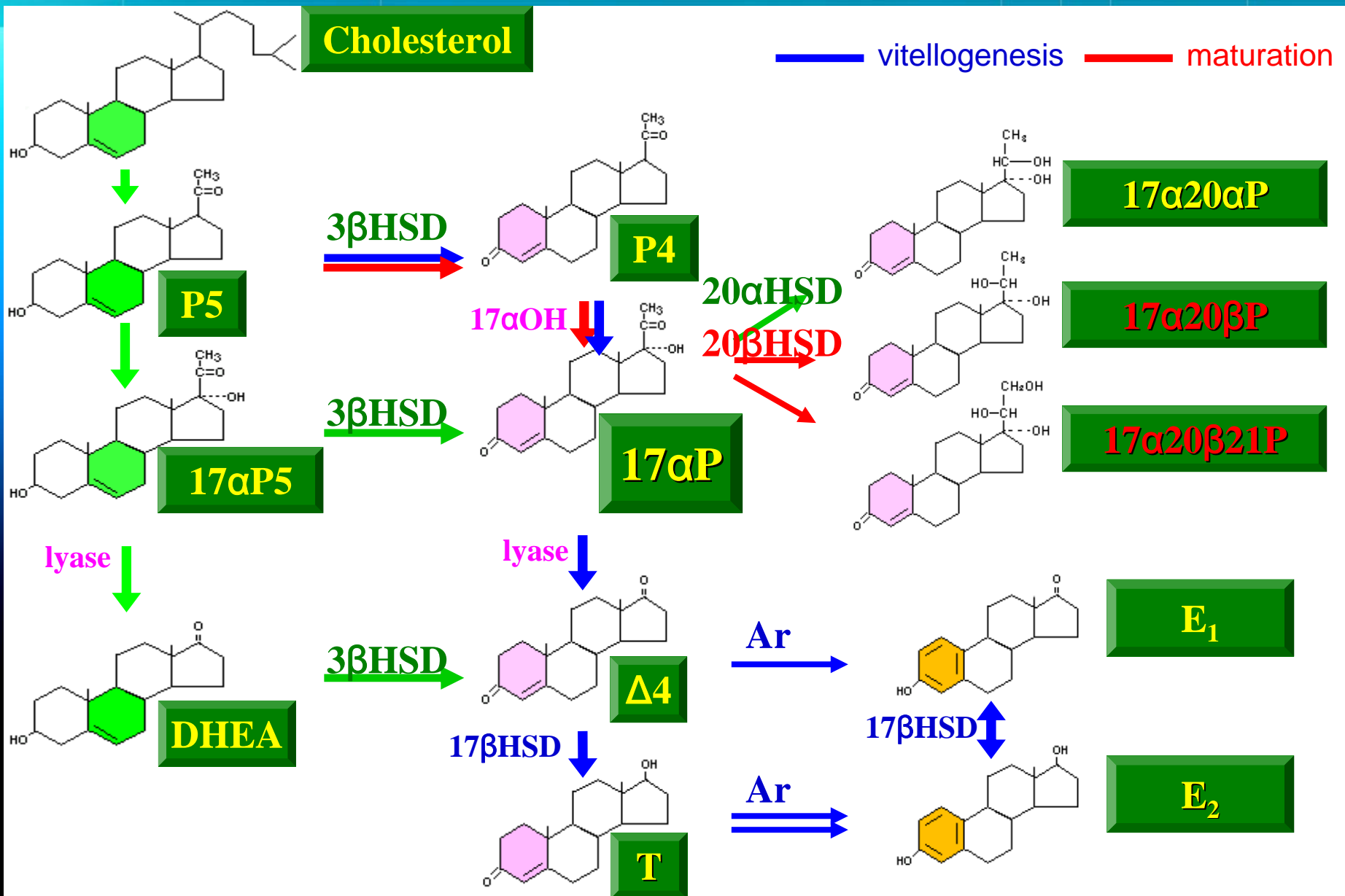
← **Long period** → ← **Short period** →

GSI increases gradually
Increase in oocyte diameter

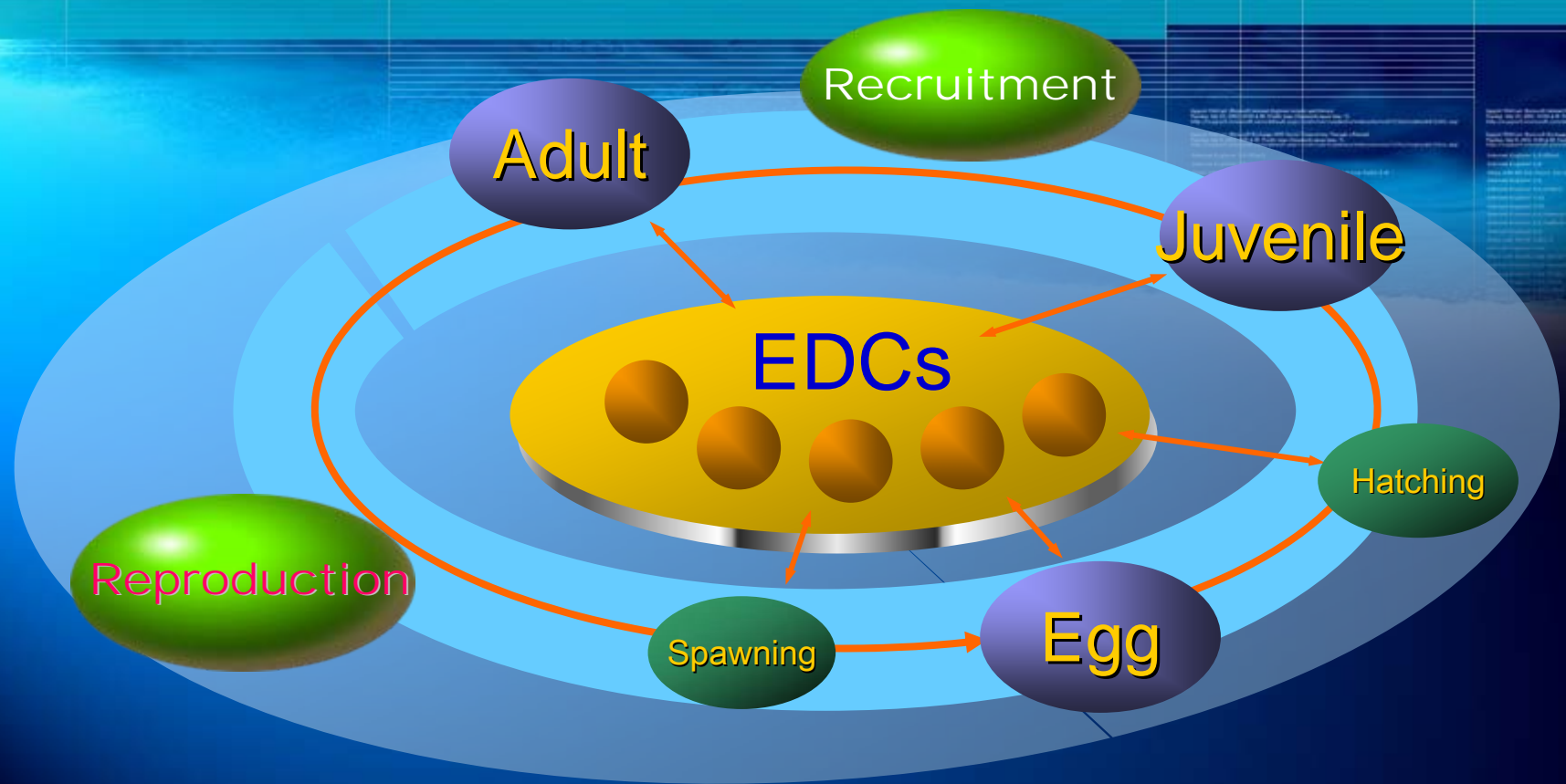
Maximum in GSI
Occurrence of GVBD and ovulation
Induction of final maturation
Ready to spawn

Prerequisite process for stock recruitment

General steroidogenic pathway of teleost



Endocrine Disrupting Chemicals and Fisheries

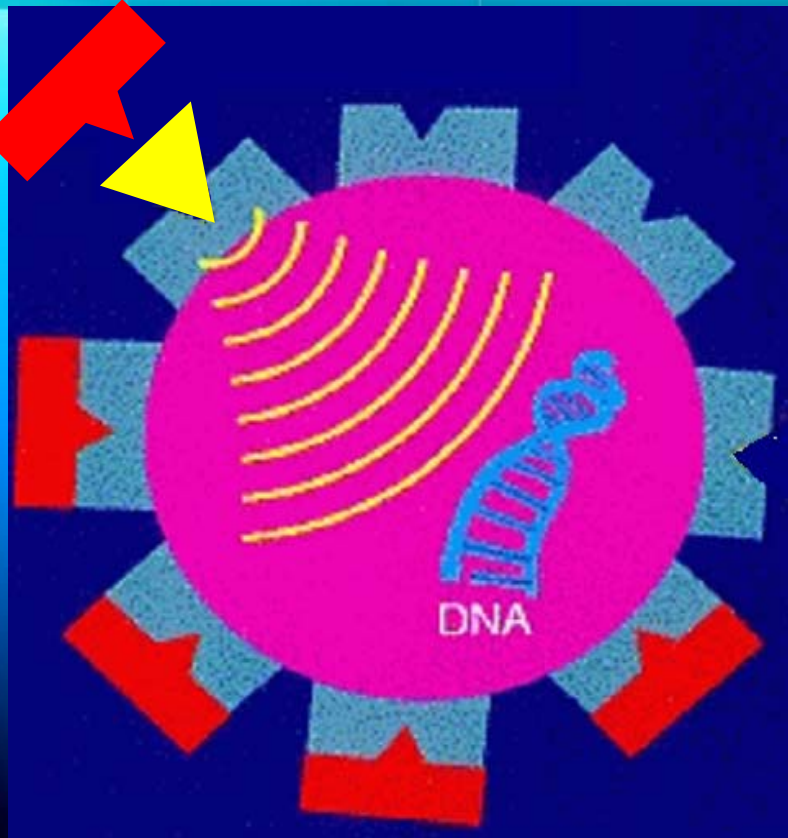


EDCs

Natural or anthropogenic compounds
that **mimic endogenous hormones**

Also known as hormone mimics,
hormonally active agents, **xenoestrogens**

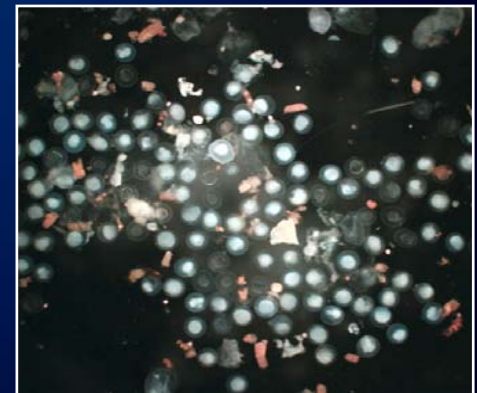
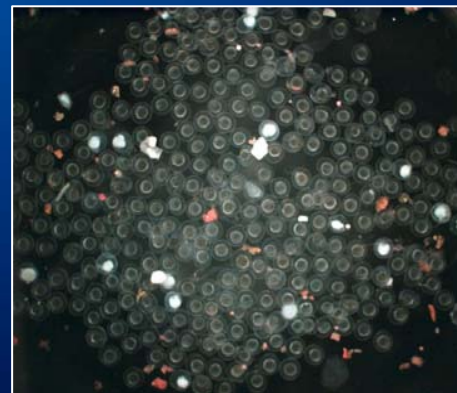
Mechanism of endocrine disrupting chemicals



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)

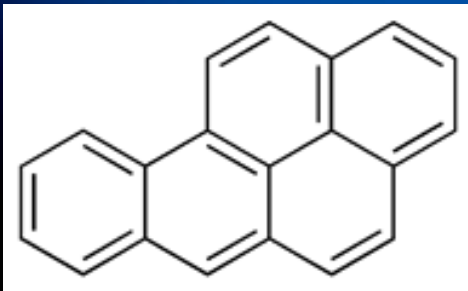
Polycyclic Aromatic Hydrocarbons (PAHs)



Byproducts of
incomplete combustion

Bioaccumulation
in lipid-rich tissues

Benzo[a]pyrene

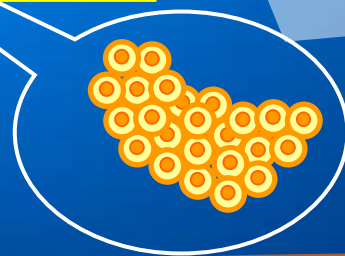


A representative PAHs that has strong toxicity

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*



In vitro exposure



GVBD

**Steroid
production**

Maturation

Ovary

Oogonium

Oocyte

Vitellogenesis

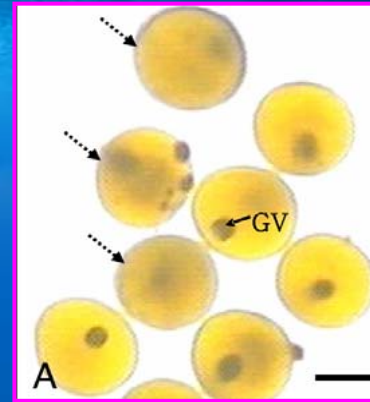
Estradiol-17 β (E2)

17 α 20 β OHHP

Final
maturation

GV disappeared

Germinal vesicle break down (GVBD)



Morphological index
of maturation

Steroid hormone production

- Maturation inducing steroid (MIS, 17 α 20 β OHHP)
- Estrogen and testosterone
- Ratio of each steroid (E2/T or E2/17 α 20 β OHHP)

Gobiid fish : Ideal organism in EDCs study



Longchin goby,
Chasmichthys dolichognathus

1

Short life cycle

2

Small size

3

Easy to handle

4

Strong tolerance

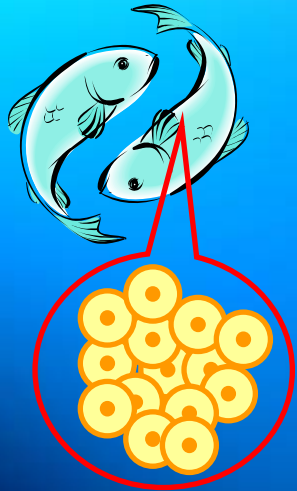
5

World wide distribution

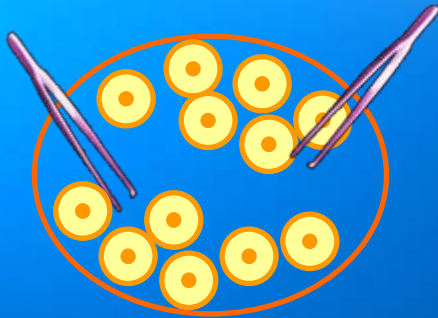
Materials and methods

Experimental protocol

Dissection



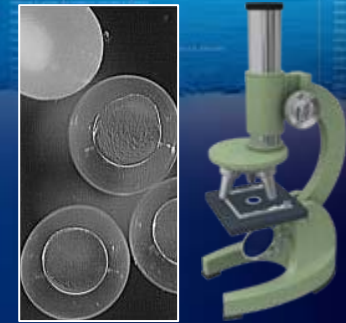
Separation of oocytes



In vitro Incubation



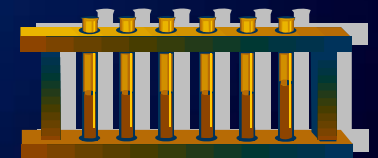
Extraction of steroids in the media



Incubated oocytes
-Observation of GVBD

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

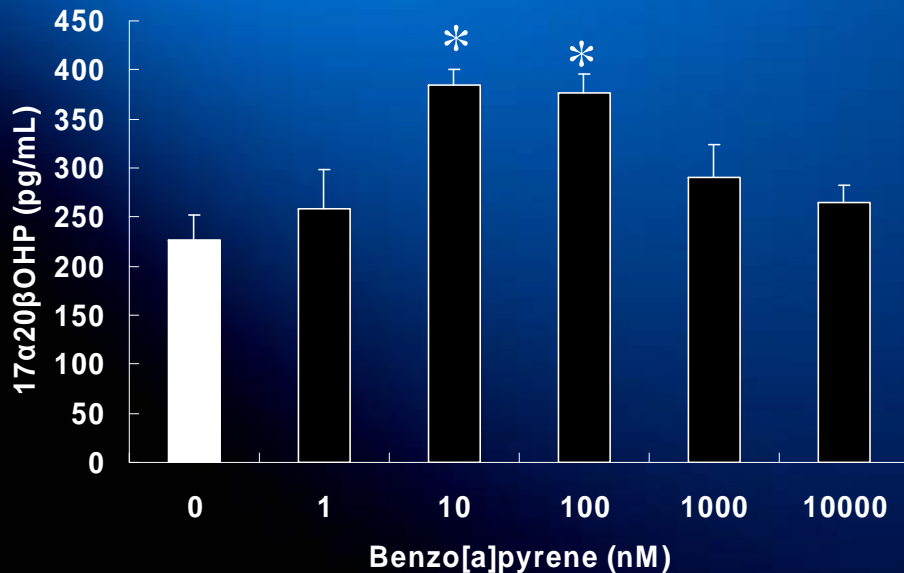
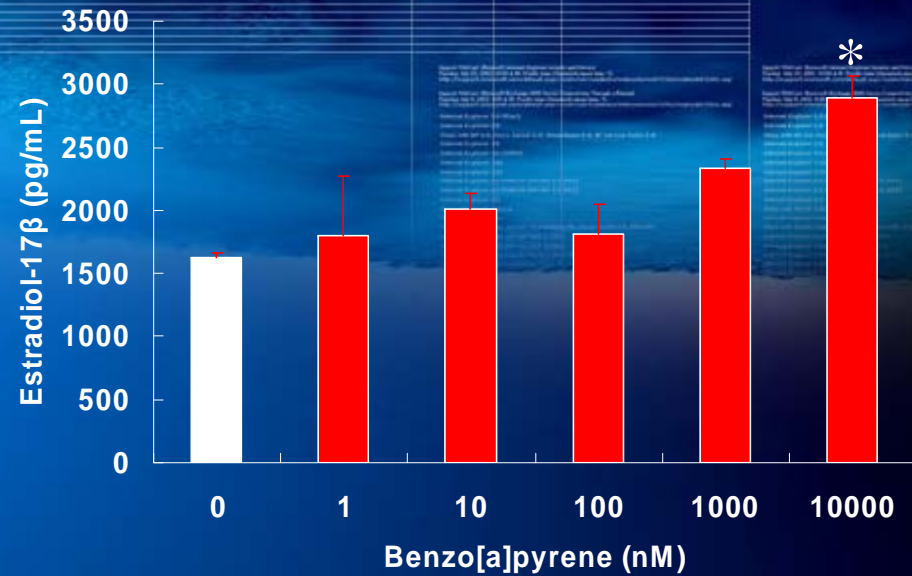
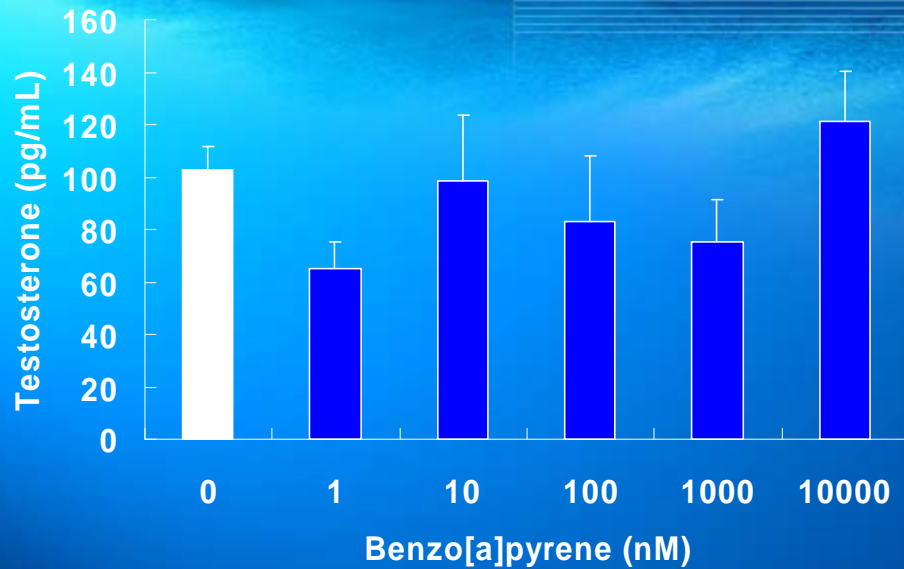
Exposure to 1, 10, 100, 1,000 and 10,000 nM of **B[a]P**



Steroids levels - **RIA**

Results and discussion

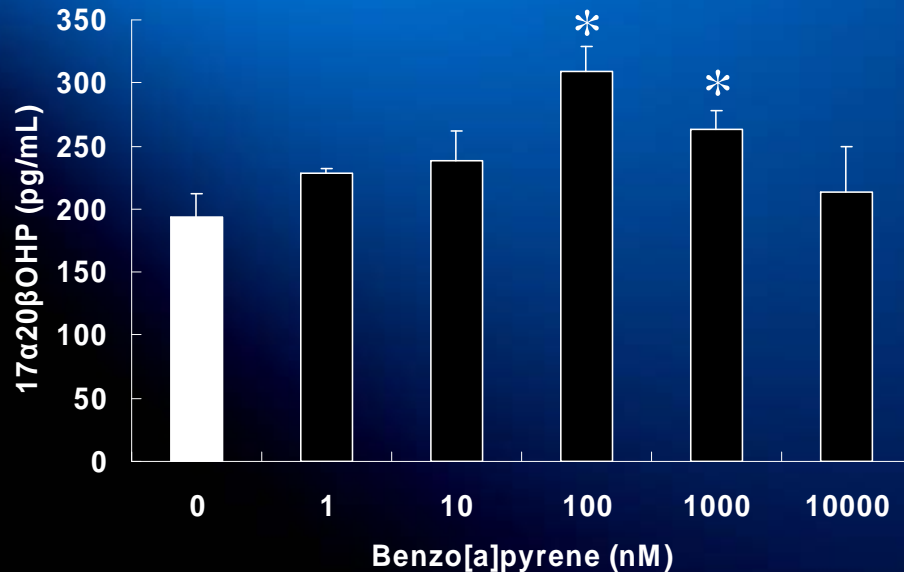
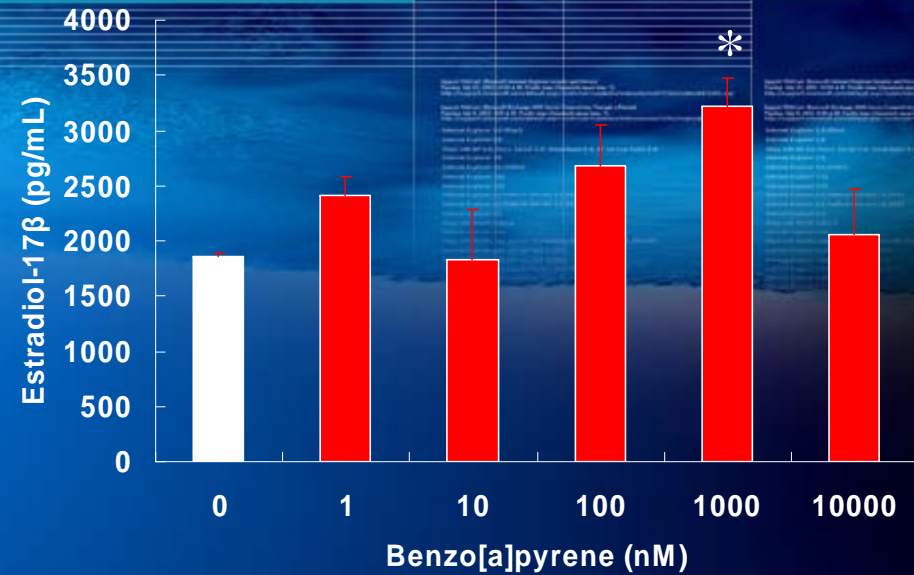
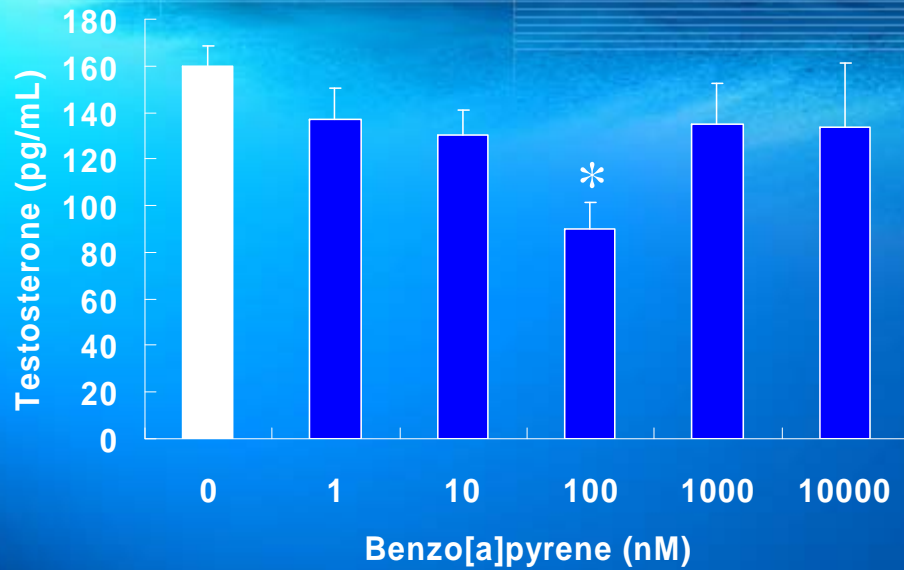
Effects of B[a]P on *in vitro* steroid production ($\Phi=0.8-0.9$ mm)



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 17 α 20 β OHP production
-related to final maturation

Effects of B[a]P on *in vitro* steroid production ($\Phi=0.9-1.0$ mm)

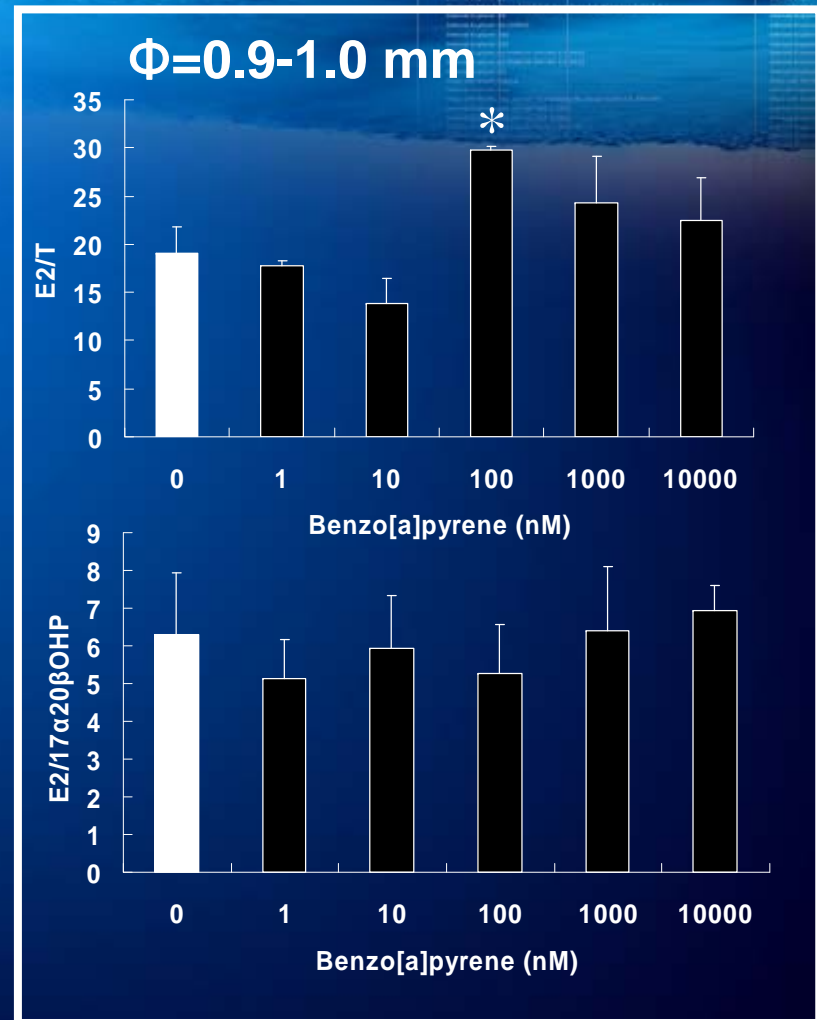
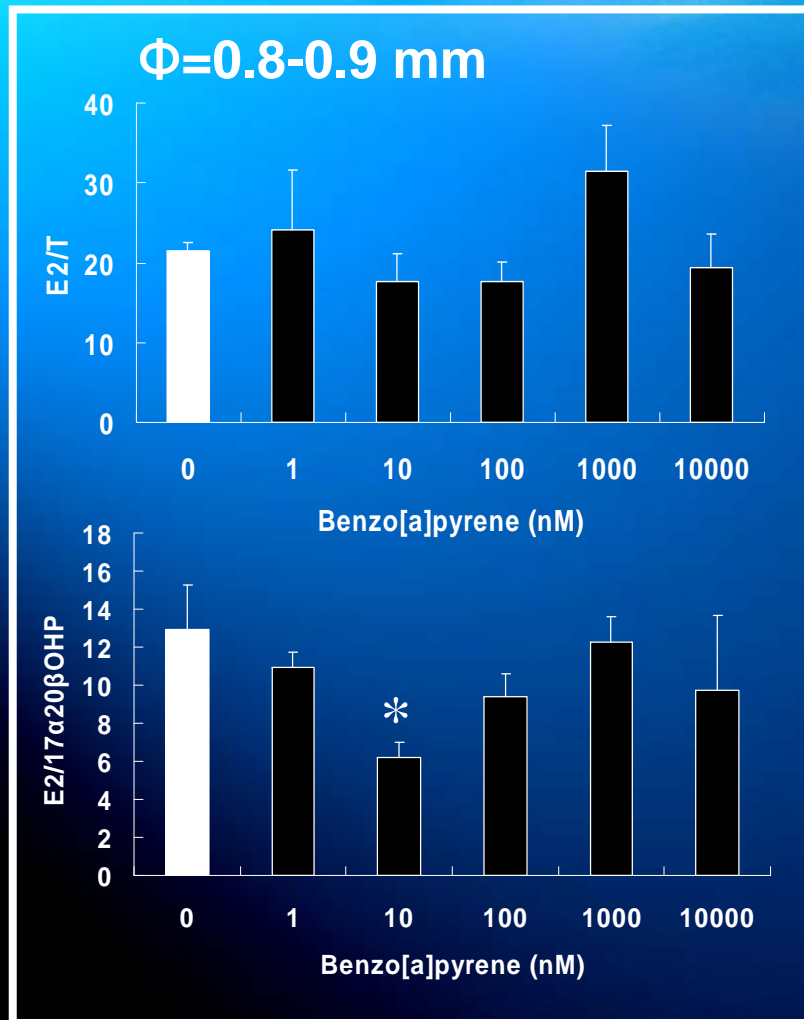


Bars represent the mean and SEM (n=3). Asterisks indicate significant differences from controls (P<0.05).

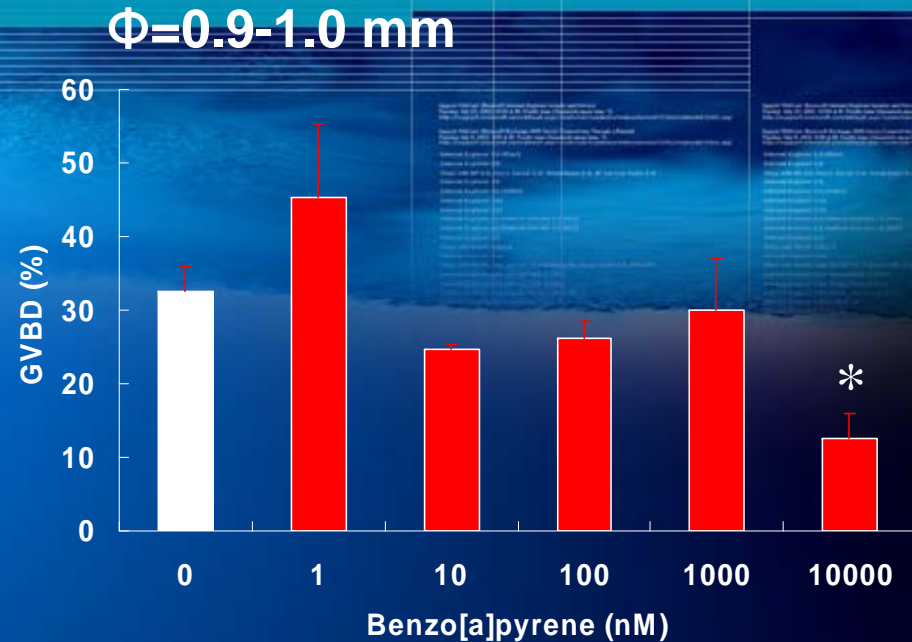
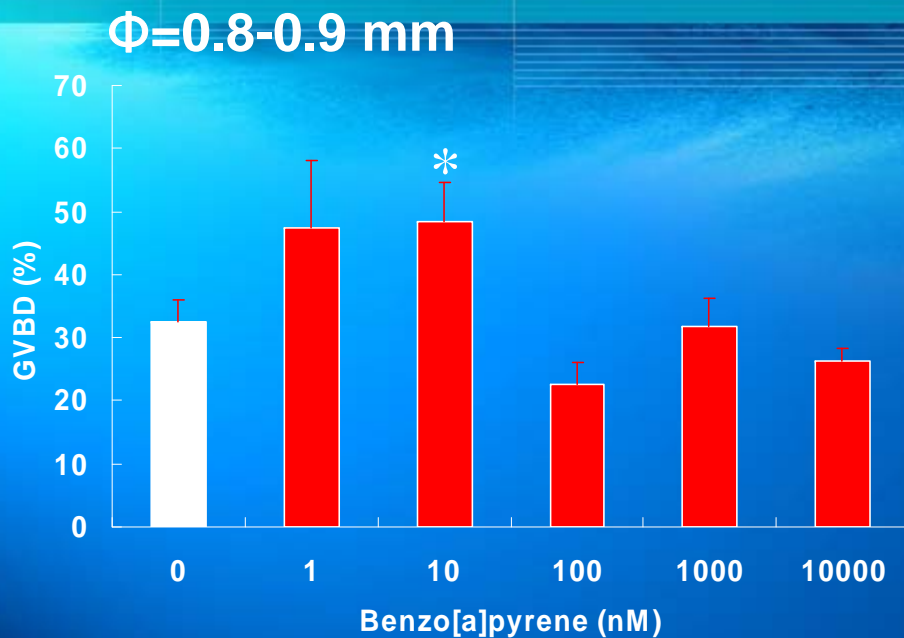
Increase in E2 production
Increase in 17 α 20 β OHP production

Effects of B[a]P on *in vitro* steroid production (E2/T and E2/17 α 20 β OHP)

High E2/T and E2/17 α 20 β OHP – Estrogenic activity or inhibition of maturation



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



But, I'm not sure.
Study more
in detail!



Exposure to
lower concentrations



Stimulation
in maturation



Exposure to
higher concentrations



Estrogenic effects
Inhibition in maturation

Investigation
of long-term effects

In vivo experiment

Molecular biological methods



Mechanism of
endocrine disruption
on oocyte maturation
by PAHs, B[a]P

Thank you.
Don't spill your oil.