In Vivo Imaging of Gonad Dynamics in Transparent *Casper* Zebrafish

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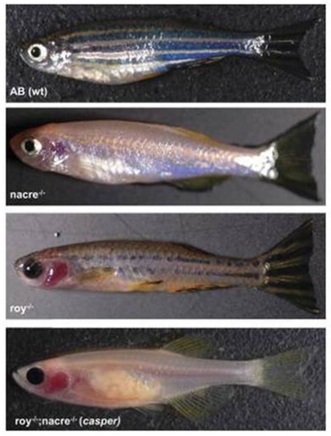
Casper Zebrafish

Double mutant

Nacre lacks melanocytes
Roy lacks iridophores
Casper – Roy/Nacre double homozygous mutant

Allows for continuous data collection over time

(White et al., 2008)



Casper Gonad in vivo

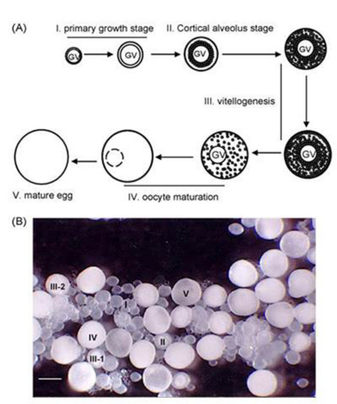
Female Gonad

Male Gonad

Oogenesis –
Process of Oocyte Production
Five Stages of oocyte Development

1. primary growth
2. cortical alveolus stage
3. vitellogenesis
4. oocyte maturation
5. mature egg

Image: Clelland and Peng, 2009



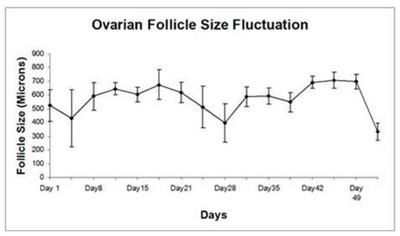
Hypotheses

The ovary is dynamic and experiences cycles of growth and regression
Large oocytes seem to have a finite lifespan and are either ovulated and oviposited or resorbed (atresia)
Exogenous hormones (synthetic estrogen) will disturb normal ovarian cycle by causing regression or drastic cycling

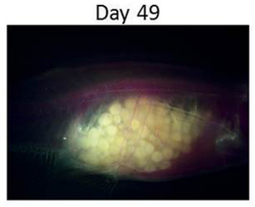
Experimental Design: Part 1

What is normal behavior of the ovary?
•Female zebrafish were observed for 6 weeks
•Animals were anesthetized and gonad imaged
•Follicle diameters were measured via ImageJ
•Thus a history of gonad dynamics was established for each animal

Female Ovary Fluctuation



Day 49



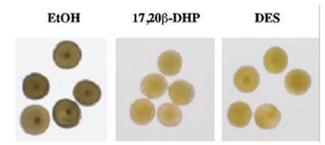
Day 53

Endocrine Disruption
Diethylstilbestrol

Preliminary data suggest the ovary experiences cycles of growth and regression
Large oocytes seem to have a finite lifespan and are either ovulated and oviposited or resorbed (atresia)
What controls ovarian oscillation? – Hormone measurement difficult in small animal (~300 mg body weight)
What effects do exogenous hormones have on the ovary?
Environmental endocrine disruptors can mimic estrogen
Pharmaceuticals are found in aquatic systems

The morphology of oocytes after 6 hours of each treatment was photographed.
Germinal vesicles were seen near the center of oocytes after EtOH treatment, whereas they disappeared after 17,20-DHP and DES treatments
GVBD indicates maturation

DES induces Oocyte Maturation



Tokumoto, 2004

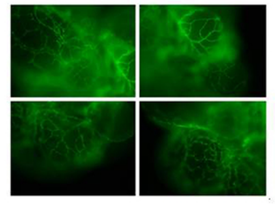
Experimental Design: Part 2

6 female *Casper* zebrafish one year old, weigh 0.25 – 0.30 grams, and have been imaged for gonad status for at least 2 weeks prior to experimental treatment
The groups will receive 0.2 nM or 0.35 nM diethylstilbestrol. The control groups will only receive the vehicle.
Compounds will be applied to the tank water and the animals will be monitored for 20 days post application.
Reproductive status will be assessed by image analysis of follicle diameters and the physical appearance of ovary, in vivo.

Future Plans

Future plans in the lab include utilizing the transgenic (Fli-1 GFP) zebrafish put into a *Casper* genetic background
Observe fluorescence changes in vascularization of the ovary over time, in vivo.

Fli-1 GFP in zebrafish ovary



Summary

Casper Zebrafish line is being developed in our lab as a model for longitudinal ovarian studies and appears useful in the study of endocrine disruptors in vivo
Diethylstilbestrol is hypothesized to elicit increased ovarian cycling and to induce oocyte maturation in vivo
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