The effects of differing ethanol concentration on zebrafish embryos

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Objective

The objective of the experiment is to determine the effects of ethanol exposure on the embryonic development of zebrafish through observation of physical defomities.

Introduction

Exposure of prenatal humans to alcohol can produce Fetal Alcohol Syndrome (FAS) which results characteristic craniofacial defects, poor muscle tone, underdeveloped fetuses, and heart defects. As a model organism, zebrafish (*Danio rerio*) have been used to investigate teratogens, including alcohol exposure, in part because they develop most of the major organ systems present in mammals in less than a week (Rubinstein, 2006).

Embryonic development of zebrafish is affected by ethanol in a manner similar to higher vertebrates (Blader and Strähle, 1998). Exposure of zebrafish embryos to ethanol causes cyclopia and craniofacial abnormalities, alters gene expression in the ventral aspects of the fore and midbrain, induces developmental abnormalities of the notochord and spinal cord, and malformation of the body trunk (Blader and Strahle, 1998). Reimers et al. (2004) also observed adverse developmental effects including cyclopia, under-developed mid, fore and hindbrains, and pericardial edema.

In order to understand the origin of the deformities, it is necessary to know how embryonic cells of zebrafish normally migrate during gatrulation. During gastrulation, blastoderm cells epibolize over the yolk. When about half the yolk cell becomes covered with blastoderm cells, the margin of epibolizing blastoderm cells form the epiblast and the hypoblast. The organizer, known as the embryonic shield, forms on the dorsal side of the embryo (Gilbert, 2010). Hypoblast cells of the embryonic shield converge, extend anteriorly, and narrow along the dorsal midline of the hypoblast (Gilbert, 2010). Some of these cells eventually form the prechordal plate and the notochord (Gilbert, 2010).

The prechordal plate cells of zebrafish exposed to ethanol form at an ectopic position of (Blader and Strähle, 1998). It is, therefore, believed that ethanol is responsible for the abnormal migration of prechordal plate cells that ultimately causes cyclopia and other deformities. The prechordal plate cells express genes like *goosecoid* and *islet-1*, which control cell differentiation in the anterior region of the embryo (Blader and Strähle, 1998). Hence, prechordal-specific genes are expressed ectopically to bring about the observed deformities.

In addition to the deformities, ethanol appears to cause abnormal cell death (Sulik *et al*., 1988). Ethanol exposure at early development stages results in significant death among the cells destined to give rise to facial structures (Sulik *et al*., 1988). Ethanol seems to achieve apoptosis by activating the cells' self-destruction machineries (Sulik *et al*., 1988). Cell death has also been proposed as a potential explanation for ethanol-dependent toxicity in developing embryos (Reimers et al., 2004). The link between cell death and ethanol in early embryos can be partially explained by an increase in oxidative stress, leading to oxidative tissue damage and in higher doses death.

Materials
Mature zebrafish

Zebrafish Embryo Medium (ZFEM; see below)

60 mm glass Petri dishes

wide-mouth glass Pastuer pipets

Siphon and fine fry net or mesh filter

Incubator (28°C)

Dissecting microscope and camera

Procedure

1. Clean algae and other debris out of tank with adult fish by vacuuming the tank with a siphon. After cleaning, place a single layer of washed marbles across the bottom of the tank. One-two hours after 'dawn', use the siphon to collect the embryos from the marbles into a mesh filter. Place the embryos on the mesh filter into a glass dish of tank water and examine using a dissecting microscope.

2. Select embryos that are at the dome/30% epiboly stage stage (Figure 1).

3. Prepare five separate petri dishes that contain either normal zebrafish embryo medium, or 1%, 2%, 2.5%, 3% ethanol in zebrafish embryo medium solution.

4. Place at least 10 of the selected embryos into each of the petri dishes and let them sit for 3 hours.

5. After 3 hours has passed, transfer embryos into petri dishes with normal zebrafish embryo solution. (Note: this step can be omitted if timing does not permit).

6. One day later, observe embryos for abnormalities and photograph them.

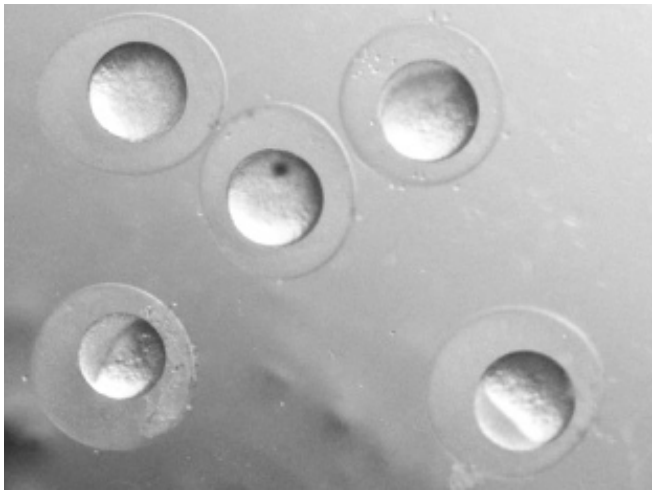

Figure 1: Zebrafish embryos at 30% epiboly.

References

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Zebrafish Embryo Medium (from Westerfield, 1993)

Hank's Stock #1

NaCl	4.0 g
KCl	0.2 g
ddH_2O	to 50 ml

Hank's Stock #2

$Na_2HPO_4*7H_2O$	0.36 g
KH_2PO_4	0.30 g
ddH_2O	to 50 ml

Hank's stock #4

$CaCl_2*2H_2O$	0.96g
ddH_2O	to 50 ml

Hank's Stock #5

$MgSO_4*7H_2O$	1.23 g
ddH_2O	to 50 ml

Embryo Medium

Stock #1	0.5 ml
Stock #2	0.05 ml
Stock #4	0.5 ml
ddH_2O	48.3 ml
Stock #5	0.5 ml
$NaHCO_3$	0.18 g

Use about 10 drops of 1 M NaOH to bring to pH 7.