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REDUCTION OF GERMINAL VESICLE BREAK DOWN IN DANIO FOLLOWING DELTAMETHRIN EXPOSURE

Dr.D.NALINI[1]

[1] LECTURER IN ZOOLOGY, THE AAS COLLEGE,ADONI.

ABSTRACT

During the course of maturation, oocytes undergo drastic morphological changes associated with the progression of the meiotic cell cycle, among which breakdown of the oocyte nuclear envelope (germinal vesicle breakdown, GVBD) occurring at the prophase/metaphase transi-tion is usually regarded as a hallmark of the progress of oocyte maturation. As the zebrafish is asynchronous, the ovary contains follicles present in different stages. The present study shows more number of oocytes at GV IV position GVBD) in control and lower concentrations of decis indicates normal progress in oocytes maturation. Less numberof oocytes in Germinal Vesicles IV position GVBD) in higher concentrations of decis indicates delay in oocytes maturation.

Key words: Germinal vesicle, maturation,oocytes.

Introduction

Water polluted by different ways is contaminated with different contaminants. Various contaminants that lead to degradation of the water quality include pathogens, chemicals and other sensory and physical changes. Most of these contaminants are either oxygen depleting, where they deplete the water body of requisite oxygen for survival of aquatic species, some of them are toxic as they produce some toxic substances that can prove fatal and harmful for life to survive, also many of them are capable of blocking the sun-light which in turn restricts the normal cycles for survival of life. Chemicals resulting in water pollution are classified as Organic and Inorganic. Organic chemicals include detergents, insecticides, pesticides etc, while inorganic include industrial bi-products, fertilizers etc., pathogens like viruses, parasitic worms' results from ill-treated sewage discharge. Pollution is a silent killer that endangers not just a person's health, but also his or her environment. Among the different types of pollutions, Water pollution has emerged as one of the gravest environmental threats around the world and particularly in India. Over the years Water Pollution has turned out to be the biggest environmental concern because nearly 14,000 deaths are recorded daily due to it.

Review of literature

Exposure to xenobiotics in the environment has been associated with a number of abnormalities, such as decreased fertility, demasculinization, feminization, decreased hatching success, alteration of the immune system, increase of cancer etc., in wild species of fish. It is widely recognized that a variety of endocrine disrupting chemicals found in the environment may affect sexual development and reproduction in wildlife, including fish (WHO, 2002).

Exposure to environmental estrogenic alkyl phenolic chemicals in rain brouw trout resulted in a decreased fertility and egg production in females, reduction of gonadal size (Jobling et al., 1996; Ashfield et al., 1998), reduced sperm production and demasculinisation of genetic male Japanese medaka, intersex gonads (Nimrod and Benson, 1998; Gimeno et al., 1998) etc. Another frequently observed effect of exposure to environmental estrogens is the induction of the yolk precursor protein vitellogenin (VTG) in male and sexually immature fish (Jobling et al., 1996; Folmar et al., 1996).

Different studies show VTG induction in a dose - and

time- dependent manner. For example, significant increase in VTG has been reported in adult common carp (Mandich et al., 2007) and medaka (Kang et al., 2002). Ishibashi et al., (2005) at 1000 mg/L BPA after 14 and 21 days exposure, respectively. Lindholst et al., (2000) observed vtg induction in rainbow trout exposed to 500mg /L BPA for 12 days through a continuous flow system. In Atlantic cod, BPA at 59mg/L induced VTG production after 21 days exposure (Larsen et al., 2006). Hatef et al., (2011) reported disruption of male reproductive physiology in goldfish exposed to environmentally relevant concentrations of BPA via alternations of androgens (T and 11-KT) and VTG synthesis and sperm motility and velocity. When medaka were exposed to the environmental estrogen, *o,p'*-DDT, for two or for 8 weeks, fertility and hatching success were reduced (Cheek et al., 2001). Males produced the yolk precursor, Vitellogenin and when exposed male cohorts were subsequently paired with control females, a 50% reduction of fecundity was seen.

Abnormal gonadal development, such as delayed maturation, high levels of atresia or intersexuality may also be detected histologically. Such parameters are frequently investigated in fish from contaminated environments or those exposed to anthropogenic chemicals (Jobling and Tyler, 2003; Bateman et al., 2004). Follicular atresia has been reported as a response to plant sterol exposures, in Japanese medaka (*Oryzias latipes*) exposed to the phytoestrogens genistein and the bioflavonoid quercetin (Kiparissis et al., 2003). Female fish from polluted areas have shown a higher incidence of oocyte atresia compared to control females (Jobling et al., 2002ab).

MAINTENANCE OF PARENTAL FISH

Wild type adult Zebrafish (*Danio rerio*) used in this study were bred in our aquarium facility for two generations. Females and males are kept in a ratio of 2:1 in an aquaria filled with filtered tap water with the oxygen saturation of more than 80% and P^H at 7.0±0.3.The water temperature was maintained at 26±1^{0}C at a 14h: 10h day and light regime. Fish were regularly provided with varied diet comprising of freshly hatched live brine shrimp (*Artemia nauplii*) once a day, supplemented with vitamin fed dried flake food twice a day.The aquarium water was aerated continuously with stone diffusers connected to mechanical air compressor. Renewal of water is done in a semi-static manner and the aquaria screens were cleaned daily. The excess amount of food and fecal matter was removed from the water and healthy environment was provided before experimentation. The water quality and cleanliness of aquaria was monitored regularly and reset to initial state. Less than 1% of the population died during acclimatization.

ZEBRAFISH EGG COLLECTION

Embryos were collected from breeding stock of healthy, unexposed mature male and female zebrafish which were above the six months. Care was taken such that the fish were free of macroscopically discernable symptoms of infection and disease. The spawning glass trays were covered with a fine nylon net with an appropriate mesh size for eggs to fall through were placed in the aquaria on the evening before the spawning was required. Plant imitations made of plastic serving as spawning substrate are fastened to the nylon mesh. The fish were left undisturbed over night. Eggs were spawned synchronously at dawn of the next morning. After the light was turned on the next morning embryos were generated by natural mating and then collected within 30 minutes after spawning. Newly fertilized eggs were collected from the spawning trays and embryos were rinsed several times with tap water and their quality was checked under the microscope being sure to select the healthy fertilized eggs for the experiment. Unfertilized eggs were identified by their milky color and discarded. The dead embryos appear white because of the coagulation of precipitation of proteins.

PREPARATION OF TEST SOLUTION

Decis EC 11% (W/W) manufactured by Bayer's company was purchased from local Agro-

Chemical stores. Using the formula $C_1V_1=C_2V_2$, the concentration of deltamethrin present in the decis was calculated. Then the stock solution was prepared by dissolving 1.9ml of decis in distilled water and made it upto 100ml standard flask.

EMBRYO-LARVAL TOXICITY TEST

Embryos at the same developmental stage (4hpf) were collected and rinsed with tap water. Exposure experiments were carried out by placing 100 embryos in 500ml of water containing glass chambers with different concentrations of decis, 0.2, 0.4, 0.6, 0.8, 1.0µg/l of decis upto 72hpf and unexposed embryos were used as controls. Exposure experiments were carried out in triplicate. Morphological and developmental endpoints were observed under an optic microscope in control and treated embryos at 24, 48, 72 and 96hpf. The toxicant was added everyday to maintain exact concentration of decis and the water quality. Occasional stirring as well as replacement of the medium were done daily to ensure even distribution of the chemical. Embryos and larvae were daily observed under a stereomicroscope (Magnus MLX) and magnification used for observation was 4X and 10X for eggs and larvae. Embryos and larvae were considered dead when no heart beat was observed and dead embryos or larvae were removed immediately and at each observation time mortality was recorded every 24h from. In the embryo-larval phase the parameters were evaluated were eye and body pigmentation, absorption of the yolk sac and hatching. After hatching, edema, trunk curvature and tail malformation were observed and recorded. From 48hpf embryos started to come out of the chorion asynchronously and the data of hatching and other parameters were recorded.

IDENTIFICATION OF GERMINAL VESICLE POSITION

Oocytes were obtained from sexually matured female zebrafish exposed to different concentrations of decis, 0.02 and 0.04µg/l. For each group six fish were exposed and control were also maintained. Oocytes collected from ovary by sacrifying the fish were separated mechanically, washed twice with water and placed in glass petridishes. Then the 20 oocytes from each group were fixed in Serris fluid (600ml of alcohol 96% + 300ml of formaldehyde +100ml of acetic acid) and immersed in Turpentine oil for transparency. Then the oocyte were fixed for germinal vesicle position and observed under the microscope.

GERMINAL VESICLE BREAKDOWN

The oocyte is the starting point for a new generation. Most of the machinery for DNA and protein synthesis needed for the developing embryo is made autonomously by the fertilized oocyte. During the course of maturation, oocytes undergo drastic morphological changes associated with the progression of the meiotic cell cycle, among which breakdown of the oocyte nuclear envelope (germinal vesicle breakdown, GVBD) occurring at the prophase/metaphase transi-tion is usually regarded as a hallmark of the progress of oocyte maturation. As the zebrafish is asynchronous, the ovary contains follicles present in different stages.

In most animals, the growing oocyte contains an enlarged nucleus called Germinal Vesicle (GV), and is active in transcribing an extensive array of genes whose products are necessary for oocyte development and for sustaining early embryonic development (Vornonia and Wessel,2003). Germinal vesicle is generally located at the centre of the oocyte. In response to hormonal stimulation it starts to migrates toward the animal pole during late vitellogenesis. The disappearance of the germinal vesicle is commonly referred to as Germinal Vesicle Break Down (GVBD).

Results

Control: 10% oocytes are present in GV I Position, 20% in GV II, 25% oocytes in GV III and finally 45% oocytes are present in GV IV position.

0.02µg/l exposure of decis: 20% oocytes are present in GV I Position, 25% in GV II, 20% oocytes in GV III

and finally 35% oocytes are present in GV IV position.

0.04µg/l exposure of decis: 40% oocytes are present in GV I Position, 25% in GV II, 20% oocytes in GV III and finally 15% oocytes are present in GV IV position.

More no. of oocytes at GV IV position GVBD) in control and lower concentrations of decis indicates normal progress in oocytes maturation. Less numberof oocytes in GV IV position GVBD) in higher concentrations of decis indicates delay in oocytes maturation.

Percent of oocytes observed at different positions after exposing adut female zebrafish to 0.02µg/l and 0.04µg/l concentrations of commercial grade deltamethrin, decis. Controls were also observed.

Concentration	GV I (%)	GV II (%)	GV III (%)	GV IV (%)
Control	10	20	25	45
0.02µg/l	20	25	20	35
0.04µg/l	40	25	20	15

DISCUSSION

Zebrafish oocyte maturation is a complex event encompassing a number of cellular changes including germinal vesicle migration (GVM) and dissolution or breakdown (GVD), ooplasmic clearing (OC) with correlated yolk protein changes (YP), development of osmoregulation (OR) in fresh water, the formation of the future embryonic pole, the blastodisc (BF) and activatibility (AC) or cortical maturation. In zebrafish, and many other teleosts, 17α, 20ß-dihydroxy-4-pregnen-3-one (17α, 20ß-DP) has been shown to be the normal inducer of oocytematuration.Oocyte maturation in fish is triggered by MIH, which acts on progestin receptors located on the oocyte membrane and induces the activation of MPF in the oocyte cytoplasm (Tokumoto et al., 2007). During the course of maturation, oocytes undergo drastic morphological changes associated with the progression of the meiotic cell cycle, among which breakdown of the oocyte nuclear envelope (germinal vesicle breakdown, GVBD) occurring at the prophase/metaphase transition is usually regarded as a hallmark of the progress of oocyte maturation.

Several endocrine-disrupting chemicals or EDCs, such as Kepon and o ,p-DDD, have been reported to antagonize MIH-induced meiotic maturation of fish oocytes in vitro (Das and Thomas, 1999). A potent inhibitory effect of pentachlorophenol (PCP) was demonstrated on oocyte maturation induced by MIH and DES (Tokumoto et al., 2005).

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