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RESEARCH PAPER

Differential Effects of Heavy Metals (Cadmium, Cobalt, Lead and Mercury) On Oocyte Maturation and Ovulation of the Catfish *Heteropneustes fossilis*: an *In Vitro* Study

Geeta J. Gautam¹, Radha Chaube^{1,*}

¹ Banaras Hindu University, Zoology Department, Mahila Mahavidyalaya, Varanasi-221005, India.

* Corresponding Author: Tel.: +91.933 6847252
E-mail: chauberadha@rediffmail.com, radhachaube72@gmail.com

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Abstract

The aim of the present study was to examine comparative effects of different concentrations of heavy metals such as cadmium (Cd), cobalt (Co), lead (Pb) and mercury (Hg) on catfish oocyte maturation and ovulation. Post-vitellogenic oocytes of the catfish *H. fossilis* were incubated with heavy metals at concentrations of 0.1, 1.0, 10 and 50ng/ml. Translucent follicles without germinal vesicle and opaque follicles with GV were scored after 4, 8, 16, and 24 hr of incubation. Percentage GV breakdown (GVBD) was calculated as an index of oocyte maturation. The results of present investigation suggested that the effect of heavy metals on oocyte maturation and ovulation was in the order of Pb > Hg > Cd > Co. Pb and Hg showed significant increases ($P < 0.05$) in GVBD and ovulation at all concentrations during 4 and 8hr of the incubation. However, there was a dose –dependent decrease at 16 and 24hr. Cd and Co showed decline in GVBD and ovulation at 16 and 24 hr of the incubation. However, mercury and lead showed a stimulatory effect on ovulation at 16 and 24 hr compared to control ($P < 0.05$). Thus, these results suggest that catfish oocytes are differentially influenced by heavy metals and are resistant to them at certain levels.

Keywords: Heavy metals, catfish, ovary, oocyte maturation, ovulation.

Introduction

In teleost, as in other non-mammalian vertebrates, the principal events responsible for the enormous growth of oocytes are due to the accumulation of yolk proteins within their cytoplasm (Devlin & Nagahama, 2002). After the oocyte completes its growth it becomes ready for the next phase of oogenesis, i.e. the resumption of meiosis (completion of the first meiotic division followed by progression to metaphase II), which is accompanied by several maturational process in the nucleus and the cytoplasm of the oocyte. This process called oocyte maturation, occurs prior to ovulation and is a prerequisite for successful fertilization; it consists of the germinal vesicle break down (GVBD), chromosome condensation, assembly of the meiotic spindle and formation of the first polar body (Nagahama & Yamashita, 2008). The endocrine regulation of oocyte maturation and ovulation has been investigated most extensively in fishes (Nagahama, Yoshikuni, Yamashita & Nagahama, 1994; Thomas, Baer & White, 1994; Nagahama, 1997). Studies using well characterized *in vitro* as well as *in vivo* systems have revealed that oocyte

maturation in fish is regulated by three mediators, gonadotropin (GTH and Luteinizing hormone, LH), maturation inducing hormone (MIH), and maturation promoting factor (MPF).

Endocrine disruptors have been reported to interfere with steroid hormone production or steroid mediating signaling in the gonads and eventually lead to serious damage of the reproductive organs and function. Fish inhabits practically all aquatic zones and are highly sensitive to changes in the environment. Hence, they play an important role in monitoring any kind of aquatic pollution and are widely used to evaluate the health of aquatic ecosystems. Heavy metals belong to undesirable substances in water environment. They are known for their ability of persistent accumulation in animal tissues. They can influence fish organism and disturb the process of reproduction (Thomas, 1993; Mukherjee, Kumar, & Chakraborti, 1994; Szczerbik *et al*, 2008). Cd is one of the most dangerous among heavy metals (Lukjanienko, 1974). Nandi, Gupta, Selvaraju, Roy and Ravindra (2010) has shown that culturing Buffalo (*Bubalus bubalis*) oocytes in different increasing concentrations of heavy metals for 24 hr had detrimental effects on cumulus

expansion and oocyte maturation. Chronic exposure to pollutants like heavy metals leads to reproductive dysfunction in aquatic species (Choubey, Chaube & Joy, 2015). Heavy metals are not only highly toxic and persistent in nature but are also reported to have an endocrine disruptive potential (Zhu, Kusaka, Sato & Zhang 2000). Different heavy metals like cadmium, mercury, lead, arsenic, manganese and zinc are known to cause effect on endocrine system (Dyer, 2007; Luszczek-Trojnar et al., 2014). It has been reported that chronic exposure to cadmium influences reproductive disruption in fish, inhibiting induction of vitellogenin (Olsson et al., 1995), delaying oogenesis in brown trout (Brown et al., 1994) enhancing luteinizing hormone (LH) secretion and decreasing parameters of gonadosomatic index (GSI) and ovulation in Prussian carp (Szczerbik et al., 2006).

Heavy metals enter into various environmental matrices (air, water and soil) from a wide variety of natural and anthropogenic sources. All the sources of pollution affect the physicochemical characteristics of the water and biological components and thus the quality and quantity of fish. Fishes are one of the most widely distributed organisms in the aquatic environment and, being susceptible to heavy metal contamination (Khillare et al., 2017). As a result, large scale mortality of fishes has been observed due to discharge of heavy metals in natural water resources (FAO, 1996.) Catfish *Heteropneustes fossilis*, which is an annual breeder and breeds during the monsoon rainfall (July–August). The fish is economically important for aquaculture in the Indian subcontinent and the flesh is highly priced, which is rich in protein but poor in lipid and fat content. At Varanasi due to heavy pollution in Ganges River and anthropogenic activities, river water is contaminated by heavy metal pollutants, which has severe impact on survivability of aquatic organisms specially fish population. Oocyte maturation and ovulation is the sensitive parameter for assessment of such pollutants. Thus, the objective of the present study was to examine the effect of heavy metals, cadmium (Cd), cobalt (Co), lead (Pb) and mercury (Hg) on catfish *H. fossilis* oocyte maturation and ovulation under *in vitro* conditions.

Materials and Methods

Chemicals

Cadmium Chloride (CdCl_2 , Mol. Wt.- 183.32); Cobalt Chloride (CoCl_2 , Mol. Wt. :- 237.93); Lead Nitrate ($\text{Pb}(\text{NO}_3)_2$, Mol.Wt.: -331.21) and Mercuric chloride (HgCl_2 , Mol. Wt.: - 271.50) were purchased from Sisco Research laboratories (SRL) Pvt. Ltd. Mumbai, Maharashtra, India. All other chemicals were of analytical grade and purchased locally.

Animal Collection and Maintenance

The experiments were performed in accordance

with the guidelines of Banaras Hindu University for experimentation in animals and all care was taken to prevent cruelty of any kind. Mature female catfish *Heteropneustes fossilis* (40-50 g) were purchased from local fish market in the pre-spawning phase (May-June) of the annual reproductive cycle. The gonado-somatic index (GSI) was 8.03 ± 0.56 . They were maintained in the laboratory under normal photoperiod (13.0L: 11.0D) and temperature ($25 \pm 2^\circ \text{C}$) until used for experiments. The fish were fed goat liver daily *ad libitum*. A few fish were sacrificed randomly to determine the maturation of ovary. The fish containing ovaries filled with dark green post-vitellogenic follicles (oocyte diameter 1.0 mm) were used in the study.

Preparation of Incubation Medium and Test Compounds

The incubation medium consisted of (in grams) NaCl 3.74, KCl 0.32, CaCl_2 0.16, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.10, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.16 and glucose 0.40 in one L of triple distilled water (Goswami & Sundararaj, 1971). As phenol red (indicator of pH) is estrogenic, it was omitted in the preparation of the medium. The pH was adjusted to 7.5 with 1N sodium bicarbonate and autoclaved. Penicillin (200,000U) and streptomycin sulphate (200 mg) were added and filtered. The medium was stored at 4°C and was prepared fresh every week. Just before the incubation, CdCl_2 , CoCl_2 , $\text{Pb}(\text{NO}_3)_2$ and HgCl_2 were dissolved in the incubation medium to give different working concentrations (0.1, 1, 10, 50ng/mL).

Effects of Heavy Metals (Cd, Co, Pb and Hg) On GVBD and Ovulation

All instruments and glassware were sterilized. Gravid female *H. fossilis* were sacrificed by decapitation and ovaries were transferred to a Petri dish containing fresh cooled incubation medium. Round, dark green post-vitellogenic ovarian follicles (1mm diameter) were separated with fine brush and watchmaker's forceps. Batches of about 30-40 follicles were incubated in embryo cups containing 3mL incubation medium or medium containing test compound (0.1, 1, 10, 50ng/mL) at $24 \pm 2^\circ \text{C}$ for 4, 8, 16 and 24 hrs. In each experiment, follicles from 5 fish ovaries were used and the incubations were done in triplicate from each ovary. The medium was changed 4 hourly and replenished with fresh medium containing required amount of the test compound. As controls, the follicles were incubated in medium containing the vehicle.

GVBD Scoring

At the end of the incubation (4, 8, 16 or 24 h), the follicles were cleared in a clearing solution (ethanol : acetic acid : formalin, 6:1:3; Trant & Thomas, 1988) and observed under a stereobinocular

microscope. Translucent follicles without germinal vesicle (GV) were scored. Percentage of GV breakdown (GVBD) was calculated from the total number of follicles incubated that underwent GVBD (Figure 1).

$$\% \text{ GVBD} = \frac{\text{Number of follicles that underwent GVBD}}{\text{Total number of follicles incubated}} \times 100$$

Total number of follicles incubated

Ovulation Response

After the termination of the experiments, the follicles were examined under a stereo-binocular for scoring percentage ovulation. The intact follicles (unovulated) and oocytes without the follicular layer (ovulated) were counted separately. The percentage of ovulation was determined from the total number of

the follicles incubated (Figure1).

$$\% \text{ Ovulation} = \frac{\text{Number of follicles that underwent Ovulation}}{\text{Total number of follicles incubated}} \times 100$$

Total number of follicles incubated

Statistical Analysis

All experiments were repeated three times using follicles of the ovaries obtained from five fish. The difference between control and exposed groups were analyzed by Two way ANOVA followed by Tukey's test using SPSS statistical software. P values of less than 0.05 were considered significant (Table 1 and Table 2). Pearson correlation was done between heavy metal duration /concentration with oocyte maturation and ovulation (Table 3, Table 4, Table 5, and Table 6).

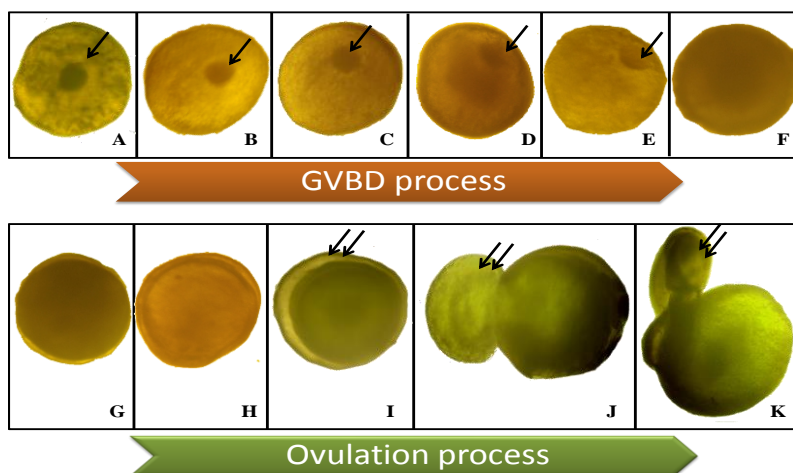


Figure 1. Showing the normal process of germinal vesicle breakdown (GVBD) and Ovulation. ✓ - Germinal vesicle (GV), ✓✓Follicular envelope, A- GV in centre; B,C,- GV migrating towards periphery ; D- GV at periphery; E- GV breakdown started; F-GVBD complete ; G,H-Ovulation process starting; I- Oocyte hydrated showing detached follicular envelope; J- Follicular envelope detached; K-Ovulation complete.

Table 1. Statistical analysis using Two-way ANOVA followed by Tukey's test of effects of heavy metals on GVBD.

	Two-way ANOVA (P<0.001); followed by Tukey's test (P<0.05)		
Heavy metals	F _{duration}	F _{concentration}	F _{duration x concentration}
Cadmium	683.42	456.61	184.22
Cobalt	732.62	1408.1	158.08
Lead	1434.9	1530.8	121.94
Mercury	3949.3	781.3	322.6

Table 2. Statistical analysis using Two-way ANOVA followed by Tukey's test of effects of heavy metals on Ovulation.

	Two-way ANOVA (P<0.001); followed by Tukey's test (P<0.05)		
Heavy metals	F _{duration}	F _{concentration}	F _{duration x concentration}
Cadmium	9.871	41.491	10.393
Cobalt	26.03	52.818	12.126
Lead	440.26	100.41	59.085
Mercury	90.574	83.267	26.342

Results

was limited to 6% which is just negligible.

Effects of Cadmium on GVBD and Ovulation

Incubation of the follicles with cadmium produced an overall significant effect on the percentage GVBD and ovulation after 4, 8, 16 and 24hr (Figure 2A; $P<0.001$). Cd significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 8hr and lowest at 24hr at 10ng/mL dose. It decreased at 16 and 24hr of incubation ($P<0.05$). On the other hand, % ovulation showed significant increase at 8 hr with 0.1,1,10 and 50ng/mL. Further, at 16 and 24 hr ovulation decreased significantly compared to control (Figure 2B; $P<0.001$). However, maximum rate of ovulation

Effects of Cobalt on GVBD and Ovulation

Incubation of the follicles with cobalt produced an overall significant effect on the percentage GVBD and ovulation after 4,8,16 and 24hr (Figure 3A; $P<0.001$). Co significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 4 and 8hr at 0.1ng/ml and lowest at 16 and 24hr at 50ng/mL. There was significant concentration and duration dependent decrease ($P<0.05$). Cobalt treatment induced ovulation only upto 8%, which was maximum at 1ng/ml at 4 and 8 hr. Further, there was decline due to death of follicles (Figure 3B; $P<0.001$).

Table 3. Pearson Correlation between heavy metals with duration of exposure for GVBD

GVBD	Duration (hr)			
	4h	8h	16h	24h
Cd	-0.04095	0.342971	-0.07826	-0.35518
Co	-0.27876	-0.09469	-0.64449	-0.59659
Pb	0.630488	0.708183	0.630299	0.483135
Hg	0.103499	0.565914	-0.10214	-0.28969

Correlation is significant at $P<0.05$ level (2 tailed)

Table 4. Pearson Correlation between heavy metals with duration of exposure for ovulation

GVBD	Duration (hr)			
	4h	8h	16h	24h
Cd	-0.42002	0.55177	0.883078	-0.10156
Co	-0.19768	0.47173	-0.10011	-0.10156
Pb	0.756954	0.67743	0.087686	0.196078
Hg	-0.2399	0.226299	0.642779	0.0883

Correlation is significant at $P<0.05$ level (2 tailed)

Table 5. Pearson Correlation between heavy metals with different concentrations of exposure for GVBD

GVBD	Concentration (ng/mL)					
	Control	0.1ng/mL	1ng/mL	10ng/mL	50ng/mL	
Cd	0.931143	0.835279	0.019667	-0.62763	-0.03707	
Co	0.931143	-0.94121	-0.87356	-0.78165	-0.77388	
Pb	0.931143	0.790449	0.814539	0.629724	0.481619	
Hg	0.931143	-0.06847	-0.67795	-0.81567	-0.80802	

Correlation is significant at $P<0.05$ level (2 tailed)

Table 6. Pearson Correlation between heavy metals with different concentrations of exposure for GVBD

GVBD	Concentration (ng/mL)					
	Control	0.1ng/mL	1ng/mL	10ng/mL	50ng/mL	
Cd	0.232129	-0.74901	-0.79805	-0.24543	-0.149	
Co	0.232129	-0.85889	-0.91141	-0.60741	-0.55971	
Pb	0.232129	-0.79247	0.914674	0.355535	-0.17274	
Hg	0.362085	-0.9092	0.065525	0.365561	0.121208	

Correlation is significant at $P<0.05$ level (2 tailed)

Effects of Lead on GVBD and Ovulation

Incubation of the follicles with lead produced an overall significant effect on the percentage GVBD

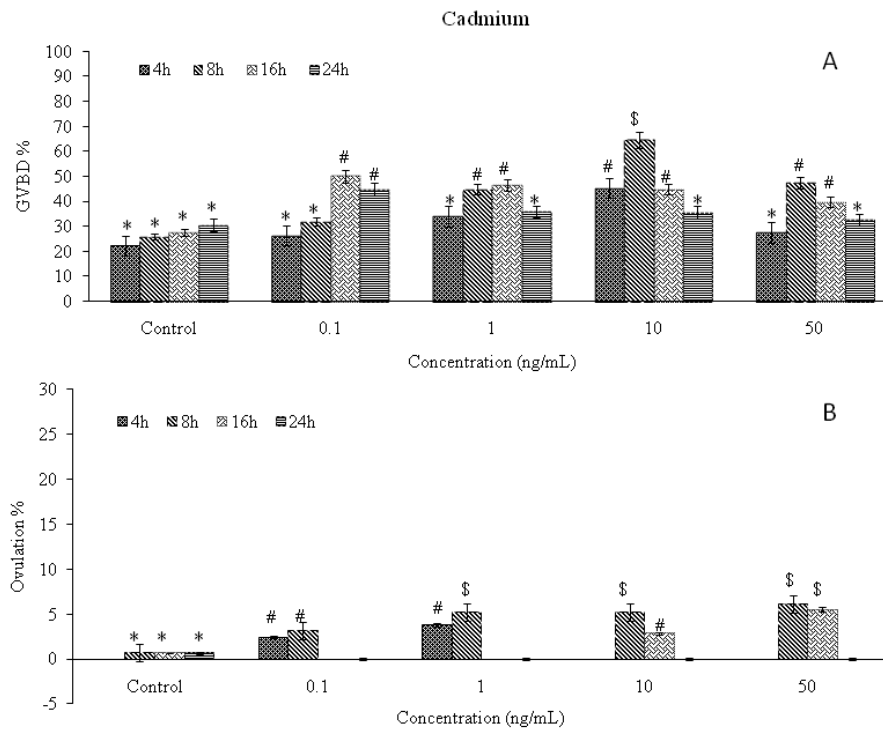


Figure 2. *In vitro* effects of different concentration of Cadmium (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24 hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean±SEM and were analyzed by two way ANOVA, followed by Tukey's test ($P<0.05$). Groups with the same letter are not significantly different.

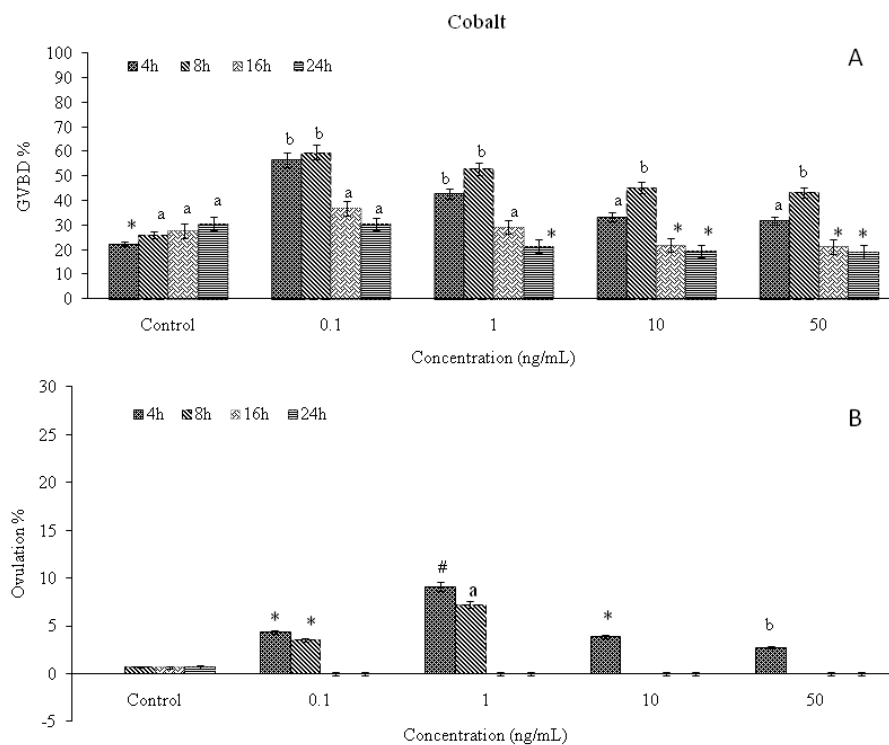


Figure 3. *In vitro* effects of different concentration of Cobalt (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean ± SEM and were analyzed by two way ANOVA, followed by Tukey's test ($P<0.05$). Groups with the same letter are not significantly different.

and ovulation after 4,8,16 and 24hr (Figure 4A; $P<0.001$). Pb significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 16hr at 10ng/ml and 50ng/ml and lowest at 4hr at 0.1ng/ml and 1ng/ml compared to control. There was significant concentration and duration dependent increase in GVBD upto 16hr in all concentration groups and later on at 24hr GVBD decreased. Lead treatment induced ovulation only upto 8%, which was maximum at 1ng/ml at 4 and 8 hr. Further, there was decline due to death of follicles (Figure 4B; $P<0.001$). Lead showed significant concentration dependent stimulation at all the durations as compared to control, maximum stimulation was seen at 16h.

Effects of Mercury on GVBD and Ovulation

Incubation of the follicles with mercury produced an overall significant effect on the percentage GVBD and ovulation after 4,8,16 and 24hr (Figure 5A; $P<0.001$). Hg significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 4, 8, 16 and 24hr at 0.1ng/ml and lowest at 16 and 24hr at 50ng/mL. There was significant concentration and duration dependent decrease. Mercury treatment induced ovulation only upto 8%, which was maximum at 1ng/ml at 4 and 8 hr. Further, there was decline due to death of follicles (Figure 5B; $P<0.001$). Mercury treated oocytes showed ovulation at all the durations, maximum effect seen at 8 and 16 h.

Correlation analysis resulted in mixed negative and positive correlation for all metal concentration

with respect to duration for GVBD and ovulation. Lead exposure showed high positive correlation at 0.1 and 1.0 ng/mL for GVBD and ovulation. The order of correlation in GVBD with respect to concentration was as follows $Pb>Cd>Hg>Co$ and for ovulation $Pb>Hg>Cd>Co$.

Discussions

The present studies demonstrate that exposure to heavy metals, i.e., cadmium, cobalt, mercury and lead, caused a decline in oocyte maturation and ovulation in a concentration and duration dependent manner. Heavy metals are endocrine disruptors with the ability to cause hormonal imbalances affecting various physiological processes such as reproduction. Chaube *et al.* (2010) showed that environmental Pb can be a potent endocrine disruptor affecting ovarian steroidogenesis, gametogenesis and ovulation, which may lead to adverse impact on fish reproduction and population density. In our study, heavy metals increased GVBD at low concentration and decreased at high concentration. Hwang, Kima, Kima, Leeb and Baeka (2010) showed that NP increased endogenous E_2 production at the vitellogenic stage and inhibited GVBD at the fully vitellogenic stage.

Studies in sheep (Leoni *et al.*, 2002), cattle and mice (Yang, Xu & Wang, 2004) have provided evidence suggesting that exposures to cadmium disrupt oogenesis *in vitro*. Work on the effect of lead on mammalian oocytes *in vitro* is limited to a single study wherein lead was reported to affect the control of both meiosis arrest and meiosis resumption of the mouse oocyte *in vitro*, at least via the PKC pathway

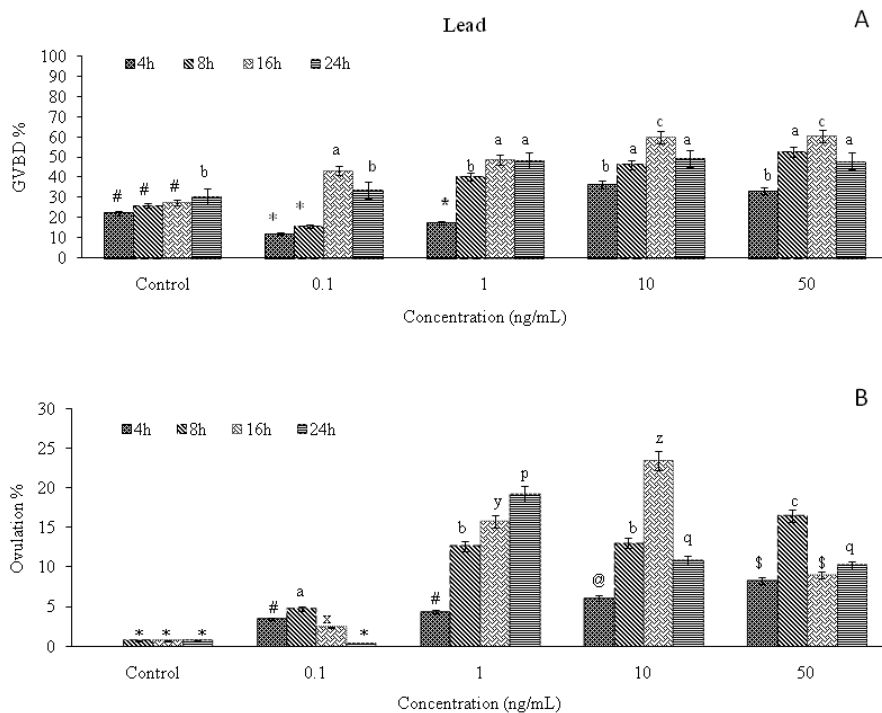


Figure 4. *In vitro* effects of different concentration of Lead (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean \pm SEM and were analyzed by two way ANOVA, followed by Tukey's test ($P<0.05$). Groups with the same letter are not significantly different.

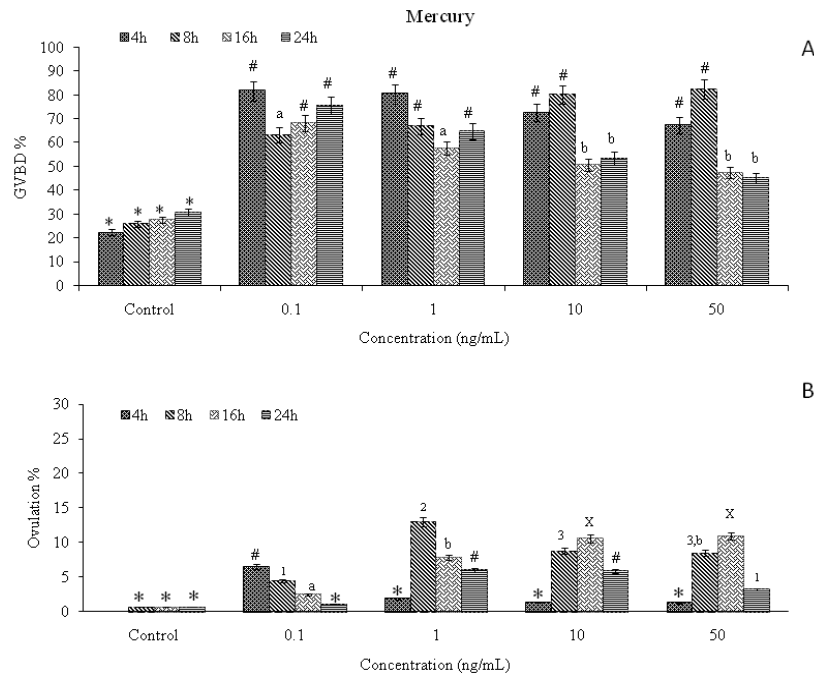


Figure 5. *In vitro* effects of different concentration of Mercury (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean \pm SEM and were analyzed by two way ANOVA, followed by Tukey's test ($P < 0.05$). Groups with the same letter are not significantly different.

(Azaveri, Denys & Lefevre, 2006). Lead also induced changes in ovarian follicular development and maturation in mice (Junaid, Chowdhuri, Narayan, Shanker, & Saxena, 1997). This was the first report on the effect of heavy metal exposure on oocyte and embryo development *in vitro* in large ruminants. Both lead and cadmium caused a significant reduction in gonadotropin binding, which altered the steroidogenic enzyme activity of granulosa cells (Nampoothiri & Gupta, 2006). Higher concentrations of cadmium inhibited activity of the P450_{scc} gene (Smida, Valderrama, Agostini, Furlan & Chedrese, 2004), inhibited progesterone synthesis (Massanyi, Uhrin, Toman, Kovacic & Biro, 1999; Zhang & Jia, 2007), and facilitated changes in cell morphology and cell death (Smida, *et al* 2004; Kondoh, Araragi, Sato, Higashimoto, Takiguchi & Sato, 2002). Thus, polluting substances, classified as endocrine disruptors, interfere with the endogenous hormone signaling system, leading to impairment of reproductive health (Caserta *et al.*, 2011; Gupta *et al.*, 2010; Zhang *et al.*, 2013).

Lead elicited biphasic effects on estradiol-17 β , testosterone, and cortisol: stimulatory at lower concentrations and inhibitory at higher concentrations. The observation of such effects led the authors to make an unusual conclusion that in trace amounts, Pb may be beneficial (Chaube, Mishra, & Singh, 2010). The physiological significance of the stimulatory response of the low concentrations of Pb on the steroid levels is not clear. It may be that Pb like other essential metals (Cu, Zn, Mn, etc.) at very low concentrations may be beneficial, if not essential for

fish metabolism (Patra, Swarup, Sharma, & Naresh, 2006; Sprocati *et al.*, 2006). But it seems very doubtful because of the lack of any evidences for the physiological role of Pb.

Cadmium alters the secretory patterns of pituitary hormones (Lafuente, Cano, & Esquifino, 2003), stimulates the progesterone synthesis at low dose (Powlin, Keng, & Miller, 1997; Massanyi *et al.*, 2000) and inhibits P4 synthesis at high doses (Jolibois, Shi, George, Henson & Anderson, 1999a; Jolibois *et al.*, 1999b). Cd is shown to down regulate 11 β -HSD by mimicking the ability of estrogen to attenuate the expression of this placental enzyme (Chowdhury & Arora, 1982). Smida *et al* (2004) reported the stimulatory effect of Cd on transcription of P450 side chain cleavage in porcine granulosa cells resulting into increase in progesterone production.

Lead is shown to cause reduction in the expression of the steroidogenic acute regulatory protein (StAR), inhibition of LH secretion (Srivastava *et al.*, 2004), increase in lipid peroxidation and ROS production (Kasperczyk *et al.*, 2008; Hsu, Hsu, Liu, Chen, & Guo, 1998). *In vivo* exposure to lead suppressed circulating LH, FSH and estradiol without affecting progesterone in cynomolgus monkey (Foster, 1992). *In utero* exposure to Pb reduced ovarian primordial follicles in mice (Wide, 1985), adult mice exposed to pb by gavage for 60 days showed alteration in the population of small, medium and large ovarian follicles (Junaid *et al.*, 1997), and when injected it decreased primordial follicles and increased growing and atretic follicles (Taupeau, Poupon, Nome, & Lefevre, 2001).

It is known that Cd can act as a reproductive disrupter in animals (Singhal, Nijayvargiya, & Shukla, 1985). It was found to interfere with ovarian steroidogenesis in mammals (Paksy, Varga, Narav, Olajos & Folly, 1992) and fish (Mukherjee *et al.*, 1994). Cd was found to stimulate the spontaneous maturation of Prussian carp oocytes in our study. We suppose that such an effect could come from the chemical similarity of Cd to calcium. Calcium is a very important regulator of ovarian steroidogenesis (Veldhuis & Klase, 1982; Tsang & Carnegie, 1983; 1984; Kleiss-San & Schuetz, 1987). The calcium channel blockers were shown to inhibit the forskolin (a direct adenyl cyclase activator) stimulated testosterone production in goldfish (Van Der Kraak, 1991). It was found that Cd affected calcium homeostasis by activating or inhibiting several calcium-related enzymes (Beyersmann & Hechtenberg, 1997).

Endocrine disruptive effect of cobalt was shown by Romani *et al.* (2014). HCG and cobalt chloride were shown to induce progesterone release, vascular endothelial growth factor release and mRNA expression in human luteal cells respectively. The estrogenicity of Hg was examined in MCF-7 cells. HgCl₂ stimulated both estrogen receptor dependent transcription and increased proliferation of MCF-7 cells (Choe *et al.*, 2003). Mondal, Mukhopadhyay, and Bhattacharya (1997) reported that HgCl₂ stimulated the activity of 3β-HSD in oocyte of fish. Both *in vivo* and *in vitro* HgCl₂ treatment showed high rate of progesterone synthesis and low rate of conversion to 17β-Estradiol in the oocytes of *Channa punctatus*. Besides this Hg was also shown to cause reduction in sperm motility and sperm count (Chowdhury & Arora, 1982) and increase in plasma levels of T₄, TSH, Estrone and Estradiol (Abdelouhaba *et al.*, 2008). Thus, in the present study, we showed that heavy metals can differentially modulate catfish oocyte maturation, ovulation. It may be related to direct or indirect effect of heavy metals on the oogenesis process, which ultimately results in reduced quantity and quality of catfish eggs and decline in population.

Conclusions

In conclusion, heavy metals inhibited or stimulated oocyte growth and development *in vitro* differentially. Toxicity of heavy metals in water may be the major threat for growth, development, reproduction and survival of the fishes as well as all the aquatic flora and fauna. This may have potential risk to catfish populations in the waters contaminated with heavy metals. The present study may serve as baseline data to monitor future anthropogenic activities in river water specific area. Further, mechanistic approach needs to be investigated

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References

- Abdelouhaba, N., Merglera, D., Takserb, L., Vaniera, C., St-Jeana, M., Baldwin, M., Spearc, P.A., & Chan, H.M. (2008). Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). *Environmental Research*, 107, 380-392. <https://doi.org/10.1016/j.envres.2008.01.006>
- Avazeri, N., Denys, A., & Lefèvre, B. (2006). Lead cations affect the control of both meiosis arrest and meiosis resumption of the mouse oocyte *in vitro* at least via the PKC pathway. *Biochimie*, 88(11), 1823-1829. <https://doi.org/10.1016/j.biochi.2006.04.011>
- Beyersmann, D., & Hechtenberg, S. (1997). Cadmium, Gene Regulation, and Cellular Signalling in Mammalian Cells. *Toxicology and Applied Pharmacology*, 144, 247-261. <https://doi.org/10.1006/taap.1997.8125>
- Brown, V., Shurben, D., Miller, W., & Crane, M. (1994). Cadmium toxicity to rainbow trout *Oncorhynchus mykiss* Walbaum and brown trout *Salmo trutta* L. over extended exposure periods. *Ecotoxicology and Environmental Safety*, 29, 38-46. [https://doi.org/10.1016/0147-6513\(94\)90028-0](https://doi.org/10.1016/0147-6513(94)90028-0)
- Caserta, D., Mantovani, A., Marci, R., Fazi, A., Ciardo, F., La, R.C., Maranghi, F., & Moscarini M. (2011). Environment and women's reproductive health. *Human Reproduction Update*, 17, 418-433. <https://doi.org/10.1093/humupd/dmq061>
- Chaube, R., Mishra, S., & Singh, R.K. (2010). *In vitro* effects of lead nitrate on steroid profiles in the post-vitellogenic ovary of the catfish *Heteropneustes fossilis*. *Toxicology in Vitro*, 24 (7), 1899-1904. <https://doi.org/10.1016/j.tiv.2010.07.021>
- Choe, S.Y., Kim, S.J., Kim, H.G., Lee, J.H., Choi, Y.H., Lee, H., & Kim, Y.H. (2003). Evaluation of estrogenicity of major heavy metals. *Science of the Total Environment*, 312, 15-21. [https://doi.org/10.1016/S0048-9697\(03\)00190-6](https://doi.org/10.1016/S0048-9697(03)00190-6)
- Choubey, M., Chaube, R. & Joy, K.P. (2015). Toxic effects of lead nitrate Pb(NO₃)₂ on the testis in the catfish *Heteropneustes fossilis*. *Pharmacologia* 6(2), 63-72. <https://doi.org/10.5567/pharmacologia.2015>
- Chowdhury, A. R., & Arora, U. (1982). Toxic effect of mercury on testes in different animal species. *Indian Journal of Physiology and Pharmacology*, 26, 246-249.
- Devlin, R.H., & Nagahama, Y. (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, 208 (3-4), 191-364. [https://doi.org/10.1016/S0044-8486\(02\)00057-1](https://doi.org/10.1016/S0044-8486(02)00057-1)
- Dyer, C.A. (2007). Heavy metals as endocrine disrupting chemicals. In: Gore AC, (Eds.), *Endocrine-Disrupting Chemicals: From Basic Research to Clinical Practice*. (pp.111-133). Totowa, NJ, Humana Press., 361pp
- Foster, W.G. (1992). Reproductive toxicity of chronic lead exposure in the female cynomolgus monkey.

- Reproductive Toxicology*, 6,123-131. [https://doi.org/10.1016/0890-6238\(92\)90113-8](https://doi.org/10.1016/0890-6238(92)90113-8).
- Goswami, S.V., & Sundararaj, B.I. (1971). *In vitro* maturation and ovulation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch): effects of mammalian hypophyseal hormones, catfish pituitary homogenate, steroid precursors and metabolites, and gonadal and adrenocortical steroids. *Journal of Experimental Zoology*, 178, 467-478. <https://doi:10.1002/jez.1401780405>
- Gupta, R.K, Singh, J.M., Leslie, T.C., Meachum, S., Flaws, J.A. & Yao, H. H. (2010). Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles *in vitro*. *Toxicology and Applied Pharmacology*, 242, 224-230. <https://doi.org/10.1016/j.taap.2009.10.011>
- Hsu, P., Hsu, C., Liu, M., Chen, L., & Guo Y.L. (1998). Lead-induced changes in spermatozoa function and metabolism. *Journal of Toxicology and Environmental Health* A,55,45-64. <http://dx.doi.org/10.1080/009841098158610>
- Hwang, I.J., Kima, H.W., Kima, J.K., Leeb, Y.D. & Baeka, H. J. (2010). Estrogenicity of 4-nonylphenol and diethylstilbestrol on *in vitro* oocyte maturation of the dusky tripletooth goby, *Tridentiger obscurus*. *Animal Cells and Systems*, 14(3), 161-167. <http://dx.doi.org/10.1080/19768354.2010.504339>
- Jolibois, L.S.Jr, Shi, W., George, W.J., Henson, M.C., & Anderson, M., (1999a). Cadmium accumulation and effects on progesterone release by cultured human trophoblast cells. *Reproductive toxicology*, 13, 215-221. [https://doi.org/10.1016/S0890-6238\(99\)00009-X](https://doi.org/10.1016/S0890-6238(99)00009-X)
- Jolibois, L.S.Jr., Burrow, M.E., Swan, K.F., George, W.J., Anderson, M., & Henson, M.C., (1999b). Effects of cadmium on cell viability, trophoblastic development, and expression of low density lipoprotein receptor transcript in cultured human placental cells. *Reproductive toxicology*, 13, 1081-1084. [https://doi.org/10.1016/S0890-6238\(99\)00041-6](https://doi.org/10.1016/S0890-6238(99)00041-6)
- Junaid, M., Chowdhuri, D.K., Narayan R., Shanker, R., & Saxena, D. K. (1997). Lead-induced changes in ovarian follicular development and maturation in mice. *Journal of Toxicology and Environmental Health* A,50, 31-40. <http://dx.doi.org/10.1080/009841097160582>
- Kasperczyk, A., Kasperczyk, S., Horak, S., Ostalowska, A., Grucka-Mamczar, E., Romuk, E., Olejek, A., & Birkner, E. (2008). Assessment of semen function and lipid peroxidation among lead exposed men. *Toxicology and Applied Pharmacology*, 228,378-384. <https://doi.org/10.1016/j.taap.2007.12.024>
- Khillare, K., Khillare, Y.K., & Wagh, W. (2017). Histological changes in gonads of fresh water fishes due to heavy metal pollution. *World Journal of Pharmacy and Pharmaceutical Sciences*. 6(7), 601-609. <https://doi.org/10.20959/wjpps20177-8743>
- Kleiss-San, F., & Schuetz, A. W., (1987). Sources of calcium and the involvement of calmodulin during steroidogenesis and oocyte maturation in follicles of *Rana pipiens*. *Journal of Experimental Zoology*, 244(1), 133-143. <https://doi.org/10.1002/jez.1402440116>
- Kondoh, M., Araragi, S., Sato, K., Higashimoto, M., Takiguchi, M., & Sato, M. (2002). Cadmium induces apoptosis partly via caspase-9 activation in HL-60 cells. *Toxicology*, 170 (1-2), 111-117. [https://doi.org/10.1016/S0300-483X\(01\)00536-4](https://doi.org/10.1016/S0300-483X(01)00536-4)
- Lafuente, A., Cano, P., & Esquifino, A. I. (2003). Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? *Biometals*, 16, 243pp. <https://doi.org/10.1023/A:1020658128413>
- Leoni, G., Bogliolo, L., Deiana, G., Berlinguer, F., Rosati, I., Pintus, P.P, Naitana, S., (2002). Influence of cadmium exposure on *in vitro* ovine gamete dysfunction. *Reproductive Toxicology* 16(4), 371-377. [https://doi.org/10.1016/S0890-6238\(02\)00040-0](https://doi.org/10.1016/S0890-6238(02)00040-0)
- Łuszczek-Trojnar, E., Drag-Kozak, E., Szczerbik, P., Socha, M., & Popek, W. (2014). Effect of long-term dietary lead exposure on some maturation and reproductive parameters of a female Prussian carp (*Carassius gibelio* B.) *Environmental Science and Pollution Research*. 21, 2465-2478. <https://doi.org/10.1007/s11356-013-2184-x>
- Łukjanienko W.J., 1974. Toksykologia ryb. PWRiL, Warszawa.
- Massanyi, P., Uhrin, V., Sirotkin, A.V., Paksy, K., Forgács, Z. S., Toman, R., & Kovacik, J. (2000). Effects of cadmium on ultrastructure and steroidogenesis in cultured porcine ovarian granulosa cells. *Acta Veterinaria Brno*, 69, 101-106. <https://doi.org/10.2754/avb200069020101>
- Massanyi, P., Uhrin, V., Toman, R., Kovacik, J., & Biro, D. (1999). Histological changes in the oviduct of rabbits after administration of cadmium, *Journal of Animal Feed Science*, 8, 255-261. <https://doi.org/10.22358/jafs/68933/1999>
- Mondal, S., Mukhopadhyay, B. & Bhattacharya, S. (1997). Inorganic mercury binding to fish oocyte plasma membrane induces steroidogenesis and translatable messenger RNA synthesis. *BioMetals*, 10, 285-290. <https://doi.org/10.1023/A:1018372332624>
- Mukherjee, D., Kumar, V., & Chakraborti, P. (1994). Effect of mercuric chloride and Cd chloride on gonadal function and its regulation in sexually mature common carp *Cyprinus carpio*. *Biomedical and Environmental Science*, 7(1), 13-24. (PMID:8024715)
- Nagahama, Y., Yoshikuni, M., Yamashita, M., & Nagahama, Y. (1994). Regulation of oocyte maturation in fish. In: Sherwood N.M, Hew C.L., Parrell A.P., Randall D.J. (eds.): *Fish Physiology: Molecular Endocrinology of Fish*. New York, 13, 393-439. [https://doi.org/10.1016/S1546-5098\(08\)60074-6](https://doi.org/10.1016/S1546-5098(08)60074-6)
- Nagahama, Y. & Yamashita, M. (2008). Regulation of oocyte maturation in fish. *Development and Growth Differentiation* 50: S195-S219. <https://doi.org/10.1111/j.1440-169X.2008.01019.x>
- Nagahama, Y., (1997). 17 α , 20 β -dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: mechanism of synthesis and action. *Steroids*, 62, 190-196. [https://doi.org/10.1016/S0039-128X\(96\)00180-8](https://doi.org/10.1016/S0039-128X(96)00180-8)
- Nampoothiri L.P., & Gupta, S. (2006). Simultaneous effect of lead and cadmium on granulosa cells: a cellular model for ovarian toxicity. *Reproductive Toxicology*, 21, 179-185. <https://doi.org/10.1016/j.reprotox.2005.07.010>
- Nandi, S., Gupta, S.P., Selvaraju, S., Roy, S.C. & Ravindra, J.P. (2010). Effects of exposure to heavy metals on viability, maturation, fertilization, and embryonic development of Buffalo (*Bubalus bubalis*) oocytes *in vitro*. *Archives of Environmental Contamination and Toxicology*, 58 (1), 194. <https://doi.org/10.1007/s00244-009-9342-7>
- Olsson, P.E., Kling, P., Petterson, C., & Silversand, C.

- (1995). Interaction of cadmium and oestradiol-17 beta on metallothionein and vitellogenin synthesis in rainbow trout (*Oncorhynchus mykiss*). *Biochemical Journal*, 307, 197-203. <https://doi.org/10.1042/bj3070197>
- Paksy, K., Varga, B., Náráy, M., Olajos, F., & Folly, G. (1992). Altered ovarian progesterone secretion induced by cadmium fails to interfere with embryo transport in the oviduct of the rat. *Reproductive Toxicology*, 6, 77-83. [https://doi.org/10.1016/0890-6238\(92\)90024-N](https://doi.org/10.1016/0890-6238(92)90024-N)
- Patra, R. C., Swarup, D., Sharma, M. C., & Naresh, R. (2006). Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. *Journal of Veterinary Medicine A, Physiology, Pathology, Clinical Medicine*, 53(10), 511-517. <https://doi.org/10.1111/j.1439-0442.2006.00868.x>
- Powlin, S. S., Keng, P. C., & Miller, R. K. (1997). Toxicity of cadmium in human trophoblast cells (JAR choriocarcinoma): Role of calmodulin and the calmodulin inhibitor, zaldaride maleate. *Toxicology and Applied Pharmacology*, 144, 225-234. <https://doi.org/10.1006/taap.1997.8135>
- Romani, F., Tropea, A., Scarinci, E., Federico, A., Russo, C. D., Lisi, L. & Catino, S. (2014). Endocrine disruptors and human reproductive failure: the in vitro effect of phthalates on human luteal cells. *Fertility and Sterility*, 102(3), 831-837. <https://doi.org/10.1016/j.fertnstert.2014.05.041>
- Singhal, R.L., Nijayvargiya, R. & Shukla, G.S. (1985). Toxic effects of cadmium and lead on reproductive functions. In: Thomas, J.A., Korach, K.S., McLachlan, J.A. (Eds.), *Target Organ Toxicology Series: Endocrine Toxicology* (pp-149-180). New York, Raven Press.
- Smida, A.D., Valderrama, X.P., Agostini, M.C., Furlan, M.A., & Chedrese, J. (2004). Cadmium stimulates transcription of the cytochrome p450 side chain cleavage gene in genetically modified stable porcine granulosa cells. *Biology of Reproduction*, 70, 25-31. <https://doi.org/10.1095/biolreprod.103.019000>
- Sprocati, A.R., Alisi, C., Segre, L., Tasso, F., Galletti, M., & Cremisini, C. (2006). Investigating heavy metal resistance, bioaccumulation and metabolic profile a metallophilic microbial consortium native to an abandoned mine. *Science of Total Environment*, 366, 649-658. <https://doi.org/10.1016/j.scitotenv.2006.01.025>
- Srivastava, V., Dearth, R.K., Hiney, J.K., Ramirez, L.M., Bratton, G.R., & Dees, W.L. (2004). The effects of low-level Pb on steroidogenic acute regulatory protein (StAR) in the prepubertal rat ovary. *Toxicological Sciences*, 77, 35-40. <https://doi.org/10.1093/toxsci/kfg249>
- Szczerbik, P., Mikołajczyk, T., Sokołowska-Mikołajczyk, M., Socha, M., Chyb, J., & Epler, P. (2006) Influence of long-term exposure to dietary cadmium on growth, maturation and reproduction of goldfish (subspecies: Prussian carp *Carassius auratus gibelio* B.). *Aquatic Toxicology*, 77(2), 126-135. <https://doi.org/10.1016/j.aquatox.2005.11.005>
- Szczerbik, P., Mikołajczyk, T., Sokołowska-Mikołajczyk, M., Socha, M., Chyb, J., & Epler, P. (2008). The influence of cadmium on Prussian carp oocyte maturation, development of eggs and hatching. *Czech Journal of Animal Sciences*, 53(1), 36-44.
- Taupeau, J., Poupon, F., Nomé, B. & Lefèvre, (2001). Lead accumulation in the mouse ovary after treatment-induced follicular atresia. *Reproductive Toxicology*, 15, 385-391. [https://doi.org/10.1016/S0890-6238\(01\)00139-3](https://doi.org/10.1016/S0890-6238(01)00139-3)
- Thomas P. (1993). Effect of cadmium on gonadotropin secretion from Atlantic croaker pituitaries incubated in vitro. *Marine and Environment Research*, 35, 141-145. [https://doi.org/10.1016/0141-1136\(93\)90028-X](https://doi.org/10.1016/0141-1136(93)90028-X)
- Thomas, P., Baer, K. N., & White, R. B. (1994). Isolation and partial characterization of metallothionein in the liver of the red-eared turtle (*Trachemys scripta*) following intraperitoneal administration of cadmium. *Comparative and Biochemical Physiology Part C. Pharmacol.*, 107(2), 221-226. [https://doi.org/10.1016/1367-8280\(94\)90044-2](https://doi.org/10.1016/1367-8280(94)90044-2)
- Trant, J.M., & Thomas, P. (1988). Structure-activity relationships of steroids in inducing germinal vesicle breakdown of Atlantic croaker oocyte in vitro. *General and Comparative Endocrinology*, 71, 307-317. [https://doi.org/10.1016/0016-6480\(88\)90259-6](https://doi.org/10.1016/0016-6480(88)90259-6)
- Tsang, B.K., & Carnegie, J.A. (1983). Calcium requirement in the gonadotrophic regulation of rat granulosa cell progesterone production. *Endocrinology*, 113, 763-769. <https://doi.org/10.1210/endo-113-2-763>
- Tsang, B.K., & Carnegie, J.A. (1984). Calcium dependent regulation of progesterone production: effects of the calcium ionophore A23187, prostaglandin E2, dl-isoproterenol and cholera toxin. *Biology of Reproduction*, 30(4), 787-794. <https://doi.org/10.1095/biolreprod30.4.787>
- Van Der Kraak, G. (1991). Role of calcium in the control of steroidogenesis in preovulatory ovarian follicles of the goldfish. *General and Comparative Endocrinology*, 81, 268-275. [https://doi.org/10.1016/0016-6480\(91\)90011-T](https://doi.org/10.1016/0016-6480(91)90011-T)
- Veldhuis, J.D., & Klase, P.A., (1982). Mechanisms by which calcium ions regulate the steroidogenic actions of luteinizing hormone in isolated ovarian cells in vitro. *Endocrinology*, 111, 1-6. <https://doi.org/10.1210/endo-111-1-1>
- Wide, M., (1985). Pb exposure on critical days of fetal life affects fertility in the female mouse. *Teratology*, 32, 375-380. <https://doi.org/10.1002/tera.1420320307>
- Yang, M.Q., Xu, D., & Wang, F. (2004). Effect of several kinds of trace elements on meiotic maturation and in vitro fertilization on mouse oocyte. *Animal Biotechnology Bulletin*, 9, 444-448.
- Zhang, W., & Jia, H. (2007). Effect and mechanism of cadmium on the progesterone synthesis of ovaries. *Toxicology*, 239, 204-212. <https://doi.org/10.1016/j.tox.2007.07.007>
- Zhang, X.F., Zhang, L.J., Li, L., Feng, Y.N., Chen, B., Ma, J.M., & Shen, W. (2013). Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. *Environmental and Molecular Mutagenesis*, 54, 354-361. <https://doi.org/10.1002/em.21776>
- Zhu, X., Kusaka, Y., Sato, K., & Zhang, Q. (2000). The Endocrine Disruptive Effects of Mercury. *Environmental Health Prevention Medicine*, 4, 174-183. <https://doi.org/10.1007/BF02931255>