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Subjection to Cadmium Chloride Compromises Oocyte Maturation, Fertilization Competence and Subsequent Embryo Development in Mature Female Mice

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Abstract

Background: Cadmium is one of the main trace elements in the environment which in high doses could have adverse effects on human health, including male and female reproduction potential. Therefore, the present study was carried out to determine the possible toxic effect of this trace element on oocyte maturation.

Materials and Methods: Mature female NMRI mice (6–8 weeks old) were divided into three groups and received 0 (control), 1 mg/kg and 2 mg/kg cadmium chloride intraperitoneally for five days. Then all mice received PMSG and after 48 hours received hCG intraperitoneally. 14 hours later, cumulus-oocyte complexes were extracted from oviducts and the percentage of degenerated, germinal vesicle, metaphase I and metaphase II was recorded. Also, after performing in vitro fertilization, the cleavage and blastocyst formation rate was assessed.

Results: The degeneration and maturation rate of oocytes was significantly higher and lower, respectively, in groups treated with different concentrations of cadmium compared with the control group ($p<0.05$). The number of fertilized, cleaved and blastocysts was significantly higher in groups that received cadmium compared with the control group ($p<0.05$).

Conclusion: Even low dose and short duration exposure to cadmium has obvious detrimental effect on oocyte maturation, fertilization competence and subsequent embryo development.

Keywords: Cadmium, Oocytes, Fertilization, Mice

1 Introduction

Nowadays, environmental pollution by human activities is a matter of concern because many pollutants released to environment are associated with the pathogenesis of a vast variety of diseases (1-3). Many studies have demonstrated adverse effects of exposure of individuals to different levels of air pollutants on the occurrence of health problems. Cadmium (Cd) is a member of the heavy metal or trace elements family which has common industrial uses in batteries, alloys, plastic stabilizers and pigments; it is also an important component of cigarettes (4-6). The effect of indoor and outdoor air pollution on the human reproductive system has been an interesting topic for a wide variety of studies and policy analysts. It has been indicated that cadmium has a detrimental effect on female fertility (7). Oocyte maturation is the final and a critical step in the development of a fertilizable oocyte defined as a re-entry into meiosis that occurs just prior to ovulation and allows oocytes to advance from prophase I to metaphase II of meiosis, determined by extrusion of the first polar body (8, 9). This process is highly sensitive and should be regulated precisely to result in successful ovulation (10). Furthermore, it has been demonstrated that the quality of the oocyte is related to subsequent fertilization and development competence of oocytes (11). Therefore, in the current study the potential toxicity of cadmium on oocyte maturation, fertilization and developmental competence was investigated by administration of low doses of cadmium chloride to mature female mice within a short period of time.

2 Materials and Methods

2.1 Experimental design and collection of oocytes

Mature female NMRI mice (6–8 weeks old) were purchased from the animal house of Shiraz University of Medical Sciences. Animals were kept on a 12 h light: 12 h dark schedule with controlled temperature condition and free access to water and food. The animal experiments were performed according to the principles of the care and use of laboratory animals established by the National Institutes of Health (12). Mature female NMRI mice (6–8 weeks old) were divided into 3 groups and received 0 (control), 1 mg/kg and 2 mg/kg cadmium chloride intraperitoneally for five days. At the 5th day, all mice received 10 IU of pregnant mare serum gonadotropin (PMSG; Gonaser, HIPRA, Spain), followed 48 h later by injecting 10 IU human chorionic gonadotropin (hCG; Organon, Oss, The Netherlands). Fourteen hours later, mice were sacrificed by cervical dislocation and the oviducts were removed and placed in handling medium (GMOPSTM, Vitrolife, Göteborg, Sweden), which was pre-incubated for 24 h at 37°C. The oviducts were dissected with two insulin syringes (Helma Teb, Baspar Sanat Fakher, Saveh, Iran) under a stereomicroscope (Nikon, Tokyo, Japan) and the released cumulus oocyte complexes were placed in a few drops of hyaluronidase (80 IU/mL) (Vitrolife, Göteborg, Sweden) for separation of cumulus cells. The oocytes were placed in drops of G-IVF medium (Vitrolife) and the number of degenerated, Germinal Vesicle (GV), Metaphase I (MI) and Metaphase II (MII) oocytes was recorded using an inverted microscope (Nikon, Tokyo, Japan). Observation of germinal vesicle or the extrusion of first polar body

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were used as the criterion for nuclear maturation of oocytes. Oocytes with extruded first polar body were used for in vitro fertilization.

2.2 IVF and in vitro development

2.2.1 Sperm capacitation

Spermatozoa were collected from the cauda epididymides of mature NMRI male mice and capacitated by preincubation at 37°C and 5% CO2 for 1 h in 200 µl of G-IVF drops (Vitrolife, Göteborg, Sweden) under mineral oil (Reproline, Rheinbach, Germany).

2.2.2 In vitro fertilization and embryo culture

Mature oocytes were inseminated *in vitro* with 1×10^6 spermatozoa/ml in 100 µl of G-IVF medium for 4 hours and the number of fertilized oocytes manifested by observation of two pronuclei (2PN) was recorded. The presumptive zygotes were cultured in G1 and then G2 medium (Vitrolife, Göteborg, Sweden) under mineral oil at 37°C in a humidified incubator with 5.0% CO2 and rates of cleavage and blastocyst formation were recorded.

2.2.3 Statistical analysis

Data were analyzed by one-way ANOVA followed by the Tukey post hoc test using SPSS 20 (IBM Corp., Armonk, N.Y., USA). Differences were considered significant at $P<0.05$.

3 Results

As shown in Table 1, the percentage of degenerated and germinal vesicle oocytes significantly increased in the two experimental groups compared to the control group ($p<0.05$). Rates of MI and MII oocytes demonstrated significant decrease in the two experimental groups compared to the control group ($p<0.05$). Administration of cadmium to mature female mice significantly decreased the fertilization, cleavage and blastocyst formation rate ($p<0.05$) (Table 2). Percentage of degenerated, germinal vesicle (GV) metaphase I (MI) and metaphase II oocytes (MII) in different groups. All experiments were repeated six times. Data are expressed as mean ± SEM. Different superscripts (a-c) show significant differences in a column and the same superscripts show no significant differences in a column ($p<0.05$). Percentage of fertilized oocytes with 2PN, cleavage rate and blastocyst formation. All experiments were repeated eight times. Different superscripts (a-c) show significant differences in a column and the same superscripts show no significant differences in a column ($p<0.05$).

4 Discussion

The adverse and toxicological effects of many pollutants on living organisms are demonstrated in a vast number of studies (13-15). Cadmium is a member of the heavy metal family, also called trace elements, which at high doses exerts toxic effects on organs; it is also classified as a human carcinogen. It is generally present in the environment at low levels; however, human activity has greatly increased its levels in the environmental (6, 16). Cadmium is able to bioaccumulate in the food chain, including fish, meat, eggs and milk. People can be exposed to cadmium through eating contaminated foods, smoking cigarettes or drinking contaminated water. Living or working near industrial facilities which release cadmium into the air is detrimental to health. Cadmium exerts its effects through different mechanisms to destroy physiological functions including reproduction (7). Studies have demonstrated that air pollution has destructive impacts on both the male and female reproductive system at the level of tissue structure, function and gametogenesis (17-19).

The findings of this research show that a low dose of cadmium even with a short duration of administration induces degeneration of oocytes and has adverse effects on the maturation process of oocytes, displayed also in fertilization and developmental potential. Many researchers have demonstrated the adverse effects of this heavy metal on the male and female reproductive systems (5-7). Mohavi et al observed that cadmium exposure through drinking water decreases the number of ovulated oocytes and impairs oocyte meiotic maturation rate both in vivo and in vitro by disrupting of meiotic spindle morphology, cytoskeletal organization, mitochondrial function and histone modifications. The embryonic development after fertilization was also impaired (20). Akar et al observed the in vitro exposure of bovine COCs to CdCl2 for 24 hours and concluded that the rate of COCs expansion, MI oocytes, post-fertilization cleavage rate in presumptive zygotes and blastocyst development decreased (21). Shimoi et al added cadmium to invitro maturation and IVF medium of mouse oocyte. Cd exposure during the maturation period disturbed the fertilization of oocytes and Cd exposure during the fertilization period, disrupting the normal development of embryos after fertilization (22). Overall, cadmium at the level of mentioned doses and duration of administration has significant detrimental effects on oocyte maturation and subsequent fertilization and development, but the precise mechanism of cadmium toxicity on the mentioned parameters remains to be clarified precisely.

Table 1: Effect of cadmium chloride on oocyte maturation

Different concentrations (mg/Kg) of cadmium chloride	Total COCs	Degenerated oocyte (%)	GV oocyte (%)	MI oocyte (%)	MII oocyte (%)
0 (Control)	198	2.19 ± 0.9^{a}	5.21 ± 3.1^{a}	19.17 ± 1.4^{a}	73.43 ± 3.6^{a}
1	210	24.96 ± 4.02^{b}	12.69 ± 5.7^{b}	14.75 ± 4.7^{b}	45.6 ± 3.6^{b}
2	206	30.7 ± 3.2^{c}	28.1 ± 1.89^{c}	12.1 ± 3.28^{c}	29.1 ± 4.8^{c}

Table 2: Effect of cadmium chloride on IVF and embryo development

Different concentrations (mg/Kg) of cadmium chloride	Number of mature oocytes	Fertilized oocytes with 2PN (%)	Cleavage (%)	Blastocyst Formation (%)
0 (Control)	95	88.2 ± 4.2^{a}	84.12 ± 2.6^{a}	70.23 ± 3.3^{a}
1	91	65.1 ± 4.1^{b}	50.7 ± 2.6^{b}	36.6 ± 1.5^{b}
2	99	54.2 ± 3.2^{c}	39.5 ± 3.2^{c}	28.1 ± 2.8^{c}

5 Conclusion

In conclusion, one of the manifestations of cadmium toxicity in the female reproductive system is in the disruption of oocyte maturation, quality, fertilization potential and subsequent embryo development, even by exposure to low dose and short period of administration.

Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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