Exposure to hexavalent chromium causes infertility by disrupting cytoskeletal machinery and mitochondrial function of the metaphase II oocytes in superovulated rats

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ABSTRACT

Previous studies from our laboratory showed that prenatal exposure to hexavalent chromium, Cr(VI), caused premature ovarian failure and decreased pregnancy rates and litter size. Exposure to the endocrine disrupting chemicals (EDCs) can cause X-chromosome aneuploidy of the oocytes, increasing chromosome missegregation and risk of infertility, autoimmune diseases, cancers, and various genetic disorders. Cr(VI) is an EDC that is widely used in numerous industries. Environmental exposure to Cr(VI) caused detrimental reproductive effects in women and health effects in infants from California. Women with occupational Cr(VI) exposure experienced infertility, pregnancy loss, spontaneous abortion, and stillbirth. However, the adverse effects of Cr(VI) on oocyte development and quality have not been reported. Mitochondrial membrane potential and function are the critical determinants of oocyte quality in natural pregnancies and successful assisted reproductive techniques. The cytoskeletal machinery of the oocytes orchestrates the meiotic division of the oocytes, whereas cortical granules (CGs) prevent polyspermy. Therefore, the objective of the current study was to examine whether the mechanism by which Cr(VI) compromises oocyte quality and morphology is by altering cytoskeleton dynamics and mitochondrial function of the metaphase II (MII) oocytes. Rats were treated with environmentally relevant doses of Cr(VI) (1 and 5 ppm potassium dichromate) in drinking water from postnatal day (PND) 22–28, followed by superovulation and retrieval of MII oocytes. The data indicate that Cr(VI) exposure disrupted F-actin structure and distribution pattern, compromised mitochondrial function, altered CGs distribution, increased dysmorphic and degenerated oocytes, delayed first polar body extrusion, and caused infertility.

1. Introduction

Infertility is estimated to affect 186 million people worldwide [1]. The prevalence of infertility in reproductive-aged women is one in every seven couples in the western world and four couples in developing countries [2]. According to a World Health Organization (WHO) study on gender distribution and infertility etiologies, female infertility was the underlying cause for 37% of infertile couples [3]. The various causes of infertility in women include ovulatory disorders, polycystic ovary syndrome, hyperprolactinemia, hypothalamic hyperprognadism, premature ovarian failure, hypothyroidism, adrenal hyperplasia, pelvic adhesive disease, and uterine abnormalities (e.g., fibroids, endometrial polyps), cervical stenosis, oocyte quality, age-related aneuploidy [4], and exposure to endocrine disrupting chemicals (EDCs) [5].

EDCs are substances that cause adverse health effects via endocrine-mediated mechanisms in an intact organism or its progeny or (sub) populations [6]. The female reproductive system is a primary target for EDCs. Exposures to EDCs such as pharmaceuticals, chemicals used in consumer goods and industrial practices, and pesticides and herbicides can cause female infertility and, in some cases, impact future generations [7]. Because the mammalian female is born with a finite number of oocytes, exposure to EDCs at any point in life can affect female fertility as an adult. Furthermore, the development of the reproductive tract is profoundly influenced by steroid hormones during development, making the developmental period a vulnerable time for EDC effects, even with low doses of EDCs. The national toxicology program’s report on EDCs states that many of EDCs, especially estrogenic compounds, can cause adverse effects on the male and female reproductive organs [8]. The endocrine system is most vulnerable to disruption by EDCs during the prenatal and early development windows, with the adverse effects
Persisting into adulthood and across generations [9]. An increase of EDCs (hexachlorocyclohexane, polychlorinated biphenyls, and dichlorodiphenyltrichloroethane) in woman’s blood is positively associated with infertility. Women with the highest PCBs levels have a 50% decline in fertility compared to women with lower PCB levels. In women farmers in Ontario, fertility decreased in proportion to the use of pesticides dicamba (49%), glyphosate (39%), 2,4-D (29%), organophosphates (25%), and thiocarbamates (24%). When infertile couples seek in vitro fertilization (IVF) treatment, those with the highest PCB levels were much more unlikely to achieve pregnancy than those with lower PCB levels [10]. The National Health and Nutrition Examination Survey (1999–2016) in the United States has reported metals (As, Pb, Hg, Cr, and Cd) detected in 83–99% of the urine samples collected from pregnant women [11]. A study of levels of metals (Cd, Pb, Hg) in people undergoing assisted reproductive technologies (ART) found metal levels were associated with decreases in pregnancy rates or IVF success [12]. Strikingly, exposure of reproductive age women to chromium through occupational or environmental exposure has been linked to infertility [13,14]. Overall, exposure to EDCs impacts the reproductive potential in women, measured by ovarian reserve and by ART/IVF outcomes. Exposure to various EDCs decreases estradiol (E2) levels (BPA), AMH concentrations (PCBs), antral follicle count (BPA, parabens, phthalates), oocyte quality (BPA, triclosan, phthalates, PCBs), fertilization rate (PFCs, PCBs), implantation (BPA, phthalates, PCBs), embryo quality (triclosan, PCBs, BPA), and rate of clinical pregnancy and live births (parabens, phthalates) [15].

The most significant contributing factor for implantation failure and early miscarriage in human embryo is aneuploidy. More than half of human embryos are affected by aneuploidy, resulting in miscarriage, or birth defects [16]. Aneuploidy increases chromosome missegregation and a broad spectrum of clinical features. X chromosome aneuploidy predisposes an individual to diseases such as autoimmune diseases, cancer, primary biliary cirrhosis, congenital defects, and genetic disorders such as X-chromosome monosomy, Turner’s syndrome, Down syndrome, and progeroid pathologies [17]. EDCs such as phthalates and BPA induce aneuploidy during oocyte meiosis [18,19]. However, how various EDCs cause aneuploidy of the X chromosome during oocyte meiosis and the clinical implications are not clearly understood.

Chromium (Cr) pollution is a significant environmental threat, severely impacting our environment and natural resources, especially water and soil worldwide. Excessive exposure due to increased usage of Cr worldwide and improper disposals could lead to higher accumulation levels in human and animal tissues, resulting in toxic and detrimental health effects, including female reproductive dysfunction [20,21]. Several epidemiological studies indicate that men and women working in the Cr industries such as tanneries and welding industries or living in a Cr (VI)-contaminated environment undergo reproductive failures [13,22–28]. Overcoming the challenges of poor oocyte quality in IVF clinics is an ongoing challenge [29]. In the current study, we investigated the basis for a novel mechanism of Cr(VI) on MII oocyte morphology, mitochondrial function, and cytoskeletal machineries in superovulated rats.
2. Materials and methods

2.1. Chemicals

The chemicals used in these studies were purchased from Sigma Chemical Company (St. Louis, MO), ABCAM Inc., (Boston, MA), Fisher Scientific Company LLC (Houston, Texas), or Life Technologies Corporation (Carlsbad, CA) unless stated otherwise.

2.2. Animals and treatments

Sprague-Dawley rats were purchased from Charles River Laboratories (Houston, Texas), maintained in AAALAC-approved animal facilities with a 12 h light/12 h dark cycle at 23–25 °C, and fed with Teklad 4% mouse/rat diet and water ad libitum. Animal Use Protocols were performed following the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and with standards established by Guiding Principles in the Use of Animals in Toxicology and specific guidelines and standards of the Society for the Study of Reproduction approved by the Animal Care and Use Committee (IACUC) of Texas A&M University. Postnatal day (PND) 22 female rats were divided into three groups: Control (n = 10), Cr(VI)- 1 ppm (n = 10) and Cr(VI)- 5 ppm (n = 10) for the MII oocytes collection. Control rats received regular drinking water, and Cr(VI) treated groups received 1.0 ppm or...
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5.0 ppm potassium dichromate in drinking water from PND 22–29.

2.3. Choosing the Cr(VI) doses relevant to human exposure

The EPA has established a maximum contaminant level (MCL) of 100 μg/L or 100 ppb for total chromium in drinking water. According to FDA, the total chromium concentration in bottled drinking water should not exceed 100 μg/L [30]. However, groundwater from Midland, Texas, contains 5.28 ppm Cr (5280 μg/L) [31]. In California, 1596 drinking water sources from different counties had Cr levels ranging from 5 to 50 μg/L. The state of California had issued a specific MCL of 10 μg/L and a public health goal (PHG) of 0.02 μg/L for Cr(VI) in drinking water [32]. A study conducted in Piedmont aquifers of North Carolina documented that 90% of the wells had detectable Cr(VI) and were above the California PHG [32]. Since Piedmont rocks extend to other states in the US, and a large population is potentially consuming drinking water with high Cr(VI) levels, Cr(VI)-induced infertility in females needs to be thoroughly studied in the light of human exposure level of Cr(VI). Therefore, we chose 1 and 5 ppm Cr(VI) for the current study.

2.4. Superovulation and oocyte collection

Superovulation and oocytes collection was performed based on the method described by Agca Y [33] with minor modifications. After one

Fig. 3. Effects of Cr(VI) exposure (5 ppm) on oocyte morphology. Prepubertal rats (n=10) were exposed to Cr(VI) as described in materials and methods. Based on the morphological, the oocytes were harvested and classified as normal (healthy) and abnormal (see materials and methods). Exposure to Cr(VI) increased dysmorphic oocytes compared to control. Representative images of various phenotypes of dysmorphic oocytes are given as follows: healthy oocyte (A), incomplete PB1 extrusion (B), enlarged PB1 (C), dark cytoplasm (D), increased cytoplasmic granularity (E), enlarged ZP (F), enlarged PVS with granules or debris (G), a rupture in the oolemma (H), condensed cytoplasmic organelles in the middle (I), an abnormal oocyte shape (J), refractile bodies in the cytoplasm (K), and condensed smooth endoplasmic reticulum (cSER) (L).
week of treatment, each female rat was injected with 10 IU of pregnant mare serum gonadotropin intraperitoneally (i.p) using a 1 mL syringe with a 27 g needle. After 48 h, rats were injected with 10 IU human chorionic gonadotropin (hCG). Animals were euthanized by cervical dislocation 14–15 h post hCG injection. After confirming the death of the animals, blood was collected for hormone assays. The oviducts were dissected after euthanasia and temporarily stored in mineral oil until processed for oocytes collection. Clutches of cumulus-oocyte complexes were removed from the oviduct and placed into hyaluronidase (1 mg/mL) using a fine Tweezer and a 27 g needle a stereomicroscope (Nikon SMZ 745 T). Cumulus cells were separated from the oocytes using hyaluronidase for 3–5 min in the M2 medium (Sigma, catalog # M7167). Oocytes were washed in washing buffer a few times using an oocyte handling pipet (Cooper surgical, Stafford, TX) before further staining for various analyses.

2.5. Determination of oocyte quality and dysmorphic oocytes

Oocyte quality and phenotypes of dysmorphic oocytes were determined per previous studies [34–40]. Good-quality mature oocytes were categorized to have the following features: a clear, moderately granular cytoplasm; a small perivitelline space (PVS), a clear, colorless zona pellucida (ZP), and a single unfragmented polar body (PB) [34]. Abnormal or dysmorphic oocytes were classified as having dark cytoplasm, cytoplasmic granularity, cytoplasmic vacuoles, refractile bodies in the cytoplasm, condensed smooth endoplasmic reticulum (cSER) in the cytoplasm, an abnormal oocyte shape, an abnormal ZP, a large PVS, debris in the PVS, or an abnormal and larger PB. These abnormal phenotypes of the oocytes were based on the criteria described in the literature [35–41].

2.6. Mitochondria distribution and activity (JC-1 staining)

Mitochondrial membrane potential (MMP) indicates mitochondrial activity, which is a marker for oocyte quality [42]. MMP was measured using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzamidazol-carboxyanine (JC-1), a cationic dye [43]. JC-1 uptake in the mitochondria is directly related to the level of mitochondrial function. A greater concentration of JC-1 aggregate is correlated with greater mitochondrial uptake resulting in a red fluorescent emission signal. In contrast, a green fluorescence indicates the presence of JC-1 as a monomer. A higher red: green ratio indicates a hyperpolarized mitochondrial inner membrane [44]. Therefore, the red/green ratio was generated by dividing red fluorescence pixels by the green fluorescence pixels (Zeiss LSM 780 NLO Multiphoton Microscope, Germany), providing the overall mitochondrial activity [45].

2.7. Fluorescent staining of F-actin and cortical granules

Cytoskeletal F-actin was stained using the rhodamine-phalloidin method as described [46]. The assessment of cortical reaction was performed by labeling the oocytes with the lectin Lens culinaris agglutinin, which binds specifically to CG content and exudate [47]. Confocal laser-scanning images were obtained by Leica Confocal microscope (Leica, Germany). According to the manufacturer’s instructions, the intensity of staining for mitochondrial membrane potential, F-actin and CGs were quantified using Image-ProPlus 6.3 image processing and analysis software (Media Cybernetics, Inc.; Bethesda, MD) and expressed as Integrated Optical Density (IOD).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey posttest pair-wise comparison was used to detect the statistical significances from the mean of all the quantitative data analyzed for control, Cr 1 ppm, and Cr 5 ppm using statistical software Sigmaplriot 14.5 (Systat Software Inc., Chicago, IL). A p < 0.05 was considered statistically significant.

3. Results

3.1. Exposure to Cr(VI) decreases oocyte quality

Cr(VI) treatment (1 ppm and 5 ppm) significantly (p < 0.05) decreased the number of healthy oocytes compared to the control group (Fig. 1A & C). There was a dose-dependent decrease in the number of healthy oocytes between the 1 ppm and the 5 ppm groups, where 5 ppm significantly (p < 0.05) decreased the number compared to 1 ppm. Cr(VI) treatment significantly (p < 0.05) increased the number of degenerated oocytes with 5 ppm and 1 ppm Cr(VI) compared to control (Fig. 1B & D). There was a dose-dependent increase in the number of degenerated oocytes between the 1 ppm and the 5 ppm groups, where 5 ppm significantly (p < 0.05) increased the number compared to 1 ppm.
3.2. Exposure to Cr(VI) increases dysmorphic oocytes

Based on morphological characteristics described in the literature [48–54], control oocytes were round in shape, had normal-looking cytoplasm with low or moderate granularity, a single and normal size PB, and appropriate ZP thickness. Control oocytes also did not have any tears in the oolemma, or vacuoles or condensed organelles in the cytoplasm (Fig. 2A). However, 1 ppm Cr(VI) exposure increased the number of dysmorphic oocytes with incomplete PB1 extrusion (Fig. 2B), enlarged PB (Fig. 2C), dark cytoplasm (Fig. 2D), increased cytoplasmic granularity (Fig. 2E), enlarged ZP (Fig. 2F), enlarged PVS with granules or debris (Fig. 2G) compared to control. It also delayed PB extrusion (Fig. 2H), condensed cytoplasmic organelles in the middle (Fig. 2I), caused an abnormal oocyte shape (Fig. 2J), caused the presence of refractile bodies in the cytoplasm (Fig. 2K), and condensed smooth endoplasmic reticulum (cSER) (Fig. 2L).

As shown in Fig. 3, oocytes from the 5 ppm Cr(VI) group exhibited dysmorphic morphology with incomplete PB1 extrusion (Fig. 3B), enlarged PB1 (Fig. 3C), darker cytoplasm (Fig. 3D), increased cytoplasmic granularity (Fig. 3E), enlarged ZP (Fig. 3F), abnormal PVS with debris (Fig. 3G), a ruptured oolemma (Fig. 3H), condensed cytoplasmic organelles in the middle (Fig. 3I), an elongated oocyte (abnormal shape) (Fig. 3J), refractile bodies (Fig. 3K), and condensed SER (Fig. 3L).

The histogram in Fig. 4 shows the percent increase in dysmorphic oocytes in Cr(VI) groups compared to control. Exposure to Cr(VI) significantly increased (p ≤ 0.05) the number of dysmorphic oocytes with the following phenotypes: incomplete PB1 extrusion (IPB1), enlarged PB1 (EPB1), delayed PB1 extrusion (DPBE), dark cytoplasm (DCP), cytoplasmic granularity (CGR), enlarged ZP (EZP), enlarged PVS (EPVS), condensed cytoplasmic organelles in the middle (CCO), condensed SER (cSER), refractile bodies (RFB), and abnormal oocyte shape (AOC). Control rats did not have EPB1, DPBE, and cSER in the oocytes.
3.3. Exposure to Cr(VI) decreases mitochondrial membrane potential of the MII oocytes

Mitochondria are one of the organelles in the cytoplasm of the oocytes. Altered MMP and decreased mitochondria function lead to poor oocyte quality, adversely affecting embryo development [55]. Oocyte mitochondria produce ATP through oxidative phosphorylation to supply the energy required for fertilization to the blastocyst stage [56]. Therefore, we localized and examined the MMP of the mitochondria using the JC-1 staining method. In the oocytes from control rats, the red fluorescence that occurs with the aggregated form of JC-1 was peripherally distributed (Fig. 5A). The green fluorescence that occurs with the monomeric form of JC-1 was homogeneously distributed through the entire cytoplasm of the oocytes (Fig. 5B). In the oocytes from Cr (VI)-treated rats, the red fluorescence intensity was decreased (Fig. 5D & G) whereas the green fluorescence was increased (Fig. 5E & H) compared to control.

Cr(VI) treatment significantly decreased the ratio of the red/green fluorescence intensity compared to control (p < 0.05) (Fig. 5J). In the oocytes from 5 ppm Cr(VI) treatment group, the green fluorescence was significantly decreased compared to control and 1 ppm Cr(VI) groups (Fig. 5G), thus showing a dose-dependent effect of Cr(VI).

3.4. Exposure to Cr(VI) inhibits cytoplasmic maturation in the MII oocytes

Redistribution of organelles, including CGs and mitochondria, is necessary for oocyte cytoplasmic maturation [55]. Therefore, we examined the effects of Cr(VI) treatment on CG distribution using confocal scanning and quantitative analysis. Cr(VI) treatment caused abnormal distributions of CGs. In the control oocytes, the CGs were homogeneously distributed in the peripheral regions except for the cortical-free region (Fig. 6A). In oocytes from the 1 ppm Cr(VI) group, the CG distribution was disrupted as evidenced by significantly decreased fluorescence intensity (Fig. 6B) compared to the control group. The CGs showed abnormal and discontinuous distribution in the oocytes from the 5 ppm Cr(VI) group (Fig. 6C). The fluorescence intensity of the CGs in 5 ppm Cr(VI) treated group was significantly decreased compared to the control group (p < 0.05) (Fig. 6D) and 1 ppm Cr(VI)-treated group.

3.5. Exposure to Cr(VI) causes the abnormal distribution of F-actin in the MII oocytes

Although microtubules are the main component of the meiotic spindle in oocytes, actin filaments associate with microtubules and permeate the spindle in various species [57]. F-actin is crucial for protecting the mammalian oocyte from chromosome segregation errors and
aneuploidy [57]. F-actin was distributed in the marginal regions of the oocytes in the control group (Fig. 7A). However, the F-actin was disrupted and distributed in an abnormal pattern in the oocytes from the Cr (VI) exposed rats compared to control (Fig. 7B & C). F-actin was significantly (p < 0.05) increased in 1 ppm and 5 ppm Cr(VI) groups showing multiple folding patterns in the actin cap and the periphery of the oocytes, compared to the control (Fig. 7D). In the oocytes from 5 ppm Cr(VI) group, the F-actin increased with an abnormal distribution pattern with increased folding of the actin filaments compared to control or 1 ppm Cr(VI) groups (Fig. 7C).

4. Discussion

Epidemiological and clinical data indicate that environmental exposure to Cr(VI) in women adversely affects pregnancy outcomes and subsequent health of the exposed women, and their immediate offspring. Cr(VI)-exposed women experienced low birth rate, higher risk of pregnancy loss, and spontaneous abortion. Children (F1 offspring) of the Cr(VI)-exposed women experienced respiratory problems, perinatal jaundice, and increased birth defects. The study was conducted using data released from the hospital records that included newborn records from 29311-unexposed (control) and 5036-exposed (Willits population), as well as 31444-unexposed (control) and 5558-exposed pregnant women (Willits population). For the first time, the detrimental reproductive effects from non-occupational Cr(VI) exposure in human females and their infants was reported in the U.S [13]. Another epidemiological study indicated that occupational exposure to Cr during pregnancy caused intrauterine growth retardation (IUGR) and resulted in low birth weight of the newborn children. Women exposed to Cr(VI) through occupational settings have infertility and pre-term labor. Therefore, the goal of the current study was to understand the direct effects of Cr(VI) exposure on the oocytes. Oocyte quality is one of the critical determinants for fertility in women [58]. The current study shows that Cr(VI) treatment decreased the number of healthy oocytes (oocyte yield) and increased degenerated and dysmorphic oocytes compared to control. IVF success rates depend upon the retrieval of a number of good-quality oocytes [59]. Our study shows for the first time that exposure to Cr(VI) increases dysmorphic oocytes, reduces oocyte quality, and decreases ovulatory response in superovulated rats (Fig. 8).

Interestingly, exposure to Cr(VI) induced dysmorphisms related to the ZP, PVS, and the PB of the mature oocyte. Oocytes with a large PVS are negatively correlated with fertilization rate and embryo quality [48, 50]. Data from the literature indicate that a large PVS may be ascribed to eggs that have shrunken, presenting a large gap between the oocyte and surrounding zona. A large PVS will also occur if a larger portion of the cytoplasm is extruded with the haploid chromosomal set during the first PB formation [52,60]. Enlarged PVS is a sign of oocyte degeneration [61], and it is also associated with overmaturity of the cytoplasm [62].

![Fig. 7. Cr(VI) exposure decreased cortical granules in the MII oocyte. Prepubertal rats (n=10) were treated with Cr(VI) in drinking water and superovulated. Cortical granules were detected using immunocytochemistry. Representative images for the control (Fig. 7A), Cr(VI) 1 ppm (Fig. 7B), and Cr(VI) 5 ppm (Fig. 7C) are shown. The histogram (Fig. 7D) represents mean ± SEM of oocytes from 10 rats. Exposure to Cr(VI) significantly (p < 0.05) decreased GC and altered the distribution pattern compared to control. IOD was measured using the Image ProPlus software. a: Control vs Cr(VI) 1 ppm or 5 ppm; b: Cr(VI) 1 ppm vs Cr (VI) 5 ppm.](image-url)
Our data clearly shows that Cr(VI) exposure caused large PVS and PB1, abnormal ZP, granular cytoplasm (hypergranulation), and blebbing of the cytoplasm in the PVS, which might predispose the oocytes to be less competent for fertilization.

Mitochondria play a significant role in producing energy for oocyte maturation and embryo development through precise cellular functions comprising of Ca²⁺ homeostasis regulation, glycolysis, amino acid, fatty acid metabolism, and regulation of apoptosis. Mitochondrial structure, content, and function determine oocyte competence, embryo viability, and implantation success during ARTs. Their defects could lead to low fertilization rates and embryonic development failure [63]. The mitochondrial distribution pattern is suggested as a prerequisite for fertilization competence [71]. Oocytes with low maturation competence express larger mitochondrial clumps throughout the cytoplasm, evident with Cr(VI) exposure. Oocytes with large mitochondrial clusters at the cytoplasm periphery express the high maturation competence of MII-stage oocytes, as noticed in the control oocytes [64].

The F-actin network has been linked to vesicle transport, nuclear positioning, spindle migration and anchorage, PB extrusion, and accurate chromosome segregation in mammalian oocytes [65]. Cr(VI) increased the intensity of F-actin accumulation at the actin cap, with an obvious pattern of multiple layers of F-actin on the oocyte membrane compared to control. Yeast cells exposed to Cr(VI) exhibited an increased number of actin patches [66], whereas human primary skin fibroblast showed disrupted F-actin fibers, accumulating at the cell surface as clumps, leading to cell shrinkage and fragmentation [67]. CGs play a crucial role in preventing polyspermy during fertilization. 2, 4-dichlorophenol and Cu disrupted the dynamics of CGs, diminishing CGs [68, 69]. Our study suggests for the first time that exposure to Cr(VI) adversely affects F-actin architecture and CGs’ distribution, contributing to the delayed PB extrusion of the MII oocytes. Perturbation of cytoskeletal machinery and mitochondrial function lead to poor quality oocytes and infertility.

5. Conclusion

The current data suggests that Cr(VI) exposure may cause infertility by compromising oocyte quality, decreasing mitochondrial function, aberrantly increasing actin accumulation, delaying PB extrusion, and altering CG distribution. Therefore, from a clinical perspective, measuring the Cr burden in the urine from women with sub-fertility, or follicular fluid in IVF women might be a helpful approach for predicting bad quality oocytes, at least in Cr-exposed patients. Based on our findings and evidence from the literature, clinical practice in reproductive medicine may benefit from monitoring EDC levels in infertile women or couples undergoing IVF as a prognostic marker for infertility.

Fig. 8. Schematic diagram of effects of Cr(VI) exposure on the MII oocytes. Exposure to Cr(VI) through drinking water results in rapid absorption of Cr(VI). Antioxidants reduce Cr(VI) into Cr(III), which increases ROS. ROS alters mitochondrial membrane potential, increases F-actin, delays PB1 extrusion, and decreases CGs, resulting in poor quality oocytes.
Author statement

Liga Wuri (first author) contributed to methodology, animal experiments, data analysis, and drafting the materials and methods section. Joe A. Arosh assisted in Image-Pro software to quantify the data, carried out part of the statistics. John z. Wu assisted Liga Wuri in experiments. Sakhila K. Banu (corresponding author) contributed to the experiments’ conceptualization, validation, writing, illustration, and supervision.

Authorship conformation form

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors report no declarations of interest.

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