Adverse effects of metal pollutants on reproductive health

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ABSTRACT
Consumption of essential metals is needed for function of certain protein to maintain normal cell integrity. Incorporation and excretion of these metals are governed by the cells, but changes in environmental levels of metals can cause stress. There are areas in which the ambient environment contains non-essential metals that function as toxicants, which alter metal homeostasis thus causing oxidative stress and epigenetic alterations. Ambient air and water, as well as food items in poorly protected areas contain toxic metals, such as arsenic, cadmium, and chromium. Often the exposure to these common metal pollutants is associated with inflammatory diseases and carcinogenesis. However, epidemiology studies and animal experiments demonstrate these metals affect fertility spermatogenesis, sperm concentration, sperm motility, oocyte integrity via epigenetic changes, and contribute to gestational complications. In this review, we present a comprehensive summary of studies demonstrating the detrimental effects of exposure to these metals commonly found in ambient environment on the reproductive health of both males and females.

KEYWORDS: toxic metals, pollution, fertility, reproductive health.

INTRODUCTION
As the global population grows, use and production of hazardous materials to advance manufacturing and food production adds to the global crisis of air pollution. There is growing evidence from multiple sources across the world that air pollution introduces exposure of various metals in both ambient and household environments that result in various maladies, such as cancer and other inflammatory diseases [1, 2]. Additionally, studies have also examined the role of contaminated environments in female and male fertility [2], as well as reproductive outcome [2, 3]. Damage to fertility and reproductive outcome may be induced by the materials in the air. Ambient and household environments contain fine particulates (PM$_{2.5}$ and PM$_{10}$) that comprise of metals, both essential and nonessential.

Essential metals participate in normal function of various proteins in the daily maintenance of cellular homeostasis. Essential metal ions such as iron, calcium, magnesium, copper, and zinc are required to carry oxygen, mediate cadherin function, and participate in the function of transcription factors. As such, metals and the balance of those metals within cells contribute to successful reproductive health. For example, iron (Fe) is essential for both female and male fertility. While free Fe is toxic and may induce oxidative stress, most of the body’s Fe is bond to proteins, such as hemoglobin, myoglobin, ferritin and transferrin [4, 5]. In humans, Fe deficiency is associated with ovulatory infertility [6], as well as shorter gestation and low birth weights [7]. Examination of rat and human ovarian tissue demonstrate that the distribution of Fe bound to transferrin is important for optimal granulosa cell function and thus proper ovarian follicle maturation and ovum development [8]. In mammalian males, certain concentrations of Fe bound to transferrin...
In vitro analysis demonstrated that oocytes exposed to 2 µg/mL of As for 2 hours resulted in chromosome misalignments during a disrupted meiosis I and severe chromosome spreading during disrupted meiosis II [19]. In vivo experiments also demonstrate the dangers of As on uterine health. In a study where rats were exposed to various doses of As (50, 100, and 200 ppm) via drinking water, the animals exposed to high As doses (100 and 200 ppm) exhibited a thinning of the uterine horns compared to the lowest As dose used and non-exposed animals, with the thinning more severe in the 200 ppm group compared to 100 ppm [20]. Moreover, gross anatomy and H&E analysis of uterine cross sections from all four groups showed that As exposure decreased the overall weight and size of the uterus, and altered epithelium invagination [20]. Further analysis demonstrated that As exposure also altered endometrium and myometrium thickness and morphology in a dose dependent manner [20]. With increased As doses, there was also a dose-dependent decrease in plasma levels of important hormones that govern the menstrual cycle and uterine health, estradiol, progesterone, FSH, and LH [20]. Exposure to As also affects embryo viability and implantation. Embryos cultured with As doses higher than 250 nM result in poor survival after 72 hr [21]. Moreover, embryos cultured in 500 nM of As for 72 hr exhibited a high number of blastomere cells containing fragmented DNA resulting in apoptosis [21], a phenomenon that may explain how As exposure at low levels in the uterine lumen may promote miscarriage, congenital defects, and still birth.

Cadmium (Cd), found in rocks and soils [22], is often found in agricultural products, such as rice and seafood [23, 24]. Cd accumulates in the body [25] and may affect reproductive health via its ability to interfere with Zinc homeostasis via displacement [26]. Cd can be excreted from the body through urine and feces, but at very low rates and is therefore predominantly stored in the body [24]. Cd is tightly bound to metallothionein and can remain in the human body for over 10 years. Exposure to Cd via drinking water (32 mg/L for 35 days) resulted in reduced ovulated oocytes and a lower percentage of oocytes in meiosis II compared to control [27]. By altering oocyte maturation, the Cd exposure rendered these
reproductive damage persisted past a generation following the plant closure may result from the effects of hexavalent Cr on fetal ovarian development. To study the adverse effects of Cr to the development of ovarian tissue, Stanley et al. exposed ovaries from rat embryos to hexavalent Cr [33]. Their study demonstrated that while control ovaries contained tightly packed germ cells in germ cell nests (GCN), the hexavalent Cr exposure had damaged the GCN resulting in damaged and dead oocytes and granulosa cells [33]. Examination of the primordial and primary follicles showed degeneration that may result in an inability to ovulate during the menstrual cycle [33]. After 9 days of hexavalent Cr exposure, the embryonic rat ovarian tissue presented high levels of Caspase 3, BAX, and p53, as well as downregulation of oocyte-specific proteins BMP-15, GDF-9 and cKit [33] that together contribute to lack of mature oocytes during ovulation that would be successfully fertilized.

The high risk of miscarriage and diminished birth rate observed in areas with high ambient Cr (as found in Willits [31]), may not be solely due to damage on the ovarian tissue, but also the uterus. Histological analyses of uterine tissue isolated from rats exposed to Cr demonstrated atrophy of endometrial glands, accumulation of fibrous tissue, and hyperplasia of the uterine epithelium [34] that may affect successful implantation or gestation, which may contribute to the increased risk of miscarriage observed in humans.

Metal pollutants and male fertility

Mammalian male fertility is also affected by diet and environment. Successful fertility depends on the integrity of the testicular tissue, generation of quality sperm (numerous and motile), various genetic predispositions. Upon reaching puberty, mammalian males can generate several million sperm per day. The number and motility of sperm, as well as the genetic content in the sperm can be altered by various external stimuli. For example, injury to testicles by change in temperature or mechanical injury can affect quality of sperm. Here we will present how exposure to non-essential metals in the ambient environments may affect testicular tissue and generation of healthy, functional sperm. Epidemiological studies examining environmental exposure to As and various aspects of male infertility
demonstrated that men exposed to As exhibited chromosomal alterations in sperm [35] and erectile dysfunction [36]. In a study examining semen quality with concentration of various As species in the urine, higher urine levels of inorganic As and total As were found in males exhibiting “unexplained infertility” compared to fertile males [37]. Rats treated with As resulted in decreased testes weight compared to control rats [38]. The As exposure reduced testosterone levels in the serum and within the testicles [38]. Moreover, compared to control rats, the testes in the As-treated rats also have lower levels of LDH, ALP, and ACP, which are markers of normal testicular activity [38]. Maintenance of testicular tissue is an important part of producing healthy sperm. Exposure to As was shown to diminish the number of sperm within the testicles and epididymis [38], which contain reserve sperm and the daily produced sperm. These diminished numbers of sperm also had limited motility and presented with increased sperm abnormalities [38]. Diminished sperm number and increased sperm abnormalities were induced by As genotoxicity. Analysis of Comet assay demonstrated that rats exposed to As exhibited damaged spermatozoa DNA [39]. Arsenic can also cause these sperm abnormalities via altered expression of 70 proteins (36 up-regulated and 34 down-regulated) and 13 metabolites (8 increased and 5 decreased) [40]. Among these, 19 proteins and 2 metabolites were specifically related with spermatogenesis, sperm function, and fertilization [40]. The As-mediated damage to testes and sperm quality contributes to the observed decreased fertility in mammalian males.

Cd exposure is also deleterious to male fertility. In a study, fifty healthy male volunteers were recruited and provided semen and blood samples. Examinations showed that the mean concentrations of Cd was $8.18 \pm 1.6 \text{ ng/ml}$ in blood samples and $2.56 \pm 0.9 \text{ ng/ml}$ in semen samples [25]. Additionally, a significant positive correlation was demonstrated between Cd blood levels, number of immotile spermatozoa, and teratozoospermia index (TZI) [25]. In 1957, Pařízek demonstrated that rats administered Cd salts exhibited damage of the testes, the seminiferous epithelium, and interstitial tissue [41], which essentially mimicked castration. Treatment with Zinc prevented the Cd-mediated damage [41]. Cd exposure in various animals also caused testicular damage resulting in diminished weight, tissue edema, areas of hemorrhage, necrosis, and loss of germ cells [42] that may all contribute to sterility. After Cd enters testicular tissue, it then accumulates in spermatogonia, spermatocytes, spermatid and spermatozoa [43]. In rats, the accumulation of Cd in the spermatogonia and spermatocytes leads to a decrease in both cells types [43]. Elevated administration of Cd and prolonged exposure results in reduced sperm concentration due to increased apoptosis of sperm cells that is comparable to what is observed in cigarette smokers (as cigarette smoke contains high levels of Cd) [43]. Cd exposure not only reduces the number of sperm in semen, but also affects sperm motility. Sperm motility is essential for travel through the female reproductive tract in order to fertilize an ovulated ovum. Sperm analyzed from rats exposed to Cd displayed a significant reduction in motility, distance travelled, and velocity [44], all of which would contribute to semen unable to achieve fertilization of ovum. Moreover, the sperm from the rats treated with Cd contained a significantly lower percentage of sperm with normal morphology, with a majority displaying sperm head defects and flagellum described as “knob-wisted” [44].

The Cr-mediated low birth rate and gestational defects observed in the Willits residents [31] may also be due to damage in male reproductive health. Occupational exposure present with decreased sperm count and motility [45]. Examination of trace metals in seminal fluid demonstrated a correlation of elevated Cr levels to donors presenting with oligospermia (few sperm in semen) and azoospermia (no sperm in semen) compared to males with normal sperm number [46]. These sperm abnormalities may be due to increased apoptosis and DNA fragmentation found in the testes [45]. The reproductive abnormalities can also persist in the second generation of males. Pregnant rats treated with Cr from gestational day 12 to 21.5 produced males with reduced fetal Leydig cells and their proliferative potential [47]. Due to advancements of in vitro fertilization, reproductive science may overcome low, immobile sperm. However, Cr exposure may also induce cytotoxicity thus reducing successful fertilization and embryo development [48]. Sperm isolated from healthy mice were treated
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During embryonic implantation and it is postulated that the miR-31 expression plays a role in immune-tolerance thereby securing successful implantation and decreasing risk of miscarriage [56].

All together, these various studies suggest that the alterations ambient, non-essential metals induce to promote carcinogenesis may also contribute to the observed reproductive adversities observed in patients and in animal studies. Understanding shared pathways may aid in improving fertility and prevention of metal-induced carcinogenesis.

CONFLICT OF INTEREST STATEMENT

Neither author has any conflicts of interest to report.

REFERENCES


