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Fertility in male rats: Disentangling adverse effects of arsenic compounds

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ABSTRACT

Arsenic impairs male reproductive functions. However, it is not clear whether different arsenic compounds similarly affect fertility. In this study, we compared the impact of sodium arsenite and arsenate on sperm quality and fertility. After 56 d exposure, male Wistar rats were mated and pregnant females were evaluated by fertility indexes. Clearly, exposure to 10 mg/L arsenite reduced daily sperm production via H_2O_2 overproduction and germ cells loss. Animals from this group also showed a decrease in epididymal sperm counts and percentage of sperm with intact membranes. Moreover, they presented low fertility potential and high preimplantation loss. In contrast, 10 mg/L arsenate caused oxidative stress in testis, mineral imbalance in epididymis, and sperm membranes damage, with no effects on fertility. Both arsenic compounds at 0.01 mg/L altered reproductive parameters. We concluded that arsenite is more harmful than arsenate to sperm quality and male fertility, with negative influences in early pregnancy.
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1. Introduction

Over the last decades, environmental toxicants have become a serious public health concern. In this scenario, arsenic is considered one of the most hazardous chemical substances based on a combination of incidence, toxicity, and potential for human exposure [1,2]. Both organic and inorganic arsenics are widely distributed in the environment through natural and anthropogenic sources [3]. Despite humans may be exposed to arsenic through environmental, agricultural, and occupational routes, drinking water has been considered the major source of contamination [1,3–5]. Consequently, many diseases have been associated with chronic arsenic poisoning, such as skin pathologies, diabetes, hepatitis, cardiovascular and respiratory diseases, cancer, developmental neurotoxicity, and genotoxicity [2–5].

Notwithstanding, arsenic is also harmful to male fertility [3–5]. Several studies have reported that arsenic toxicity involves the inhibition of spermatogenesis and steroidogenesis, as well as the generation of reactive oxygen species (ROS) [6–10]. Specifically, the exposure to high concentrations of arsenic (more than 5 mg/Kg) might cause a decrease in sperm production, epididymal sperm number, and sperm quality, being associated with infertility in men and reproductive toxicity in male animals [6,11,12]. To our knowledge, two studies described the effects of arsenic at 0.01 mg/L in male reproductive parameters [13,14]. This value corresponds to the maximum arsenic concentration tolerated in drinking water [15,16]. In those reports, animals exposed to 0.01 mg/L arsenic presented alterations in testis, epididymis and sperm outcomes after 56 d exposure [13,14].

The toxicity of this metalloid varies across its different chemical form and valence state [17]. Overall, organic arsenic is less toxic than inorganic arsenic. The latter is found in drinking water as pentavalent arsenate (iA^V) and trivalent arsenite (iA^{III}) [3,4,18]. The most toxicologically potent compound is the arsenite due its reactivity with cysteine residues, thiol groups and sulphur containing compounds. While arsenite is commonly used in studies involving arsenic-induced reproductive toxicity [3,6,12], arsenate

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is still poorly studied [13,14,19]. In mice, arsenate may produce an elevation of interstitial cell hyperplasia incidence in testis with no tubular degeneration [19]. Moreover, high doses of arsenate impaired the testicular tissue integrity and sperm quality due to ROS generation [4]. In rats, sodium arsenite showed to be more harmful than sodium arsenate on male reproductive functions [14]. The latter compound, however, was able to cause alterations in histomorphometric parameters and antioxidant defense system in the testis, as well as epididymal sperm count [13,14].

Although there are several studies reporting arsenic effects on male reproductive functions [1,4–6,9,10,19], it is not clarified yet whether arsenite and arsenate adversely affect fertility indexes. Male fertility potential is primarily assessed by the ability to produce good quality sperm. For that, spermatozoa undergo a variety of sequential modifications involving morphophysiological, metabolic, and biochemical changes. Maturational changes occur in developing germ cells and maturing sperm [20,21]. The maturation process, in turn, is strongly dependent upon sperm-protein and trace elements interactions [22], and it might be affected by arsenic exposure. In fact, the influence of this metalloid in mineral dynamics has been reported in testis, kidney and liver, with no data in epididymal tissue [23]. Although the low requirement of minerals for body homeostasis, their imbalance may cause disturbances in reproductive organs functions [23].

Based on that, the purpose of the present study was to give a step forward to the better understanding of the reproductive toxicity of inorganic arsenics. Therefore, we compared the effect of sodium arsenite and arsenate in fertility indexes and sperm quality in order to highlight the specific role of each chemical form in these endpoints. Moreover, we evaluated testis and epididymis environments in which spermatozoa are produced and matured, respectively, after exposure to arsenic compounds. Finally, our findings clarified whether the concentrations of arsenic compounds used in the present study (0.01 and 10 mg/L) have an effect on reproductive outcomes.

2. Materials and methods

2.1. Animals

Sexually mature male ($n = 25$, 70 days old, 255–300 g) and female ($n = 50$, 65 days old, 214–270 g) Wistar rats were provided by the Central Animal Facility of the Center of the Biological and Health Sciences of the Federal University of Viçosa (UFV). Animals were housed individually in polypropylene cages, under controlled photoperiod (12 h light/dark) and temperature (21 °C). The provision of feed was controlled, being 30 g of balanced rat feed (Nuvilab) per day. While male rats were provided drinking water as oral route treatment, female rats were provided filtered tap water with no arsenic. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental procedures were reviewed and approved by the Committee on the Ethics and Use of Animal Experiments of UFV (CEUA; protocol no. 19/2011).

2.2. Experimental design

Male rats were weighed and randomly divided into five groups ($n = 6$ animals/group). While animals from the control group received saline solution (0.9% NaCl), the others were exposed to arsenic in the form of sodium arsenate or arsenite ($Na_2HAsO_4 \cdot 7H_2O$; $AsNaO_2$; Sigma-Aldrich Co., St. Luis, MO) at the concentrations of 0.01 and 10 mg/L. The lower concentration used in the present study corresponds to the maximal quantity of this element tolerated in drinking water [15,16], whereas 10 mg/L is based on published reports of arsenic-induced toxicity following oral exposure [13,14,24]. The animals were provided 30 mL arsenic solutions daily through drinking water during 56 d. This exposure period was determined using the duration of one cycle of the seminiferous epithelium in rats [20]. Clinical signs were observed during the experimental period. In addition, saline was provided to control animals to keep the taste of the drinking water similar to arsenic solutions and avoid influences on the amount of solution ingested by this group.

2.3. Natural mating and fertility

Natural mating was performed with non-exposed females in a 1:2 ratio up to 72 h. Vaginal smears were collected at 24, 48 and 72 h mating. The detection of sperm in the vaginal smear of each female confirmed mating, and it was used for establishing the Day 0 of gestation (GD0). Males and females were separated at the end of this period.

On the GD17, females were sedated with xylazine hydrochloride (10 mg/kg/ip), anesthetized with ketamine hydrochloride (150 mg/kg/ip), and euthanized. Their reproductive tract was removed and dissected. Ovaries were weight and evaluated for the presence and number of corpora lutea under gross morphology, whereas uterine horns were used for quantifying areas of implantation sites, and number of fetuses. Thereafter, the fertility indexes were determined: female mating index (number of females mated/number of females × 100), male mating index (number of males mated/number of males × 100), pregnancy index (number of females pregnant/number of females mated × 100), male fertility index (number of males impregnating females/number of males mated × 100), fertility potential (number of implants/number of corpora lutea × 100), preimplantation loss (number of corpora lutea – number of implants/number of corpora lutea × 100), and postimplantation loss (number of implants – number of viable fetuses/number of implants × 100) [25]. Viable fetuses are considered animals with no structural malformations and skeletal anomalies related to arsenic exposure, such as spina bifida, anencephaly, rib and vertebral malformation, and tail defects [25,26].

2.4. Collection of organs from male rats

Male rats were weighed and euthanized on the 3rd day after the mating period, when sperm reserves in the epididymis cauda are fully reestablished [27]. The mean body-weight gain for each group was calculated from the final weight minus the initial weight of the animals. The testes, epididymis, seminal vesicle (without coagulating gland), and ventral prostate were extracted, dissected, and weights (absolute and relative to the body weights) were recorded.

2.5. Sperm analyses

Freshly dissected portions of the epididymis cauda were cut three times and placed in a petri dish containing 500 μL tris-citric acid-fructose (Tris 3.025 g, citric acid 1.7 g, fructose 1.25 g, distilled water 100 mL) for 5 min at 37 °C to enable release of spermatozoa. Aliquots of this fluid were collected for sperm analyses.

Sperm motility was evaluated immediately post-collection using computer-assisted semen analysis (CASA; Sperm Class Analyzer® program, v.4, Microptics *S.L*, Barcelona, Spain). For that, the equipment was adjusted for rodent spermatozoa, and the following endpoints were determined: total motility, progressive motility, curvilinear velocity, progressive velocity, path velocity, linearity, and straightness.

For sperm morphology analysis, epididymal fluid (50 μL) was fixed in 100 μL 4% buffered formaldehyde. Two hundred cells were

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