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Prepubertal arsenic exposure alters phosphoproteins profile, quality, and fertility of epididymal spermatozoa in sexually mature rats

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ABSTRACT

Arsenic intoxication affects male reproductive parameters of prepubertal rats. Besides, morphological and functional alterations in their testis and epididymis may remain after withdrawal of arsenic insult, causing potential impairment in male fertility during adulthood. In this study, we aimed at analyzing the effect of prepubertal arsenic exposure on the fecundity of epididymal sperm from sexually mature Wistar rats, assessing fertility indexes, sperm parameters, and sperm phosphoproteins content. Male pups on postnatal day (PND) 21 received filtered water (controls, $n = 10$) and 10 mg L^{-1} arsenite ($n = 10$) daily for 30 days. From PND52 to PND81, rats from both groups received filtered water. During this period, the males mated with non-exposed females between PND72 and PND75. Our results showed that sexually mature rats presented low sperm production, epididymal sperm count, motility, and quality after prepubertal arsenic exposure. These findings possibly contributed to the low fertility potential and high preimplantation loss. Epididymal sperm proteome detected 268 proteins, which 170 were found in animals from both control and arsenic groups, 27 proteins were detected only in control animals and 71 proteins only in arsenic-exposed rats. In these animals, SPATA 18 and other five proteins were upregulated, whereas keratin type II cytoskeletal 1 was downregulated ($q < 0.1$). The results of KEGG pathway analysis demonstrated an enrichment of pathways related to dopaminergic response, adrenergic signaling, protein degradation, and oocyte meiosis in arsenic-exposed animals. Moreover, 26 proteins were identified by phosphoproteomic with different phosphorylation pattern in animals from both groups, but SPATA18 was phosphorylated only in arsenic-exposed animals. We concluded that prepubertal exposure to arsenic is deleterious to sperm quality and male fertility, altering the sperm phosphoproteins profile.

1. Introduction

Arsenic exposure has attracted the attention of scientific and public communities owing to its environmental and toxicological issues (Liu et al., 2014; Renu et al., 2018; Coelho et al., 2020a, b). Over the last decades, humans are contaminated chronically with inorganic arsenic in drinking water, which is considered a relevant source of arsenic intake. Exposure to this metalloid may lead to cancer development and other

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systemic complications, including the reproductive tract (Hughes, 2002; Hughes et al., 2003; Abdul et al., 2015; Huang et al., 2016). Epidemiological studies have associated the adverse effect of arsenic with male reproductive disorders in humans, such as erectile dysfunction, sperm damages, and prostate cancer (Benbrahim-Talla and Waalkes, 2008; Hsieh et al., 2008; Xu et al., 2012). Likewise, studies demonstrated that arsenic impairs rat fertility by disturbing spermatogenesis, and reducing the motility, number, and viability of epididymal spermatozoa (Reddy et al., 2011; Lima et al., 2018; Chen et al., 2020).

So far, few studies have reported reproductive alterations caused by arsenic in young individuals (Perobelli, 2014; Medeiros et al., 2018). Prepuberty is a critical lifetime period for testis and epididymis development since spermatogenesis, steroidogenesis, and epithelial differentiation are not fully completed (Sharpe, 1994; Robaire and Hinton, 2015). Such developmental events are remarkably responsive to xenobiotic-induced endocrine disorders (Perobelli, 2014). A recent study described damages to spermatogenesis and inactivation of androgen receptors in the testis of juvenile rats exposed to arsenic during prepuberty (Medeiros et al., 2018). Later on, our group showed that most of the arsenic damages observed in pubertal rats remained in sexually mature males, such as damages in testis and epididymis tissues and alterations in antioxidant enzymes' activity, with diminishing serum testosterone levels 30 d after the arsenic insult (Couto-Santos et al., 2020). Veeramachaneni et al. (2001) cited that long-term sequela of infantile exposure to toxic agents could remain undetected for many years until manifestation as idiopathic subfertility or testicular pathology in adult men.

In this light, it is reasonable to hypothesize that prepubertal exposure to arsenic may impair sperm fecundity. The acquisition of such ability occurs during sperm transit throughout the epididymis. This organ is a highly convoluted duct lined by a pseudostratified epithelium that creates a unique luminal environment for sperm maturation (Breton et al., 2019). This process involves several biochemical and structural membrane changes caused by the removal, translocation, and modification of proteins (Naz and Rajesh, 2004; Jankovičová et al., 2018). In the end, matured sperm gain the motility and fertile ability required for fertilizing the egg (Robaire and Hinton, 2015). The addition of phosphate groups into serine, threonine, and tyrosine residues is one of the most common post-translational modifications (PTM) in proteins (Manning et al., 2002). Several studies have focused on the description of sperm protein phosphorylation in many species (Kalab et al., 1998; Aitken et al., 1995; Flesch et al., 1999; Jankovičová et al., 2018) and, for most of them, the flagellum is the main component of the spermatozoon that undergoes phosphorylation (Barth and Oko, 1989).

Notwithstanding, the protein activity or content is strongly affected by exogenous stresses (O'Flaherty and Matsushita-Fournier, 2017; Xie et al., 2020). The inorganic arsenite, for instance, enters the cell and binds to proteins through thiol groups, and consequently alters their conformation and function (Hughes et al., 2011; Gervasi and Visconti, 2017; Hirano, 2020). In this framework, we aimed to evaluate the effects of prepubertal exposure to arsenic on the reproductive competence of sexually mature rats. To this extent, we assessed fertility indexes and functional parameters of epididymal sperm, including motility, morphology, and protein content, with emphasis on phosphoproteins. Identification of molecular elements present in toxicological response may help to elucidate fertility alterations of spermatozoa often related to arsenic exposure (Guvvala et al., 2016; Lima et al., 2018).

2. Material and methods

2.1. Animals

This study is part of a comprehensive work concerning the effect of prepubertal exposure to arsenic in adult rats (Couto-Santos et al., 2020). Male (21 d old, $n = 20$) and female (40 d old, $n = 20$) Wistar rats were obtained from the Central Biotherium of the Universidade Federal de Viçosa. The animals were individually housed in polypropylene cages and were maintained under controlled conditions (21 °C; 70 % air humidity, 12−12 h light/dark cycle) with food and water *ad libitum*. The experimental procedures were conducted following the ethical principles in animal research adopted by the Ethics Committee of Animal Use of the Universidade Federal de Viçosa, Brazil (protocol no. 96/2017).

2.2. Experimental design and treatment

Male pups on postnatal day (PND) 21 were randomly divided into two groups ($n = 10$ animals/group). The control animals received filtered water *ad libitum* for 60 d, from PND 21 to PND 81. The rats from the arsenic group, in turn, were exposed to sodium arsenite ($AsNaO_2$; Sigma-Aldrich Co., St. Louis, MO) at the concentration of 10 mg L^{-1} in drinking water *ad libitum* from PND 21 to PND 51. From PND 52 to PND 81, they were maintained alive receiving filtered water *ad libitum* (Medeiros et al., 2018; Couto-Santos et al., 2020). The arsenic exposure period used here corresponded to the prepubertal phase of rats' development as our previous study (Couto-Santos et al., 2020). At PND 72, control and arsenic-exposed animals were mated with female non-exposed rats by natural mating. Later on, male rats were euthanized on PND 82, age in which they are considered sexually mature due to the maximum daily sperm production (Perobelli, 2014; Picut et al., 2018).

The arsenic solution was prepared daily and provided *ad libitum* in drinking water to male rats from the arsenic group. A concentration of 10 mg L^{-1} of arsenic was used, diluting 17,33 mg sodium arsenite per liter of distilled water. This concentration is based on published studies evaluating arsenic-induced toxicity in rodents following oral exposure (Pant et al., 2001; Chang et al., 2007; Souza et al., 2016a). When normalized for body surface area, this concentration corresponds to 1.6 mg L^{-1} in humans (Reagan-Shaw et al., 2008), which is a dose found in sites affected by arsenic contamination (Mukherjee et al., 2006).

2.3. Natural mating and fertility

At PND 72, one male rat was mated with a healthy female on PND 70 ($n = 10$ animals/group). Vaginal smears were collected at 24, 48 and, 72 h mating to detect the presence of sperm in the vagina and confirm the mounting. This day was then considered Day 0 of gestation (GD0). Ultimately, males and females were separated at the end of this period (Lima et al., 2018).

On the GD19, females were sedated with xylazine hydrochloride (10 mg Kg^{-1} intraperitoneal [ip]), anesthetized with ketamine hydrochloride (150 mg Kg^{-1} ip), and euthanized to enable fertility evaluation. The female reproductive organs were removed and dissected. Ovaries were used to account the number of corpora lutea, implants, fetuses, and resorptions. Also, the following endpoints were determined: male mating index (number of males mated/ number of males x 100), male fertility index (number of males impregnating females/ number of males mated x 100), fertility potential (number of implants/ number of corpora lutea x 100), preimplantation loss (number of corpora lutea - number of implants/ number of corpora lutea x 100), and postimplantation loss (number of implants - number of viable fetuses/ number of implants x 100). Viable fetuses were 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 (Hood, 2012).

2.4. Euthanasia and collection of organs

Male rats were euthanized on PND 82, one week after the end of the mating period. This period is enough to re-establish the sperm reserves in the cauda epididymis (Jones, 1999). First, the animals were weighed, sedated with xylazine hydrochloride (10 mg Kg^{-1} ip), and euthanized by deep anesthesia using ketamine hydrochloride (150 mg Kg^{-1} ip). The left testis and epididymis were dissected and stored at −20 °C for

evaluating the daily sperm production, epididymal sperm counting, and sperm transit time. Spermatozoa from the right epididymis were collected and immediately analyzed under microscopy. Aliquots of the epididymal fluid containing sperm were separated for proteomic analysis.

2.5. Daily sperm production (DSP), sperm number and transit time in epididymis

The left testis (n = 5/group) was decapsulated, weighed, and homogenized in 5 mL 0.9 % NaCl containing 0.05 % Triton X-100. After a 10-fold dilution, the sample was transferred to Neubauer chambers for counting the number of homogenization-resistant testicular spermatids (four fields per animal). Daily sperm production was assessed by dividing the number of spermatids per testis at stage 19 by 6.1, according to Robb et al. (1978). Likewise, caput/corpus and cauda epididymis (n = 5/group) were cut into small fragments with scissors, homogenized, and the sperm count was performed as described for testis. The transit time of sperm through the epididymis was determined by dividing the number of sperm in each portion of the epididymis by the DSP (Robb et al., 1978).

2.6. Sperm analyses

Portions of cauda epididymis (n = 5/group) were cut into small pieces and added into 500 μL BWW medium (Aitken et al., 2016) at 37 °C for 5 min to enable the release of spermatozoa. Thereafter, sperm motility was evaluated using 10 μL of epididymal fluid placed between a slide and coverslip, previously heated to 37 °C, and analyzed using phase-contrast microscope (Bioval L-1000B, Brazil) at 400x magnification. Two hundred of cells were counted per animal and classified as motile (progressive and non -progressive motility) or immotile (with no motility). Morphological features of gametes were assessed using 50 μL of epididymal fluid fixed in 100 μL 4% buffered formaldehyde. Normal and abnormal (*e.g.*, defects in the head, midpiece and tail) sperm morphologies were analyzed in 200 cells under phase contrast microscopy (Bioval L-1000B, Brazil) at 400x magnification. Finally, 10 μL of epididymal fluid were used to evaluate the structural integrity of plasma and acrosomal sperm membranes. For that, sperm cells were incubated in a solution of 4% buffered formaldehyde plus buffer citrate, carboxyfluorescein diacetate (CFDA), and propidium iodide (PI) for 8 min at 37 °C (Harrison and Vickers, 1990). Two hundred sperm cells were analyzed under EVOS FL fluorescence microscope (Advanced Microscopy Group, Bothell, WA, USA) at 400x magnification. These cells were classified into two categories: sperm with intact plasma and acrosome membranes ($CFDA^{+}/PI^{-}$) and damaged plasma and acrosomal membranes ($CFDA^{-}/PI^{+}$). All the results were expressed as percentage.

2.7. Proteomic analysis of epididymal spermatozoa

There were harvested sperm from cauda epididymis (n = 5/group) by chopping the tissue with a scalpel in 1 mL BWW medium (Aitken et al., 2016) with bovine serum albumin (BSA; 3 mg L^{-1}) in a petri dish, kepping in heat trail (Menezes et al., 2018). They were filtered in 100 μm cell strainers. After that, the samples were laid in 1 mL over 3 mL of 30 % Percoll (Sigma-Aldrich Co., St. Louis, MO), followed by centrifugation at 600 x g for 20 min. The samples were resuspended in 2 mL of RBC Lysis buffer 10 mg EDTA (NH4Cl 2.075 g/NaHCO3 0.5 g in 250 mL pH 7.4) and followed by centrifugation at 1,200 x *g* for 20 min. The latter process was repeated more two times. The pellet was resuspended in 1 mL BWW and centrifuged at 600 x *g* for 20 min. Ultimately, the solution was examined in a light microscope to verify the presence of sperm cells and the absence of red blood cells. Samples were then centrifuged, and the pellet was resuspended in acetic acid and acetonitrile solution and then stored at -20 °C.

2.8. Immobilized metal-ion affinity chromatography (IMAC)

Immobilized metal ion affinity chromatography (IMAC) has been used for the enrichment of phosphorylated proteins and peptides. PHOS-SelectTM Iron Affinity Gel (Sigma-Aldrich Co., St. Louis, MO) was used according to the manufacturer's instructions. Briefly, a 500 μL slurry of PHOS-Select iron affinity gel beads was washed three times with 250 mMol acetic acid in 30 % acetonitrile (pH 2.5). Then, 500 μL of sample diluted in the same buffer solution was loaded onto each spin column (about 2 mg total protein per 500 μL of bead slurry) and incubated for 15 min at room temperature with gentle shaking. Phosphoproteins bound to the IMAC columns were eluted three times with 500 μL of elution buffer (150 mMol Ammonium hydroxide in 25 % acetonitrile) each time incubating at room temperature for 10 min with gentle shaking and then centrifuged at 8,200 ×*g* for 1 min. The two eluates were pooled and dried in a speed vac and frozen at −20 °C for further analysis.

2.9. Protein digestion

Protein digestion was performed according to Viana et al. (2018). Briefly, sperm proteins were suspended in 0.02 M ammonium bicarbonate and 0.5 M DTT (dithiothreitol), followed by incubation at 55 °C for 25 min. Further, 0.014 M IAA (iodoacetamide) was added and maintained at 21 °C in the dark for 40 min. All samples were incubated with trypsin (Promega, Fitchburg, WI, USA) at 37 °C for 18 h, with a 1/50 (w/w) enzyme/substrate ratio. Then, a solution of 1% TFA (trifluoroacetic acid) was added to stop the tryptic activity.

2.10. Orbitrap MS

Tryptic peptides were fractionated on Easy nLC II (Thermo Scientific) nanoflow HPLC system connected to an LTQ Orbitrap XL mass spectrometer (MS) (Thermo, Bremen, Germany) equipped with a nanoelectrospray ion source. Peptides samples from sperm of each animal were split in aliquots to generate two technical replicates. Peptides were initially loaded onto a trap column 2.0 cm long (100 μm internal diameter) packed in-house with C18 resin (5 μm, 100 Å pore, Magic C18 AQ, Bruker-Michrom, Auburn, CA) and fractionated on a RP-HPLC column 30 cm long (75 μm internal diameter). The gradient conditions were as follows: 2–40% of 0.1 % trifluoroacetic acid (v/v) in acetonitrile solution for 162 min and up to 80 % of this solution for 4 min. This concentration was maintained for 2 min before the column was re-equilibrated. The eluted peptides were directly introduced to an LTQ Orbitrap XL MS for analysis.

Precursor scans were performed in Orbitrap mass detector at a resolution of 60,000 in a mass range from 300 m/z to 1700 m/z, while MS/MS scans were acquired in a linear trap analyzer. With the exclusion of singly charged ions, up to ten of the most intense precursor ions were subjected to produce ion scans using collision-induced dissociation (CID) with a normalized collision energy of 35.0. Moreover, MS/MS scans were only triggered for precursor ions having a minimum signal threshold of 10,000 counts. Precursors selected for MS/MS scans were dynamically excluded for 30 s from a repeated product ion scan within a ±10 ppm mass error.

2.11. Database search

Database searches of the tandem mass spectra acquired on LTQ Orbitrap XL mass spectrometer were performed using Peaks Studio 8.5. There was applied database searching from Uniprot and NCBI repositories in September 2019, filtered for the species *Rattus norvegicus*, *Mus musculus*, *Homo sapiens,* and *Bos taurus*. The search parameters for monoisotopic peptide masses allowed two missed enzymatic cleavage. Carbamidomethylation of the cysteine residues was defined as fixed modification and the oxidation (M), whereas phosphorylation (P) as variable modifications. A fragment ion mass tolerance of 0.60 Da and a

parent ion tolerance of 20 ppm were used. To choose from variables modifications, the PEAKS PTM algorithm WARE tool was used, with the same parameters described above, and modifications with AScore 1000 were selected for the searches. False discovery rates (FDR) were estimated through the PEAKS decoy fusion approach. A peptide-spectrum match FDR of 1% and protein identifications with at least two unique peptides were the criteria used to establish FDR values at peptide and protein levels ≤ 1 %.

2.12. Protein identification and analysis of functional clusters

The protein identification was performed using a conservative criterion of at least two unique peptides for each protein (Carr et al., 2004), and proteins present in at least three from five animals (60 %) of each group. There were considered genes of the species *Rattus norvegicus*, *Mus musculus*, *Homo sapiens,* and *Bos taurus.* Functional clusters associated with proteins from rat sperm were analyzed through the DAVID platform (DAVID Functional Annotation Bioinformatics Analysis - https://david.ncifcrf.gov; Huang et al., 2009). This analysis was conducted for each protein detected in the control and arsenic groups separately, and they were compared with the annotated genome and functional terms. Gene Ontology annotated terms for biological process, molecular function, and cellular component, as well as metabolic pathways annotated in KEGG (Kyoto Encyclopedia of Genes and Genomes) database, were clustered and defined according to enrichment scores and p-values.

2.13. Statistical analysis

The normality of the results was assessed by Shapiro-Wilk test. All the variables analyzed herein were compared between control and arsenic groups using Student's T-test. Differences were considered significant when $p < 0.05$. All tests and graphics were performed using the GraphPad Prism 6.0 statistical software (GraphPad Software Inc., San Diego, CA, USA). Data were expressed as mean ± standard error mean (SEM). Finally, an unpaired *t*-test with false discovery rates (FDR) and $\log_2$-fold change (FC) were carried out to identify differentially expressed sperm proteins between control and arsenic-exposed animals ($q < 0.1$; Zhu et al., 2020).

3. Results

3.1. Prepubertal exposure to arsenic affects fertility indexes of sexually mature rats

Ten animals from the arsenic group were able to mate with non-exposed females in contrast to the eight from the control group. The male mating index reflected this difference (Table 1). However, eight rats of each control and arsenic group impregnated their respective females. This fact influenced the low male fertility index of arsenic-exposed rats compared to control animals (Table 1). Consequently, the fertility potential of intoxicated rats was lower than the control animals ($p < 0.05$; Table 1). The number of corpora lutea, implants, resorptions, viable fetuses, and ovary weight did not differ between groups ($p > 0.05$; Table 1). While the percentage of preimplantation loss was higher in arsenic-exposed animals compared to their controls ($p < 0.05$), the postimplantation loss did not differ between groups ($p > 0.05$; Table 1).

3.2. Prepubertal exposure to arsenic reduces sperm count and daily sperm production in sexually mature rats

The number of mature spermatids per testis and per gram testis, as well as the daily sperm production was diminished in arsenic-exposed animals compared to control animals ($p < 0.05$; Table 2). Similarly, the number of sperm count in caput/corpus and cauda epididymis reduced in animals previously exposed to arsenic compared to their controls ($p < 0.05$; Table 2). The sperm transit time in epididymal regions, in turn, did not alter after prepubertal arsenic exposure ($p > 0.05$; Table 2).

Table 1
Fertility indexes of sexually matured rats ($n = 10$ animals/group) exposed to 10 mg L^{-1} sodium arsenite between postnatal day (PND) 21 and 51, after natural mating with non-exposed females.

Parameters	Control	Arsenic
Number of females	10	10
Number of females mated[a]	8	10
Number of males mated[a]	8	10
Number of males impregnating females	8	8
Number of females pregnant	8	8
Number of corpora lutea[b]	14.17 ± 1.01	16.43 ± 0.99
Number of implants[b]	12.17 ± 0.60	11.57 ± 0.68
Number of resorptions[b]	0.50 ± 0.18	0.75 ± 0.41
Number of viable fetuses[b]	11.83 ± 0.79	10.71 ± 0.80
Ovary weight (g) [b]	0.18 ± 0.012	0.17 ± 0.008
Male mating index (%)	80	100
Male fertility index (%)	100	80
Fertility potential (%)[b]	87.13 ± 4.90	71.47 ± 4.91*
Preimplantation loss (%)[b]	17.62 ± 2.62	30.70 ± 4.22*
Postimplantation loss (%)[b]	9.05 ± 0.67	17.66 ± 3.53

[a] Sperm presence on vaginal smears.
[b] Values expressed as mean ± SEM. *Significant differences ($p < 0.05$) between control and arsenic groups by Student's *t*-test.

Table 2
Sperm count in testis and epididymis, and sperm parameters of sexually matured rats exposed to 10 mg L^{-1} sodium arsenite between postnatal day (PND) 21 and PND 51.

Parameters	Control	Arsenic
Sperm count		
Spermatid number (x10^6/testis)	175.30 ± 4.63	115.30 ± 3.34*
Spermatid number (x10^6/g testis)	122.60 ± 1.09	90.65 ± 4.80*
Daily sperm production (x10^6/testis/d)	28.73 ± 1.69	18.90 ± 0.54*
Caput/corpus epididymis sperm number (x10^6/organ)	94.83 ± 3.08	60.37 ± 2.38*
Caput/corpus epididymis sperm number (x10^6/g organ)	290 ± 6.60	194 ± 3.50*
Sperm transit time in the caput/corpus epididymis (d)	3.09 ± 0.21	3.21 ± 0.18
Cauda epididymis sperm number (x10^6/organ)	126.40 ± 9.03	69.43 ± 4.73*
Cauda epididymis sperm number (x10^6/g organ)	619 ± 23.72	389 ± 9.76*
Sperm transit time in the cauda epididymis (d)	4.38 ± 0.22	3.70 ± 0.32
Sperm parameters		
Sperm motility (%)	76.46 ± 2.28	62.00 ± 4.83*
Normal sperm morphology (%)	93.80 ± 2.39	92.60 ± 2.50
Sperm with intact membranes (%)	32.30 ± 1.25	8.10 ± 1.23*

Mean ± SEM.*Significant differences ($p < 0.05$) between control and arsenic groups by Student`s *t*-test. $n = 5$ animals/group.

3.3. Sperm parameters were altered in sexually mature males after prepubertal exposure to arsenic

The percentage of motile spermatozoa and cells with intact plasma and acrosomal membranes was lower in arsenic-exposed animals than the control rats ($p < 0.05$; Table 2). Exposure to arsenic did not alter the percentage of sperm with normal morphology in those animals ($p > 0.05$; Table 2).

3.4. Proteome of epididymal sperm from control and arsenic-exposed rats after prepubertal contamination

A total of 268 proteins were identified from epididymal spermatozoa

(Supplementary Table 1). From those, 170 proteins were found in rats of both control and arsenic groups, 27 were detected only in control animals, and other 71 proteins were present in the epididymal sperm of rats exposed to arsenic (Fig. 1). Although we identified a large number of sperm proteins, 19 proteins exhibited differences in their abundance between control and arsenic-exposed rats ($q < 0.1$; Table 3). Mitochondria-eating protein (MIEAP), also known as spermatogenesis-associated protein 18 (SPATA 18), showed the highest fold change of the proteins upregulated in spermatozoa of arsenic-exposed rats. In addition, mitochondrial ATP synthase subunit beta, peroxiredoxin-like 2A, 14-3-3 protein zeta/delta, calmodulins, and mitochondrial cytochrome c oxidase subunit 5A were also upregulated in epididymal sperm from arsenic group. By contrast, only keratin type II cytoskeletal 1 (KRT1) was downregulated in spermatozoa of rats from arsenic exposed group (Table 3).

Functional clustering analyses were performed to evaluate the insight into biological processes, molecular function, and cellular components of proteins detected in each group. The 27 genes from proteins found in control rats corresponded to one single cluster with no significance ($p > 0.05$; data not shown). In arsenic group, genes that corresponded to the 71 proteins matched into four clusters (Supplementary Table 2), and the enriched terms ($p < 0.05$) ranked according to p-value [-log (p-value)] for each category are shown in Fig. 2. Biological processes involved in the regulation of circadian rhythm, glycogen metabolic process, protein dephosphorylation, and cell cycle were enriched in animals exposed to arsenic (Fig. 2). The enriched terms related to molecular functions were phosphatase activities and metal ion binding. Regarding cellular components, there were described enriched proteins in PTW/PP1 phosphatase and MLL5-L complexes, telomeric region of nuclear chromosome, and lysosome (Fig. 2). The results of KEGG pathway analysis demonstrated an enrichment of terms related to dopaminergic response, adrenergic signaling, protein degradation, and oocyte meiosis in arsenic-exposed animals (Fig. 2).

3.5. Arsenic affected the phosphorylation pattern of protein SPATA18

A total of 26 proteins were found phosphorylated in animals from control and arsenic groups. The proteins cyclin 2, osteopontin, glutathione peroxidase 3, spermatogenesis-associated protein 19 mitochondrial, and A-kinase anchor protein 4 (AKAP4) were phosphorylated in most of the animals (Table 4[1–5]). The protein AKAPA4 was identified in both mouse and rat species. Interestingly, the protein SPATA 18 showed no phosphorylation pattern in epididymal sperm from control rats and

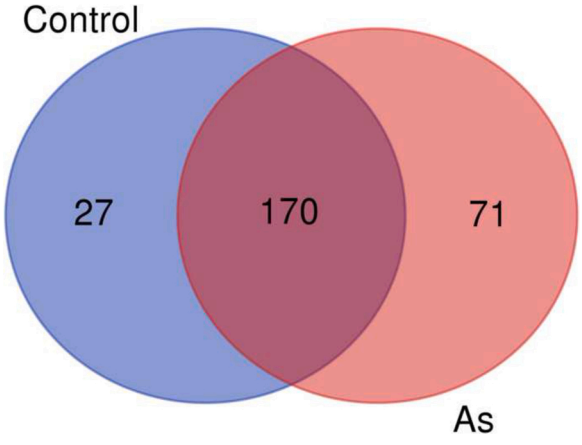

Fig. 1. Venn diagram generated by the Bioinformatics & Evolutionary Genomics platform (http://bioinformatics.psb.ugent.be/webtools/Venn showing the proteome of epididymal sperm of sexually mature rats. From a total of 268 proteins, 27 were identified in animals from control group and 71 were identified in rats exposed to 10 mg L^{-1} sodium arsenite during prepuberty (As group).

Table 3
List of up and down regulated proteins ($n = 19$; $q < 0.1$) of rat sperm harvested from cauda epididymis of sexually mature rats exposed to 10 mg L^{-1} sodium arsenite between postnatal day (PND) 21 and PND 51, identified by mass spectrometry.

Accession number	Protein name	Regulation	log_2 FC	q-value
Q6AYL6	Spermatogenesis-associated protein 18	Up	1.38	0.021
P00829	ATP synthase subunit beta, mitochondrial	Up	1.27	0.018
Q6AXX6	Peroxiredoxin-like 2A	Up	1.25	0.029
P63104	14-3-3 protein zeta/delta	Up	1.13	0.097
P63103	14-3-3 protein zeta/delta	Up	1.13	0.097
P0DP29	Calmodulin-1	Up	0.85	0.033
P0DP26	Calmodulin-1	Up	0.85	0.033
P04264	Keratin, type II cytoskeletal 1	Down	−0.85	0.089
P62157	Calmodulin	Up	0.83	0.033
P0DP23	Calmodulin-1	Up	0.83	0.033
P0DP30	Calmodulin-2	Up	0.83	0.033
P0DP27	Calmodulin-2	Up	0.83	0.033
P0DP24	Calmodulin-2	Up	0.83	0.033
P0DP31	Calmodulin-3	Up	0.83	0.033
P0DP28	Calmodulin-3	Up	0.83	0.033
P0DP25	Calmodulin-3	Up	0.83	0.033
P11240	Cytochrome c oxidase subunit 5A, mitochondrial	Up	0.66	0.050
P12787	Cytochrome c oxidase subunit 5A, mitochondrial	Up	0.66	0.050
P00426	Cytochrome c oxidase subunit 5A, mitochondrial	Up	0.66	0.050

FC: Fold change.

was phosphorylated in three from five animals of the arsenic group (Table 4[6]). Moreover, 20 phosphorylated proteins were detected in only one animal relying upon the group (Table 4[7-26]).

4. Discussion

To the best of our knowledge, the current study is the first focused on the analysis of sperm parameters, phosphoprotein content, and fertility of animals exposed to arsenic during prepuberty. In this scenario, adult rats exposed to this metalloid exhibited low sperm quality 30 d after arsenic withdrawal. Moreover, these animals presented a reduction in their fertility potential with alteration in the epididymal sperm proteome. Despite the detrimental effects of arsenic on the reproductive competence of adult rats are well documented (Sarkar et al., 2003; Reddy et al., 2011; Souza et al., 2016a, b; Lima et al., 2018), its impact during a critical period of development as puberty is still poorly understood (Ferreira et al., 2012; Adedara et al., 2017; Medeiros et al., 2018). Puberty occurs around PND 46 in Wistar rats and is known as the period in which the spermatogenic process first concludes its whole cycle and sperm enter the epididymis (Senger, 2003). One week later, fertile sperm are present in the epididymis cauda. The sperm production/g of testis increases until PND 75, supporting the sperm reserves in rat epididymis. Then, males can express their fertility potential and achieve sexual maturity (Robb et al., 1978; Picut et al., 2018).

Our findings showed that prepubertal contamination with arsenic impairs sperm production in the testis of sexually mature rats. The low number of spermatids in testis and per gram of testis and the reduced daily sperm production have been previously described in adult rats 24 h after the end of arsenic intake (Souza et al., 2016a; Lima et al., 2018). Here, we verified that this effect remains in poisoned animals 30 d after the arsenic removal. The loss of germ cells is often reported in intoxicated animals by arsenicals, which accumulate in testis tissue undergoing oxidative stress even after exposure withdrawal (Souza et al., 2019; Couto-Santos et al., 2020). The number of sperm produced by testis influenced the epididymal sperm count observed here. Altogether, these parameters are relevant indicators of male fertility potential

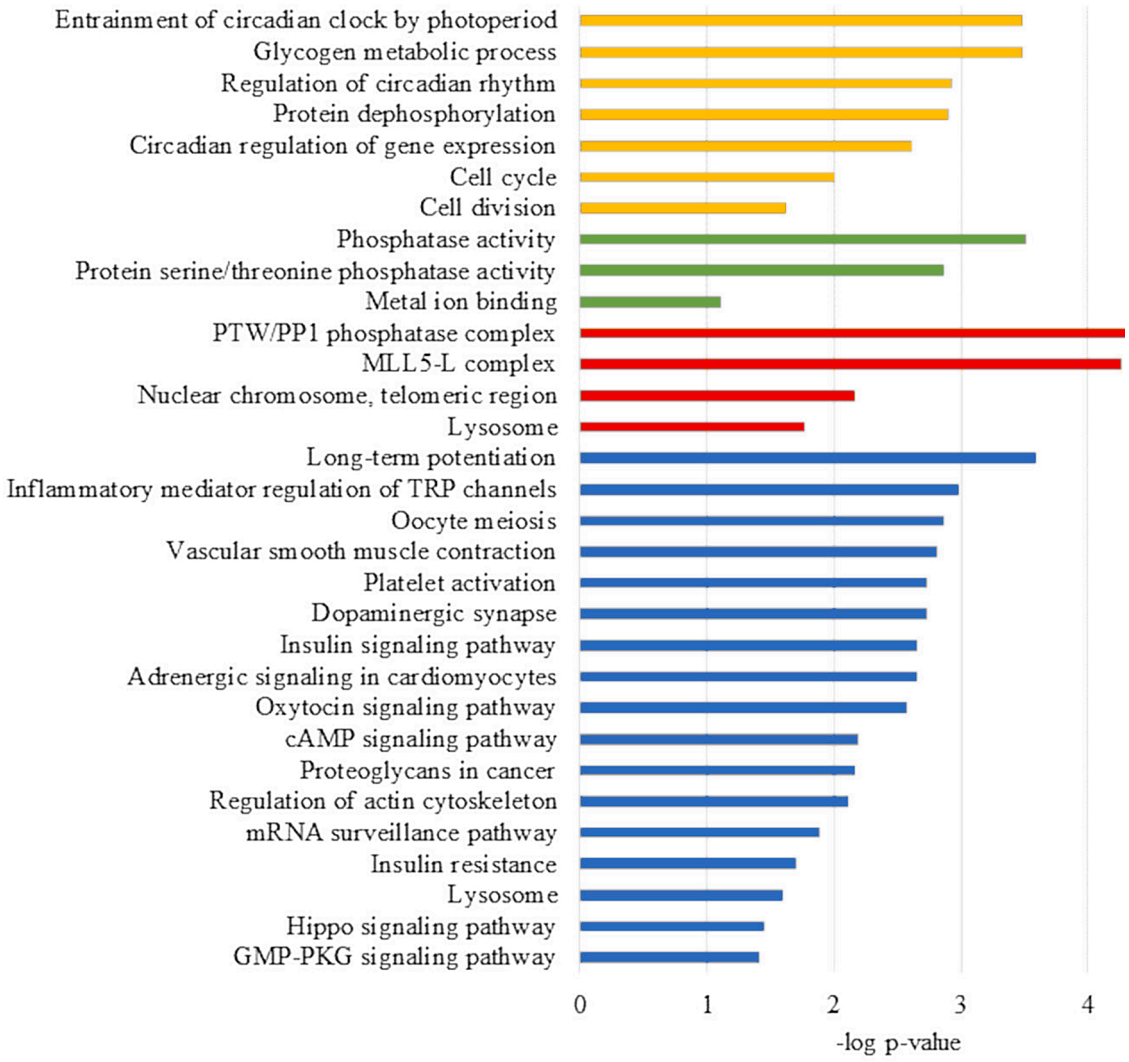

Fig. 2. Functional annotations [-log (p-value)] of proteins' clusters identified in the epididymal sperm of sexually mature rats exposed to 10 mg L^{-1} sodium arsenite during prepuberty (arsenic group) according to biological process, molecular functions, cellular components, and KEGG pathways. Protein data were clustered and defined according to enrichment scores and p-values using the DAVID platform (DAVID Functional Annotation Bioinformatics Analysis - https://david.ncifcrf.gov).

(Mangelsdorf et al., 2003; Fernandes et al., 2007; Sanabria et al., 2016).

On the other hand, intoxicated animals did not show alteration in the percentage of sperm with normal morphology after 30 d of arsenic insult. Studies have reported no alterations in this parameter when assessed 24 h after arsenic exposure (Souza et al., 2016a,b; Lima et al., 2018) in contrast to others that described a higher occurrence of tail defects, followed by head and midpiece defects (Ferreira et al., 2012; Bashandy et al., 2016; Prathima et al., 2008). These controversies may result from the acceleration of the sperm transit time, the high osmolarity of dilution media, heat stress, cold shock, and human manipulation during the sperm analysis, particularly those related to the distal cytoplasmic droplet, coiled tail, broken tail, hook different angles, and detached head (Barth and Oko, 1989). Typically, sperm abnormalities are mainly related to alteration in the spermatogenic process. A recent study reported that arsenic influences spermiogenesis by disorganizing the elongation of spermatids in arsenic-intoxicated animals (Han et al., 2020). This abnormality, however, needs to be assessed by electronic microscopy.

Furthermore, epididymal sperm quality was disrupted by prepubertal arsenic exposure according to our findings of their motility and structural membranes integrity. Once epididymis is the organ responsible for sperm maturation, spermatozoa should display forward motility and fertility ability after the transit throughout the entire duct (Klinefelter and Hess, 1998; Sullivan et al., 2007; Robaire and Hinton, 2015). Two factors may influence these parameters, the sperm transit time and the quality of luminal microenvironment within the epididymal duct. There are controversial findings concerning the capacity of this metalloid to retard the sperm transit throughout the epididymis when this variable is assessed 24 h after the last exposure (Souza et al., 2016b; Altoé et al., 2017; Souza et al., 2019). Herein, the concentration of 10 mg L^{-1} did not alter the sperm transit time 30 d after arsenite removal. Thus, we might speculate that the withdrawal exposure was enough to reestablish any possible alteration in the sperm transit time caused by arsenic poisoning. With respect to the luminal microenvironment, its quality conditions after arsenic exposure are still poorly studied. Notwithstanding, structural and functional alteration in the epididymal epithelium along with the oxidative stress driven by arsenic may disturb the optimal environment created within the epididymal duct. It is known that arsenite can access the intracellular environment by the aquaporin 9 highly expressed in the apical membrane of the principal cells present in the epididymal epithelium (Hughes, 2002; Da Silva et al., 2006). Within the cell, this trivalent arsenical can inhibit enzymes or alter protein structures by reaction with their thiol groups (Thomas, 2007). For instance, the inactivation of thiol-containing proteins may destabilize structures in the sperm flagellum by disulfide bond formation (Hughes, 2002; Ijiri et al., 2014; Gervasi and Visconti, 2017).

Table 4
List of phosphoproteins of rat (R) sperm harvested from cauda epididymis of sexually mature animals exposed to 10 mg L^{-1} sodium arsenite during prepuberty, identified by mass spectrometry after IMAC enrichment.

	Phosphorylated proteins ($n = 26$)			Control					Arsenic				
n	Acession number		Description	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
1	Q28092	CYLC2_BOVIN	Cylicin-2	x	x	x			x	x			
2	P31096	OSTP_BOVIN	Osteopontin	x	x	x			x	x			
3	P37141	GPX3_BOVIN	Glutathione peroxidase 3	x	x	x		x	x	x			
4	Q3SZQ3	SPT19_BOVIN	Spermatogenesis-associated protein 19 mitochondrial		x	x			x		x		
5	O35774 Q60662	AKAP_RAT AKAP_MOUSE	A-kinase anchor protein 4			x	x	x	x	x	x	x	x
6	Q6AYL6	MIEAP_RAT	Spermatogenesis-associated protein 18								x	x	x
7	Q80XH4	WSCD1_MOUSE	WSC domain-containing protein 1	x									
8	Q49AM1	MTEF2_HUMAN	Transcription termination factor 2 mitochondrial						x				
9	Q9H6K5	PRR36_HUMAN	Proline-rich protein 36						x				
10	Q4U2R1	HERC2_MOUSE	E3 ubiquitin-protein ligase HERC2		x								
11	Q8VHX6	FLNC_MOUSE	Filamin-C							x			
12	Q80V94	AP4E1_MOUSE	AP-4 complex subunit epsilon-1							x			
13	Q8BGZ7	K2C75_MOUSE	Keratin type II cytoskeletal 75							x			
14	Q6S8J3	POTEE_HUMAN	POTE ankyrin domain family member E							x			
15	A5A3E0	POTEF_HUMAN	POTE ankyrin domain family member F							x			
16	P0CG38	POTEI_HUMAN	POTE ankyrin domain family member I							x			
17	Q96F45	ZN503_HUMAN	Zinc finger protein 503			x							
18	P50851	LRBA_HUMAN	Lipopolysaccharide-responsive and beige-like anchor protein			x							
19	P54652	HSP72_HUMAN	Heat shock-related 70 kDa protein 2			x							
20	Q6AY30	SCPDL_RAT	Saccharopine dehydrogenase-like oxidoreductase								x		
21	P00342	LDHC_MOUSE	L-lactate dehydrogenase C chain								x		
22	Q9UI47	CTNA3_HUMAN	Catenin alpha-3								x		
23	A2A870	FBF1_MOUSE	Fas-binding factor 1				x						
24	O09046	OXLA_MOUSE	L-amino-acid oxidase				x						
25	Q3ZBH0	TCPB_BOVIN	T-complex protein 1 subunit beta				x						
26	Q5JR59	MTUS2_HUMAN	Microtubule-associated tumor suppressor candidate 2										x

Aside from enzyme inactivation, the lipid peroxidation driven by arsenic intake in the epididymis of adult rats, previously described by Couto-Santos et al. (2020), probably caused the damages observed in the acrosomal and sperm membranes. Collectively, these findings indicated that prepubertal arsenic exposure is hazard to sperm quality in sexually mature rats, with possible implications to their progressive motility as well as their ability to capacitate and interact with oocytes during the fertilization process.

Herein, the low fertility potential added to the high percentage of preimplantation loss indicated a possible failure of fertilization ability of epididymal spermatozoa from arsenic-exposed rats. Lima et al. (2018) also observed a reduction in the fertility potential and an increased preimplantation loss in adult rats exposed to 10 mg L^{-1} arsenic. Da Silva et al. (2017) demonstrated that arsenic caused adverse effects on sperm fertilizing ability by downregulating proteins linked to sperm motility, such as sperm mitochondrial associated cysteine-rich protein. The interaction of these molecules may cause a decrease in motility and impair the capacitation events of the sperm that, in turn, may disturb male fertility (Makker et al., 2009). Indeed, our results showed three types of mitochondrial proteins upregulated in arsenic-exposed rats, SPATA 18, ATP synthase subunit beta, and cytochrome c oxidase subunit 5A. The latter two proteins belong to the electron transport chain responsible for ATP and ROS production, located on the inner mitochondrial membrane (Lobo-Jarne and Ugalde, 2018). SPATA18, in turn, is a cytoplasmic protein that mediates either mitochondrion repairing or degradation in response to mitochondrial damage, probably caused by oxidative stress (Kitamura et al., 2011). It is known that arsenic induces oxidative stress through reactive oxygen species (ROS) generation that cause direct cellular oxidative injuries (Aitken, 2011; Souza et al., 2019). Spermatozoa undergo a cascade of biochemical and physiological events, as known as capacitation, before fertilization. Such events increase ROS production (Aitken, 2011), which may be detrimental to those cells that are suffering oxidative stress due to arsenic intoxication. Taking these findings into account, we may suggest that pubertal arsenic exposure leads to increased expression of mitochondrial proteins in response to the ongoing ROS overproduction once mitochondrion is a target organelle for arsenic toxicity.

In addition to the above, peroxiredoxin-like 2A, calmodulin, and 14-3-3 zeta/delta protein were also upregulated in arsenic-exposed rats. Peroxiredoxin-like 2A belongs to a family of very conserved proteins named peroxiredoxins (PRDXs). PRDXs are thiol-dependent proteins that play a vital role in sperm function and male fertility by mediating antioxidant system (Rahman et al., 2015; Ozkosem et al., 2016; Ryu et al., 2017). The inhibition of PRDXs in mice spermatozoa reduces sperm quality and increases ROS levels, DNA fragmentation, and mitochondrial membrane potential disruption (Ryu et al., 2017). Rahman et al. (2015) also reported an upregulation of PRDXs, specifically PRDX 5, in mice spermatozoa under stressed conditions. Calmodulin belongs to a family of very conserved proteins and is highly abundant in mammal spermatozoa (Chin and Means, 2000). It regulates intracellular Ca^{2+} homeostasis and modulates cell signaling. The upregulation of calmodulin in epididymal sperm from rats exposed to arsenic during its pubertal phase might be a response to the breakdown of Ca^{2+} homeostasis induced by this metalloid (Zhang et al., 2016). Finally, the upregulated 14-3-3 zeta/delta protein belongs to a family of 14-3-3 homologous proteins with several isoforms. This protein interacts with hundreds of proteins containing phosphorylated serine or threonine motif and contributes to the regulation of several biological processes (Muslin et al., 1996). Moreover, 14-3-3 proteins play a wide range in all eukaryotic cells as a part of the regulatory system with kinases and phosphatases (Johnson et al., 2010; Munier et al., 2021). In bull spermatozoa, 14-3-3 zeta/delta protein plays a crucial role in sperm motility by modulating the presence of protein phosphatase 1 gamma (PP1gamma) in the flagellum (Huang et al., 2002). Thus, the upregulation of 14-3-3 zeta/delta protein in sperm of arsenic-exposed rats may be feedback to the increase in phosphatase activities, reported here through functional clustering analysis. On the other hand, KRT1 was the only protein downregulated in sperm of arsenic-exposed rats. KRT1 is one of the components of perinuclear ring of spermatid head (Kierszenbaum, 2002). In male mice's knock out for KRT1 gene, there were reported 35 % spermatids with ectopic head and 40 % of epididymal sperm with abnormal tail morphology (Rivkin et al., 2005).

Looking into both biological process and molecular function analyses, it was possible to observe an enrichment of phosphatase activity that results in an altered phosphorylation pattern in epididymal sperm of arsenic-exposed rats. Functional clustering analysis aims to build a meaningful cluster of proteins with similar functions or that participate in the same signaling pathway. Therefore, applying this analysis to proteins from epididymal sperm of arsenic-exposed rats may bring us insights into the molecular changes that exposure to this metalloid can cause to male fertility. Regarding KEGG pathway analysis, terms related to dopaminergic response, adrenergic signaling, protein degradation, and oocyte meiosis were found enriched in sperm of rats exposed to arsenic due to the specific presence of subunits of serine/threonine-protein phosphatase PP1 and cAMP-dependent protein kinase catalytic. Authors suggested that dopamine acts as a modulator of sperm viability after experimentally incubating stallion and boar sperm with high doses of dopamine, reducing sperm motility, acrosomal integrity, and tyrosine phosphorylation (Ramírez et al., 2009; Urra et al., 2014). Starovlah et al. (2020), in turn, showed that stress can decrease rat sperm viability through a disorder mediated by sperm adrenergic receptors, leading to alteration in mitochondrial dynamics and ultrastructure.

Among the 26 phosphorylated proteins, cyclin 2, osteopontin (OPN), glutathione peroxidase 3 (GPX3), spermatogenesis-associated protein 19 (SPATA 19), and A-Kinase Anchoring Protein 4 (AKAP 4) were present in the spermatozoa of most rats from control and arsenic groups. Cyclin is a family of proteins that form a complex with cyclin-dependent kinases. They are constantly activated and inactivated to regulate the cell cycle (Pines, 1993). For instance, cyclins phosphorylation is essential for their function and activation during spermiogenesis (Wolgemuth et al., 2013). OPN is a phosphoprotein identified in several tissues, including the testis, epididymis, and spermatozoon of rats (Siiteri et al., 1995). This protein was previously described in the membranes of rat sperm retrieved from the testis and epididymis (Luedtke et al., 2002). In bovine, the OPN secreted into the seminal plasma is associated with sperm fertility through the increment of sperm capacitation (Cancel et al., 1999). Also, OPN influences the intracellular calcium concentration, which is crucial for sperm capacitation and acrosome reaction (Denhadrt and Guo, 1993). Moreover, OPN plays a role in the sperm-egg interaction by association with integrins (Gonçalves et al., 2007), and in embryo implantation as a mediator of cell extracellular matrix (Johnson et al., 2003). GPX3, in turn, is one of the enzymes involved in cell protection against oxidative damage (Halliwell and Gutteridge, 2007). The SPATA 19 belongs to the family of spermatogenesis-associated proteins. It is related to the maintenance of sperm mitochondria, androgen secretion, and male fertility (Suzuki-Toyota et al., 2007; Nourashrafeddin et al., 2014; Mi et al., 2015). Finally, AKAP4 is the main component of sperm fibrous sheath that targets protein kinase A and other subcellular enzymes (Ben-Navi et al., 2016). AKAP4 are phosphorylated at serine and tyrosine residues during sperm capacitation, playing a putative role in sperm motility (Carrera et al., 1996). Knockout rats for AKAP4 showed no progressive motility and were infertile (Miki et al., 2002).

Interestingly, SPATA 18 was the only phosphorylated protein found in three (60 %) arsenic-exposed animals and absent in all rats from the control group. SPATA18 is a protein that, in its phosphorylated state, induces lysosome-like organelles within the mitochondria contributing to mitochondrial quality control (Kitamura et al., 2011). In males, this protein has been identified in rats' testis, specifically in the residual body of elongated spermatids (steps 15–18), playing a role in spermiogenesis. During this process, the gamete undergoes a decrease of cytoplasm volume and retention of SPATA18 in the flagellar portion after spermiation, suggesting a role in flagellum structure (Iida et al., 2004; Bornstein et al., 2011). Therefore, we may suggest that the exposure to arsenic leads to phosphorylation of SPATA18, indicating an attempt of the cell to repair the mitochondrial structure to maintain sperm energy source for motility. The mechanism underlying this phosphorylation might be related to the ROS overproduction in sperm mitochondria during the arsenic attack. Despite this metalloid can also act on proteins modifying the bonds of their thiol groups, it is not possible to affirm that this mode of action is involved in the SPATA18 phosphorylation. To our knowledge, this is the first study describing the SPATA18 phosphorylation in epididymal spermatozoa of arsenic-exposed rats. Further investigations should evaluate the signaling pathway mediated by arsenic toxicity in the early phosphorylation of crucial proteins, such as SPATA18. If confirmed the role of arsenic in this process, this finding could be a potential biomarker of infertility in exposed mammals.

5. Conclusion

In summary, our findings showed that prepubertal arsenic intoxication caused relevant damages to sperm parameters in sexually mature rats. The reduced sperm production, number, and quality may have contributed to the low fertility potential and the high percentage of preimplantation loss. Moreover, the epididymal sperm phosphoproteome revealed a possible mechanism involved in arsenic-induced male reproductive toxicity. Our results showed the importance of kinases and phosphatases specifically expressed in animals exposed to arsenic and how they contributed to the enrichment of biological pathways. The excessive ROS generation driven by arsenic may lead to alteration in the phosphorylation pattern of sperm proteins, mainly SPATA 18 phosphorylation, which is deleterious to its viability before fertilization. Therefore, early arsenic exposure impacts male reproductive capacity and fertility over a lifetime. Our findings are the first pieces of information about the arsenic toxicity to sperm proteome. They create a baseline for studies investigating the detrimental effects of this metalloid on the functionality of epididymal sperm.

Credit authorship contribution statement

F. Couto-Santos: Conceptualization, Investigation, Formal analysis, Writing. **A.G.A. Viana:** Investigation, Data curation, Validation, Writing. **A.C.F. Souza:** Conceptualization, Investigation, Data curation, Writing. **A.A.A. Dutra:** Investigation, Data curation. **A.T.S. Ferreira:** Investigation. **T.A.O. Mendes:** Data curation, Validation, Writing. **J.E. P. Aguilar:** Investigation. **L.L. Oliveira:** Data curation. **M. Machado-Neves:** Conceptualization, Methodology, Resources, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tox.2021.152886.

References

Abdul, K.S., Jayasinghe, S.S., Chandana, E.P., Jayasumana, C., De Silva, P.M., 2015. Arsenic and human health effects: a review. Environ. Toxicol. Pharmacol. 40, 828–846. https://doi.org/10.1016/j.etap.2015.09.016.

Adedara, I.A., Abolaji, A.O., Awogbindin, I.O., Farombi, E.O., 2017. Suppression of the brain-pituitary-testicular axis function following acute arsenic and manganese co-exposure and withdrawal in rats. J. Trace Elem. Med. Biol. 39, 21–29. https://doi.org/10.1016/j.jtemb.2016.07.001.

Aitken, R.J., 2011. The capacitation-apoptosis highway: oxysterols and mammalian sperm function. Biol. Reprod. 85, 9–12. https://doi.org/10.1095/biolreprod.111.092528.

Aitken, R.J., Paterson, M., Fisher, H., Buckingham, D.W., van Duin, M., 1995. Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. J. Cell. Sci. 108, 2017–2125.

Aitken, R.J., Gibb, Z., Baker, M.A., Drevet, J., Gharagozloo, P., 2016. Causes and consequences of oxidative stress in spermatozoa. Reprod. Fertil. Dev. 28, 1–10. https://doi.org/10.1071/RD15325.

Altoé, L.S., Reis, I.B., Gomes, M.L.M., Dolder, H., Monteiro Pirovani, J.C., 2017. Could vitamin C and zinc chloride protect the germ cells against sodium arsenite? Hum. Exp. Toxicol. 36, 1049–1058. https://doi.org/10.1177/0960327116679714.

Barth, A.D., Oko, R.J., 1989. Abnormal Morphology of Bovine Spermatozoa. Iowa State University Press, IA, Ames, pp. 19–88.

Bashandy, S.A., El Awdan, S.A., Ebaid, H., Alhazza, I.M., 2016. Antioxidant potential of spirulina platensis mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats. Oxid. Med. Cell. Longev. 2016, 7174351. https://doi.org/10.1155/2016/7174351.

Benbrahim-Tallaa, L., Waalkes, M.P., 2008. Inorganic arsenic and human prostate cancer. Environ. Health Perspect. 116, 158–164. https://doi.org/10.1590/S1413-81232009000100037.

Ben-Navi, L.R., Among, T., Yao, Z., Seger, R., Naor, Z., 2016. A-Kinase Anchoring Protein 4 (AKAP4) is an ERK1/2 substrate and a switch molecule between cAMP/PKA and PKC/ERK1/2 in human spermatozoa. Sci. Rep. 6, 37922. https://doi.org/10.1038/srep37922.

Bornstein, C., Brosh, R., Molchadsky, A., Madar, S., Kogan-Sakin, I., Goldstein, I., Chakravarti, D., Flores, E.R., Goldfinger, N., Sarig, R., Rotter, V., 2011. SPATA18, a spermatogenesis-associated gene, is a novel transcriptional target of p53 and p63. Mol. Cell. Biol. 31, 1679–1789. https://doi.org/10.1128/MCB.01072-10.

Breton, S., Nair, A.V., Battistone, M.A., 2019. Epithelial dynamics in the epididymis: role in the maturation, protection, and storage of spermatozoa. Andrology 7, 631–643. https://doi.org/10.1111/andr.12632.

Cancel, A.M., Chapman, D.A., Killian, G.J., 1999. Osteopontin localization in the Holstein bull reproductive tract. Biol. Reprod. 60, 454–460. https://doi.org/10.1095/biolreprod60.2.454.

Carr, S., Aebersold, R., Baldwin, M., Burlingame, A., Clauser, K., Nesvizhskii, A., 2004. The need for guidelines in publication of peptide and protein identification data: Working group on publication guidelines for peptide and protein identification data. Mol. Cell Proteomics 3, 531–533. https://doi.org/10.1074/mcp.T400006-MCP200.

Carrera, A., Moss, J., Ning, X.P., Gerton, G.L., Tesarik, J., Kopf, G.S., Moss, S.B., 1996. Regulation of protein tyrosine phosphorylation in human sperm by a calcium/calmodulin-dependent mechanism: identification of A kinase anchor proteins as major substrates for tyrosine phosphorylation. Dev. Biol. 180 https://doi.org/10.1006/dbio.1996.0301, 284-196.

Chang, S.I., Jin, B., Youn, P., Park, C., Park, J.D., Ryu, D.Y., 2007. Arsenic induced toxicity and the protective role of ascorbic acid in mouse testis. Toxicol. Appl. Pharmacol. 218, 196–203. https://doi.org/10.1016/j.taap.2006.11.009.

Chen, H., Liu, G., Qiao, N., Kang, Z., Hu, L., Liao, J., Yang, F., Pang, C., Liu, B., Zeng, Q., Li, Y., Li, Y., 2020. Toxic effects of arsenic trioxide on spermatogonia are associated with oxidative stress, mitochondrial dysfunction, autophagy and metabolomic alterations. Ecotoxicol. Environ. Saf. 190, 110063. https://doi.org/10.1016/j.ecoenv.2019.110063.

Chin, D., Means, A.S., 2000. Calmodulin: a prototypical calcium sensor. Trends Cell Biol. 10, 322–328. https://doi.org/10.1016/s0962-8924(00)01800-6.

Coelho, D.G., Marinato, C.S., de Matos, L.P., de Andrade, H.M., da Silva, V.M., Santos-Neves, P.H., Araújo, S.C., Oliveira, J.A., 2020a. Is arsenite more toxic than arsenate in plants? Ecotoxicology 29, 196–202. https://doi.org/10.1007/s10646-019-02152-9.

Coelho, D.G., Marinato, C.S., de Matos, L.P., de Andrade, H.M., da Silva, V.M., Neves, P. H.S., de Oliveira, J.A., 2020b. Evaluation of heavy metals in soil and tissues of economic-interest plants grown in sites affected by the Fundão dam failure in Mariana. Brazil. Integr. Environ. Assess. Manag. https://doi.org/10.1002/ieam.4253.

Couto-Santos, F., Souza, A.C.F., Bastos, D.S.S., Ervilha, L.O.G., Dias, F.C.R., Araújo, L.S., Guimarães, S.E.F., Oliveira, L.L., Machado-Neves, M., 2020. Prepubertal exposure to arsenic alters male reproductive parameters in pubertal and adult rats. Toxicol. Appl. Pharmacol. 27, 115304. https://doi.org/10.1016/j.taap.2020.115304. Online ahead of print.

Da Silva, N., Silberstein, C., Beaulieu, V., Piétrement, C., Van Hoek, A.N., Brown, D., Breton, S., 2006. Postnatal expression of aquaporins in epithelial cells of the rat epididymis. Biol. Reprod. 74, 427–438. https://doi.org/10.1095/biolreprod.105.044735.

Da Silva, R.F., Borges, C.S., Lamas, C.A., Cagnon, V.H.A., Kempinas, W.G., 2017. Arsenic trioxide exposure impairs testicular morphology in adult male mice and consequent fetus viability. J. Toxicol. Environ. Health 80, 1166–1179. https://doi.org/10.1080/15287394.2017.1376405.

Denhadrt, D.T., Guo, X., 1993. Osteopontin: a protein with diverse functions. FASEB J. 7, 1475–1482. https://doi.org/10.1096/fasebj.7.15.8262332.

Fernandes, G.S., Arena, A.C., Fernandez, C.D., Mercadante, A., Barbisan, L.F., Kempinas, W.G., 2007. Reproductive effects in male rats exposed to diuron. Reprod. Toxicol. 23, 106–112. https://doi.org/10.1016/j.reprotox.2006.09.002.

Ferreira, M., Matos, R.C., Oliveira, H., Nunes, B., Pereira, M.L., 2012. Impairment of mice spermatogenesis by sodium arsenite. Hum. Exp. Toxicol. 31, 290–302. https://doi.org/10.1177/0960327111405862.

Flesch, F.M., Colenbrander, B., van Golde, L.M., Gadella, B.M., 1999. Capacitation induces tyrosine phosphorylation of proteins in the boar sperm plasma membrane. Biochem. Biophys. Res. Commun. 262, 787–792. https://doi.org/10.1006/bbrc.1999.1300.

Gervasi, M.G., Visconti, P.E., 2017. Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. Andrology 5, 204–218. https://doi.org/10.1111/andr.12320.

Gonçalves, R.F., Wolinetz, D.C.C.D., Killian, G.J., 2007. Influence of arginine-glycine-aspartic acid (RGD), integrins (alphaV and alpha5) and osteopontin on bovine sperm-egg binding, and fertilization in vitro. Theriogenology 67, 468–474. https://doi.org/10.1016/j.theriogenology.2006.08.013.

Guvvala, P.R., Sellapan, S., Parameswaraiah, R.J., 2016. Impact of arsenic (V) on testicular oxidative stress and sperm functional attributes in Swiss albino mice. Environ. Sci. Pollut. Res. 23, 18200–18210. https://doi.org/10.1007/s11356-016-6870-3.

Halliwell, B., Gutteridge, J.M.C., 2007. Free Radicals in Biology and Medicine. Oxford University, Oxford, p. 110 p..

Han, Y., Liang, C., Manthari, R.K., Yu, Y., Gao, Y., Liu, Y., Jiang, S., Tikka, C., Wang, J., Zhang, J., 2020. Arsenic influences spermatogenesis by disorganizing the elongation of spermatids in adult male mice. Chemosphere 238, 124650. https://doi.org/10.1016/j.chemosphere.2019.124650.

Harrison, R.A.P., Vickers, S.E., 1990. Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. J. Reprod. Fertil. 88, 343–352.

Hirano, S., 2020. Biotransformation of arsenic and toxicological implication of arsenic metabolites. Arch. Toxicol. 94, 2587–2601. https://doi.org/10.1007/s00204-020-02772-9.

Hood, R.D., 2012. Developmental and Reproductive Toxicology: a Practical Approach, 3rd ed. CRC Press, p. 1168.

Hsieh, F.I., Hwang, T.I., Hsieh, Y.C., Lo, H.C., Su, C.T., Hsu, H.S., Chiou, H.Y., Chen, C.J., 2008. Risk of erectile dysfunction induced by arsenic exposure through well water consumption in Taiwan. Environ. Health Perspect. 116, 532–536. https://doi.org/10.1289/ehp.10930.

Huang, Z., Khatra, B., Bollen, M., Carr, D.W., Vijayaraghavan, S., 2002. Sperm PP1g2 is regulated by a homologue of the yeast protein phosphatase binding protein sds22. Biol. Reprod. 67, 1936–1942. https://doi.org/10.1095/biolreprod.102.004093.

Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57. https://doi.org/10.1038/nprot.2008.211.

Huang, Q., Luo, L., Alamdar, A., Zhang, J., Liu, L., Tian, M., Eqani, S.A., Shen, H., 2016. Integrated proteomics and metabolomics analysis of rat testis: mechanism of arsenic-induced male reproductive toxicity. Sci. Rep. 6, 32518. https://doi.org/10.1038/srep32518.

Hughes, M.F., 2002. Arsenic toxicity and potential mechanisms of action. Toxicol. Lett. 133, 1–16. https://doi.org/10.1016/S0378-4274(02)00084-X.

Hughes, M.F., Kenyon, E.M., Edwards, B.C., Mitchell, C.T., Del Razo, L.M., Thomas, D.J., 2003. Accumulation and metabolism of arsenic in mice after repeated oral administration of arsenate. Toxicol. Appl. Pharmacol. 191, 202–210. https://doi.org/10.1016/S0041-008X(03)00249-7.

Hughes, M.F., Beck, B.D., Chen, Y., Lewis, A.S., Thomas, D.J., 2011. Arsenic exposure and toxicology: a historical perspective. Toxicol. Sci. 123, 305–332. https://doi.org/10.1093/toxsci/kfr184.

Iida, H., Ichinose, J., Kaneko, T., Mōri, T., Shibata, Y., 2004. Complementary DNA cloning of rat spetex-1, a spermatid-expressing gene-1, encoding a 63 kDa cytoplasmic protein of elongate spermatids. Mol. Reprod. Dev. 68, 385–393. https://doi.org/10.1002/mrd.20101.

Ijiri, T.W., Vadnais, M.L., Huang, A.P., Lin, A.M., Levin, L.R., Buck, J., Gerton, G.L., 2014. Thiol changes during epididymal maturation: a link to flagellar angulation in mouse spermatozoa? Andrology 2, 65–75. https://doi.org/10.1111/j.2047-2927.2013.00147.x.

Jankovičová, J., Michalková, K., Sečová, P., Horovská, L., Maňásková-Postlerová, P., Antalíková, J., 2018. Evaluation of protein phosphorylation in bull sperm during their maturation in the epididymis. Cell Tissue Res. 371, 365–373. https://doi.org/10.1007/s00441-017-2705-x.

Johnson, G.A., Burghardt, R.C., Bazer, F.W., Spencer, T.E., 2003. Osteopontin: roles in implantation and placentation. Biol. Reprod. 69, 1458–1471. https://doi.org/10.1095/biolreprod.103.020651.

Johnson, C., Crowther, S., Stafford, M.J., Campbell, D.G., Toth, R., MAcKinstosh, C., 2010. Bioinformatic and experimental survey of 14-3-3-binding sites. Biochem. J. 427, 69–78. https://doi.org/10.1042/BJ20091834.

Jones, R.C., 1999. To store or mature spermatozoa? The primary role of the epididymis. Int. J. Andro. 22, 57–67. https://doi.org/10.1046/j.1365-2605.1999.00151.x.

Kalab, P., Peknicova, J., Geussova, G., Moos, J., 1998. Regulation of protein tyrosine phosphorylation in boar sperm through a cAMP dependent pathway. Mol. Reprod. Dev. 51, 30–314. https://doi.org/10.1002/(SICI)1098-2795(199811)51:3<304.AID-MRD10>3.0.CO;2-2.

Kierszenbaum, A.L., 2002. Intramanchette transport (IMT): managing the making of the spermatid head, centrosome, and tail. Mol. Reprod. Dev. 63, 1–4. https://doi.org/10.1002/mrd.10179.

Kitamura, N., Nakamura, Y., Miyamoto, Y., Miyamoto, T., Kabu, K., Yoshida, M., Futamura, M., Ichinose, S., Arakawa, H., 2011. Mieap, a p53-inducible protein, controls mitochondrial quality by repairing or eliminating unhealthy mitochondria. PLoS One 6, e16060. https://doi.org/10.1371/journal.pone.0016060.

Klinefelter, G.R., Hess, R.A., 1998. Toxicology of the male excurrent ducts and accessory sex glands. In: Korach, K.S. (Ed.), Reproductive and Developmental Toxicology. Marcel Dekker, New York, pp. 553–591.

Lima, G.D.A., Sertorio, M.N., Souza, A.C.F., Menezes, T.P., Mouro, V.G.R., Gonçalves, N. M., Oliveira, J.M., Henry, M., Neves, M.M., 2018. Fertility in male rats: disentangling adverse effects of arsenic compounds. Reprod. Toxicol. 78, 130–140. https://doi.org/10.1016/j.reprotox.2018.04.015.

Liu, S., Guo, X., Wu, B., Yu, H., Zhang, X., Li, M., 2014. Arsenic induces diabetic effects through beta-cell dysfunction and increased gluconeogenesis in mice. Sci. Rep. 4, 6894. https://doi.org/10.1038/srep06894.

Lobo-Jarne, T., Ugalde, C., 2018. Respiratory chain supercomplexes: structures, function and biogenesis. Sem. Cell Develop. Biol. 76, 179–190. https://doi.org/10.1016/j.semcdb.2017.07.021.

Luedtke, C.C., McKee, M.D., Cyr, D.G., Gregory, M., Kaartinen, M.T., Mui, J., Hermo, L., 2002. Osteopontin expression and regulation in the testis, efferent ducts, and epididymis of rats during postnatal development through to adulthood. Biol. Reprod. 66, 1437–1448. https://doi.org/10.1095/biolreprod66.5.1437.

Makker, K., Agarwal, A., Sharma, R., 2009. Oxidative stress & male infertility. Indian J. Med. Res. 129, 357–367.

Mangelsdorf, I., Buschmann, J., Orthen, B., 2003. Some aspects relating to the evaluation of the effects of chemicals on male fertility. Regul. Toxicol. Pharmacol. 37, 356–369. https://doi.org/10.1016/S0273-2300(03)00026-6.

Manning, G., Whyte, D.B., Martinez, R., Hunter, T., Sudarsanam, S., 2002. The protein kinase complement of the human genome. Science 298, 1912–1934. https://doi.org/10.1126/science.1075762.

Medeiros, P.C., Samelo, R., Silva, A.P., Santiago, G., Duarte, F., Castro, I., Perobelli, J., 2018. Prepubertal exposure to low of sodium arsenite impairs spermatogenesis and epididymal histophysiology in rats. Envirom. Toxicol. 34, 83–91. https://doi.org/10.1002/tox.22660.

Menezes, T.P., Hill, E., de Alencar Moura, A., Lobo, M.D.P., Monteiro-Moreira, A.C.O., Breton, S., Machado-Neves, M., 2018. Pattern of protein expression. In the epididymis of *Oligoryzomys nigripes* (Cricetidae, Sigmodontinae). Cell Tissue Res. 372, 135–147. https://doi.org/10.1007/s00441-017-2714-9.

Mi, Y., Shi, Z., Li, J., 2015. Spata19 is critical for sperm mitochondrial function and male fertility. Mol. Reprod. Dev. 82, 907–913. https://doi.org/10.1002/mrd.22536.

Miki, K., Willis, W.D., Brown, P.R., Goulding, E.H., Fulcher, K.D., Eddy, E.M., 2002. Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. Dev. Biol. 248, 331–342. https://doi.org/10.1006/dbio.2002.0728.

Mukherjee, A., Sengupta, M.K., Hossain, M.A., Ahamed, S., Das, B., Nayak, B., Lodh, D., Rahman, M.M., Chakraborti, D., 2006. Arsenic contamination in groundwater: a global perspective with emphasis on the Asian scenario. J. Health Popul. Nut. 24, 142–163.

Munier, C.C., Ottmann, C., Perry, M.W.D., 2021. 14-3-3 modulation of the inflammatory response. Pharmacol. Res. 163, 105236. https://doi.org/10.1016/j.phrs.2020.105236.

Muslin, A.J., Tanner, J.W., Allen, P.M., Shaw, A.S., 1996. Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. Cell 84, 889–897. https://doi.org/10.1016/s0092-8674(00)81067-3.

Naz, R.K., Rajesh, P.B., 2004. Role of tyrosine phosphorylation in sperm capacitation/acrosome reaction. Reprod. Biol. Endocrinol. 2, 75. https://doi.org/10.1186/1477-7827-2-75.

Nourashrafeddin, S., Ebrahimzadeh-Vesal, R., Modarressi, M.H., Zekri, A., Nouri, M., 2014. Identification of Spata-19 new variant with expression beyond meiotic phase of mouse testis development. Rep. Biochem. Mol. Biol. 2, 89–93.

O'Flaherty, C., Matsushita-Fournier, D., 2017. Reactive oxygen species and protein modifications in spermatozoa. Biol. Reprod. 97, 577–585. https://doi.org/10.1093/biolre/iox104.

Ozkosem, B., Feinstein, S.I., Fisher, A.B., O'Flaherty, C., 2016. Absence of peroxiredoxin 6 amplifies the effect of oxidant stress on mobility and SCSA/CMA3 defined chromatin quality and impairs fertilizing ability of mouse spermatozoa. Biol. Reprod. 94, 68. https://doi.org/10.1095/biolreprod.115.137646.

Pant, N., Kumar, R., Murthy, R.C., Srivastava, S.P., 2001. Male reproductive effect of arsenic in mice. Biometals 14, 113–117. https://doi.org/10.1023/A:1016686113763.

Perobelli, J., 2014. The male peripubertal phase as a developmental window for reproductive toxicology studies. Curr. Pharm. Des. 20, 5398–5415. https://doi.org/10.2174/1381612820666140205150059.

Picut, C.A., Ziejewski, M.K., Stanislaus, D., 2018. Comparative aspects of pre- and postnatal development of the male reproductive system. Birth Defects Res. 110, 190–227. https://doi.org/10.1002/bdr2.1133.

Pines, J., 1993. Cyclins and cyclin-dependent kinases: take your partners. Trends Biochem. Sci. 18, 195–197. https://doi.org/10.1016/0968-0004(93)90185-p.

Prathima, P., Pavani, R., Sukeerthi, S., Sainath, S.B., 2008. α-Lipoic acid inhibits testicular and epididymal oxidative damage and improves fertility efficacy in arsenic-intoxicated rats. J. Biochem. Mol. Toxicol. 32 https://doi.org/10.1002/jbt.22016.

Rahman, M.S., Kwon, W., Lee, J., Yoon, S., Ryu, B., Pang, M., 2015. Bisphenol-A affects male fertility via fertility-related proteins in spermatozoa. Sci. Rep. 5, 9169. https://doi.org/10.1038/srep09169.

Ramírez, A.R., Castro, M.A., Angulo, C., Ramió, L., Rivera, M.M., Torres, M., Rigau, T., Rodríguez-Gil, J.E., Concha, I.I., 2009. The presence and function of dopamine type 2 receptors in boar sperm: a possible role for dopamine in viability, capacitation, and modulation of sperm motility. Biol. Reprod. 80, 753–761. https://doi.org/10.1095/biolreprod.108.070961.

Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. FASEB J. 22, 659–661. https://doi.org/10.1096/fj.07-9574LSF.

Reddy, P., Rani, G., Sainath, S., Meena, R., Supriya, C., 2011. Protective effects of N-acetylcysteine against arsenic-induced oxidative stress and reprotoxicity in male mice. J. Trace Elem. Med. Biol. 25, 247–253. https://doi.org/10.1016/j.jtemb.2011.08.145.

Renu, K., Madhyastha, H., Madhyastha, R., Maruyama, M., Vinayagam, S., Valsala, Gopalakrishnan, A., 2018. Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. Life Sci. 212, 37–58. https://doi.org/10.1016/j.lfs.2018.09.045.

Rivkin, E., Eddy, E.M., Willis, W.D., Goulding, E.H., Suganuma, R., Yanagimachi, R., Kierszenbaum, A.L., 2005. Sperm tail abnormalities in mutant mice with neo (r) gene insertion into an intron of the keratin 9 gene. Mol. Reprod. Dev. 72, 259–271. https://doi.org/10.1002/mrd.20335.

Robaire, B., Hinton, B.T., 2015. The epididymis. In: Plant, T., Zeleznik, A.J. (Eds.), Knobil and Neill's Physiology of Reproduction. Elsevier, New York, pp. 691–772.

Robb, G., Ammann, R.P., Killian, G.J., 1978. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J. Reprod. Fertil. 54, 103–107. https://doi.org/10.1530/jrf.0.0540103.

Ryu, D., Kim, K., Kwon, W., Rahman, M.S., Khatun, A., Pang, M., 2017. Peroxiredoxin activity is a major landmark of male fertility. Sci. Rep. 7, 17174. https://doi.org/10.1038/s41598-017-17488-7.

Sanabria, M., Cucielo, M.S., Guerra, M.T., Dos Santos Borges, C., Banzato, T.P., Perobelli, J.E., Leite, G.A., Anselmo-Franci, J.A., Kempinas, W.D.G., 2016. Sperm quality and fertility in rats after prenatal exposure to low doses of TCDD: a three-generation study. Reprod. Toxicol. 65, 29–38. https://doi.org/10.1016/j.reprotox.2016.06.019.

Sarkar, M., Chaudhuri, G.R., Chattopadhyay, A., Biswas, N.M., 2003. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J. Androl. 5, 27–31.

Senger, P.L., 2003. Pathways to pregnancy and parturition. Washington Current Conceptions, 2nd revision edited, p. 381.

Sharpe, R.M., 1994. Regulation of spermatogenesis. In: Knobil, E., Neil, J.D. (Eds.), The Physiology of Reproduction, second edition. Haven Press Ltd, New York.

Siiteri, J.E., Ensrud, K.M., Moore, A., Hamilton, D.W., 1995. Identification of osteopontin (OPN) mRNA and protein in the rat testis and epididymis, and on sperm. Mol. Reprod. Dev. 40, 16–28. https://doi.org/10.1002/mrd.1080400104.

Souza, A.C.F., Marchesi, S.C., Lima, G.D.A.L., Ferraz, R.P., Santos, F.C., da Matta, S.L.P., Machado-Neves, M., 2016a. Effects of sodium arsenite and arsenate in testicular histomorphometry and antioxidants enzymes activities in rats. Biol. Trace Elem. Res. 171, 354–362. https://doi.org/10.1007/s12011-015-0523-0.

Souza, A.C.F., Marchesi, S., Ferraz, R., Lima, G.D.A.L., Oliveira, J.A., Machado-Neves, M., 2016b. Effects of sodium arsenate and arsenite on male reproductive functions in Wistar rats. J. Toxicol. Environ. Health 6, 274–286. https://doi.org/10.1080/15287394.2016.1150926.

Souza, A.C.F., Bastos, D.S., Sertorio, M.N., Santos, F.C., Ervilha, L.O.G., Oliveira, L.L., Machado-Neves, M., 2019. Combined effects of arsenic exposure and diabetes on male reproductive functions. Andrology 7, 730–740. https://doi.org/10.1111/andr.12613.

Starovlah, I.M., Pletikosic, S.M.R., Kostic, T.S., Andric, S.A., 2020. Reduced spermatozoa functionality during stress is the consequence of adrenergic-mediated disturbance of mitochondrial dynamics markers. Sci. Rep. 10, 16813. https://doi.org/10.1038/s41598-020-73630-y.

Sullivan, R., Frenette, G., Girouard, J., 2007. Epididymosomes are involved in the acquisition of new sperm proteins during epididymal transit. Asian J. Androl. 9, 483–491. https://doi.org/10.1111/j.1745-7262.2007.00281.x.

Suzuki-Toyota, F., Ito, C., Toyama, Y., Maekawa, M., Yao, R., Noda, T., Iida, H., Toshimori, K., 2007. Factors maintaining normal sperm tail structure during epididymal maturation studied in Gopc$^{-/-}$ Mice. Biol. Reprod. 77, 71–82. https://doi.org/10.1095/biolreprod.106.058735.

Thomas, D.J., 2007. Molecular processes in cellular arsenic metabolism. Toxicol. Appl. Pharmacol. 222, 365–373. https://doi.org/10.1016/j.taap.2007.02.007.

Urra, J.A., Villaroel-Espíndola, F., Covarrubias, A.A., Rodríguez-Gil, J.E., Ramírez-Reveco, A., Concha, I.I., 2014. Presence and function of dopamine transporter (DAT) in stallion sperm: dopamine modulates sperm motility and acrosomal integrity. PLoS One 9, e112834. https://doi.org/10.1371/journal.pone.0112834.

Veeramachaneni, D.N.R., Palmer, J.S., Amann, R.P., 2001. Long-term effects on male reproduction of early exposure to common chemical contaminants in drinking water. Hum. Reprod. 16, 979–987. https://doi.org/10.1093/humrep/16.5.979.

Viana, A.G.A., Martins, A.M.A., Pontes, A.H., Fontes, W., Castro, M.S., Ricart, C.A.O., Sousa, M.V., Kaya, A., Topper, E., Memmili, E., Moura, A.A., 2018. Proteomic landscape of seminal plasma associated with dairy bull fertility. Sci. Rep. 8, 16323. https://doi.org/10.1038/s41598-018-34152-w.

Wolgemuth, D.J., Manterola, M., Vasileva, A., 2013. Role of cyclins in controlling progression of mammalian spermatogenesis. Int. J. Dev. Biol. 57, 159–168. https://doi.org/10.1387/ijdb.130047av.

Xie, J., Yu, J., Fan, Y., Zhao, X., Su, J., Meng, Y., Wu, Y., Uddin, M.B., Wang, C., Wang, Z., 2020. Low dose lead exposure at the onset of puberty disrupts spermatogenesis-related gene expression and causes abnormal spermatogenesis in mouse. Toxicol. Appl. Pharmacol. 393, 114942. https://doi.org/10.1016/j.taap.2020.114942.

Xu, W., Bao, H., Liu, F., Liu, L., Zhu, Y.-G., She, J., 2012. Environmental exposure to arsenic may reduce human semen quality: associations derived from a Chinese cross-sectional study. Environ. Health 11, 46. https://doi.org/10.1186/1476-069X-11-46.

Zhang, J., Sun, G., Wang, M., Ping, L., Du, Y., Yang, K., Sun, X., 2016. Arsenic trioxide triggered calcium homeostasis imbalance and induced endoplasmic reticulum stress-mediated apoptosis in adult rat ventricular myocytes. Toxicol. Res. 5, 682. https://doi.org/10.1039/c5tx00463b.

Zhu, W.Z., Olson, A., Portman, M., Ledee, D., 2020. Sex impacts cardiac function and the proteome response to thyroid hormone in aged mice. Proteome Sceince 18, 11. https://doi.org/10.1186/s12953-020-00167-3.