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Quantitative assessment of heavy metal effects on sperm function using computer-aided sperm analysis and cytotoxicity assays

Farren Hardneck, Gadieja Israel, Edmund Pool and Liana Maree

Abstract

One known environmental risk factor impacting on human reproduction is heavy metal pollution. Although some metals (e.g., Cu, Se and Zn) have protective effects on the male reproductive system in low doses, heavy metals can accumulate to toxic levels and result in poor semen quality and decreased sperm function. We investigated the effect of $CuSO_4$ and $CdCl_2$ (10, 50, 100 and 250 μg/ml or 500 μg/ml) on human sperm motility and vitality by using computer-aided sperm analysis (CASA) and two cytotoxicity assays (WST-1 and XTT). Several sperm motility parameters were significantly reduced after 5 hr of exposure to the highest concentrations of $CuSO_4$ (250 μg/ml) and $CdCl_2$ (500 μg/ml). The WST-1 assay also revealed significantly lower absorbance values for 50, 100 and 250 μg/ml $CuSO_4$ and for 500 μg/ml $CdCl_2$; however, no significant effect was seen with XTT. The calculated average IC50 value was 50.31± 4.34 μg/ml for $CuSO_4$ and 392.32 ±76.79 μg/ml for $CdCl_2$. The effects of these metals were confirmed with $MgCl_2$, a positive control. This study provides threshold concentrations for the harmful effect of $CuSO_4$ and $CdCl_2$ on human spermatozoa and recommends the use of WST-1 as vitality assay in future in vitro studies.

1 | Introduction

Male fertility has deteriorated over the past few decades with male factor infertility being accountable for 40%–50% of human infertility (Brugh & Lipshultz, 2004; Kumar & Singh, 2015). This decline in semen quality is assumed to have a direct relation with environmental conditions rather than pre-existing factors (Maartens, Aboua, & du Plessis, 2015) and therefore should be taken into account when assessing male infertility (Mukhopadhyay, Varghese, Nandi, Banerjee, & Bhattacharyya, 2010).

Environmental risk factors impacting on human reproduction mainly originate from land and water resources that have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities (Lone, He, Stoffella, & X-e, 2008). Pollution includes both point sources such as emission, vehicle exhaustion and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), insecticides and pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilisers (Lone et al., 2008; Maartens et al., 2015). Each source of contamination has its own damaging effects to plants, animals and human health, with those

adding heavy metals to soils and waters being a serious concern due to its persistence in the environment (Lone et al., 2008). People are exposed to heavy metals either voluntarily through supplementation or involuntarily through the intake of contaminated food and water or contact with contaminated soil, dust or air (Meeker & Rossano, 2008). Aquatic heavy metal pollution usually represents high levels of Hg, Cr, Pb, Cd, Cu, Zn and Ni in the water system (Zhou, Zhang, Fu, Shi, & Jiang, 2008).

Although heavy metals such as Mn, Cu, Cr, Mb, Se and Zn are important for maintaining good health by serving as cofactors for numerous enzymes, these metals can accumulate to toxic concentrations that may be harmful to humans (Adejuwon, Ilesanmi, & Akindade, 1996; Yuyan, Junqing, Wei, Weijin, & Ersheng, 2008). Occupational as well environmental exposure to metals such as Pb, Cd, Cr, Co, Ni and As may pose both short-term and long-term health risks (Zhou et al., 2016) and has been divided mainly into carcinogenic and noncarcinogenic effects (Wang, Sato, Xing, & Tao, 2005). Low doses of metals such as Cu, Se and Zn have protective effects on the male reproductive system and may assist in counteracting the effects of other metals (Meeker & Rossano, 2008). However, several of these heavy metals (e.g., Pb and Cd) are considered reproductive toxicants and endocrine disruptor compounds (Mendiola et al., 2011) due to causing hypothalamic-pituitary axis disruption or by its direct affect on Sertoli cells, Leydig cells and spermatogenesis, ultimately resulting in poor semen quality (Chowdhury, 2009; Mendiola et al., 2011; Pizent, Tariba, & Zivkovic, 2012).

Significant decreases in total sperm count in epididymal samples or sperm concentration in semen have been reported when mice, rats and humans were exposed to Cd (Akinloye, Arowojolu, Shittu, & Anetor, 2006; Hew, Ericson, & Welsh, 1993), Pb (Fatima et al., 2010), Hg (Choy et al., 2002) and As (Chiou, Chu, Tzeng, Huang, & Liao, 2008) respectively. Additionally, these studies as well as in a study on fish sperm exposed to Hg (Abascal, Cosson, & Fauvel, 2007) found a decrease in the percentage normal sperm morphology. Both in vitro exposure of water buffalo sperm to copper sulphate (Tabassomi & Alavi-Shoushtari, 2013) and subcutaneous injections of arsenic trioxide in mice (Chiou et al., 2008) caused a decrease in sperm viability. All the above-mentioned studies reported a significant decrease in total sperm motility or the percentage progressive swimming spermatozoa (Abascal et al., 2007; Akinloye et al., 2006; Chiou et al., 2008; Choy et al., 2002; Fatima et al., 2010; Hew et al., 1993; Tabassomi & Alavi-Shoushtari, 2013).

Numerous studies have underlined the importance of sperm motility evaluation, in particular forward or progressive motility, in order to define fertilising capacity, to evaluate the effects of medical and/or surgical treatments and to study the follow-up of kinematic parameters in subjects affected by andrological pathologies (Björndahl, 2010; Elia et al., 2010; van der Horst, Maree, & du Plessis, 2018). Computer-aided sperm analysis (CASA) is a useful tool for analysing the effects of heavy metals on sperm motility parameters (Dietrich et al., 2010; Mukhopadhyay et al., 2010). CASA not only provides a fast and objective tool for assessing sperm quality, but also allows for the detection of subtle changes in sperm motion that cannot be identified by conventional, manual sperm motility analysis

(Rijsselaere et al., 2005; van der Horst et al., 2018). This permits the detailed analysis of sperm kinematics and sperm motility subpopulations, especially to indicate possible effects of different environmental conditions on male fertility (Abascal et al., 2007; Maree & van der Horst, 2013; Mortimer & Mortimer, 2013; Mortimer, van der Horst, & Mortimer, 2015).

Sperm vitality is usually assessed by manual or CASA evaluation of the sperm plasma membrane integrity after staining with eosin-nigrosin (dye exclusion test) or fluorescent probes (Sybr-14 or Hoechst and propidium iodide) (van der Horst & du Plessis, 2017; WHO, 2010). However, since the harmful effect of heavy metals on spermatozoa is due to its interference with the cell's proteins and enzymes (Chowdhury, 2009; Sharma, Goloubinoff, & Christen, 2008), it should be useful to assess sperm vitality using tests for enzyme function, and thus indirectly a cell's viability, such as the WST-1 and XTT cytotoxicity assays. These assays measure the cleavage of tetrazolium salts by active enzymes (mitochondrial dehydrogenases), producing a formazan dye detectable only in metabolic active cells (Roche Diagnostics, 2006, 2011).

The objective of this study was to determine the effect of various concentrations of heavy metals (cadmium chloride and copper sulphate) on human sperm motility and vitality by utilising CASA and two cytotoxicity assays (WST-1 and XTT). Additionally, we aimed to identify which sperm functional test can be used to calculate IC_{50} values for the two mentioned heavy metals.

2 | Materials and methods

2.1 | Sample collection and evaluation

Thirteen normospermic human semen samples were acquired from seven healthy donors by masturbation, after two to three days of sexual abstinence, as part of a donor programme (Division of Physiology, Department of Biomedical Sciences, Stellenbosch University). To be considered for use, semen samples were selected according to the following WHO criteria: semen volume ≥1.5 ml, sperm concentration $\geq 15\times10^6$/ml, total motile sperm ≥40% and a pH range of 7.2–8.0 (WHO, 2010). After collection, semen samples were kept at 37°C for 30–60 min in order for liquefaction to occur before semen was processed. Ethical clearance for this study was obtained from the Ethics Committees of the University of the Western Cape (Ref. no. 13/10/90) and Stellenbosch University (Ref. no. N14/06/074). The Helsinki Declaration governing research on humans has been adhered to (Christie, 2000) and each human donor gave written consent.

2.2 | Selection of motile spermatozoa

Human tubal fluid (HTF) (Mortimer, 1994) was used as medium for all experimental procedures. HTF was supplemented with 1% human serum albumin (HSA). All chemicals were supplied by Sigma, Cape Town, South Africa.

A double density gradient centrifugation technique was employed to select motile spermatozoa by using PureSperm (Kat Medical Laboratories, Johannesburg, South Africa).

This procedure involved layering 400 µl PureSperm 80 into a 1.7 ml conical centrifuge tube, followed by 400 µl PureSperm 40 and finally adding 400 µl liquefied semen onto the PureSperm 40. The preparation was centrifuged at 300 *g* for 20 min, followed by removal of the supernatant. The sperm pellet was then washed by resuspending it in 200 µl HTF (without HSA) and centrifugation at 500 *g* for 10 min, followed by removal of the supernatant. The remaining pellet was resuspended in 100 µl HTF (with HSA), with or without a specific metal concentration, to result in a final concentration of $10–20\times10^6$/ml motile spermatozoa.

2.3 | Exposure of spermatozoa to heavy metals

Sperm preparations were exposed to different concentrations of the two specified heavy metals, namely copper sulphate ($CuSO_4$) and cadmium chloride ($CdCl_2$). Initially, a dose-response was used to determine which metal concentrations to expose the spermatozoa to (data not shown). The final concentrations selected were 10, 50, 100 and 250 µg/ml for $CuSO_4$ and 10, 50, 100 and 500 µg/ml for $CdCl_2$ respectively. Similar concentrations of magnesium chloride ($MgCl_2$) were used as positive control. All metal concentrations were prepared using HTF supplemented with 1% HSA.

Motile sperm preparations were exposed to these metal concentrations for up to 5 hr while being incubated at 37°C. The sperm motility and vitality of the prepared samples were assessed every hour for 5 hr, as it was shown by Roblero, Guadarrama, Lopez, and Zegers-Hochschild (1996) that sperm motility, vitality and acrosome reaction were significantly affected by 100 µg/ml copper ion (Cu^{2+}) after 5 hr of incubation. All experiments were done in triplicate (n = 3) with three different donors' samples used for each experiment.

2.4 | Sperm motility analysis

Sperm motility was assessed after 15 min and then after 1, 2, 3, 4 and 5 hr of incubation using the Motility module of the Sperm Class Analyzer® (SCA) (Microptic S.L., Barcelona, Spain) CASA system, Version 5.1. Capturing of the data involved a Basler avA 1,000–100gc digital camera (Microptic S.L., Barcelona, Spain) that was mounted on an Olympus CH2 microscope (Wirsam, Cape Town, South Africa), equipped with a 10 × phase contrast objective and a heated stage.

Sperm motility analysis involved pipetting 2–3 µl of sperm preparation into 20 µm deep prewarmed (37°C) four or eight chamber Leja slides (Leja Products B.V., Nieuw-Vennep, The Netherlands). The percentages total motility, progressive motility, rapid, medium and slow swimming spermatozoa and eight kinematic parameters were analysed by capturing sperm tracks at 40 frames/s until a total of 200 motile spermatozoa were analysed. The kinematic parameters included curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), linearity (LIN=VSL/VCL), straightness (STR =VSL/VAP), wobble (WOB =VAP/VCL) and beat-cross frequency (BCF). The three sperm subpopulations were determined using VCL cut-off values of 22 < 60 > 90

μm/s to identify slow, medium and rapid swimming spermatozoa (Maree & van der Horst, 2013). Fields were captured randomly to eliminate bias towards higher sperm motility; however, fields containing debris or clumps of spermatozoa were avoided to limit incorrect analysis.

2.5 | Sperm vitality analysis

Sperm vitality was assessed using two colorimetric assays, namely XTT (sodium 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium inner salt; Cell Proliferation Kit II; Cat. No. 11 465 015 001) and WST-1 (sodium 5-(2,4-disulfophenyl)-2-(4-iodophenyl)-3-(4-nitrophenyl)-2Htetrazoliuminner salt; Cell Proliferation Agent; Cat No. 11 644 807 001), which is commonly used it tissue culture studies for cell proliferation, viability and cytotoxicity assessments (Roche, Mannheim, Germany).

The WST-1 labelling reagent was prepared according to the manufacturer's protocol by mixing 200 μl WST-1 reagent with 800 μl phosphate-buffered saline (PBS). The XTT labelling reagent was prepared by mixing 1 ml XTT reagent with 20 μl PMS electron-coupling reagent. For both assays, 50 μl sperm preparation and 50 μl labelling reagent were added to the wells of a microtitre plate in duplicate for each metal concentration. A zero-minute reading was obtained where after plates were incubated at 37°C and 5% CO_2 until the next analysis. Measurements of the absorbance were taken after 1, 2, 3, 4 and 5 hr of incubation using an ELISA reader (Microplate reader, Multiskan EX, Thermo Scientific) set at 450 nm with a reference wavelength of 650 nm.

Using these absorbance measurements, IC_{50} values, defined as the concentrations of the heavy metals required for 50% inhibition of sperm vitality, were determined as a parameter for the toxicity of $CuSO_4$ and $CdCl_2$

2.6 | Statistical analysis

MedCalc® version 10.4.0.0 (Mariakerke, Belgium) was used for basic statistical analyses. Levene's test for equality of variances was applied and when $p > 0.05$, one-way analysis of variance analysis (ANOVA) was performed for parametric data distributions. Any significant differences ($p < 0.05$) as indicated in the ANOVA table between groups were furthermore analysed using the Student-Newman-Keuls test for pairwise comparisons. In subsets of data that appeared to have nonparametric data distributions, the Kruskal–Wallis test was employed and further elaborated for individual differences using the Mann–Whitney test for independent samples. Data are represented as the mean ±SD in figures and $p < 0.05$ was considered significant using the analyses above.

3 | Results

Exposure of motile human spermatozoa ($n = 3$) to different concentrations of $CuSO_4$ and $CdCl_2$ had a negative impact on several motility parameters over the 5-hr incubation period. However, significant decreases ($p < 0.05$) were only found at the highest concentration of

$CuSO_4$ (250 µg/ml) and $CdCl_2$ (500 µg/ml). After 2 hr of $CuSO_4$ exposure, percentage progressive motility, sperm velocity (VSL and VAP) and linearity (LIN) started to decrease and after 3 hr of incubation, percentage total motility and VCL also showed significantly lower values (Figure 1a-c). The three swimming speed classes (rapid, medium and slow sperm) and other kinematic parameters were not significantly affected by $CuSO_4$ exposure. For $CdCl_2$ exposure, similar results were found in terms of decreased motility parameters for $CuSO_4$ exposure (Figure 1d-f). Interestingly, these decreases were already apparent after only 15 min to I hr of incubation.

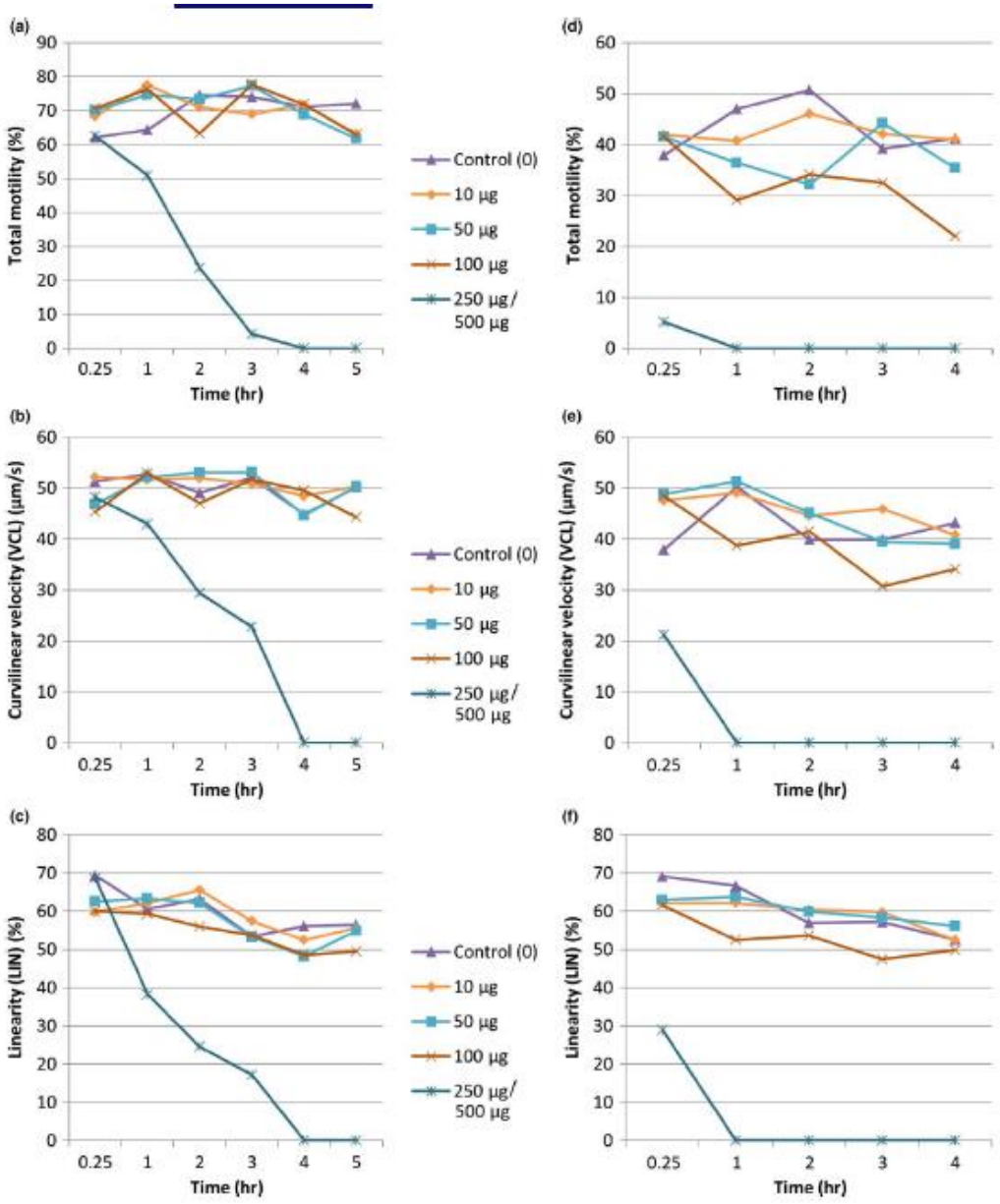

FIGURE 1 Examples of the effect of various concentrations of $CuSO_4$ (0–250 µg/ml) and $CdCl_2$ (0–500 µg/ml) on sperm motility parameters after 4–5 hr of incubation, including (a,d) total motility, (b,e) curvilinear velocity and (c,f) linearity. Each graph represents the data for one donor exposed to these heavy metal concentrations

The effect of the two heavy metals on sperm vitality was assessed by using the XTT and WST-1 cytotoxicity assays (n = 3). After both $CuSO_4$ and $CdCl_2$ exposure, there was no significant effect on vitality when only XTT results were considered (Figure 2a and c). On the other hand, WST-1 results revealed significantly lower average absorbance values for 50, 100 and 250 µg/ml $CuSO_4$ after 4 hr of incubation (Figure 2a). Although the WST-1 average absorbance (0.23) at 500 µg/ml $CdCl_2$ after 5 hr of incubation (Figure 2c) was 56% less than the control (0.53), this decrease was not significant (p = 0.12). Significantly lower absorbance values were, however, found in verification experiments (see Figure 3d). Due to the XTT assay not revealing any effect of the two heavy metals on sperm vitality, IC_{50} values were calculated using only WST-1 average absorbance values. The average IC_{50} value for $CuSO_4$ was 50.31± 4.34 µg/ml and for $CdCl_2$, it was 392.32± 76.79 µg/ml. Examples of the calculation of IC_{50} values for individual donor samples are indicated in Figure 2b ($CuSO_4$) and Figure 2d ($CdCl_2$).

In order to verify the negative effect of $CuSO_4$ and $CdCl_2$ on sperm motility and vitality, a third mineral salt, namely $MgCl_2$, was used as positive control to compare its effect on these sperm functional parameters. On its own, similar concentrations (10, 50, 250, 500 µg/ml) of $MgCl_2$ had no significant effect on sperm motility or vitality over a 5-hr incubation period (Figure 3). A significant decrease in percentage total sperm motility and WST-1 absorbance, compared to the control and $MgCl_2$, was observed after 5 hr of incubation when motile sperm was exposed to 250 µg/ml $CuSO_4$ (Figure 3a,b). Similar significant decreases in sperm motility and WST-1 absorbance were also found for motile sperm exposed to 500 µg/ml $CdCl_2$

4 | Discussion

In this study, the negative effect of both heavy metals was illustrated by a significant decrease in percentage total motility and progressive motility as well as VCL, VSL, VAP and LIN. These CASA-derived sperm velocity parameters have been shown to be closely related to fertility prediction and pregnancy outcomes in humans (Shibahara et al., 2004) and animals (Ahmed, Andrabi, Anwar, & Jahan, 2017; Nagy, Polichronopoulos, Gáspárdy, Solti, & Cseh, 2015). A decrease in these parameters could thus be a contributing factor to male infertility.

Our results corroborate the findings of previous studies, reporting reductions in sperm motility and sperm velocity parameters after exposure to Cd or Cu in humans (Mukhopadhyay et al., 2010), rabbit (Roychoudhury & Massanyi, 2008), turkey (Slanina, Miškeje, Petrovičová, Lukáč, & Massányi, 2015), common carp (Chyb, Sokolowska-Mikolajczyk, Kime, Socha, & Epler, 2001) and African catfish (Kime, Ebrahimi, Nysten, Roelants, & Ollevier, 1996). Mukhopadhyay et al. (2010) found a significant decline in VCL and STR in a tobacco-exposed group, whereas a heavy metal-exposed group had a significant reduced VCL and ALH. High concentrations of Cu in

seminal plasma are also correlated with reduced sperm motility and may render sperm immotile (Roblero et al., 1996; Wong et al., 2001). Aberrant sperm motility caused by heavy metals such a Pb and Cd is correlated with altered expression of L-type voltage-dependent calcium channel isoforms found on the sperm tail, which regulate calcium influx required for flagellar bending (Benoff, Auborn, Marmar, & Hurley, 2008).

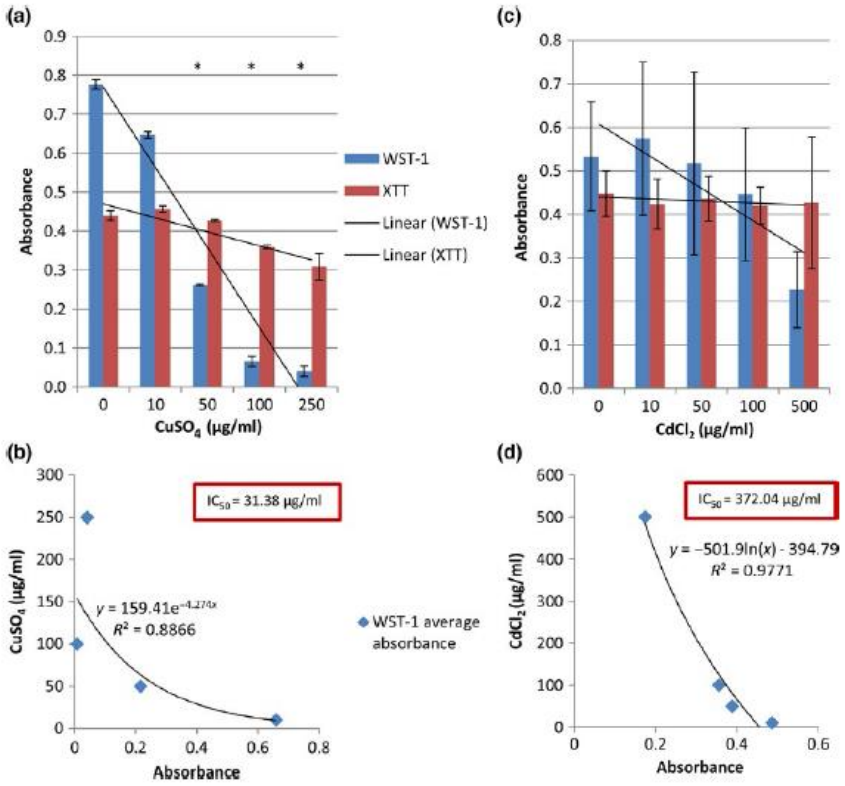

FIGURE 2 Effect of various concentrations of (a,b) $CuSO_4$ (0–250 µg/ml and 5-hr incubation) and (c,d) $CdCl_2$ (0–500 µg/ml and 4-hr incubation) on sperm vitality ($n = 3$). IC_{50} values for $CuSO_4$ (b) and $CdCl_2$ (d) for individual donors were calculated using only WST-1 average absorbance values $^*p < 0.05$

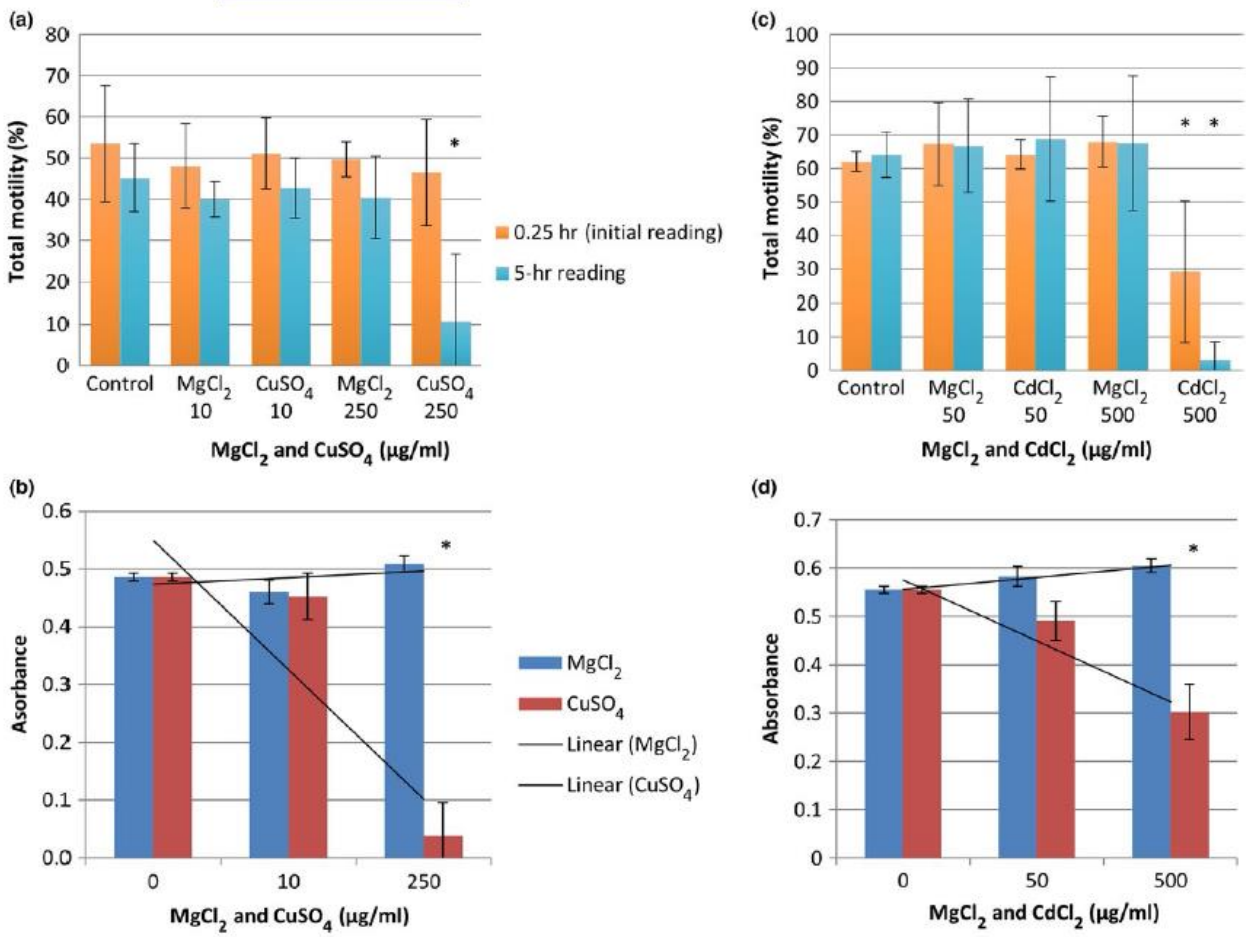

FIGURE 3 Comparison of the effect of similar concentrations of $MgCl_2$ to (a,b) $CuSO_4$ and (c,d) $CdCl_2$ on percentage total motility (a,c) and WST-1 reduction (b,d) after 15 min and 5 hr of incubation. *$p < 0.05$

Our in vitro study only revealed significant negative effects on sperm motility and vitality at the highest concentration of $CuSO_4$ (250 µg/ml) and CdCl2 (500 µg/ml) selected for exposure. There are, however, species differences in the concentration of these heavy metals that will result in deleterious effect on spermatozoa. Additionally, in vivo and longer duration studies indicated a severely negative effect on sperm parameters at much lower heavy metal concentrations than reported here. An in vivo study by Benoff et al. (2008) demonstrated a dose-dependent decrease in sperm motility of rats after exposure to 5 or 50 µg/ml cadmium in drinking water for four weeks.

Interestingly, the adverse effect of $CdCl_2$ on sperm motility in our study was already seen within 1 hr of exposure, while for $CuSO_4$, an evidential decrease was only seen after 2–3 hr. Similar results were reported for Holstein bulls where $CdCl_2$ was reported to decrease sperm motility with increasing Cd concentrations (20–700 µmol) after 60 min (Arabi, 2006). Tabassomi and Alavi-Shoushtari (2013) also indicated a significant decrease in water buffalo sperm motility percentages after 60–120 min of exposure to 0.064 µg/ml $CuSO_4$. It is possible that the concentration of $CdCl_2$ used in our study was too high, and it thus had an almost immediate shock effect on sperm motility. However,

sperm vitality was only significantly reduced at 500 µg/ml $CdCl_2$. Alternatively, this almost instant effect of $CdCl_2$ can be explained by the fact that Cd is highly toxic for mammalian cells (Telišman et al., 2000). Copper on the other hand occurs naturally, is an essential trace element and plays a vital part of several enzymes, such as ferroxidases, cytochrome c oxidase and superoxide dismutase (Slanina et al., 2015; Van Niekerk & Van Niekerk, 1989).

Since heavy metals often interfere with the structure and function of proteins and enzymes (Chowdhury, 2009; Sharma et al., 2008), its adverse effects should be easily detected by assessing changes in metabolic activity. Likewise, Telišman et al. (2000) found better correlations with reproductive parameters when enzymatic activity (biomarker for Pb exposure) rather than blood and seminal fluid levels of Pb was used to assess its effect on reproductive function. In the current study, a significant decrease in WST-1 average

absorbance values was seen for 50, 100 and 250 µg/ml $CuSO_4$ after 4 hr of incubation and for 500 µg/ml $CdCl_2$ after 5 hr of incubation. The XTT assay, however, did not detect significant effects of these two heavy metals on sperm vitality and thus proved to be less sensitive than the WST-1 assay. Aitken, Ryan, Curry, and Baker (2003) also indicated that human spermatozoa have the ability to reduce the WST-1 reagent, and this significant effect was seen after 2 hr of incubation. The advantages of WST-1 are that it is more stable and more sensitive than MTT-, XTT- or MTS-based assays (Abcam plc (1998–2017; Berridge, Herst, & Tan, 2005) and WST-1 also has a wider linear range and shows accelerated colour development compared to XTT (Roche Diagnostics, 2006). Comparing the WST-1 results with the motility results in our study, it also seems that the WST-1 assay is more sensitive to detect the adverse effects of at least $CuSO_4$ on sperm function.

Previous studies reported similar decreases in absorbance values and sperm vitality when spermatozoa were exposed to various Cu and Cd concentrations. Buffalo spermatozoa exposed to 3.9–1,000 µmol/l Cu resulted in a significant decrease in sperm motility and viability (MTT assay) at all concentrations of Cu (Knazicka, Tydra, Bardos, & Lukac, 2012). Several earlier studies found that seminal plasma Cd levels (Dawson, Ritter, Harris, Evans, & Powell, 1998) and cigarette smoking were inversely correlated with the percentage live sperm (Chia, Xu, Ong, Tsakok, & Lee, 1994; Emad, Azza, Sohair, Nagwa, & Taymour, 2012). Copper and cadmium toxicities lead to ROS production and oxidative stress (Liu et al., 2010), followed by DNA, protein and lipid oxidation (Kaur & Sharma, 2015; Olivari, Hernandez, & Allende, 2008), which are negatively correlated with sperm motility and viability (Tvdra et al., 2013). Any excess of Cu in a cell is reduced to cuprous ions that readily bind with sulfhydryl groups (Vlarengo, Pertica, Mancinelli, Zanicchi, & Orunesy, 1980), interfering with electron transport and inhibiting ATP production (Wimalasena, Wiese, & Wimalasena, 2007). Sharma et al. (2008) proved that Cd, Hg and Pb affect spontaneous and chaperone-assisted folding of proteins by forming multidendate complexes with thiol, imidazole and carboxyl groups, which are all present in several intracellular enzymes.

With the WST-1 assay being more sensitive than XTT in revealing any effect of the two heavy metals, the IC_{50} values were calculated using the average absorbances from the WST-1 assay. The average IC_{50} value for $CuSO_4$ and $CdCl_2$ in this study was calculated to be 50.31 µg/ml and 392.32 µg/ml respectively. Knazicka et al. (2012) found that buffalo sperm viability, via MTT assay, was decreased significantly after copper administration (3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1,000 µmol/l). The concentrations 250 µmol/l and 500 µmol/l Cu^{2+} are equivalent to 37.96 µg/ml and 75.53 µg/ml, which places our IC_{50} value of 50.31 µg/ml $CuSO_4$ within this range. Arabi (2006) found that concentrations of 100–700 µmol $CdCl_2$ significantly lowered Holstein bull sperm viability after 60 min of incubation. The 700 µmol/l concentration is equivalent to 105.74 µg/ml $CdCl_2$, which is far less than the calculated IC_{50} value obtained from the current study.

We used $MgCl_2$ as a positive control since a previous study reported that $MgCl_2$ significantly improved the velocity of paddlefish spermatozoa (Linhart, Cosson, Mims, Shelton, & Rodina, 2002). However, as $MgCl_2$ had no significant effect on human sperm motility and vitality, it might be a suitable heavy metal control for future studies. Magnesium is an important cation found in nearly all enzymatic systems and is a vital cofactor in more than 300 enzymatic reactions involving energy metabolism (ATP) and nucleic acid synthesis (Wong et al., 2001).

5 | Conclusion

Albeit many previous studies have investigated the effect of heavy metals on spermatozoa, it is important to note that the effect of each heavy metal is species specific and dependent on route of administration, dosage and duration. Our findings provide threshold concentrations for the harmful effect of $CuSO_4$ and $CdCl_2$ on human spermatozoa for use in future in vitro studies or as bioindicator for heavy metal toxicity. We recommend the use of WST-1 assays rather that XTT assays for evaluation of human sperm vitality. Future studies on these heavy metals should include the evaluation of additional sperm functional parameters such as acrosome reaction, DNA integrity, hyperactivation, cervical-mucus penetration and zona pellucida binding.

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ORCID

Liana Maree http://orcid.org/0000-0003-4147-4221

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