REVIEW

Copper-Induced Changes in Reproductive Functions: In Vivo and In Vitro Effects

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Received April 29, 2015
Accepted July 10, 2015
On-line November 24, 2015

Summary

The goal of this study is to summarize the current knowledge on the effects of one of the essential metals, copper (Cu) on the reproductive system. The development of past four decades addressing effects of Cu on reproductive organs is reviewed. The most relevant data obtained from *in vivo* and *in vitro* experiments performed on humans and other mammals, including effects of copper nanoparticles (CuNPs) on the reproductive functions are presented. Short term Cu administration has been found to exert deleterious effect on intracellular organelles of rat ovarian cells *in vivo*. *In vitro* administration in porcine ovarian granulosa cells releases insulin-like growth factor (IGF-I), steroid hormone progesterone (P_4), and induces expression of peptides related to proliferation and apoptosis. Adverse effect of Cu on male reproductive functions has been indicated by the decrease in spermatozoa parameters such as concentration, viability and motility. Copper nanoparticles are capable of generating oxidative stress *in vitro* thereby leading to reproductive toxicity. Toxic effect of CuNPs has been evident more in male mice than in females. Even though further investigations are necessary to arrive at a definitive conclusion, Cu notably influences the reproductive functions by interfering with both male and female reproductive systems and also hampers embryo development in dose-dependent manner.

Key words

Copper • Effect • Reproductive function • *In vivo* • *In vitro* • Nanotoxicity

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Introduction

Copper (Cu) is necessary in maintaining the functioning of living organisms, being an essential trace element (Michaluk and Kochman 2007, Roychoudhury *et al.* 2008, 2009, Uauy *et al.* 2008, Yunus *et al.* 2015). The widespread use of this metal in electronic industry, building materials, water pipes, wood preservatives, transportation sectors, intrauterine contraceptive devices resulted in its adverse effects, including toxicity (CDA 2013, Roychoudhury and Massanyi 2014, Zhang *et al.* 2012).

Copper is readily absorbed after ingestion, inhalation and dermal exposure (Bentur *et al.* 1988). Following both acute and chronic ingestion of Cu compounds significant absorption takes place through the gastrointestinal tract (Cross *et al.* 1979, Nagaraj *et al.* 1985, Spitalny *et al.* 1984). Dietary Cu is absorbed across

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the mucosal membrane in the small intestines, but also to a limited extent in the stomach in mammals (Pena *et al.* 1999). Active transport mechanisms are believed to be involved at lower dietary levels, while passive diffusion may occur at higher levels (Varada *et al.* 1993). Most absorbed Cu is retained within the mucosal cells, bound mainly to metallothionein or glutathione (Tapiero *et al.* 2003). It is stored primarily in the liver, brain, heart, kidney and muscles. In serum, Cu is normally about 98 % bound to ceruloplasmin with the remainder in association with albumin. In acute intoxication, when the serum concentration of Cu rises rapidly, the metal binds to albumin rather than to ceruloplasmin (Piscator 1979). Under normal physiological conditions, approximately 98 % of the Cu excretion is through the bile and the remaining 2 % is through the urine (Wijmenga and Klomp 2004). The post-Golgi vesicular compartment of the hepatocyte is localized in close vicinity to the biliary canalicular membrane and is thereby involved in the biliary excretion (Langner and Denk 2004). The elimination of Cu in the urine may be greatly enhanced in the Cu-poisoned patient if the body storage sites are saturated (Walsh *et al.* 1977). Minimal amounts of Cu are eliminated in the skin, hair, and sweat (Turnlund *et al.* 1990). Small amounts of Cu are secreted daily by salivary, gastric, pancreatic and duodenal excretions. The dietary Cu biological half-life is reported to be 13 to 33 days, with biliary excretion being the main route of elimination (Barceloux 1999).

Human economic activities, involving the production and usage of Cu and its compounds, as well as the consumption of materials that contain amounts of Cu, result in its re-distribution in different environmental media. Because it is an essential metal, an adequate supply is necessary for normal metabolism (Georgopoulos *et al.* 2001). The usual routes by which humans receive toxic exposure to Cu are through skin or eye contact, as well as by inhalation of powders and dusts (USEPA 1986). In the cases of acute exposure, the inhalation of Cu-containing mists can cause congestion of the mucous membranes in the nose and pharynx, and possibly also ulceration with perforation of the nasal septum (Scheinberg 1983). If the toxicant reaches the gastrointestinal tract, there may be irritation including salivation, nausea, vomiting, gastric pain, hemorrhage, gastritis and diarrhea (Sittig 1985). Ingestion leaves metallic taste in mouth, burning sensation in the throat, nausea, vomiting, epigastric pain, diarrhea, hypotension, hematemesis, melena, hemolytic anemia and gastrointestinal hemorrhage, pallor, oliguria, anuria, jaundice, delirium, coma, hepatic failure, respiratory failure and convulsions are the features of poisoning in the cases of acute exposure. There is centrilobular necrosis and biliary stasis in the liver. In some cases hypotension leading to shock develops, indicating a poor prognosis (Chugh *et al.* 1975, Cole and Lirenman 1978, Nagaraj *et al.* 1985, Thirumalaikolundusubramanian *et al.* 1984). Acute exposure to Cu salts may cause irritation to the skin (Scheinberg 1983), itching, erythema and an allergic contact dermatitis (Sittig 1985). Metallic Cu may cause keratinization of the hands and soles of the feet, but not normally dermatitis (Sittig 1985).

Several mechanisms have been proposed to explain Cu-induced cellular toxicity. Copper can exist in both oxidized, cupric (Cu^{2+}), or reduced, cuprous (Cu^{+}), state. In living cells, Cu acts as catalyst in the production of superoxide radicals, hydroxyl radicals and hydrogen peroxide *via* the Haber-Weiss reaction (Bremner 1998, Kadiiska *et al.* 1993), which can cause oxidative damage and induce adverse effects (Gaetke and Chow 2003). High concentrations of Cu may cause increased oxidative damage to lipids, proteins, and DNA. Recently, it has been suggested that copper-oxide nanoparticles (CuONPs) are toxic to skin-associated cells and that extracellular signal-regulated kinase (Erk) and p53 may be the key factors regulating the cytotoxicity (Luo *et al.* 2014). CuONPs also induced oxidative stress and apoptosis in HaCaT human keratinocytes (Alarifi *et al.* 2013). In another study, human MCF-7 cells were treated with Cu^{2+} in a dose-response manner and used attenuated total reflection Fourier transform infrared microspectroscopy combined with computational analysis to examine cellular alterations. Cupric ions induced bimodal dose-response effects on cells, while lipids and proteins seemed to be the main cell targets (Llabjani *et al.* 2014).

Reproductive and developmental effects of Cu have been well-documented in both *in vivo* and *in vitro* experiments (Chattopadhyay and Biswas 2013, Kolesarova *et al.* 2010, Roychoudhury *et al.* 2010, 2014). During the last decade a particular rise (from 16-40 %) has been noted in the research relating to reproductive effects of Cu (Fig. 1A). The goal of this review is to summarize the results of previously performed *in vivo* and *in vitro* experiments. The *in vivo* experiments appear to have attracted more attention from researchers in comparison to *in vitro* experiments in recent times. Among different targets a large number of *in vivo* works focused on

developmental defects (35 %), whereas *in vitro* research focused more on neuroendocrine effects (25 %). Investigated data is presented into two broad categories: (1) *in vivo* experiments, and (2) *in vitro* experiments, which are further subdivided based on the affected targets. The subdivisions mainly include effects of Cu on neuroendocrine system, ovarian function, spermatozoa, testis, fetal development, and nanotoxicity (Fig. 1B).

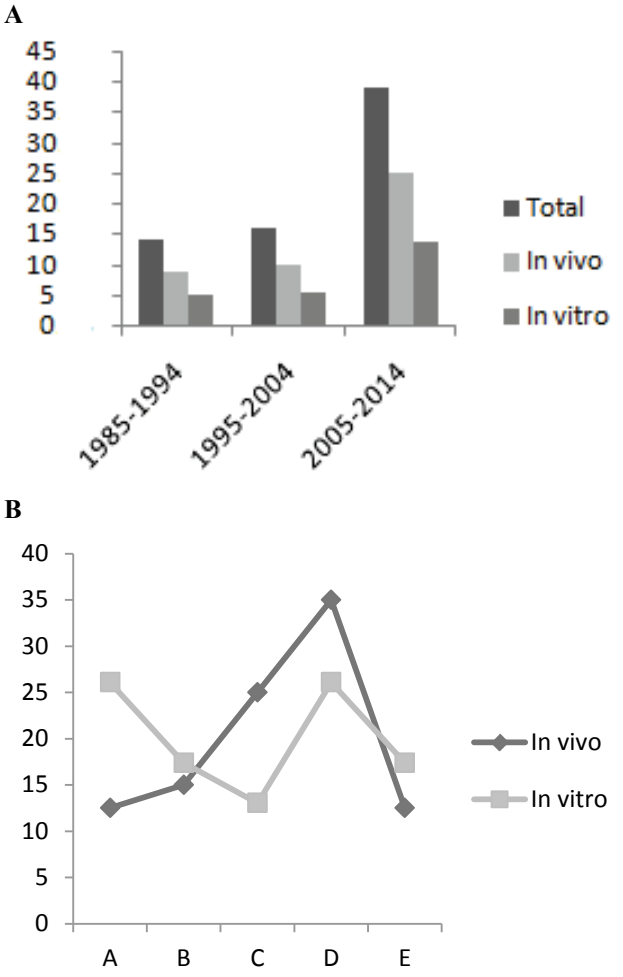

Fig. 1. *In vivo* and *in vitro* studies on effects of Cu on reproductive functions. **A**: Trend of research in the field of effects of Cu on reproduction during last three decades and comparison between *in vivo* and *in vitro* studies; **B**: Percentage of studies on effected targets *in vivo* and *in vitro*: A – Neuroendocrine effects, B – Effects on ovarian function, C – Effects of spermatozoa and testis, D – Developmental defects, E – Nanotoxicity.

In vivo experiments

Neuroendocrine effects

In the development and regulation of reproductive system hypothalamic-pituitary gonadal (HPG) axis plays a critical role (Forgacs *et al.* 2012). There are certain classes of compounds which are capable of affecting pituitary function directly by altering the hormone secretion and cellular activity. While some compounds affect pituitary function indirectly by modifying central nervous system (CNS) and gonadal hormone stimulation, many other compounds have both direct and indirect effects (Cooper *et al.* 1986).

Copper plays an important role in the activity of dopamine β-monooxygenase by catalyzing hydroxylation of dopamine to noradrenaline, which is an essential neurotransmitter involved in the secretion of gonadotropin releasing hormone (GnRH). Binding of GnRH with a specific receptor on the gonadotrope cell membrane is responsible for the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary (Michaluk and Kochman 2007). Complexes of Cu with GnRH reportedly evoked the release of FSH more effectively than LH (Kochman *et al.* 2005), but complex of Cu^{2+} with luteinizing hormone releasing hormone (LHRH) brought about a high release of LH and even higher release of FSH (Yu *et al.* 2008). In the presence of Cu^{2+}, secretion of growth hormone by incubated pituitary cells of 32-35 days old pigs was stimulated *in vivo* (Kochman *et al.* 2005). Hazum (1983) reported induction of ovulation by Cu and the reduction in serum LH when reducing agents are injected.

Effects on ovarian function

As early as 1936, Fevold and colleagues were the first to report that ovulation could be induced by intravenous injection of Cu salts. It was observed that the number of antral follicles in mice ovaries decreased by 100 mg/kg copper sulfate ($CuSO_4$) administration following 14 days and at a dose of 200 mg/kg after 35 days showed significantly lower quantities of all follicular classes including primordial, primary, growing, secondary, and antral follicles and also *corpus luteum* (Babaei *et al.* 2012). Checking of vaginal smear has been the most widespread method to test ovarian function in laboratory rodents, but certain biochemical parameters from ovarian tissue or blood sample could be more specific functional parameter and histological examination may be performed to check morphological changes (Forgacs *et al.* 2012). Buffalo-cows were clinically and gynecologically examined and blood samples were collected to study the correlation between Cu status and ovarian function. Results revealed that 19.12 % of the examined animals showed clear clinical signs of Cu deficiency (hypocuprosis) and 21.84 % of these hypocupremic buffalo-cows suffered

from ovarian inactivity and low serum progesterone level, during the luteal phase of the estrous cycle (Ahmed *et al.* 2009). Short term administration of Cu (14 days) even with low dose (100 mg/kg) was found to exert deleterious effects on intracellular organelles of mouse ovarian cells (Babaei *et al.* 2012). Recently, the most typical ovarian follicle of a rodent bank vole (*Myodes glareolus*) was presented for the first time. High dose of Cu was found to exert negative effect on morphological development whereas low dose relatively increased the uterus weight, but Cu had no effect on the number of follicles (Schramm *et al.* 2014). Studies on the effect of Cu on ovarian function have not remained limited to mammals only. Copper exposure has been linked with altered ovarian function in a crustacean, estuarine crab (*Chasmagnathus granulata*), wherein although 14 days exposure to 0.1 mg/l of Cu showed no significant change of the gonadosomatic index the eyestalk ablated exposed females showed significantly lower gonadosomatic index values than the control (Medesani *et al.* 2004).

Effects on spermatozoa and testis

The effect of Cu has been investigated on quality of spermatozoa and testicular histopathology (Sakhaee *et al.* 2012). The primary functions of the testicles are to produce spermatozoa, androgens, and male sex hormone, testosterone (Forgacs *et al.* 2012). A significant decrease in spermatozoa concentration, viability and motility indicated the possibility of adverse effect of Cu on male fertility (Roychoudhury *et al.* 2008, 2010). Copper was found to play an essential role in spermatogenesis and male infertility in Wistar albino rats (Sakhaee *et al.* 2012). Copper intake even with low dose (100 mg/kg) showed adverse effects on testis morphology in male mice 14th day of exposure onwards (Babaei *et al.* 2012). The role of Cu in the spermatozoa is unclear, but it appears to be involved in spermatozoa motility and may also act at the pituitary receptors which control the release of LH (Yunus *et al.* 2015). Fertility is adversely affected by Cu, specifically a decline in male reproductive capacity had been suggested in a number of studies (Roychoudhury *et al.* 2008, 2010, Sakhaee *et al.* 2012). In immature male rat a dose of 2000 and 3000 µg/kg body weight for 26 days resulted in reduction of serum testosterone, FSH and LH whereas 1000 µg/kg caused rise in their levels (Chattopadhyay *et al.* 1999). Bank voles, when exposed to 150 and 600 mg/kg Cu for 12 weeks showed low sperm count and spermatozoa head abnormalities, while higher dose compromised spermatozoa tail membrane integrity, viability and motility (Schramm *et al.* 2014).

Among men, symptoms of adverse effect of Cu usually include prostate enlargement, prostate infections, erectile dysfunction, depression, anxiety, testicular pain and testicular cancer (Badiye *et al.* 2013). At any stage of cell differentiation the disruption of spermatogenesis may result in the decrease of total sperm count (Sharpe *et al.* 2003). Moreover, progressive spermatozoa motility is impaired due to the accumulation of metals in the epididymis, prostate, vesicular seminalis or seminal fluid (Hess 1998). Seminal plasma Cu concentrations found in oligozoospermic, asthenozoospermic and azoospermic groups was significantly higher than normozoospermic group (Eidi *et al.* 2010).

Developmental effects

Copper present in either excess or deficient amount during the developmental stage plays an important role. Developmental effects of Cu relate more to its deficiency rather than toxicity. Development of the CNS was found to be affected by reduced Cu availability (Danks 1988). Deficiency of Cu during embryonic and fetal development can result in numerous gross structural and biochemical abnormalities. Evidence for the importance of Cu for prenatal development arose from studies of enzootic ataxia, a disease in lambs. Neonatal ataxia and brain abnormalities have been reported among Cu deficient newborn goats, swine, guinea pigs and rats (Keen *et al.* 1998). Developmental defects were observed in rats, mice, and chickens in response to Cu deficiency (Hurley and Keen 1979, Opsahl *et al.* 1984, Phillips *et al.* 1991, Vulpe 1995). In addition to brain defects, Cu-deficient fetuses and neonates were characterized by connective tissue abnormalities and cardiac hemorrhages in sheep, rats, guinea pigs and mice (Hurley and Keen 1979, Rucker *et al.* 1998, Tinker and Rucker 1985).

The average intake of Cu by women during childbearing age is lower than the daily intake for adults, which is 1.5-3.0 mg Cu (NRC 1989). A correlation between low Cu in drinking water and the occurrence of neural tube defects was reported (Morton *et al.* 1976), with an implication that deficiency of Cu could result in birth defects. It has been found that Cu increases the incidence of fetal resorptions and induces malformations in the offspring of pregnant hamsters when administered high intravenous doses of Cu (Ferm and Hanlon 1974). A daily diet supplemented with >6 mg Cu/kg as $CuSO_4$ impaired lactation in female minks (*Neovison vison*)

(Lecyk 1980). Increased mortality was observed in the fetuses of pregnant mice fed 104 mg Cu/kg/day as $CuSO_4$ during gestation, and developmental abnormalities at 155 mg Cu/kg/day (Aulerich *et al.* 1982).

Nanotoxicity

Recently, nanoparticles (NPs) have been found to exert adverse effect on reproductive organs, as they are able to penetrate through biological barriers (Singh *et al.* 2009). Severe toxic symptoms have been observed in male mice suffering more from copper nanoparticles (CuNPs) than females after they were exposed to the same mass of particles (Chen *et al.* 2006). Copper oxide (CuO) was found to reduce the GSH content and inhibit the catalase (CAT) and superoxide dimutase (SOD) activities, which caused embryo oxidative damage and changes in the physiology of zebrafish, including hatching failure, shorter body length, and lower reproduction (Liu *et al.* 2014). Copper NPs were effective in decreasing the reproduction in red worms (*Eisenia fetida*), too (Alahdadi and Behboudi 2015). Copper oxide NPs significantly reduced the body length of zebrafish. The hatching rates of the embryos exposed to CuONPs decreased with the increasing concentrations of 1 mg/dm^3 to 25 mg/dm^3 (Liu *et al.* 2014).

Table 1 summarizes the main *in vivo* effects of Cu compounds on reproductive functions.

Table 1. *In vivo* studies on the effects of Cu compounds on reproductive functions.

Test System	Exposure	Effect	References
Female mice	100 mg/kg $CuSO_4$ for 14 days 200 mg/kg $CuSO_4$ for 35 days	Decrease in number of antral follicles Lower quantities of all follicular classes including primordial, primary, growing, secondary, antral and also corpus luteum	Babaei *et al.* 2012
C. granulate (Estuarine crab)	100 mg/kg Cu for 14 days	Cu produced no significant effect while eyestalk ablated crabs showed significantly lower gonadosomatic index	Medesani *et al.* 2004
Female mouse	100 mg/kg for 14 days	Deleterious effects on intracellular organelles of mouse ovarian cell	Babaei *et al.* 2012
Male mice	100 mg/kg for 14 days	Toxic effect from 14^{th} day of exposure on testis	Babaei *et al.* 2012
Immature male rats	2000 and 3000 µg/kg bw for 26 days	Reduction of serum testosterone, FSH and LH, whereas 1000 µg/kg bw causes rise in their levels	Chattopadhyay *et al.* 1999
Bank vole	150, 600 mg/kg Cu for 12 weeks	Low spermatozoa count and sperm head abnormality	Schramm *et al.* 2014
Female mink	>6 mg Cu/kg/day as $CuSO_4$	Impaired lactation	Lecyk *et al.* 1980
Pregnant mice	104 mg Cu/kg/day	Increased mortality rate was observed	Aulerich *et al.* 1982
	155 mg Cu/kg/day	Developmental abnormalities are observed	
Zebrafish	1 mg/dm^3 to 25 mg/dm^3 of CuNPs	Decrease in hatching rate of embryos	Liu *et al.* 2014

In vitro experiments

Neuroendocrine effects

Lorenson *et al.* (1983) investigated the effect of divalent metal ions on *in vitro* release of GH and prolactin (PRL) from bovine adenohypophysial secretory granules. Complexes of Cu with GnRH (Cu-GnRH) bind with the GnRH receptors. The effect of Cu-GnRH was

found to be dose-dependent in porcine pituitary cells to modulate cyclic adenosine monophosphate synthesis and phosphoinositols formation apparently increasing LH release (Kochman *et al.* 2005). Copper ions stimulate both basal and GnRH-stimulated LH release from pituitary cells of immature female rats (Hazum 1983). Copper was reported as a potent releaser of GnRH from isolated hypothalamic granules (Burrows and Barnea 1982), supporting the hypothesis that it influences GnRH neurons and Cu action only occurs in GnRH granules.

Effects on ovarian function

Roychoudhury *et al.* (2014) for the first time demonstrated the effect of Cu on IGF-I release by porcine ovarian granulosa cells. Results indicated that the release of insulin like growth factor I (IGF-I) is stimulated by 2 µg/m $CuSO_4$ concentration used, but lower concentrations (0.33-1 µg $CuSO_4$/ml) did not have any influence on IGF-I release (Kolesarova *et al.* 2010, Roychoudhury *et al.* 2014). It was observed that Cu administration in granulosa cells released IGF-I, progesterone (P_4) and induced expression of peptides related to proliferation and apoptosis. High amounts of Cu in the follicular fluid and granulosa cells of goat have been detected from small, medium, and large antral atretic follicles, respectively (Bhardwaj and Sharma 2011, Misro *et al.* 2008). Bhardwaj and Sharma (2011) reported potential use of Cu as atretic marker and for fertility improvement plans in *in vitro* studies. The effect of Cu on porcine ovarian granulosa cells proved to be concentration dependent. A dose of 2 µg/ml $CuSO_4$ was found to enhance the monolayer of porcine ovarian granulosa cells (Kolesarova *et al.* 2010, Roychoudhury *et al.* 2014).

Effects on spermatozoa and testis

Misro *et al.* (2008) demonstrated the release of Cu and its effect on functional integrity of human spermatozoa following co-incubation of semen with CuT 380A (intra-uterine device). High release of Cu from CuT 380A drastically lowered spermatozoa motility and viability but only marginally affected the acrosome status or nuclear chromatin condensation in short term incubations. Cultured rabbit spermatozoa showed negative influence of high Cu concentrations in semen, particularly on parameters of spermatozoa motility (Roychoudhury *et al.* 2008). Decrease of total motility of rabbit spermatozoa was reported within the concentration range of 3.70-4.85 µg/ml $CuSO_4$, beyond which no significant change could be detected (Roychoudhury *et al.* 2010, Roychoudhury and Massanyi 2008). After 2 h, an increase was noted for both the parameters for evaluation of spermatozoa distance and velocity, i.e. distance curved line and velocity curved line in concentrations 3.63 and 3.57 µg/ml $CuSO_4$, respectively whereas after 24 and 48 h almost all the spermatozoa including those of control were found to be dead recording no motility at all concentrations. At a concentration of 3.63 µg/ml $CuSO_4$ motility and progressive motility of spermatozoa remained unaltered (Roychoudhury *et al.* 2010).

Developmental effects

Fetus stores almost ten times more Cu than the adult organism per unit of body mass (Michaluk and Kochman 2007). It was shown that Cu and ceruloplasmin (a Cu-binding protein) concentrations rise significantly during pregnancy, and Cu is accumulated in brain of fetus (Uauy *et al.* 2008). Copper is reportedly involved in development of mouse preimplantation embryos *in vitro*, when exposed to 100 mM concentration for 24 h at the 1-cell, 2-cell, 4-cell, 6-8-cell, morula and blastocyst stages (Vidal and Hidalgo 1993). It was reported that during *in vitro* maturation, the optimal embryo development up to the blastocyst stage was partially dependent on the presence of adequate concentration of Cu (Picco *et al.* 2012). Percentages of matured oocytes that developed to the blastocyst stage were found to be the highest (33.2±1.6 %) in oocytes matured with 6 µg/ml Cu exposure. *In vitro* post-implantation development of mouse embryos from Swiss and NMRI strains were investigated for teratogenic potential of Cu. Embryos were cultured in rat serum for 48 h and supplied concentrations of $CuCl_2$ in culture medium in order to study its direct effects. The embryos from NMRI strain showed failure of closure of neural tube in head region, and significant retardation of embryonic development (Checiu *et al.* 2008). Development of 2-cell and 8-cell mouse preimplantation embryos to the blastocyst stage was completely inhibited by Cu concentrations of 13.3 µg/ml and higher (Whittingham 1972).

Nanotoxicity

Nanoparticles were found to cause pulmonary injury, hepatotoxicity, renal toxicity, immunotoxicity, neurotoxicity, and reversible testis damage in animals (Bai *et al.* 2010, Bartneck *et al.* 2012, Chou *et al.* 2008, Derfus *et al.* 2004, Lin *et al.* 2008, Schipper *et al.* 2008,

Wu *et al.* 2011). Recently it was reported that the small size of CuNPs is responsible for its toxic effect (Meng *et al.* 2007). Copper nanoparticles were found to be capable of generating oxidative stress *in vitro* (Ahamed *et al.* 2010, Fahmy and Cormier 2009), which in turns leads to reproductive toxicity. Exposure to CuONPs leads to increase in size of lipid droplets. Copper sulfate salt was more toxic than the CuONPs in freshwater flea *Daphnia magna* (Tavares *et al.* 2014).

Table 2 summarizes the main *in vitro* effects of Cu compounds on reproductive functions.

Table 2. *In vitro* studies on the effects of Cu compounds on reproductive functions.

Test System	Exposure	Effect	References
Porcine ovarian granulosa cells	0.33-1 µg/ml $CuSO_4$	2 µg/ml stimulates IGF-I release but lower concentration (0.33-1 µg/ml) did not have any influence	Kolesarova *et al.* 2010, Roychoudhury *et al.* 2014
Porcine ovarian granulosa cells	2 µg/ml $CuSO_4$	Enhance the monolayer of porcine granulosa cells	Kolesarova *et al.* 2010, Roychoudhury *et al.* 2014
Human spermatozoa	Co-incubation of semen with CuT 380A	Release of Cu from CuT 380A was found to be 9.2 to 40 times higher compared to control incubation with PBS	Misro *et al.* 2008
Rabbit spermatozoa	3.70-4.85 µg/ml $CuSO_4$	Decrease of total spermatozoa motility, beyond 4.85 no significant change could be detected	Roychoudhury *et al.* 2010
Rabbit spermatozoa	3.63 µg/ml $CuSO_4$	Motility and progressive motility remains unaltered	Roychoudhury *et al.* 2010
Mouse preimplantation embryo	100 µM for 24 h at 1-cell, 4-cell, 6-8 cell morula and blastocyst stage	Cu, affect the developmental stages	Vidal and Hidalgo 1993
Mouse embryo (Swiss and NMRI strain)	9^{th} day embryo cultured in rat serum for 48 h and supplied $CuCl_2$	Embryo from NMRI strain presented failure of closure of neural tube in head region of the embryo	Checiu *et al.* 2008
Mouse embryo (2-cell and 8-cell)	13.3 µg/ml Cu and greater	Blastocyst stage was completely inhibited by Cu	Whittingham *et al.* 1972

Conclusions

The results of previous investigations indicate that the hormonal effects may play an important role in the effects of Cu on reproductive functions both at the neuroendocrine and gonadal levels in the HPG axis (Cooper *et al.* 1986, Forgacs *et al.* 2012). Complexes of Cu with GnRH induce the release of FSH and LH (Cooper *et al.* 1986). Targets of effects include the neuroendocrine system, spermatozoa, and development of embryos, testicular and ovarian functions. Copper plays an important role in the activity of dopamine β-monooxygenase, which participates in tyrosine metabolism (Michaluk and Kochman 2007). Adequate amount Cu is needed during the development of embryo (Danks 1988), the lack of which may bring about serious developmental defects in the offspring and may even result in fetal resorption (Ferm and Hanlon 1974). Copper NPs, at its infancy cause toxicity at levels of regulation due to their small size (Ahamed *et al.* 2010). They readily cross the biological barrier resulting in reproductive toxicity (Singh *et al.* 2009). In human Cu transport, Cu is shuttled from one protein to another to eventually become loaded on Cu-dependent enzymes (Festa and Thiele 2011, O'Halloran and Culotta 2000). To avoid toxicity of Cu^{+}, the intracellular concentration of Cu is regulated *via*

dedicated proteins that facilitate its uptake, efflux as well as distribution to target Cu-dependent proteins and enzymes (Festa and Thiele 2011, O'Halloran and Culotta 2000, Robinson and Winge 2010). In humans, the 68-residue Cu^+ chaperone Atox1 picks up Cu^+ that has entered the cell *via* CTR1 and delivers the metal to cytoplasmic metal-binding domains in ATP7A and ATP7B (also called Menke's and Wilson disease proteins, respectively), two homologous multidomain P1B-type ATPases located in the trans-Golgi network (Festa and Thiele 2011, O'Halloran and Culotta 2000, Robinson and Winge 2010). During gestation, copper transfer across the placenta increases (McArdle and Erlich 1991). Uptake is through a high affinity carrier, Ctr1. Ctr1 is expressed early in pregnancy, and homozygous mutant embryos die early in gestation (Lee *et al.* 2001). Once taken up by the placenta, Cu is bound to one of a series of chaperone proteins, which deliver the metal to its target molecule. In placenta, ATP7A is located in several different cell types, whereas ATP7B is found only in syncytiotrophoblast (Hardman *et al.* 2004). Intriguingly, protein levels do not appear to change during gestation, which implies that the increase in transfer seen as development progresses (McArdle and Erlich 1991) is related to localization of the protein. In a study conducted during the first trimester and at term in 216 mothers in Finland, low copper concentrations in placenta were connected to higher birth weights (Kantola *et al.* 2004). Impaired placental Cu trafficking has been associated with the development of preeclampsia (Iseminger *et al.* 2010). Even though further investigations are necessary to arrive at a definitive conclusion, Cu notably influences reproduction by interfering with both male and female reproductive functions and also hampers embryo development in dose-dependent manner.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the Ministry of Education, Science, Research and Sports of the Slovak Republic (1/0022/13, 1/0532/11), Slovak Research and Development Agency of the Slovak Republic (APVV-0304-12) and the Department of Biotechnology, Government of India.

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