Elemental Copper: Metabolism and Impact on Reproduction

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Abstract

The role of elemental copper in reproduction is not fully understood. Copper is required for life. It is a cofactor for a number of enzymes. If there is a deficiency or an excessive accumulation of elements, serious changes can occur in the body, affecting the activities of enzymes that are directly or indirectly dependent on them. In the human, copper is known to play a role in spermatogenesis in the male. Numerous human and animal studies show that as copper concentration rises, so does the testis structure and the process of spermatogenesis (the production, maturation, motility, and fertilizing capacity of the spermatozoa). Numerous human and animal studies show that as copper concentration rises, so does the testis structure and the process of spermatogenesis (the production, maturation, motility, and fertilizing capacity of the spermatozoa. Copper deficiency is uncommon in humans. Copper deficiency causes higher levels of ROS, iron toxicity, and downregulation of enzymatic activity in the human spermatozoa, which can lead to male infertility. Copper-deficient animals had smaller ejaculates, lower sperm concentrations, and poorer sperm motility and morphology. Seminal plasma copper concentrations in oligozoospermic, asthenozoospermic, and azoospermic patients are significantly higher than in normozoospermic individuals. Copper acts as a catalyst in the formation of reactive oxygen species, which can result in oxidative stress and lipid peroxidative damage. Several aspects of male infertility may be caused by such damage. Human spermatozoa contain a high concentration of polyunsaturated fatty acids. These polyunsaturated fatty acids are particularly vulnerable to peroxidative damage. Human spermatozoa are protected from peroxidative damage by superoxide dismutase a copper-containing intracellular enzyme which catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. Superoxide anion radicals act as mediators of inflammation and tissue injury. Ceruloplasmin, a copper-containing plasma protein and acute-phase reactant, can mimic the action of cytoplasmic superoxide dismutase to scavenge superoxide and thus inhibit superoxide-mediated reactions. The role of copper in female fertility apears almost uninvestigated and much remains to be elucidated. The precise molecular mechanisms of copper affecting the human reproductive system remain unknown after 30 years of research. Much remains to be achieved in this area of reasearch.

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Introduction

Copper is required for life. If there is a deficiency or an excessive accumulation of elements, serious changes can occur in the body, affecting the activities of enzymes that are directly or indirectly dependent on them. As a result, there is an optimum range of concentrations for each element to perform vital functions (Mehri, 2020). Copper is involved in the regulation of metabolism, tissue respiration, and the biosynthesis of hormones and cofactors that promote Iron absorption. It is an essential trace element that is a structural component of

Copper containing enzymes	Functions
Cytochrome c oxidase	Electron transfer from Cytochrome C to molecular oxygen in tissue respiration
Dopamine-β-monooxygenase	Synthesis of noradrenaline
Cu,Zn-superoxide dismutase	Superoxide anion radical dismutation to hydrogen peroxide
Lysiloxidase	Maturation of collagen and elastin
Aminooxidases	Oxidative deamination of biogenic amines
Tyrosinase	Biosynthesis of melanin
Peptidylglycine α-amidating monooxygenase	Neuropeptide transformation
Ceruloplasmin	Dismutation of superoxide anion radical to hydrogen peroxide; Iron metabolism
Glycosylphosphatidylinositol (GLI)-ceruloplasmin	Iron removal from macrophages
Hephestin	Iron removal from enterocytes
Zyklopen	Iron removal from the placenta

Table 1. Copper containing enzymes and its functions in the human body

several enzymes (Table 1), and disruptions in copper metabolism have a wide range of negative health consequences. As a result, maintaining an adequate copper level in the body is critical to a person's health (Anke, 2004; Oberleas et al., 2008).

Iron (Fe) and copper (Cu) are trace elements that are an important ecophysiological component of the male reproductive system's cells and tissues. Cu, based on its physiologic functions, may be necessary, useful, or harmless within a certain range of concentrations but unnecessary or harmful beyond that range. Excessive accumulation may cause metabolic disruption, compromising male fertility, if it exceeds its range (Yuyan et al., 2008).

Cu sulphate is used in medicine as an antimicrobial agent and a cauterant. Cu sulphate was used to treat anemia and hypotrophy in the sixth and eighth centuries (nowadays, Cu chelates are used more widely). Cu salt preparations are used externally for irrigation, syringing, as liniments, and in physiotherapy. In addition, Cu is used in conjunction with Fe to treat hypochromic anemia, hypotrophy, and other diseases. Cu-containing medications and food supplements are also used to treat and prevent musculoskeletal diseases and hypothyroidism. Furthermore, the Cu Lippes loop is widely used as a contraceptive device, and the 64Cu isotope is used in radioisotope diagnostics of brain tumors in radiobiology (Olatunbosun et al, 1976).

The average adult human body contains 100-150 mg of Cu. Cu concentrations in the brain and liver are the highest; Cu concentrations in the central nervous system and heart are also high. About half of the Cu content is stored in bones and muscles (skeletal muscle accounts for about 25%), 15% in skin, 15% in bone marrow, 8 to 15% in the liver, and 8% in the brain (Gibson, 2005). The recommended daily Cu intake is 2-3 mg. When intake is less than 1 mg/day and the toxicity level is 200-250 mg/day, nutritional deficiency can develop (Skalnaya & Skalny, 2018).

Cu has a high redox potential in biological systems. Cu is essential for cellular respiration, Fe metabolism, neurotransmitter and pigment synthesis, connective tissue biology, and immunity (Ghayour-Mobarhan et al., 2009). However, at high concentrations, Cu can be toxic (Saha et al., 2008; Wilson, 2011).

Copper homeostasis in humans

Copper homeostasis in the human body is associated with the rapid binding of Cu by organic molecules, with very little unbound Cu. The system of Cu transport proteins determines the efficiency of homeostasis. In the active sites of more than ten enzymes, Cu atoms form complexes with amino acids.

Cytochrome oxidase, for example, is essential for cellular energy. Cytochrome C oxidase generates an electrical gradient as it catalyzes the reduction of molecular oxygen (O_2) to water (H_2O), which is used by mitochondria to create vital energy for the organism and stored in ATP molecules (Uauy et al., 1998).

Dopamine-hydroxylase, converts dopamine to noradrenaline in both the CNS and the adrenal glands, and its deficiency results in hypotension and decreased physical activity tolerance (Senard & Rouet, 2006).

Superoxide dismutase (SOD) is an antioxidant that catalyses the conversion of superoxide radicals (free radicals) into hydrogen peroxide, which can then be reduced to water by other antioxidant enzymes (Murthy et al., 1987). Cu-containing SOD comes in two varieties: 1) Cu/zinc SOD is found in almost all cells of the body, including red blood cells; and 2) extracellular SOD is a Cu-containing enzyme found in high concentrations in the lungs and in low concentrations in plasma (Ross et al., 2012).

Lysiloxidase is another Cu enzyme that aids in the cross-linking of the connective tissue proteins collagen and elastin. Lysiloxidase aids in the maintenance of connective tissue integrity and elasticity in the heart and blood vessels. Furthermore, it aids in bone formation (Ross et al., 2012).

The Cu-containing proteins secreted by vascular smooth muscle cells, adipocytes, and endothelial cells are related to amino oxidase (AOC_3; Bligt-Lindén et al., 2013). This enzyme is a multifunctional molecule with adhesive as well as enzymatic properties (Murata et al., 2012). Rheumatoid arthritis, psoriasis, systemic sclerosis, respiratory diseases, diabetes, and its complications have all been linked to VAP-1 and Cu (Aaseth et al., 1978; Bligt-Lindén et al., 2013; Cheng & Li, 2000; Zackheim & Wolf, 1972; Zlatkov et al., 1973).

Tyrosinase, a Cu-enzyme, is required for the formation of melanin pigment. Melanin is produced by melanocytes, which have a variety of biological functions including hair pigmentation, skin pigmentation, and UV protection for the eyes and skin (Ross et al., 2012).

Dopamine-beta-mono oxidase catalyzes the conversion of dopamine into norepinephrine (Combs, 2013), whereas Cu, Zn-superoxide dismutase (SOD1) is a ubiquitary cytosolic dimeric carbohydrate-free molecule that belongs to a family of isoenzymes involved in the scavenging of superoxide anions (Mondola, Damiano, Sasso, & Santillo, 2016). Lysyl oxidase is a Cu-amine oxidase that initiates covalent cross-linkage formation in elastin and collagen by oxidizing peptidyl lysine in these proteins to aminoadipic semialdehyde. If Cu is removed from lysyl oxidase preparations, the enzyme loses activity and is exposed to Cu chelating agents. As a result, Cu deficiency causes a decrease in the cross-linking of elastin and collagen, resulting in faulty connective

tissue formation (Wiggs, 2015). Cu-containing amino oxidases are also involved in the detoxication of putrescin, 1-phenylethylamine, serotonin, and spermine (Thomas & Thomas, 2003).

Tyrosinase is also required for the formation of melanin pigment. Tyrosinase is involved in the synthesis of melanin in the skin and hair as well as neuromelanin in the CNS. This enzyme catalyzes two steps in the melanogenesis process: tyrosine hydroxylation to DOPA and DOPA conversion to dopaquinone. (BoisseauGarsaud et al., 2002; Ross et al., 2012; Komori et al., 2014).

Peptidylglycine, an amidating monooxygenase (PAM) is involved in the conversion of a variety of peptide hormones such as thyrotropin, neuropeptide Y, and vasopressin. The efficiency with which peptide hormones interact with their receptors is improved by amidation. However, patients with hyperthyroidism and hypothyroidism have impaired Cu metabolism (Bousquet-Moore et al., 2009).

Superoxide anion radicals are primarily protected by a Cu-containing intracellular enzyme called superoxide dismutase (SOD), which catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. Superoxide anion radicals have recently been identified as mediators of inflammation and tissue injury. Ceruloplasmin, a Cu-containing plasma protein and acute-phase reactant, can mimic the action of cytoplasmic SOD to scavenge superoxide and thus inhibit superoxide-mediated reactions.

Cu and Fe ions are powerful free radical neutralizers, and ceruloplasmin facilitates the catalyzed oxidative process of free radicals. Superoxide anion radicals have been identified as mediators of inflammation and tissue injury. A Cu-containing intracellular enzyme (superoxide dismutase) (SOD) that catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen can provide protection from superoxide anion (Goldstein et al., 2006; Sampath et al. 2003).

While ceruloplasmin (Cp) is a ferroxidase that converts highly toxic ferrous Fe to non-toxic ferric Fe, astrocytes in the mammalian central nervous system express a glycosyl-phosphatidylinositol (GPI)-anchored form of this enzyme, whereas the secreted form is expressed by the liver and found in serum. In the absence of this enzyme (Jeong & David,2003), Fe accumulates in the brain, resulting in neurodegeneration.

The primary function of hephestin is to regulate Fe transport. Hephestin participates in the release of Fe from enterocytes, hepatocytes, and macrophages in collaboration with ferroportin. Cu-deficiency anemia, on the other hand, is caused by a decrease in the production of hephestin, which regulates Fe absorption in the gastrointestinal tract and its retention by enterocytes (Anke, 2004).

Copper metabolism

Copper-rich foods include shellfish, seeds and nuts, organ meats, wheat-bran cereals, whole-grain products, and chocolate. The average human diet contains approximately 1400 mcg/day for men and 1100 mcg/day for women, the majority of which is absorbed in the upper small intestine (Coates et al., 2010; King & Cousins, 2014; Prohaska, 2011). Cu can be found in a wide range of plant and animal foods, and the skeleton and muscles contain nearly two-thirds of the body's Cu (King & Cousins, 2014; Trumbo, Yates, Schlicker, & Poos, 2001).

The average adult's total body Cu content ranges between 50 and 120 mg, and only trace amounts of Cu are stored in the body (King & Cousins, 2014; Prohaska, 2011). The majority of Cu is excreted in bile, with only a trace excreted in urine. King and Cousins, 2014; Prohaska and Broderius, 2012). Cu levels in the body are homeostatically maintained to protect against Cu deficiency and toxicity by Cu absorption from the intestine and Cu release by the liver into bile (Trumbo et al., 2001). Because people with known Cu deficiencies frequently have low blood levels of Cu and ceruloplasmin, human studies typically measure Cu and cuproenzyme activity in plasma and blood cells (Prohaska, 2011). Cu serum concentrations are typically 10-25 mcmol/L (63.5-158.9 mcg/dL) and CP concentrations are 180-400 mg/L. (Thiamin, 1998).

Cu is absorbed in the gastrointestinal tract and transported to the liver bound to albumin. It is excreted in bile after entering the bloodstream via the plasma protein ceruloplasmin (Gubler, 1956). Ceruloplasmin is a serum ferroxidase that accounts for 90% of Cu transport (Lopez et al., 2021).

Cu membrane transporter 1-CMT1 transports Cu inside the cells, where some is bound to metallothioneins and another part is carried to the trans-Golgi network by Cu transport protein-ATOX1 (Cu chaperone for ATP7A and ATP7B). In response to rising Cu concentrations, an enzyme called ATP7A releases Cu into the portal vein of the liver.The CMT1 protein is also found in liver cells, and metallothionein and ATOX1 bind Cu within the cells, but it is ATP7B that links Cu to ceruloplasmin and releases it into the bloodstream, as well as removes excess Cu by secreting it into bile (Harris et al., 1998).

Approximately 90% of the Cu in the blood is incorporated into ceruloplasmin, which is in charge of transporting Cu to tissues that require it (Gropper et al., 2013; Harris et al., 1998). Because Cu excretion is so slow (10% in 72 hours), an excessive dose of Cu is a lingering issue (Gubler, 1956). As a result, a proper balance of zinc and manganese will aid in Cu absorption and metabolism. People who consume an excessive amount of zinc while consuming a low amount of Cu may increase their risk of Cu deficiency because zinc can compete with Cu in the small intestine and interfere with its absorption (Gropper et al., 2013; Harris et al., 1998). Ceruloplasmin is a transport protein that also functions as an enzyme, catalyzing the oxidation of minerals, most notably Fe. Fe must be oxidized before it can be bound to its transport protein-transferrin. Fe deficiency anemia, on the other hand, may be a symptom of Cu deficiency.

Ceruloplasmin functions as both a transport protein and an enzyme, catalyzing the oxidation of minerals, most notably Fe (Gubler, 1956). Since Fe must be oxidized by ceruloplasmin before it can be bound to its transport protein-transferrin, Fe deficiency anemia may be a symptom of Cu deficiency (Araya et al., 2006; Gropper et al., 2013). Mutations in the Cu transport proteins ATP7B and ATP7A can render these transport systems inoperable, resulting in Wilson's disease and Menkes disease, respectively (Schaefer & Gitlin, 1999; Strausak et al., 2001).

Copper and animal infertility

Many studies, both in vivo and in vitro, have been conducted on the reproductive and developmental effects of Cu, particularly on animals. In vivo experiments receive more attention from researchers than in vitro experiments in which in vivo research concentrated on developmental defects (35%), whereas in vitro research concentrated on neuroendocrine effects (25 %). According to Chattopadhyay & Biswas (2013), effects of copper on the neuroendocrine system, ovarian function, spermatozoa, testis, fetal development, and nanotoxicity, are all studied in vivo and in vitro.

Aquatic habitats such as lakes, rivers, and oceans are particularly vulnerable to metal pollution because all industrial waste, soil weathering, and urban mining is discharged into bodies of water, affecting aquatic biota (Malhotra et al., 2020). This was supported by Lee and Johnston (2007), who discovered that Cu was clearly harmful to flatworms, S. pygmaeus. Exposing flatworms to 0-25 ug/L Cu concentration reduces their ability to produce eggs. Flatworms of the Stylochidae family play an important role in an ecosystem because they are common pests of commercial bivalves and can cause significant economic losses (e.g., Newman et al., 1993; O'Connor and Newman, 2001). They can also decrease their prey and alter the community structure.

Furthermore, zebrafish Danio rerio exposed to 0, 10, 20, 40 g/L Cu for 30 days results in growth suppression and significantly affects reproductive biology in both sexes by damaging the structure of the gonads, altering steroid hormone levels, and altering the expression of endocrine-related genes in zebrafish HPG. As a result, this study shows that Cu has a negative effect on the reproductive endocrine system in zebrafish and may pose a risk to fish populations living in Cu-contaminated waters (Cao et al., 2019).

Copper and human infertility

According to Huang and colleagues (2000), there is still no complete understanding of the role of Cu in male reproduction. It impact on female reproduction appears even less or in fact, remains uninvestigated. Cu is well known to play a role in spermatogenesis in the human male. However, the precise molecular mechanisms of Cu impacting the male reproductive system remain unknown after 30 years. Numerous human and animal studies show that as Cu concentration rises, so does the testis structure and the process of spermatogenesis (the production, maturation, motility, and fertilizing capacity of the spermatozoa) (Akinloye et al., 2011; Aydemir et al., 2006; Chen et al., 2020; Kheirandish et al., 2014; Kowal et al., 2010; Tvrda et al., 2015). In contrast, Cu deficiency is uncommon in humans and primarily affects infants (Uauy et al., 1998). Thus, the effects of Cu deficiency on spermatogenesis in humans have not been reported (Gabrielsen & Tanrikut, 2016) although copper deficiency is anticpated to harm spermatogenesis (Garratt et al., 2013).

Copper deficiency and male infertility

Cu deficiency causes higher levels of ROS, Fe toxicity, and downregulation of enzymatic activity in the sperm, which can lead to male infertility (Garratt et al., 2013). Cu-deficient animals had smaller ejaculates, lower sperm concentrations, and poorer sperm motility and morphology. In rats, a large study on experimentally induced Cu deficiency was conducted. Sertolli cell inactivity resulted in less developed and less active seminiferous tubules in the deficient animals. However, once the Cu deficiency was corrected, the above parameters returned to normal (Van Niekerk & Van Niekerk, 1989a, 1989b). Histomorphological examination of cu-deficient goat testes revealed inactive germinal epithelium with signs of mild testicular degeneration (Aupperle et al., 2001).

Poor semen quality in male mice with Cu deficiency resulted in decreased in vivo oocyte fertilization, according to Tsunoda and colleagues (2012). According to Lee et al. (2002), low levels of ceruloplasmin cause Fe toxicity, whereas low levels of cytochrome C oxidase and catalase may result in high levels of ROS in Cu-deficient murine embryos. Furthermore, a lack of Cu alters the structure and, as a result, the functionality of metallothionein and glutathione, both ROS scavengers, and causes DNA damage in cells, most notably in Cu-deficient animals (Picco et al., 2001). Moreover, in Cu deficiency, testes-specific Cyc knockout mice with the final electron transfer step removed had lower ATP concentrations, spermatozoa motility, and overall fertility (Narisawa et al., 2002).

Uauy et al. (1998), reported that Cu deficiency is uncommon in humans and primarily affects infants. Hence, the effects of Cu deficiency on spermatogenesis in humans have not been studied. Meanwhile, anemia is the most common pathology caused by Fe deficiency. Anemia was also caused by low Cu levels, which had a negative impact on ceruloplasmin and its ferroxidase properties, followed by low Cu and Fe levels released throughout the organism (Evans & Abraham, 1973). Anemia creates a significant hypoxic environment in the testes. The testicular PO_2 is relatively low in anemic conditions, oxygen diffusion is slow, and the testicle has little capacity to increase total blood flow.

Spermatogenesis occurs at a high proliferation rate under normal conditions, requiring a lot of oxygen. As a result, males with Fe or Cu deficiency-related anemia may have poor sperm parameters, according to Tvrda et al. (2015). Supplementation or intravenous therapy improves the patients' hormonal and sperm profiles and can help with common anemia (Alleyne, Horne, & Miller, 2008).

Male infertility due to copper toxicity

Seminal plasma Cu concentrations in oligozoospermic, asthenozoospermic, and azoospermic patients are significantly higher than in normozoospermic individuals, according to Roychoudhury et al. (2016). Besides, exposure to high environment concentrations is associated with increased oxidative stress manifested by an increase in total oxidant value in seminal plasma (Kasperczyk et al., 2016).

On the other hand, there was a positive relationship between blood concentrations and sperm motility (Wong et al., 2001). This was supported by a study by Jockenhövel et al (1990), whereby sperm concentration, progressive motility, and normal morphology all had significant correlations. In contrast,study by Kasperczyk et al., (2016), demonstrated that there are no relationship between Cu levels and sperm volume, count, motility, or normal morphology was discovered. Interestingly, Aydemir et al. (2006), discovered higher levels of plasma Cu in a subfertile male group compared to a fertile male group. The presented study suggests that the disparity between the results of the preceding studies may be due to Cu's redox activity.

Copper and lipid peroxidation

Mammalian sperm cells have a highly specific lipid composition that includes a high concentration of polyunsaturated fatty acids, plasmalogen, and sphingomyelin. The sperm membrane's unusual structure is responsible for its flexibility and the functional ability of sperm cells. The lipid component of spermatozoa, on the other hand, is the main substrate for peroxidation, which can cause severe functional disorders in sperm. Low (physiological) levels of lipid peroxidation, on the other hand, reflect the effect of reactive oxygen species (ROS) on sperm metabolism, enhancing the ability of human spermatozoa to interact with zona pellucida (Aitken et al.,1989).

Oxidative stress and copper

Sperm preparation procedures are critical in assisted reproduction etchnology (ART) because the quality of the sperm directly affects the pregnancy outcome. The most important factor that reduces sperm capacity in ART is oxidative damage.

In vitro sperm preparation procedures are critical in ART because sperm quality has a direct impact on pregnancy outcome (F. Zhao, Yang, Shi, Luo, & Sun, 2016). The most important factor affecting sperm reproductive capacity in ART is oxidative damage (Du Plessis, et al., 2015).

Cu acts as a catalyst in the formation of ROS, which can result in oxidative stress and lipid peroxidative damage (Stohs & Bagchi, 1995). Human spermatozoa contain a high concentration of polyunsaturated fatty acids and are capable of producing reactive oxygen species (ROS), primarily superoxide anion and hydrogen peroxide. As a result, they are particularly vulnerable to peroxidative damage due to high polyunsaturated fatty acid concentrations, even though conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen (Aitken et al., 1995). Several aspects of male infertility may be caused by such damage.

In addition, human spermatozoa are protected from peroxidative damage by superoxide dismutase. Oxidative stress caused by accumulated ROS is involved in a number of pathological processes. Germ cells are as vulnerable to the potentially harmful effects of ROS as other cells at sites of gamete production, maturation, storage, and embryo implantation and may thus require antioxidant protection (Taylor, 2001).

ROS will cause mitochondrial membrane potential loss, lipid oxidation, and DNA oxidative damage. As a result of the abnormal intracellular environment, sperm motility and viability were lost (Robert John Aitken, Gibb, Baker, Drevet, & Gharagozloo, 2016). As a result, it is critical to avoid oxidative damage during sperm preparation procedures.

Despite the fact that artificial insemination and in vitro fertilization achieve a high success rate without any treatment to in vitro prepared sperm, there are many clinical cases of failed pregnancy due to oxidative stress of sperm (Aitken & Baker, 2013). Many methods based on antioxidant treatment have been reported to alleviate sperm oxidative stress, according to Ahmad et al. (2017). Ascorbic acid, for example, reduces redox potential and oxidative damage in human spermatozoa. Kopper et al. (2008) reported that the antioxidant Vitamin E has been shown to reduce sperm mitochondrial ROS generation and improve sperm motility. Cu, according to Aydemir et al. (2006), may be a mediator of the effect of oxidative damage and play an important role in spermatogenesis and

male infertility. Meanwhile, Abuja and Albertini (2001) discovered that Cu increases lipoprotein oxidation in vitro. As a result, increased lipid peroxidation and altered membrane function can impair sperm metabolism, motility, acrosome reaction reactivity, and fusogenic capacity, as well as cause oxidative damage to sperm DNA (Cummins et al., 1994).

In contrast, all-lipid components found in sperm membranes are involved in the regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction, and, eventually, membrane fusion (Sanocka & Kurpisz, 2004).

The use of copper in assisted reproductive technology

The failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse is defined as infertility (Zegers-Hochschild et al., 2009). Male factor infertility, according to Mascarenhas et al. (2012), contributed significantly to couples' ability to have children, despite the fact that research and surveillance efforts have primarily focused on female factor infertility. The assessment of sperm parameters, such as sperm concentration, progressive motility, and morphology, is an important part of the diagnostic workup for investigating male fertility status in Assisted Reproductive Technology (ART).

Many microelements, including zinc, Cu, Fe, manganese, cadmium, and selenium, appear to have an impact on sperm quality (Yuyan et al., 2008). Cu plays a role in sperm motility. Although the role of Cu in male reproductive capacity appears to be largely unknown, it may act on pituitary receptors, which control the release of LH (Slivkova et al., 2009).

However, the role of Cu in preimplantation development is still unknown. It is unclear whether the Cu levels in embryo/spermatozoa culture media (both synthetic protein-free culture media (PFM; Ali et al., 2000) and commercial conventional embryo culture media (CCEM) are adequate to maintain normal embryo/motility, vitality, and viability of spermatozoa in vitro. Hence, it is critical to determine whether CCEM/PFM should be supplemented with elemental Cu to improve embryo and spermatozoa development in vitro. The principal author is currently engaged in elucidating the requirement for Cu in embryo and sperm media.

Cu is an essential element in cell culture that contributes to the development of serum-free media. However, traditional media do not typically include it in their base formulation because serum at 5 to 10% concentrations provides adequate Cu for cell growth and survival (approximately 50 to 100 ng/mL). Certain media, such as Iscove's Modified Dulbecco's Medium (IMDM), which is frequently used as a foundation for the development of serum-free hybridoma media, do not contain Cu in their base formulations but are frequently supplemented with albumin. Albumin is a physiological Cu transport molecule, and purified natural albumins are likely to contain enough Cu to support cells in culture at least partially.

In addition, Cu's redox properties, which make it biologically essential, allow it to generate reactive oxygen species and cause oxidative damage to cells (Gaetke & Chow, 2003). Cu is an essential micronutrient for aerobic organisms because it is a cofactor for a number of enzymes that play critical biochemical roles in various metabolic pathways (Kim, Nevitt, & Thiele, 2008). As a result, optimizing the Cu concentration in chemically defined culture media is critical. Cu can mediate a shift toward net lactate consumption in certain Chinese hamster ovary (CHO) cell lines, according to Yuk et al. (2015). (Luo et al., 2012; Qian et al., 2011; Yuk et al., 2015).

Furthermore, Yuk et al. (2015) reported that the shift in lactate metabolism is dependent on the availability of a minimum level of Cu in the culture environment while different Cu concentrations had no effect on cell culture performance, they did affect product quality profiles. For the recombinant CHO tested, the various Cu levels below the postulated threshold produced no further improvements in cell culture performance. Hence, it is critical to optimize the Cu concentration based on the needs of the cell line during cell culture process development to ensure a balance between supplying adequate

Cu and achieving desired product quality attributes.

Therefore, determining the optimal Cu concentration for spermatozoa motility and viability in vitro is desirable. The total Cu concentration in human plasma ranges from 90 to 145 g/dl (0.9-1.45 g/L) (Cayli et al., 2003). Concentrations less than 60 g/dl indicate Wilson's disease or malnutrition; those greater than 150 g/dl may be indicative of cirrhosis, Hodgkin's disease, or acute lymphocytic leukemia (Delves, 1973), and may also be found in women who use oral contraceptives (Hohnadel et al., 1973). The principal author and her coworkers will be publishing their work on the effect of graded concentrations of elemental Cu in ART culture systems in the near future.

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