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Article

Antioxidant Supplementation During in Vitro Maturation

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A B S T R A C T

During in vitro maturation (IVM), oocytes are exposed to different situations from in vivo which may cause oxidative stress.Supplementation of antioxidants to the culture media is effective in as antioxidant defences against reactive oxygen species (ROS). Antioxidant is safe, it has some side effects. This is reviewed in this paper. Studies reported, supplementation antioxidant with different dose showed different effects.Double-edged effects of exogenous antioxidants on cellular responses during in vitro maturation depending potentially on their concentrations. Physiologic doses leading to beneficial effects whereas high doses may result in harmful effects.

I. INTRODUCTION

The Clinical World Health Organisation (WHO) definition of the infertility is a "disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" (Elhussein et al., 2019). According to the WHO, infertility affects up to 15% of reproductive-aged couples worldwide (Dornelles et al., 2016).A systematic analysis of national, regional, and global trends in infertility in more than 190 countries and regions around the world estimated that, in 2010, 48.5 million couples worldwide were infertile. However, according to the Demographic and Health Survey, one in every four couples in low-middle income countries are affected by infertility (Silva et al., 2019). In Indonesia, the number translates to 21.3% of couples, affecting roughly one in every couple (Harzif et al., 2019).

Estimates of infertility rates vary, with the most conservative rates being between 10% and 15% of the reproductive aged population. Alternatively, based on data collected in Indonesia's Demographic Health Survey - 2002, the World Health Organization (WHO) has estimated the female infertility rate (combining primary and secondary infertility) to be 22.3% of married women between the ages of 15 and 45. Based on the current age structure of the population there are approximately 39.8 million Indonesian women of childbearing age. Even when applying the conservative estimate of a 10% infertility rate this translates to a sub-population of around four million women experiencing infertility (Bennett et al., 2012).

Infertility is defined as inability to conceive after 12 months of regular unprotected sexual intercourse. As a disease, many of these will seek medical help to become parentsand many treatment efforts have been suggested (Dornelles et al., 2016).In vitro maturation (IVM) is a modified version of traditional in vitro fertilisation (IVF), whereby oocytes are collected from smaller follicles following little or no exogenous gonadotrophin stimulation. Oocytes are collected when they are immature, and the final stages of maturation are completed in vitro under the influence of culture media additives (Walls and Hart, 2018).

Unfortunately, the quantity and quality of embryos produced from in vitro-matured oocytes is much less than those developing in vivo. Successful oocyte IVM depends on the culture conditions and medium composition.IVM conditions usually increase reactive oxygen species (ROS), which have been implicated as one of the major causes for reduced embryonic development. Therefore, the supplementation of IVM media with antioxidants could improve the survival and development of the oocytes (Barros et al., 2019).Double-edged effects of exogenous antioxidants on cellular responses including oxidative depending potentially on their concentrations. Physiologic doses leading to beneficial effectswhereas high doses may result in harmful effects (Bouayed and Bohn, 2010).

II. METHODS

Studies of antioxidant supplementation during in vitro maturation were identified usingPubMed, ScienceDirect, Scopus, and Portal Garuda to search for all related articles. The searching data using keyword antioxidant, in vitro maturation, infertility, oocyte. The related papers and abstracts were downloaded and reviewed.

III. RESULT

In vitro maturation

Assisted Reproductive Technologies (ARTs) such as in vitro maturation (IVM) was first introduced in patients with polycystic ovary syndrome (PCOS) and patients who had severe ovarian hyperstimulation syndrome (OHSS), but the indications were expanded in recent years and in almost all areas of infertility (Hatirnaz et al., 2018).Since its introduction in the 1990s, IVM

has emerged as an attractive infertility treatment.Early experience with IVM yielded limited success, but advances in IVM protocols and improvements in maturation methods as well as culture media have led to satisfactory pregnancy rates in appropriately selected patient groups (Kyung et al., 2013).

The success of in vitro embryo production (IVP) depends on the proper IVM of an oocyte that determines its competence to develop into an embryo following in vitro fertilization (IVF) and culture (IVC) (Javvaji et al., 2019).Oocyte maturation rates are highly influenced by interactions between the oocyte and the cumulus cells (CCs); these interactions are bidirectional and essential for generating embryos with high developmental potential. Numerous cytoplasmic projections between the CCs and the oocyte facilitate the transfer of molecules that participate in cell metabolism, development and the regulation of meiosis resumption. Consequently, the amount and quality of CCs in each COC play critical roles in oocyte growth, development and maturation (Emanuelli et al., 2019).Maturation is defined in two parts of an oocyte: nuclear maturation visualized by the extrusion of the second polar body and cytoplasm maturation (Salimi et al., 2014).

Successful oocyte IVM depends on the culture conditions and medium composition (Zhiqiang et al., 2017; Salimi et al., 2014). It has been frequently documented that culture condition impaired oocyte maturation and embryonic development by inducing a series of events related to oxidative stress, including accumulation of reactive oxygen species (ROS), increased apoptosis, DNA damage, disordered mitochondria functions and glutathione-glutathione peroxidase (GSH/GPx) system (Xiao et al., 2019). This may decrease the embryo production process in vitro and has a negative effect on the viability and development oocyte (Sovernigo et al., 2017).

Production of reactive oxygen species and generation of oxidative stress

Oxidative stress is caused by an imbalance between pro-oxidants and antioxidants. This ratio could change with increased levels of pro-oxidants, such as reactive oxygen species (ROS), or a decrease in antioxidant defense mechanisms. ROS represents a wide class of molecules that indicate the collection of free radicals (superoxide anion (O2-), hydroxyl radical (OH-), etc.), non-radicals (ozone, single oxygen, lipid peroxides, hydrogen peroxide (H2O2)) and oxygen derivatives (Khazaei and Aghaz, 2017).These radicals are extremely reactive and unstable and therefore may interact with several molecules to acquire electrons in an attempt to become stable. These interactions may, in turn, induce a cascade of chain reactions that can eventually lead to cellular damage, including lipid peroxidation (mainly membrane phospholipids) and the oxidation of amino acids and nucleic acids (Rocha-Frigoni et al., 2016).

Antioxidant

Antioxidant is defined as any substance able to eliminate reactive oxygen species (ROS), directly or indirectly, acting as an antioxidant defense regulator, or reactive species production inhibitor (Salehi et al., 2018).Bioactive compounds, phytochemicals present mainly in fruits, beverages, vegetables and whole grains and they can be synthesized in laboratories(Abdel-Daim et al., 2018).Under physiological conditions, the human antioxidative defensesystem including e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and others.However, our endogenous antioxidant defense systems are incomplete without exogenous originating reducing compoundssuch as vitamin C, vitamin e, carotenoids and polyphenols, playing an essential role in many antioxidant mechanisms in living organisms (Bouayed and Bohn, 2010).

Antioxidant defense system act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Two principle mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical

present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst (Lobo et al., 2010).Recently, researchers understood that toxicity is a complicated process affected by many factors, including developmental exposures, genetic predisposition, and doses. Paracelsus (the father of toxicology) assumed that all chemicals and drugs as well as natural products, including antioxidants, could induce toxicity when received in high doses(Abdel-Daim et al., 2018). Antioxidant can also display prooxidant activities under certain conditions, such as at high dosesor in the presence of metal ions. The prooxidant or antioxidant activity intimately depends on their concentration (Bouayed and Bohn, 2010).

IV. DISCUSSION

In vitro conditions, the gametes and embryos must be manipulated during maturation, fertilization and embryo development in environments that generate oxidative stress. The conditions causing this stress include high oxygen concentration (20 %) compared to the in vivo environment (3 to 5%), exposure to light, culture medium composition, changes in pH, centrifugation processes, and many others. These can negatively affect both gametes and embryos, altering the functionality of biomolecules such as lipids, proteins, and DNA, and thus reduce the quality of embryos and influencing embryo development (Chowdhury et al., 2017; Torres-Osorio et al., 2019). ROS can diffuse and pass through cell membranes and alter most types of cellular molecules, leading to mitochondrial alterations, meiotic arrest in the oocytes, embryonic block, and cell death (Khazaei and Aghaz, 2017).

During oocyte nuclear maturation, gap junctions (GJs) are vital systems of communication between cumulus granulosa cells (CGCs) and oocytes, which mediate a rapid transfer of small metabolites and regulatory molecules among them (Khajeh et al., 2017). This coupling permits granulosa cells to provide the growing oocyte with nucleotides, amino acids, and energy substrates that it is unable to obtain itself. Communication with granulosa cells is also necessary for the growing oocyte to maintain a stable intracellular pH in vitro and promotes chromatin remodeling and acquisition of meiotic competence (El-Hayek and Clarke., 2015).

Excessive generation of ROS in oocytes can overwhelm the antioxidant capacity of the in vitro culture system and thus result in damage to the oocytes, although oocytes can protect themselves from free radical damage by increasing the production of enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) (Junyu et al., 2020). To improve the quality of oocytes derived from IVM, special attention must be paid to the antioxidant capacity of the IVM medium (Junyu et al., 2020). Exogenous antioxidant supplementation can balance within pro-oxidant and anti-oxidant. Supplementation of antioxidants to the culture media is effective in supporting embryo development in vitro as antioxidant defences against reactive oxygen species (Chowdhury et al., 2017).

Understanding the mechanism of drug- and chemical-induced toxicities is the primary interest of many researchers worldwide to develop enhanced preventive and therapeutic strategies. Recently, researchers understood that toxicity is a complicated process affected by many factors, including developmental exposures, genetic predisposition, and doses (Abdel-Daim et al., 2018). At high doses, it has been demonstrated that quercetin (50 µM) can potentiate superoxide radical (O2•-) generation within isolated mitochondria and cultured cells. At higher concentrations (>50 µM), antioxidant decreased cell survival and viability, thiol content, total antioxidant capacity and activities of SOD, and CAT (Bouayed and Bohn, 2010). Over the past decades, beneficial effects of supplementing various antioxidant in IVM medium on oocyte maturation have been demonstrated.

In previous study, the antioxidant activity of quercetin was observed only at low doses (0.1–20 µM) (Bouayed and Bohn, 2010). In another study, supplementation of the medium with low

resveratrol concentrations (0.2, 0.5, 1.0 μM) had beneficial effects on total cell number of blastocysts (Gaviria et al., 2019; Zabihi et al., 2018). 1.0 μM kaempferol can be used as the single antioxidant present in the base medium, maintaining follicular survival, increasing active mitochondria levels, and promoting the oocyte meiotic resumption (Santos et al., 2019). Treatment with 2.0 μM lupeol significantly ($P < 0.05$) improved blastocyst development (Khan et al., 2018). Therefore, antioxidants at high doses could, despite acting as prooxidants, also disrupt the redox balance following their potential to interact with ROS present at physiological concentrations required for optimal cellular functioning, leading to cellular dysfunction (Bouayed and Bohn, 2010).The controversial issue surrounding the benefits of dietary antioxidants for health promotion is the lack of clinical evidence and specific molecular markers able to measure the impact of dietary antioxidants, not only on oxidative stress status, but on health status (Huang, 2018).

V. CONCLUSION

Infertility affects up to 15% of reproductive-aged couples worldwide and they search for treatment. Studies of antioxidant supplementation during in vitro maturation need to research about clinical evidence. Overall antioxidant supplementation in medium IVM is safe, however, proper dosage needs to be considered.

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