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The Adjuvant Impacts of Antioxidant Micronutrients on Ovarian Follicle Development, Oocyte Maturation and Embryo Development of Mammalian Species: A Review

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ABSTRACT

The antioxidant micronutrients refers to minerals (zinc, selenium and copper) and vitamins (A, C and E). Antioxidant micronutrients has been shown to play pivotal roles in development of ovarian follicle, maturation of oocytes and development of embryos, pregnancy and live birth. Antioxidant micronutrients supplemented to mammalian species and *in vitro* culture media were followed. Antioxidant micronutrients are involved in a variety of cellular processes, including protein synthesis, gene expression and cell signaling. They are cofactors for many enzymes involved in metabolism and cell growth. Early ovarian follicle development regulates through antioxidant micronutrients. Antioxidant micronutrients supplementation to the maturation and culture media could improve oocyte maturation and embryo development, leading to higher rates of pregnancy and live birth. Antioxidant micronutrients supplementation had been shown to reduce the risk of chromosomal abnormalities. Antioxidant micronutrients supplementation to vitrification medium of oocytes improves subsequent *in vitro* maturation and fertilization *in vitro* of oocytes. In conclusion, antioxidant micronutrients supplementation are promising supplements for improving the success of assisted reproductive technologies by optimizing the culture environment. This review is designed to discuss the progress in antioxidant micronutrients supplementation on development of ovarian follicle, maturation of oocytes and development of embryos, pregnancy and live birth.

Key words: Blastocyst, Culture, Embryo, Fertilization, Maturation, Oocytes.

The antioxidant micronutrients refers to minerals (zinc, selenium and copper) and vitamins (A, C and E) (Adjepong *et al*., 2016; Opara and Rockway 2006; Mohammed *et al*., 2024 a,b). The progresses in antioxidant micronutrient functions has made over the past decades for the purposes of increasing reproductive performance or treatment of dysfunction (Bouayed and Bohn 2010; Senosy *et al*., 2017, 2019; Vašková *et al*., 2023). The antioxidant micronutrients have pivotal roles for protecting body function from damage caused by free radicals (Gasselin *et al*., 2020; Rathor *et al*., 2023). Therefore, their supplementation to live mammalian species or in vitro culture media might improve ovarian follicle development, oocyte maturation and the further embryo and live birth development (Kassab and Mohammed 2014a,b) (Fig 1).

Antioxidant micronutrients improve reproductive performances through the significant increases in reproductive hormones values, sizes and numbers of ovarian follicles in addition to increases of ovulation rates and corpora lutea development (Mumford *et al*., 2016; Senosy *et al*., 2017, 2019; Chen *et al*., 2023). In addition, antioxidant micronutrients supplementation to maturation media of oocytes promotes maturation and the subsequent embryo development (Yao *et al*., 2023). The antioxidant micronutrients improved embryos' quality and stimulated their mitochondrial function (Gomes da Silva *et al*., 2023). Hence, this article is designed to discuss the potential impact of antioxidant micronutrient supplementation on development of ovarian follicle, maturation of oocytes, development of embryos, pregnancy and live birth.

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Ovarian follicle development

Ovarian structures' development is a complex process that involves the growth and maturation of ovarian follicles.

At birth, ruminant species have about 1-2 million follicles in their ovaries. However, only a few hundred of these follicles will ever develop into mature oocytes and be released over ovulation. The development of ovarian follicles is stimulated by follicle stimulating hormone and luteinizing hormone. The stages of ovarian follicles include primordial follicle, primary follicle, secondary follicle, graafian follicle and corpora lutea (Gordon 2003; Mohammed *et al*., 2022; 2024c,d). Ovarian follicle development is a critical process for successful of reproduction (Fig 2). It allows the females

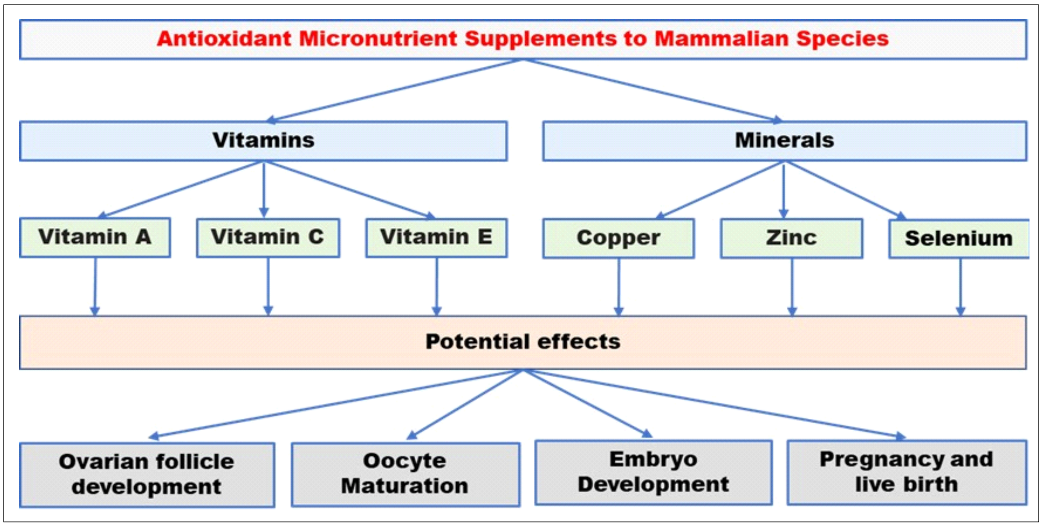
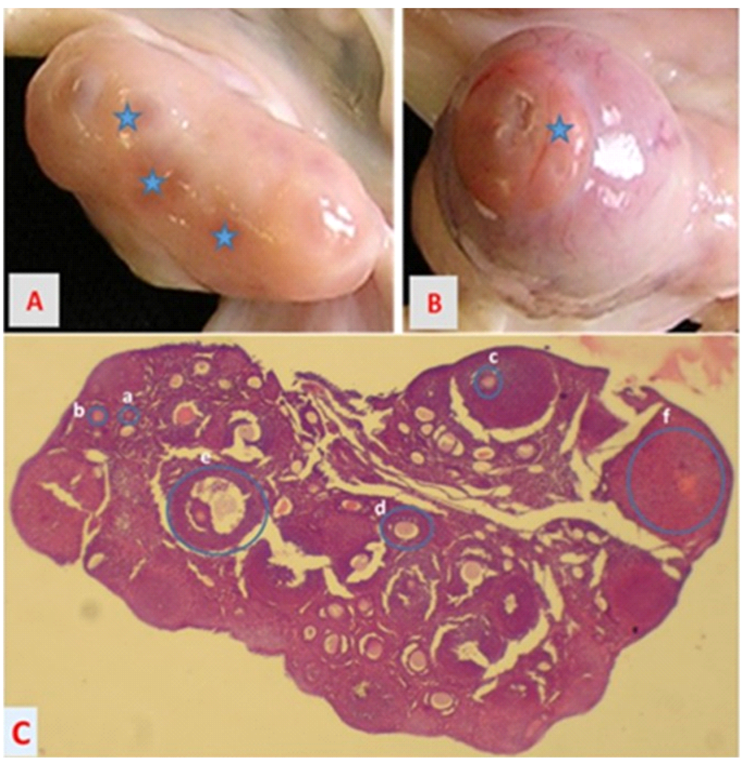


Fig 1: Potential adjuvant influences of antioxidant micronutrient supplements to mammalian species on development of ovarian follicle, maturation of oocytes and development of embryos, pregnancy and live birth.



(A) ovine ovarian antral follicles (B) ovine corpus luteum (C) histology of mouse ovarian follicle structures including primordial follicle (a), primary follicle (b), secondary follicle (c), antral follicle (d) graafian follicle (e) and corpora lutea (f).

Fig 2: Ovarian follicle structures.

to produce oocytes that can be fertilized and develop into live births. The higher sizes of ovarian follicles the higher developmental competence of the resulting oocytes and embryos (Al-Zeidi *et al*., 2022a,b; Aljubran *et al*., 2023).

Antioxidant micronutrients improve reproductive performances through the increases in reproductive hormones' values, sizes and numbers of ovarian follicles in addition to increase of ovulation rates and embryo development during oestrous or menstrual cycles (Senosy *et al*., 2017, 2019; Mohammed 2018; Ali *et al*., 2021; Chen *et al*., 2023; Mohammed *et al*., 2024a,b). Antioxidant micronutrients (minerals and vitamins) protect the follicles from degeneration by free radicals. In addition, the higher levels of antioxidant micronutrients are more likely to ovulate ovarian follicles and promote the chance of pregnancy. Therefore, it is expected, upon ovarian transplantation (Fig 3), stimulation of ovarian follicle development due to antioxidant micronutrient supplementation.

It has been assumed that antioxidant micronutrients are unlikely to be toxic since they are often natural products. Over fifty percent of athletes consumed doses of antioxidant supplements higher than the recommended daily allowance. It is important to note that exogenous antioxidant supplements should not be taken in excessive amounts, which potential harms of supplementation with high doses had been indicated in athletes (Li *et al*., 2022). The high dose harmful effects of antioxidant supplements in athletes include increased oxidative stress, increased plasma monoaldehyde levels and reduced mitochondrial biogenesis (Bryant *et al*., 2003; Yfanti *et al*., 2012; Paulsen *et al*., 2014).

Oocyte quality and maturation

Oocyte quality could be determined through morphological and biochemical features (Mohammed *et al*., 2005; Mohammed *et al*., 2024d). Oocyte morphology, or the physical appearance of an oocyte, is considered one of the most important factors in determining its quality. Good oocyte quality is essential for successful fertilization, embryo development and pregnancy. Oocyte quality is determined through morphological and biochemical features, which is essential for successful fertilization, embryo development and pregnancy. The morphological features include cumulus-enclosed cells, diameter, uniformity of zona pellucida, homogeneous and translucent cytoplasm, appropriate size polar body, perivitelline space and meiotic spindle (Gordon, 2003; Mohammed *et al*., 2005, 2008, 2010, 2019) (Fig 4).

In addition, the biochemical features that have been associated with oocyte quality can be found in the follicular fluid, the cumulus cells and the oocyte. These features might include metabolites, reactive oxygen species, mitochondrial function, gene expression (Gordon 2003, Mohammed *et al*., 2019). Gomes da Silva *et al.* (2023) concluded that feeding trace mineral supplements in proteinate form and selenium-yeast to transition cows promote oocyte quality.

Oocyte maturation is a complex process that involves several key events including cytoplasmic and nuclear changes as germinal vesicle breakdown and progress to metaphase II stages (Gordon 2003; Mohammed *et al*., 2005; Hatirnaz *et al*., 2024) (Fig 5). Oocyte maturation is essential for successful fertilization, zygote formation and the subsequent embryonic development. It allows the oocyte to reduce its chromosome number through meiotic division, which is necessary for the formation of a diploid zygote after fertilization. The zygote then develops into an embryo, fetus and eventually a new individual.

The germinal vesicle nucleus is not visible in the cytoplasts of ruminants oocytes whereas is it visible in rodent, rabbit and human oocytes (Mohammed *et al*., 2005, 2022). To visualize the GV nucleus in ruminant oocytes used in assisted reproductive techniques, ultracentrifugation at 15,000 rpm for 15 min is required. The time required for the oocyte to reach GVBD stage is 2-3 h of mouse oocytes and 5-6 h of ruminant and human oocytes as well. In addition, the time required for the oocyte to reach metaphase II stage is 15-17 h of mouse oocytes and 24 h of ruminant and human oocytes (Gordon 2003; Mohammed *et al*., 2005).

Concerning to antioxidants effects, Yao *et al.* (2023) concluded that zinc supplementation enhances ovine oocyte maturation and the following embryo development. Lai *et al.* (2023) showed that zinc deficiency compromises the maturational competence of porcine oocyte through apoptosis induction. Tripathi *et al.* (2023) showed that antioxidants in matured oocytes caused alterations in mRNA related gene expression of growth, stress and apoptosis. They reduced oxidative stress in oocytes by decreasing ROS levels, thus leading to improvement of embryo quality and quantity. Collectively, antioxidant micronutrients supplementation to *in vivo* live organism or

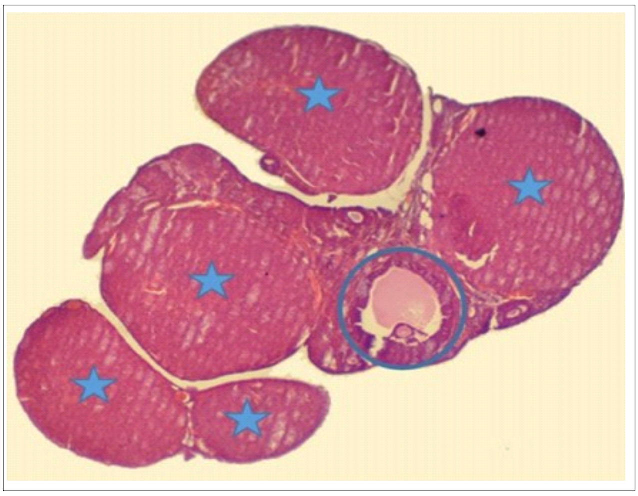


Fig 3: Rat ovarian follicle structures of transplanted ovarian tissue during luteal phase indicating copora lutea (★) and large antral follicles (O) .

in vitro culture media is indicated to promote oocyte maturation and resulting embryo development.

Embryo development

Antioxidant micronutrients are essential for several physiological processes in embryos of humans and animals (Arhin *et al*., 2017). The improvement of oocyte maturation through antioxidant micronutrients is known to play a significant role in embryo developmental competence (Yao *et al*., 2023). Improvement of cytoplasm maturation promotes developmental competence of embryos produced (Mohammed *et al*., 2005; Córdova *et al*., 2010). Antioxidants improve the quality of embryos produced *in vitro*. Castillo-Martín *et al*. (2014) found enhancement of

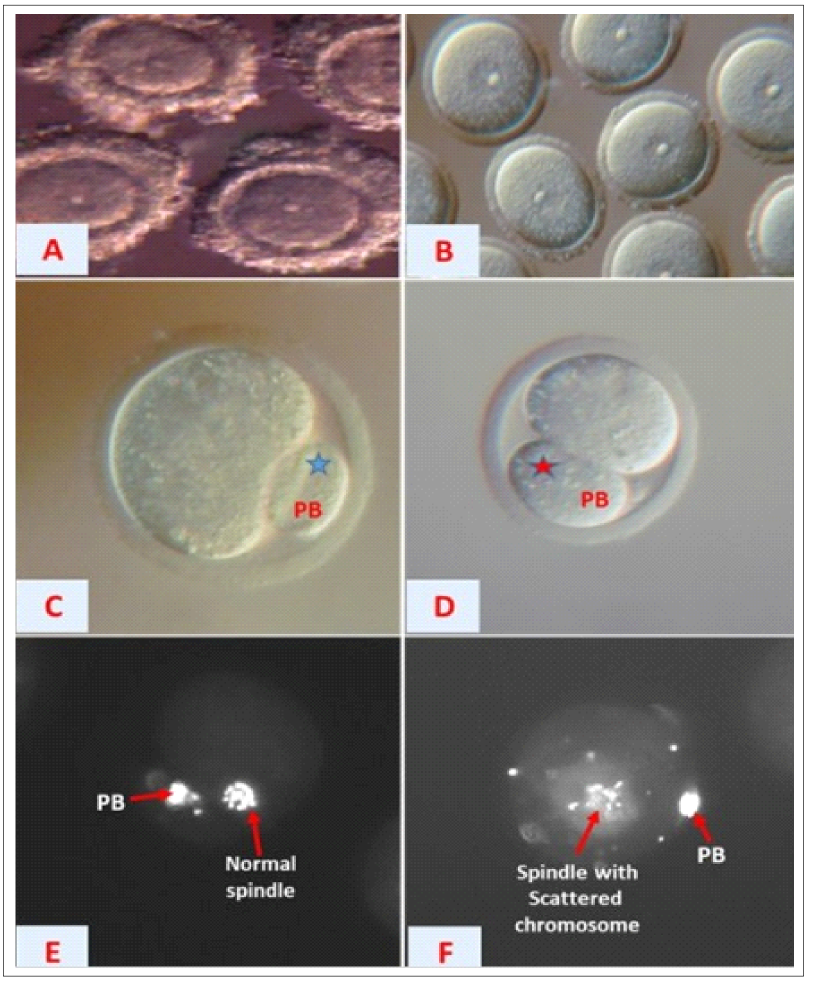


Fig 4: Mouse oocytes (A) Cumulus-enclosed GV oocytes (B) Denuded GV oocytes (C) Normal polar body (PB) (D) Large polar body (PB) Normal meiotic spindle (E) Scattered chromosome meiotic spindle (F).

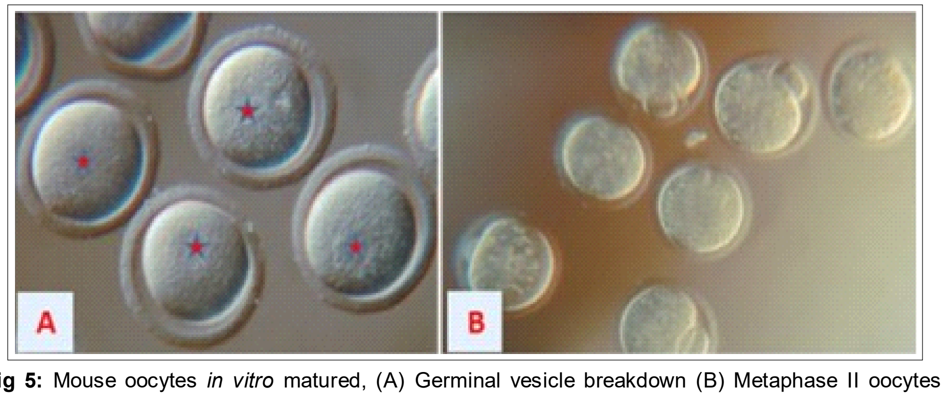


Fig 5: Mouse oocytes *in vitro* matured, (A) Germinal vesicle breakdown (B) Metaphase II oocytes.

survival rate and redox status of *in vitro* produced porcine blastocysts upon supplementing L-ascorbic acid. Additionally, zinc supplementation to vitrification medium improves oocyte maturation and fertilization *in vitro* (Geravandi *et al*., 2017). Antioxidant micronutrients can protect embryo cells from damage caused by free radicals and can protect the embryo from oxidative stress. Supplementing oocytes and embryos with a combination of antioxidants improved the quality and quantity of embryos produced through protecting the embryo's DNA from damage, reducing inflammation, improving blood flow to the uterus and supporting cell growth and development.

Fertility and reproduction

Antioxidant micronutrients are essential and played important roles in the normal growth and reproduction in animals and humans as well (Hiten and Kurlak, 2015). They play pivotal roles in protecting sperms and oocytes from damage, improving hormonal production and supporting the development of the embryos and feti (Al-Gubory *et al*., 2010). Vitamin C is essential for the production of collagen, which is important for the development of the placenta and uterus (Lo *et al*., 2015). Vitamin E and zinc is important for hormonal production as testosterone and progesterone in addition to sperm development and function (Jalali *et al*., 2020; Sánchez-Rubio *et al*., 2020; Weiss, 2022). Selenium is well-known to support maximal expression of the selenoenzymes, sperm development and function (Zhou *et al*., 2023; Zhu *et al*., 2023). Women supplementing during pregnancy vitamin C, vitamin E and beta-carotene had higher pregnancy rates (Menard, 1997). In addition, supplementation of vitamin C, vitamin E, selenium and zinc before assisted reproductive technology had a higher sperm quality including sperm count and motility (Majzoub and Agarwal 2018; Khalil *et al*., 2019). Hence, supplementing the requirements of antioxidants is essential to maintain essential functions within the body, specially fertility and reproduction. On the other hand, insufficient stores or intake of antioxidant micronutrients can have adverse effects on the pregnancy and fetus development. Numerous studies implicate antioxidant micronutrients deficiency in several reproductive and obstetric complications including female and male infertility (Mangione *et al*., 2023).

CONCLUSION

Antioxidant micronutrients supplementation are promising supplements for improving the success of assisted reproductive technologies. By optimizing the requirements of antioxidant micronutrients to live organisms and *in vitro* culture conditions, the supplementation can help to improve oocyte quality, maturation, embryo development and pregnancy and delivered offspring.

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Conflict of Interest

The authors declare no conflicts of interest.

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