Received: 27 July 2016 | Accepted: 28 September 2016

DOI: 10.1002/rmb2.12013

REVIEW ARTICLE

WILEY Reproductive Medicine and Biology

Equilibrium between anti-oxidants and reactive oxygen species: a requisite for oocyte development and maturation

Manika Kala[1,2] | Muhammad Vaseem Shaikh[1,2] | Manish Nivsarkar[1]

[1]Department of Pharmacology and Toxicology, B. V. Patel Pharmaceutical Education and Research Development Centre, Ahmedabad, India

[2]Faculty of Pharmacy, NIRMA University, Ahmedabad, India

Correspondence
Manish Nivsarkar, B. V. Patel Pharmaceutical Education and Research Development Centre, Ahmedabad, Gujarat, India.
Emails: manishnivsarkar@gmail.com; perd@perdcentre.com

Abstract

Reactive oxygen species (ROS) are required for cellular functioning and are controlled by anti-oxidants. The ROS influence the follicles, oocytes, endometrium, and their environment. The luteinizing hormone surge initiates a massive recruitment of ROS that modulates major reproductive functions namely, oocyte maturation, ovarian steroidogenesis, corpus luteal function, and luteolysis. The anti-oxidant system balances ROS generation and maintains the cellular functions. Both enzymatic and non-enzymatic anti-oxidants namely, vitamins and minerals are present in the follicles and protect the oocytes from the damaging effects of ROS. The overproduction of ROS leads to oxidative stress that affects the quality of oocytes and subsequent anovulation. Although researchers have tried to establish the role of ROS and anti-oxidants in oocyte development, still this aspect needs to be revisited. This review discusses the importance of the ROS and anti-oxidant balance that is required for the development and maturation of oocytes. There are increasing data on the activity of ROS and anti-oxidants in supporting oocyte development and maturation. However, extensive research is required to identify the safe physiological concentration and duration of both the ROS and anti-oxidants that are required to facilitate oocyte development and maturation during in vitro and in vivo conditions.

KEYWORDS

anovulation, anti-oxidants, oocyte maturation, ovulation, reactive oxygen species

1 | INTRODUCTION

During the human reproductive life span, each month several oocytes undergo the process of development and maturation to ovulate one successfully matured and healthy oocyte for fertilization. Approximately 300-400 oocytes become available for fertilization and the rest undergoes follicular atresia. The same process of development and selection is observed in other mammals. The process ensures successful fertilization by regulating the health and quality of oocytes and avoids the chance of multiple pregnancies.

The follicular micro-environment plays an important role for successful oocyte differentiation and subsequent fertilization. A bidirectional regulatory loop exists between the oocyte and its surrounding follicular somatic cells, which regulate the development of competent oocytes.[1] The importance of the quality of the oocyte released was realized by the fact that only 7% of the oocytes retrieved by in vitro fertilization (IVF) develop into a normal embryo that yields a live birth.[2] This could be because ovarian stimulation in IVF has detrimental effects on oogenesis, with the production of aneuploidity,[3] lower endometrial receptivity, reduced embryo quality, and probably perinatal outcomes,[4] thus reducing the success rate of IVF. In women with polycystic ovary syndrome (PCOS) or cancer, the in vitro maturation (IVM) technique was used to preserve fecundity in the form of immature oocytes that were obtained without any hormonal stimulation. However, the IVM success rate was low as the quality, fertilization, implantation, and pregnancy rate of embryos that were derived by IVM was less compared to in vivo matured oocytes.[5,6]

This can be attributed to the scarcity of information regarding the specific biochemical factors that are required for the oocytes during the relevant developmental windows. With advanced research on all factors that are likely to influence oocyte maturation, it might be possible to develop a culture system that is optimal for IVM. Hence, there is a need for quality research of these factors that ensure the quality of the oocyte.

Recently, much attention has been paid to the role of reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in IVM of the oocyte.[7] The ROS are produced during metabolic processes in all living beings. They act as a signaling molecule in follicular fluid and regulate oocyte maturation, meiotic arrest, and resumption.[8–11] The plausible mechanism underlining these effects of ROS is linked with the stimulation of 5′ adenosine monophosphate-activated protein kinase (AMPK) and/or the Ca^{2+}-mediated pathway that induces meiotic resumption from diplotene arrest in mammalian oocytes.[9,12–14] Also, the suppression of ROS by anti-oxidants reversibly inhibits the resumption of meiosis in rat oocytes in vitro.[15]

Excessive ROS generation also can be detrimental to cellular function and can lead to oxidative stress, which results in enzyme inactivation, lipid peroxidation, ATP depletion, and mitochondrial perturbation. A high level of ROS and low anti-oxidant activity in follicular fluid has resulted in a reduced pregnancy outcome by IVF.[16,17] An increased ROS level during meiotic maturation in vitro can induce chromosomal errors and affect the oocyte's developmental competence.[18] Increased ROS generation has been shown to cause alteration in the microtubule organization and chromosomal alignment of the metaphase II meiotic spindles in the oocytes of mouse in vitro[19] and inhibit their maturation.[20] The oxidative damage can induce the release of cytochrome C and other apoptogenic factors from the mitochondria, which eventually lead to cellular apoptosis.[21] Thus, it can be implied that equilibrium is required between ROS and anti-oxidant generation for the development of a competent oocyte.

In the reproductive tract, increased ROS generation is limited by a variety of anti-oxidant enzymes, such as superoxide dismutase (SOD), catalase, and various peroxidases. The SOD causes a dismutation of superoxide radicals to H_2O_2, which is further detoxified to water and oxygen by catalase or glutathione peroxidase (GPx). Catalase is found primarily within the peroxisomes of most cells and catalyzes the conversion of H_2O_2 into water and oxygen, while GPx catalyzes the degradation of lipid peroxides, as well as H_2O_2. These mechanisms regulate the ROS concentration in the reproductive tract, thus ensuring normal functions. Recently, a correlation was observed between increased mRNA expression of SOD subtypes and a decreased quality of matured oocytes that might result in anovulation in psychologically stressed mice.[22] This indicates that an over-activity of these protective mechanisms also might alter the process of oocyte development and maturation. Thus, it could be implicit that the generation of ROS is beneficial for the maturation of oocytes,[23] but that the over-production of ROS or a depletion in the anti-oxidant system leads to oxidative stress that could affect oocyte quality, thereby leading to anovulation.[20,22,24] In light of these studies, further research is required in order to identify the physiological concentration of ROS and anti-oxidant enzymes that is necessary for successful oocyte maturation and development.

The present review elaborates on the role of ROS and its scavengers as a regulator of oocyte development, maturation, and follicular atresia and examines the importance of this critical balance between anti-oxidants and ROS. The relevant data were searched through the computer-based literature by full-text search in Pubmed, ScienceDirect, CrossRef Search, and Embase. The functions of pro-oxidants and anti-oxidants during the development and release of oocytes is reviewed and thereby gives direction to the further research that is required in this area.

2 | PHYSIOLOGICAL RELEVANCE OF REACTIVE OXYGEN SPECIES IN OOCYTE MATURATION

Several reports suggest that ROS are allegedly involved in the maturation and quality of the oocytes that are released (Table 1). In humans, the maturation of ovarian follicles includes various stages. Inside the ovary, the oocyte is present in the primordial follicle and is surrounded by epithelial-like somatic cells. Eventually, some of the primordial follicles are recruited and matured into primary, secondary, and antral follicles. Many of these follicles undergo atresia and the rest develops into fully grown Graafian follicles. Finally, following a surge of luteinizing hormone, the oocyte matures and is released into the oviduct (Fig. 1). The process of oocyte maturation requires a rigorous supply of energy in the form of adenosine triphosphate (ATP) to fuel the transcription process, to increase the size of the follicles, as well as that of the oocyte.[25] In the human oocyte, the content of ATP at metaphase II (MII) arrest is positively correlated with a successful fertilization and IVF outcome.[26] The ATP generation by the mitochondrial electron transport chain during the maturation process results in the production of ROS. This high level of ROS is oxidized by anti-oxidant enzymes; namely, catalase, SOD, glutathione transferase (GST), paraoxonase, heat shock protein 27, and protein isomerase, which are present in the follicular fluid and eventually protect the oocytes from the harmful effects of ROS.[27]

In follicles, apart from mitochondrial respiration and oxidative phosphorylation, some other mechanisms also contribute to ROS release. Luteinizing hormone (LH) signals the corpus luteum to produce and secrete progesterone.[28] The process is highly complex and regulated at several stages enlisted below. Initially, after LH signaling, cholesterol is converted to pregnenolone in the mitochondria by cytochrome P450 side-chain cleavage that releases ROS.[29] Pregnenolone is further processed to progesterone by 3β-hydroxysteroid dehydrogenase at the endoplasmic reticulum that requires oxidized nicotinamide adenine dinucleotide (NAD^+) as the hydrogen acceptor. The NAD^+ is generated by the oxidation of NADH by ascorbic radicals through a free radical mechanism that is also regulated by ROS.[30] This suggests the important role that ROS plays during the process of LH-induced progesterone release. However, a study reported that LH also stimulates the intraluteal expressions of Copper/Zinc-dependent SOD (Cu/Zn-SOD), manganese-dependent SOD (Mn-SOD), and catalase to maintain the luteal cell viability and ROS balance.[28] This study indicates that LH not only induces ROS production but also regulates it by stimulating the anti-oxidant enzymes' expression, thus maintaining the redox state.

TABLE 1 Studies reviewed about the role of reactive oxygen species in oocyte maturation and development

Study references	Experimental model	Significant findings
Chaube et al.[8,9] Tripathi et al.[10] Martin-Romero et al.[11]	Rat/human oocytes	Concentration-dependent role of H_2O_2 on meiotic resumption: (a) lower concentration induces resumption from diplotene arrest in oocytes and (b) increase of ROS is associated with meiotic cell cycle arrest and apoptosis
Chaube et al.[9]	Rat	Calcium ionophore-induced activation and apoptosis are associated with the generation of intracellular H_2O_2
Choi et al.[12]	NIH-3T3 cells	AMPK was transiently and concentration-dependently activated by H_2O_2
Chen et al.[13]	Mouse	AMPK activation provides a potent meiosis-inducing signal in vitro
Lee et al.[14]	Rat-2 fibroblasts	Intracellular H_2O_2 was responsible for the EGF-stimulated elevation of Ca^{2+}
Takami et al.[15]	Oocyte–cumulus complexes of rat	Cell-permeant anti-oxidants inhibit the spontaneous resumption of meiosis
Kawaguchi et al.[28]	Bovine luteal cells	LH-increased anti-oxidant enzymes resulted in an increase in cell viability during the luteal phase
Attaran et al.[31]	Humans undergoing IVF	Women who became pregnant had a high ROS level in their follicular fluid
Yuan and Krisher[34]	Porcine oocytes	An adequate ROS balance is important for oocyte quality
Tripathi et al.[35]	Rat	Melatonin reduces oxidative stress by scavenging H_2O_2, slowing down meiotic cell cycle progression, and protecting against apoptosis in eggs
Tiwari and Chaube[36]	Rat	A moderate increase in ROS in the ovary is beneficial for meiotic resumption from diplotene arrest
Goud et al.[37]	Mouse	O_2^-, H_2O_2, and HOCl augment oocyte aging
Fu et al.[40]	Porcine	Alterations in the balance of pro- and anti-apoptotic gene expression can lead to follicular atresia
Stanley et al.[41]	Rat	Lactational exposure to chromium accelerates follicular atresia and decreases steroidogenesis by altering the ratio of ROS to anti-oxidants in the ovary
Sugino et al.[43]	Rat	The ROS level increased during the regression phase
Margolin et al.[44]	Rat	Peroxides act as a mediator of luteolysis by inhibiting LH-sensitive cAMP and progesterone production
Oyawoye et al.[45]	Humans undergoing IVF	ROS play a role in ovulation, fertilization, and conception

AMPK, 5′ adenosine monophosphate-activated protein kinase; cAMP, cyclic adenosine monophosphate; EGF, Epidermal growth factor; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; IVF, in vitro fertilization; LH, luteinizing hormone; ROS, reactive oxygen species.

The follicular fluid contains leukocytes, macrophages, and cytokines that can produce ROS and enhance the flow of blood to the ovary for the development of the oocyte.[31,32] The physiological concentration of ROS has an essential role in follicular rupture and acts by modulating the expression of the genes that govern the processes of oocyte maturation.[33] Clinically, it was observed that the concentration of ROS in the follicular fluid is a deciding factor for success in patients (with tubal diseases, endometriosis, and idiopathic infertility) undergoing IVF. It has been reported that those patients who got pregnant had a significantly high ROS level, as compared to those who did not get pregnant.[31] An adequate ROS balance is also important for IVM and embryonic development of the oocyte as the addition of extra anti-oxidants is detrimental to blastocyst formation.[34] Studies reported that an increased level of H_2O_2, as well as ROS, and decreased catalase activity trigger meiotic resumption from diplotene arrest in rat follicular oocytes, suggesting the importance of ROS in the maturation of oocytes.[8,10,35,36] The ROS also augment aging in the relatively old oocytes, rather than the young oocytes. This might suggest a ROS-regulated mechanism to maintain the quality of oocytes and ensure fertilization.[37] Most of these studies are limited to exploring the effect of ROS on oocyte maturation and development, without focusing on the physiological concentration of ROS that is required. Also, studies are required to explore other factors that could act alone or along with the ROS that are generated in the body and play an important role during the process. Moreover, more specific and advanced techniques are required for measuring the effect of ROS on oocyte development.

3 | REACTIVE OXYGEN SPECIES INVOLVEMENT IN FOLLICULAR ATRESIA

During reproductive life, a small fraction of the ovarian follicles that are present at birth complete their way to ovulation, while the rest undergoes a degenerative process called “atresia.” Studies have demonstrated that follicular atresia is caused by the apoptosis of granulosa cells.[38] Apoptosis is a cell-specific mechanism for the discrete elimination of cells during follicular atresia that ensures the regression of the follicle without inciting an inflammatory response. It is recognized as a hallmark and contributing factor of the atresia of antral follicles.[39] The

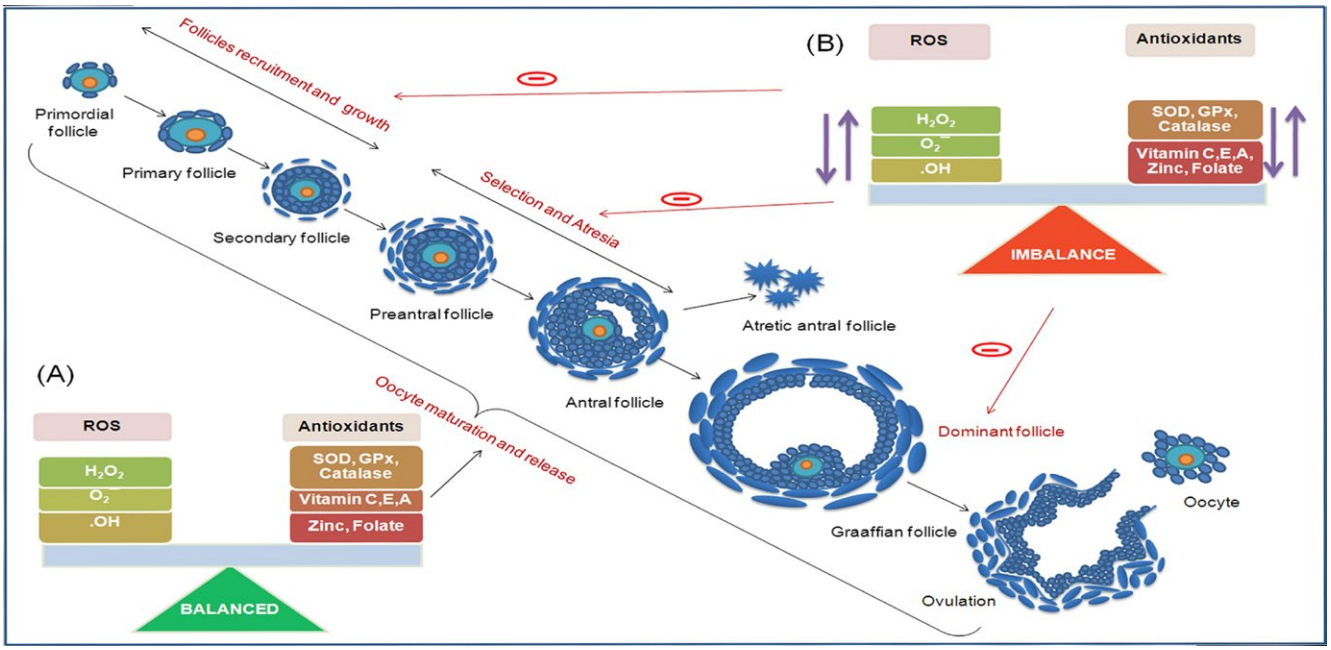

FIGURE 1 Follicular growth during the process of ovulation. (A) A balance in reactive oxygen species (ROS) and anti-oxidants is required during follicular maturation, follicular atresia, and the growth of the dominant follicle and (B) an imbalance in ROS and anti-oxidant generation lead to ovulatory dysfunction. GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; O_2^-, superoxide; SOD, superoxide dismutase

expression level of various apoptotic and anti-apoptotic genes was studied in RNA that was isolated from atretic follicles. An expression study in a single follicle showed that an increase in anti-apoptotic gene transcription could prevent apoptosis by protecting against the effects of pro-apoptotic gene expression. However, alterations in the balance of pro- and anti-apoptotic gene expression could lead to follicular atresia.[40] The lactational exposure to hexavalant chromium accelerated follicular atresia and decreased steroidogenesis in F1 rat female offspring by altering the ratio of ROS and anti-oxidants in the ovaries.[41]

Accumulating evidence shows that excessive ROS generation triggers antral follicular atresia by causing granulosa cell apoptosis.[42] The ROS are also involved in the loss of sensitivity of the granulosa cells to gonadotrophin hormones and in the loss of steroidogenic function, which are characteristics of follicular atresia. Moreover, in the rat ovarian corpus luetum, concentrations of ROS increased during the regression phase.[43] Thus, ROS might initiate apoptosis in the luteal cells and inhibit their function at an appropriate time during the female menstrual cycle. This evidence suggests the role of ROS in the atretic regression of all the follicles, except one for ovulation.[44] Indeed, studies to elucidate the ROS-regulated pathways that are involved in the process of atresia are required.

4 | ANTI-OXIDANTS

The ROS are inevitably generated during physiological processes; however, an excess of them results in oxidative stress, which is balanced by anti-oxidants. These act as the first line of defense against ROS and protect the cells from any damaging effect. Both the ROS and the anti-oxidants are required for ovulation and an imbalance between them could lead to the production of an incompetent oocyte. Although both non-enzymatic and enzymatic anti-oxidants are required, enzymatic detoxification is more efficient. Studies that underline the role of anti-oxidants in oocyte maturation are mentioned in Table 2.

4.1 | Enzymatic anti-oxidants involved in follicular development and oocyte maturation

Follicular fluid contains high concentrations of anti-oxidants,[45] which protects oocytes from ROS-induced damage. An imbalance in the pro- and anti-oxidant systems in the follicular fluid could lead to abnormal development of the oocytes and impaired fertility, as well as damage to the oocyte's DNA, cytoskeleton, or membrane. The cytoskeleton ensures that meiosis occurs in the oocyte before fertilization for formation of the haploid gamete.[46]

Anti-oxidant defense systems, like SOD, GPx, catalase, and non-enzymatic anti-oxidants like vitamin E, vitamin C, glutathione, uric acid, and albumin are present in the follicles.[24,34] The SOD, GPx, and catalase activity have been detected in bovine oocytes and cumulus cells that might regulate the ROS levels during IVM.[47] Also, transcripts corresponding to GPx, Cu/Zn-SOD, Mn-SOD, and γ-glutamylcysteine synthetase have been shown to be present in mature MII mouse and human oocytes, with higher expression of Cu/Zn-SOD in humans.[48] Anti-oxidants like cysteine showed a significant improvement in the proportion of oocytes undergoing morula and blastocyst development in bovine oocytes during IVM.[49] The increase in catalase activity in follicle-stimulating hormone (FSH)-treated granulose cells suggests its role in the development and selection of the dominant follicle.[50] Mature MII oocytes of hamster showed a higher concentration of glutathione, which might aid in meiotic spindle formation and pronucleus

TABLE 2 Studies reviewed about the importance of anti-oxidants in oocyte maturation and development

Study references	Experimental model	Significant findings
Cetica et al.[47]	Bovine oocytes	Enzyme activity diminished in the cumulus cells and increased in the oocytes due to maturation (specifically SOD)
El Mouatassim et al.[48]	Mouse and human oocytes	Maturation-specific polyadenylation of transcripts encoding Cu/Zn-dependent SOD, Mn-dependent SOD, GPx, and γ-glutamylcysteine synthetase was observed
Behl and Pandey[50]	Goat	Enzyme catalase might have a functional role in goat ovarian follicular development under endocrine regulation
Zuelke et al.[51]	Hamster oocytes	GSH plays important roles in oocyte spindle function and pronucleus development
Tatemoto et al.[52]	Porcine follicular fluid	Porcine follicular fluid had a high level of SOD activity, compared to that of FBS, and this activity was markedly blocked by the Cu/Zn-dependent SOD inhibitor
Dharmarajan et al.[53]	Rabbit	Gonadotropin-mediated inhibition of apoptosis in rabbit luteal cells enhanced the expression of Mn-dependent SOD, which protects from the ROS and/or down-regulating the expression of Bax (a pro-oxidant member of the Bcl-2 protein family)
Paszkowski et al.[54]	Humans undergoing IVF	Reduced level of selenium-dependent GPx was reported in the follicular fluid of women with unexplained infertility
Byrd et al.[58]	Hen	Sodium ascorbate could be associated with LH-stimulated progesterone biosynthesis
Crha et al.[59]	Human	Vitamin C supplementation increased the number of pregnancies observed in women undergoing IVF
Tarrin et al.[61]	Mice	Oral administration of Vitamin C and E improved the quality of oocytes retrieved from aged mice
Barzegari et al.[64]	Mice	α-Tocopherol increases the maturation rate of follicles and enclosed oocytes
Tareq et al.[65]	Porcine	A combination of selenium and vitamin E could play important roles in increasing the rate of maturation of porcine oocytes and fertilization, as well as in the development of the blastocyst and use of glucose in IVM, fertilization, and culture to the blastocysts of porcine oocytes
Lisboa et al.[66]	Cattle	α-Tocopherol maintains the survival of cattle pre-antral follicles and promotes the activation of primordial follicles in in vitro culture
Tian and Diaz[68]	Mice	Acute zinc deficiency causes profound defects during the peri-ovulatory period
Picco et al.[70]	Bovine oocytes	Zinc significantly affected intracellular GSH content and DNA integrity of cumulus cells during oocyte maturation and improved pre-implantational embryo development
Szymanski et al.[71]	Human	Supplementation of folic acid diminishes a concentration of homocysteine, resulting in better-quality, and higher degree of maturity of, oocytes
Bahadori et al.[72]	Mice	A dose-dependent response to melatonin treatment was observed on IVM, fertilization, and embryo development of mouse oocytes

FBS, fetal bovine serum; GPx, glutathione peroxidase; GSH, glutathione; IVF, in vitro fertilization; IVM, in vitro maturation; LH, luteinizing hormone; ROS, reactive oxygen species; SOD, superoxide dismutase.

development.[51] The increased level of SOD that was observed in follicular fluid efficiently reduced the DNA damage that was caused by oxidative stress in porcine oocytes and cumulus cells, resulting in successful fertilization and development to the blastocyst stage after in vitro insemination; however, these capabilities were interrupted by the Cu/Zn-SOD inhibitor.[52] Meanwhile, Mn-SOD expression suppresses apoptosis in the rabbit corpus luteum in vitro, indicating that Mn-SOD is responsible for the gonadotropin-mediated inhibition of apoptosis.[53] A reduced anti-oxidant enzyme level of selenium-dependent GPx was reported in the follicular fluids of women with unexplained infertility.[54] Follicles with fertilized oocytes had a higher level of GPx activity than the follicles with unfertilized oocytes. This decreased anti-oxidant activity negatively affects the fertilization ability of the oocyte.[54]

Despite the extensive work, some aspects need to be explored further: (1) the physiological concentration of the anti-oxidants that are required; (2) the molecular mediator of the anti-oxidants assisting oocyte development and maturation; and (3) the regulators of the anti-oxidants that are required for oocyte maturation and development.

4.2 | Role of vitamin- and mineral-based anti-oxidants

Many non-enzymatic anti-oxidant vitamins and minerals, such as vitamin C, vitamin E, selenium, zinc, and beta carotene, are ordinarily present in nutrients.[55] Both enzymatic and non-enzymatic anti-oxidants are present in the follicles and protect the oocytes from the damaging effects of ROS. The ovary is considered as the primary site of ascorbic acid accumulation and turnover, with the highest concentration in the theca interna, granulose cells, and luteal compartment.[56] A change in the ovarian content of ascorbic acid that occurs at mid-cycle in women is associated with LH secretion and appears as a biphasic response with increased excretion in the late follicular phase that declines immediately

prior to ovulation and increases again immediately after the rise in body temperature.[57] In vitro studies in chicken granulose cells showed that sodium ascorbate is stimulatory to progesterone secretion.[58] Vitamin C supplementation led to a high ascorbic acid concentration in the follicles and had an impact on the number of pregnancies in non-smoking women.[59] Also, a deficiency in vitamin C showed marked ovarian atrophy, along with widespread follicular atresia and the premature resumption of meiosis, in guinea pigs.[60] The oral administration of a pharmacological dose of vitamins C and E improves the number and quality of oocytes that are retrieved from female aged mice.[61] The role of ascorbic acid in follicular growth, repair of the ovulated follicle, and corpus luteum development has been reviewed elsewhere.[60,62] Vitamin A and its metabolites that have been used in artificial reproductive techniques (including superovulation, ovum pick-up, and IVM) in cattle provides evidence for its role in promoting cytoplasmic maturation.[63] The role of α-tocopherol in improving folliculogenesis, oocyte quality, fertilization rates, and embryo development have been reported in previous studies.[64,65] The dietary supplementation of α-tocopherol maintains the survival of cattle pre-antral follicles and promotes the activation of primordial follicles in in vitro culture.[66]

Zinc is one of the most important minerals with anti-oxidant properties that is required to protect oocytes from free radicals and ROS.[67] It is required for follicular rupture and the completion of meiosis.[68] A deficiency in zinc resulted in the altered synthesis and secretion of FSH or LH, ovarian development, estrous cycle disruption, frequent abortion, an extended gestation period, teratogenicity, stillbirths, complexity in parturition, pre-eclampsia, toxemia, and inferior infant birth weights.[69] During oocyte maturation, the concentration of zinc affects the intracellular glutathione content and DNA integrity of the cumulus cells, thus improving pre-implantation embryo development.[70] Folic acid supplementation in the diet of women showed a higher quality and maturity of oocytes, compared with those who did not receive folic acid supplementation.[71] Melatonin, the popular free radical scavenger, has been shown to promote cumulus cell expansion, in vitro oocyte maturation, and embryo development.[72,73] Non-enzymatic anti-oxidants are also required for oocyte development and maturation. However, whether they assist the enzymatic anti-oxidants or whether they solely ensure the developmental competence of the oocyte still needs to be explored further.

5 | REACTIVE OXYGEN SPECIES AND ANTI-OXIDANT BALANCE: QUESTIONS STILL UNANSWERED

The ROS and anti-oxidant balance is a prerequisite for ovulation. Elevation in either of them disturbs the redox balance, which might be the underlying cause of ovulatory dysfunction in women with unexplained infertility. The relationship between the ROS and anti-oxidant balance during follicular development is shown in Figure 1. Both the ROS and anti-oxidants work synchronously to carry out a successful development and maturation of the oocytes. However, the concentration and duration of both that are required is largely unknown. A high level of ROS (more than the physiological concentration) could trigger membrane permeability factor destabilization and a reduction in the survival factors, leading to mitochondria-mediated oocyte apoptosis and cell cycle arrest.[8,33] Both death-receptor and mitochondria-mediated pathways are involved in inducing oocyte apoptosis. The oxidative stress-induced mitochondria caspase-mediated pathway plays a major role in the elimination of germ cells from the cohort in the ovary and deteriorates oocyte quality even after ovulation.[74]

Anti-oxidants like vitamin C at higher concentrations might act as pro-oxidants. Vitamin C acts as a reducing agent and in higher doses has been postulated as a cause of infertility.[75] Studies in animals have reported that the administration of oral anti-oxidants can counteract the negative effect of female aging on the number and quality of oocytes.[61] However, vitamin C and E might induce some side-effects on reproductive fitness and impair the ovarian and uterine functions of women.[62] Thus, there is a need to identify the safe physiological concentration of the anti-oxidants that are required for reproductive functions. In a clinical trial, N-acetyl cysteine (NAC), a precursor of glutathione, showed promising results in women with PCOS. Treatment with a combination of clomiphene citrate and NAC in women with PCOS showed significant improvements in the number of follicles that were >18 mm, mean endometrial thickness, ovulation rate, and pregnancy rate. No adverse effect was observed and no case of ovarian hyperstimulation was reported in the study.[76] However, multicentric randomized trials are required to prove the effectiveness of this treatment further.

Oxidative stress causes an upsurge in the level of oxidants, rather than the depletion of anti-oxidants. The specific source that alters the crucial balance between ROS and anti-oxidants is still unclear and needs to be identified. The conventional treatment strategies focus on the use of anti-oxidants to overcome the oxidative stress; however, no work has concentrated on alternative methods to reduce excess oxidant generation. Moreover, ROS can be measured by a number of indirect methods, yet no method has been accepted universally. A uniform and reliable method is required for oxidative stress estimation, which could avoid errors while comparing the results from studies that used different measures of oxidative stress. Also, studies should focus on examining the molecular and cellular mechanisms of pro-oxidants that might influence oocyte development and maturation.

The ROS and anti-oxidant systems are very important not only for the reproductive system, but for the whole body. The body uses a combination of anti-oxidants, rather than single anti-oxidant supplementation, in order to maintain a balance with the ROS. Indeed, the key components that can be targeted are largely unexplored. Studies should be focused on the development of new treatment strategies, with an objective to re-establish the oxidant and anti-oxidant balance in the ovaries by strengthening the body's protective mechanisms that can overcome the excess ROS levels.

6 | CONCLUSION

The studies that have been published to date vary broadly in their conclusion about the role of ROS and anti-oxidants in ovulation. The ROS and anti-oxidants act synchronally in ovulatory functions;

however, the physiologically active concentrations that are required are not yet defined. The production of ROS is high in reproductive tissue because of active metabolism and steroidogenesis, which are kept under control by the body's anti-oxidant system. But, sometimes this balance gets disturbed, which might lead to ovulatory dysfunction. The mechanism by which both ROS and anti-oxidants work in equilibrium in the process of oocyte development and maturation have been reviewed in this article and need to be investigated critically.

ACKNOWLEDGEMENT

The authors wish to acknowledge B. V. Patel Pharmaceutical Education and Research Development Centre for providing the facilities to carry out this work and NIRMA University, Ahmedabad, India.

DISCLOSURE

Conflict of interest: The authors declare no conflict of interest. *Human and animal studies*: This article does not contain any study with human or animal participants that was performed by any of the authors.

REFERENCES

1. Fair T. Mammalian oocyte development: checkpoints for competence. *Reprod Fertil Dev*. 2010;22:13–20.
2. Patrizio P, Sakkas D. From oocyte to baby: a clinical evaluation of the biological efficiency of in vitro fertilization. *Fertil Steril*. 2009;91:1061–1066.
3. Baart EB, Martini E, Eijkemans MJ, et al. Milder ovarian stimulation for *in vitro* fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod*. 2007;22:980–988.
4. Santos MA, Kuijk EW, Macklon NS. The impact of ovarian stimulation for IVF on the developing embryo. *Reproduction*. 2010;139:23–34.
5. Trounson A, Anderiesz C, Jones G. Maturation of human oocytes in vitro and their developmental competence. *Reproduction*. 2001;121:51–75.
6. Jaroudi KA, Hollanders JM, Elnour AM, Roca GL, Atared AM, Coskun S. Embryo development and pregnancies from in-vitro matured and fertilized human oocyte. *Hum Reprod*. 1999;14:1749–1751.
7. Combelles CM, Gupta S, Agarwal A. Could oxidative stress influence the in-vitro maturation of oocytes? *Reprod Biomed Online*. 2009;18:864–880.
8. Chaube SK, Prasad PV, Thakur SC, Shrivastava TG. Hydrogen peroxide modulates meiotic cell cycle and induces morphological features characteristic of apoptosis in rat oocytes cultured in vitro. *Apoptosis*. 2005;10:863–875.
9. Chaube SK, Khatun S, Misra SK, Shrivastav TG. Calcium ionophore-induced egg activation and apoptosis are associated with the generation of intracellular hydrogen peroxide. *Free Radic Res*. 2008;42:212–220.
10. Tripathi A, Khatun S, Pandey AN, et al. Intracellular levels of hydrogen peroxide and nitric oxide in oocyte at various stages of meiotic cell cycle and apoptosis. *Free Radic Res*. 2009;43:287–294.
11. Martin-Romero FJ, Miguel-Lasobras EM, Dominguez-Arroyo JA, Gonzalez-Carrera E, Alvarez IS. Contribution of culture media to oxidative stress and its effects on human oocytes. *Reprod Biomed Online*. 2008;17:652–661.
12. Choi S, Kim S, Lee K, Kim J, Mu J. The regulation of AMP-activated protein kinase by H_2O_2. *Biochem Biophys Res Commun*. 2001;287:92–97.
13. Chen J, Hudson E, Chi MM, et al. AMPK regulation of mouse oocyte meiotic resumption in vitro. *Develop Biol*. 2006;291:227–238.
14. Lee ZW, Kweon SM, Kim SJ, et al. The essential role of H_2O_2 in the regulation of intracellular Ca^{2+} by epidermal growth factor in rat-2 fibroblasts. *Cell Signal*. 2000;12:91–92.
15. Takami M, Preston SL, Toyloy VA, Behrman HR. Antioxidants reversibly inhibit the spontaneous resumption of meiosis. *Am J Physiol*. 1999;276(4 Pt 1):E684–E688.
16. Bedaiwy MA, Elnashar SA, Goldberg JM, et al. Effect of follicular fluid oxidative stress parameters on intracytoplasmic sperm injection outcome. *Gynecol Endocrinol*. 2012;28:51–55.
17. Palini S, Benedetti S, Tagliamonte MC, et al. Influence of ovarian stimulation for IVF/ICSI on the antioxidant defence system and relationship to outcome. *Reprod Biomed Online*. 2014;29:65–71.
18. Tarin JJ, Vendrell FJ, Ten J, Blanes R, van Blerkom J, Cano A. The oxidizing agent tertiary butyl hydroperoxide induces disturbances in spindle organization, c-meiosis, and aneuploidy in mouse oocytes. *Mol Hum Reprod*. 1996;2:895–901.
19. Choi WJ, Banerjee J, Falcone T, Bena J, Agarwal A, Sharma RK. Oxidative stress and tumor necrosis factor-alpha-induced alterations in metaphase II mouse oocyte spindle structure. *Fertil Steril*. 2007;88:1220–1231.
20. Tamura H, Takasaki A, Miwa I, et al. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *J Pineal Res*. 2008;44:280–287.
21. Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. *Annu Rev Pharmacol Toxicol*. 2007;47:143–183.
22. Kala M, Nivsarkar M. Role of cortisol and superoxide dismutase in psychological stress induced anovulation. *Gen Comp Endocrinol*. 2016;225:117–124.
23. Tripathi A, Kumar KVP, Chaube SK. Meiotic cell cycle arrest in mammalian oocytes. *J Cell Physiol*. 2010;223:592–600.
24. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. 2005;3:28.
25. Chappel S. The role of mitochondria from mature oocyte to viable blastocyst. *Obstet Gynecol Int*. 2013;2013:Article ID 183024.
26. Van Blerkom J, Davis PW, Lee J. ATP content of human oocytes and development potential and outcome after in-vitro fertilization and embryo transfer. *Hum Reprod*. 1995;10:415–424.
27. Ambekar AS, Nirujogi RS, Srikanth SM, et al. Proteomic analysis of human follicular fluid: a new perspective towards understanding folliculogenesis. *J Proteomics*. 2013;87:68–77.
28. Kawaguchi S, Sakumoto R, Okuda K. Induction of the expressions of antioxidant enzymes by luteinizing hormone in the bovine corpus luteum. *J Reprod Dev*. 2013;59:219–224.
29. Hanukoglu I. Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. *Drug Metab Rev*. 2006;38:171–196.
30. Agrawal P, Laloraya MM. Induction of peroxidase in corpora lutea of rat ovary by luteinizing hormone. *Biochem J*. 1977;166:205–208.
31. Attaran M, Pasqualotto E, Falcone T, Goldberg JM, Miller KF, Agarwal A. The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization. *Int J Fertil Womens Med*. 2000;45:314–320.
32. Du B, Takahashi K, Ishida GM, Nakahara K, Saito H, Kurachi H. Usefulness of intraovarian artery pulsatility and resistance indices measurement on the day of follicle aspiration for the assessment of oocyte quality. *Fertil Steril*. 2006;85:366–370.
33. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev*. 2002;82:47–95.

34. Yuan Y, Krisher RL. Effect of reactive oxygen species during in vitro maturation on porcine oocyte nuclear maturation and developmental competence. *Reprod Fertil Dev*. 2011;23:235–236.
35. Tripathi A, Premkumar KV, Pandey AN, et al. Melatonin protects against clomiphene citrate-induced generation of free radicals and egg apoptosis in rat. *Eur J Pharmacol*. 2011;667:419–424.
36. Tiwari M, Chaube SK. Moderate increase of reactive oxygen species triggers meiotic resumption in rat follicular oocytes. *J Obstet Gynaecol Res*. 2016;42:536–546.
37. Goud AP, Goud PT, Diamond MP, Gonik B, Abu-Soud HM. Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide, and hypochlorous acid. *Free Radic Biol Med*. 2008;44:1295–1304.
38. Hsueh AJW, Billig H, Tsafriri A. Ovarian follicle atresia: a hormonally controlled apoptotic process. *Endocr Rev*. 1994;15:707–724.
39. Tilly JL, Kowalski KI, Johnson AL, Hsueh AJW. Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. *Endocrinology*. 1991;129:2799–2801.
40. Fu Y, Lin F, Liu H. Changes in the messenger RNA expression levels of Bcl-2 family members and caspase-8 and -3 in porcine ovarian follicles during follicular atresia. *Anim Sci J*. 2012;84:222–230.
41. Stanley JA, Sivakumar KK, Nithy TK, et al. Postnatal exposure to chromium through mother's milk accelerates follicular atresia in F1 offspring through increased oxidative stress and depletion of antioxidant enzymes. *Free Radic Biol Med*. 2013;61:179–196.
42. Devine PJ, Perreault SD, Luderer U. Roles of reactive oxygen species and antioxidants in ovarian toxicity. *Biol Reprod*. 2012;86:27.
43. Sugino N, Nakamura Y, Takeda O, Ishimatsu M, Kato H. Changes in activities of superoxide dismutase and lipid peroxide in corpus luteum during pregnancy in rats. *J Reprod Fertil*. 1993;97:347–351.
44. Margolin Y, Aten RF, Behrman HR. Antigonadotropic and antisteroidogenic actions of peroxide in rat granulosa cells. *Endocrinology*. 1990;127:245–250.
45. Oyawoye O, Abdel Gadir A, Garner A, Constantinovici N, Perrett C, Hardiman P. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. *Hum Reprod*. 2003;18:2270–2274.
46. Agarwal A, Allamaneni SR. Role of free radicals in female reproductive diseases and assisted reproduction. *Reprod Biomed Online*. 2004;9:338–347.
47. Cetica PD, Pintos LN, Dalvit GC, Beconi MT. Antioxidant enzyme activity and oxidative stress in bovine oocyte in vitro maturation. *IUBMB Life*. 2001;51:57–64.
48. El Mouatassim S, Guerin P, Menezo Y. Expression of genes encoding antioxidant enzymes in human and mouse oocytes during the final stages of maturation. *Mol Hum Reprod*. 1999;5:720–725.
49. Ali AA, Bilodeau JF, Sirard MA. Antioxidant requirements for bovine oocytes varies during in vitro maturation, fertilization and development. *Theriogenology*. 2003;59:939–949.
50. Behl R, Pandey RS. FSH induced stimulation of catalase activity in goat granulosa cells in vitro. *Anim Reprod Sci*. 2002;70:215–221.
51. Zuelke KA, Jones DP, Perreault SD. Glutathione oxidation is associated with altered microtubule function and disrupted fertilization in mature hamster oocytes. *Biol Reprod*. 1997;57:1413–1419.
52. Tatemoto H, Muto N, Sunagawa I, Shinjo A, Nakada T. Protection of porcine oocytes against cell damage caused by oxidative stress during in vitro maturation: role of superoxide dismutase activity in porcine follicular fluid. *Biol Reprod*. 2004;71:1150–1157.
53. Dharmarajan AM, Hisheh S, Singh B, Parkinson S, Tilly KI, Tilly JL. Antioxidants mimic the ability of chorionic gonadotropin to suppress apoptosis in the rabbit corpus luteum in vitro: a novel role for superoxide dismutase in regulating bax expression. *Endocrinology*. 1999;140:2555–2561.
54. Paszkowski T, Traub AI, Robinson SY, McMaster D. Selenium dependent glutathione peroxidase activity in human follicular fluid. *Clin Chim Acta*. 1995;236:173–180.
55. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci*. 2008;4:89–96.
56. Hofmann KD, Wagner F, Preibsch W, Koob G, Niedner W. Ascorbic acid contents of human ovary during vital and cyclic phases in women. *Zentralbl Gynakol*. 1970;92:1481–1484.
57. Loh HS, Wilson CWM. Relationship of human ascorbic acid metabolism to ovulation. *Lancet*. 1971;1:110–113.
58. Byrd JA, Pardue SL, Hargis BM. Effect of ascorbate on luteinizing hormone stimulated progesterone biosynthesis in chicken granulosa cells in vitro. *Comp Biochem Physiol*. 1993;104A:279–281.
59. Crha I, Hrubá D, Ventruba P, Fiala J, Totusek J, Visnova H. Ascorbic acid and infertility treatment. *Cent Eur J Public Health*. 2003;11:63–67.
60. Sekhon LH, Gupta S, Kim Y, Agarwal A. Female infertility and antioxidants. *Curr Womens Health Rev*. 2010;6:84–95.
61. Tarín JJ, Pérez-Albalá S, Cano A. Oral antioxidants counteract the negative effects of female aging on oocyte quantity and quality in the mouse. *Mol Reprod Dev*. 2002;61:385–397.
62. Luck MR, Jeyaseelan I, Scholes RA. Ascorbic acid and fertility. *Biol Reprod*. 1995;52:262–266.
63. Ikeda S, Kitagawa M, Imai H, Yamada M. The roles of vitamin A for cytoplasmic maturation of bovine oocytes. *J Reprod Dev*. 2005;51:23–35.
64. Barzegari Firouzabadi F, Javid A, Rezaei ZS. An in vitro study of follicle stimulating hormone (FSH) and α-tocopherol effects on the maturation of preantral follicle-enclosed oocytes from immature mice. *Razi J Med Sci*. 2010;17:7–15.
65. Tareq K, Akter QS, Khandoker MA, Tsujii H. Selenium and vitamin E improve the in vitro maturation, fertilization and culture to blastocyst of porcine oocytes. *J Reprod Dev*. 2012;58:621–628.
66. Lisboa LA, Andrade ER, Hertel MF, et al. Viability and growth of cattle preantral follicles after in vitro culture of ovarian fragments in α-tocopherol. *Reprod Fertil Dev*. 2009;22:318–319.
67. Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging*. 2007;2:219–236.
68. Tian X, Diaz FJ. Zinc depletion causes multiple defects in ovarian function during the periovulatory period in mice. *Endocrinology*. 2012;153:873–886.
69. Bedwal RS, Bahuguna A. Zinc, copper and selenium in reproduction. *Experientia*. 1994;50:626–640.
70. Picco SJ, Anchordoquy JM, de Matos DG, et al. Effect of increasing zinc sulphate concentration during in vitro maturation of bovine oocytes. *Theriogenology*. 2010;74:1141–1148.
71. Szymanski W, Kazdepka-Zieminska A. Effect of homocysteine concentration in follicular fluid on a degree of oocyte maturity. *Ginekol Pol*. 2003;74:1392–1396.
72. Bahadori MH, Ghasemian F, Ramezani M, Asgari Z. Melatonin effect during different maturation stages of oocyte and subsequent embryo development in mice. *Iranian J Reprod Med*. 2013;11:11–18.
73. Tamura H, Takasaki A, Taketani T, et al. The role of melatonin as an antioxidant in the follicle. *J Ovarian Res*. 2012;5:5.
74. Tiwari M, Prasad S, Tripathi A, et al. Apoptosis in mammalian oocytes: a review. *Apoptosis*. 2015;20:1019–1025.
75. Briggs MH. Vitamin C and infertility. *Lancet*. 1973;2:677–678.
76. Salehpour S, Sene AA, Saharkhiz N, Sohrabi MR, Moghimian F. N-Acetylcysteine as an adjuvant to clomiphene citrate for successful induction of ovulation in infertile patients with polycystic ovary syndrome. *J Obstet Gynaecol Res*. 2012;38:1182–1186.

How to cite this article: Kala, M., Shaikh, M.V. and Nivsarkar, M. (2017), Equilibrium between anti-oxidants and reactive oxygen species: a requisite for oocyte development and maturation. Reproductive Medicine and Biology, 16: 28–35. doi: 10.1002/rmb2.12013