ROS

REVIEW ARTICLES

Involvement of Reactive Oxygen Species in Meiotic Cell Cycle Regulation and Apoptosis in Mammalian Oocytes

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ABSTRACT | The mammalian ovary is a metabolically active organ and generates a large amount of reactive oxygen species (ROS) during final stages of folliculogenesis. ROS modulate physiological arrest (i.e., diplotene arrest) in follicular oocytes as well as metaphase-II (M-II) arrest in ovulated oocytes in most of the mammalian species. A moderate increase in the level of ROS could be beneficial for meiotic resumption from diplotene and M-II arrest in oocytes. The increased production of ROS, decreased antioxidant system, drug treatment, pathological conditions, stress, and several other factors may lead to accumulation of ROS in the ovary. Increased levels of ROS may generate oxidative stress (OS), which could induce meiotic cell cycle arrest in oocytes. OS triggers granulosa cell apoptosis and thereby reduces the transfer of nutrients and survival factors to the oocytes, leading to apoptosis. In vitro culture conditions, reduced survival factors, and destabilized maturation promoting factor (MPF) may generate ROS and thereby OS in follicular and ovulated oocytes. OS induces apoptosis in diplotene- and M-II-arrested oocytes through mitochondria-mediated pathway. The deterioration in oocyte quality resulting from ROS-mediated apoptosis may negatively impact the outcome of assisted reproductive technology (ART) in several mammalian species, including humans.

KEYWORDS | Apoptosis; Mammalian oocytes; Meiotic cell cycle arrest; Oxidative stress; Reactive oxygen species; Redox signaling

ABBREVIATIONS | ART, assisted reproductive technology; Cdk1, cyclin-dependent kinase 1; M-II, metaphase-II; MPF, maturation promoting factor; OS, oxidative stress; ROS, reactive oxygen species

CONTENTS

1. INTRODUCTION

In mammals, free radicals are produced as by-products of normal cellular metabolism and serve as signal molecules in various physiological and pathological processes [1–4]. Superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) are major reactive oxygen species (ROS) produced in the mammalian ovary [1, 2, 5]. Superoxide is generated when electrons leak from the electron transport chains, whereas dismutation of superoxide results in the formation of H_2O_2 [1]. Hydroxyl radical is highly reactive, which can modify purine and pyrimidine bases, leading to oxidative DNA damage [1].

ROS are generated in the body either by non-enzymatic (e.g., Fenton reaction in the presence of transition metal ions) or by the enzymatic (e.g., xanthine dehydrogenase) reactions [6]. Biological reactions, which utilize oxygen molecule as a substrate, also generate a large amount of ROS [7]. The mitochondrial respiratory chain is a main oxygen consuming site in a wide variety of cell types; hence, the majority of ROS are produced from this site under physiological conditions [7–9].

Drug treatment, pathological conditions, and several other factors may generate a high level of ROS in the ovary [10–12]. In addition, depletion of antioxidant system in the ovary may also result in the accumulation of ROS [1, 10–13]. The increased accumulation of ROS could lead to oxidative stress (OS), which directly or indirectly deteriorates oocyte quality by inducing apoptosis of granulosa cells as well as oocytes [10, 14–18].

2. GENERATION OF ROS IN THE OVARY

The ovary is a metabolically active organ and generates excess amounts of ROS during final stages of follicular development and ovulation [1, 2]. The bimodal role of ROS in mammalian ovary has already been documented [1]. Generation of a tonic level of ROS typically acts as a signal mechanism that modulates ovarian physiology [2], while overproduction of ROS results in OS that may cause detrimental effects on the ovary [1].

ROS are generated within the follicles during folliculogenesis. This notion is supported by previous studies showing that growing follicles, granulosa cells of graafian follicles, ovulated follicles, and luteal cells are the major sites for the generation of ROS [2, 19, 20]. The OS biomarkers, such as conjugated dienes, lipid hydroperoxide, and thiobarbituric acid-reactive substances, have been reported in human preovulatory follicles [1]. The increased levels of ROS have been reported in the follicular fluid of preovulatory follicles of swines and cows [21, 22]. ROS are also produced within the follicles at the time of follicular rupture just prior to ovulation [2, 23]. Certain amount of ROS within the physiological range is necessary for the normal development of oocytes and it could be used as a marker for healthy developing oocytes [1, 24–26]. Recent studies suggest that a moderate increase of ROS mediates human chorionic gonadotropin (hCG)-induced meiotic resumption in rat oocytes within the follicular microenvironment [27]. The higher level of ROS in human follicular fluid is associated with an increased fertilization rate and implantation [28, 29]. The level of ROS in the follicular fluid may serve as a potential marker for predicting the successful outcomes of in vitro fertilization [30].

ROS and antioxidants are known to intervene with the reproductive physiology of females [31]. The ovary has its own antioxidant system through which it maintains the redox status during final stages of folliculogenesis [1, 2]. Follicular fluid and cumulus oophorus cells contain superoxide dismutase that protects oocytes from oxidative damage. The activities of superoxide dismutase, catalase, and glutathione peroxidase, as well as the presence of non-enzymatic antioxidants have been reported in the swine follicular fluid [21]. Studies from our laboratory suggest that melatonin increases catalase activity, reduces the ROS level in the ovary, and protects against clomiphene citrate-induced adverse effects at the level of the ovary [12].

Previous studies from our laboratory also suggest that clomiphene citrate reduces the catalase activity and increases the ROS levels and apoptosis in encircling granulosa cells of preovulatory follicles [32, 33]. The granulosa cell apoptosis disrupts gap junctions and affects the supply of nutrients and maturation-enabling factors required for the achievement of meiotic competency in follicular oocytes [32, 33]. Under this condition, oocytes become more susceptible towards apoptosis which leads to the deterioration of oocyte quality after ovulation. The bioactive ingredients of neem leaf induce ROS-mediated apop-

tosis in granulosa cells as well as oocytes [17, 18, 34, 35]. The generation of ROS within the follicles and its negative impact on oocyte quality have recently been reviewed [33, 35, 36].

3. ROS-MEDIATED REGULATION OF MEIOTIC CELL CYCLE AT THE DIPLOTENE STAGE AND APOPTOSIS

Diplotene arrest is the longest phase of meiotic cell cycle, which may last for several months to several years depending on the mammalian species [3, 4, 37]. These diplotene-arrested oocytes (also known as dictyate stage oocytes) are morphologically identified by the presence of germinal vesicle (GV) [3, 4, 38, 39]. Meiotic resumption from diplotene arrest could be triggered by ROS within the preovulatory follicles. The beneficial role of ROS comes from the observations that non-enzymatic antioxidants, such as ascorbic acid and 3-*tert*-butyl-4-hydroxyanisole (BHA), inhibit spontaneous meiotic resumption from diplotene arrest [40, 41]. Our recent studies suggest that a moderate increase in the level of ROS is associated with spontaneous resumption of meiosis from diplotene arrest in rat oocytes cultured in vitro [41]. The increased level of ROS induces Thr14/Tyr15 phosphorylation of cyclin-dependent kinase 1 (Cdk1) and reduces the levels of Thr161 phosphorylated Cdk1 and cyclin B1. Increased phosphorylation of Thr14/Tyr15 and dephosphorylation of Thr161 residues of Cdk1 and reduced cyclin B1 levels destabilize maturation promoting factors (MPF), which eventually results in spontaneous resumption of meiosis in oocytes [41].

ROS act as a double-edged sword and serve as key signal molecules in meiotic cell cycle progression and also have a role in the pathological processes, such as cell cycle arrest and apoptosis [1, 33, 35]. Our previous studies suggest that less than 60 ng/oocyte is a physiological level of ROS present in diplotene-arrested oocytes [10, 11]. Further, generation of a tonic level of ROS or a small accumulation of ROS (i.e., a relatively higher physiological range) may trigger meiotic resumption in follicular oocytes [10, 11]. Our quantitative analysis suggests that a level of ≥60 ng/oocyte (i.e., 60–80 ng/oocyte) may be considered as a moderate increase of ROS, which is beneficial for meiotic resumption from diplotene arrest [10, 11]. On the other hand, due to drug treatment or pathological conditions, or stress and several other factors, ROS levels may increase beyond the physiological range (>80 ng/oocyte), which could induce cell cycle arrest and thereby apoptosis in follicular oocytes [10–12] (**Figure 1**). Based on these studies, we propose that the increased levels of ROS and the resulting OS could be one of the reasons for the deterioration of oocyte quality and depletion of germ cells from the ovary just prior to ovulation under various pathophysiological conditions.

4. ROS-MEDIATED REGULATION OF MEIOTIC CELL CYCLE AT THE METAPHASE-II STAGE AND APOPTOSIS

In freshly ovulated oocytes, metaphase-II (M-II) arrest is a physiological event in mammals [3, 38]. However, spontaneous exit from M-II arrest has been reported in several mammalian species [42–45]. Recent studies from our laboratory suggest that a moderate increase of ROS is associated with spontaneous exit from M-II arrest [16]. A transient increase of ROS or a small rise in the level of ROS modulates specific phosphorylation status of Cdk1 [16, 46]. ROS increase Thr14/Tyr15 phosphorylation and decrease Thr161 phosphorylated Cdk1 levels [16, 46]. The modulation in specific phosphorylation status of Cdk1 may result in dissociation and degradation of cyclin B1, leading to MPF destabilization. The destabilized MPF in turn triggers meiotic exit from M-II arrest [16, 46].

Handling of ovulated oocytes under in vitro culture conditions during various ART programs may increase the ROS level to the extent which could lead to OS [47]. Such a high level of ROS could induce meiotic cell cycle arrest at M-II stage and apoptosis under in vitro culture conditions. This possibility is further supported by our studies demonstrating that verapamil (a non-specific L-type calcium channel blocker that causes increased formation of ROS in cells) induces meiotic cell cycle arrest and apoptosis in rat oocytes [48, 49]. Likewise, the calcium ionophore A23187 induces generation of ROS, which also results in meiotic cell cycle arrest and apoptosis in rat oocytes [14, 49, 50]. Further, roscovitine, a specific Cdk1 inhibitor, induces generation of ROS, maintenance of M-II arrest, and apoptosis in rat oocytes cultured in vitro [16]. These studies suggest that increased levels of ROS due to drug treatment

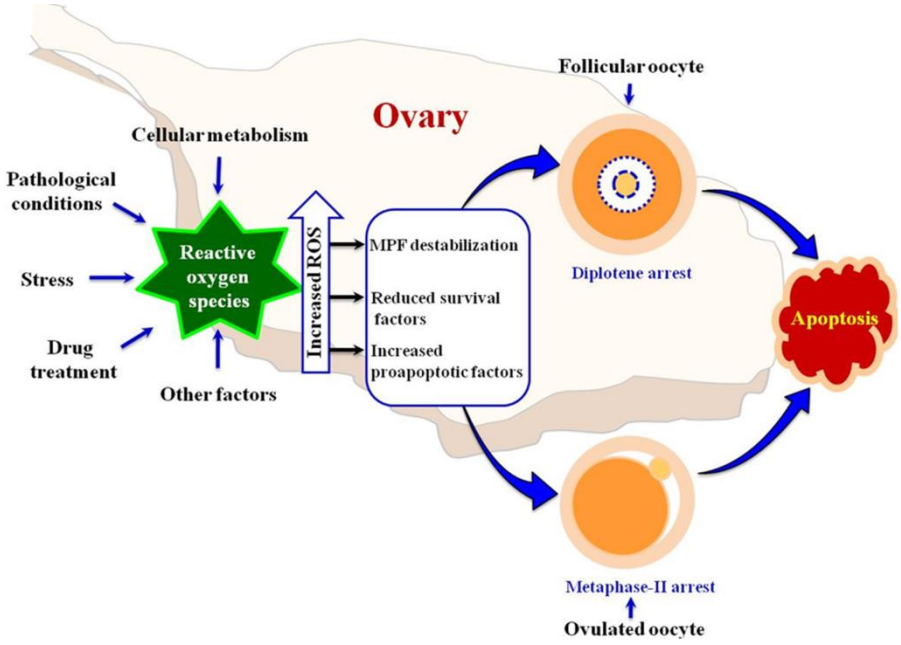

FIGURE 1. Reactive oxygen species in meiotic cell cycle regulation and apoptosis in oocytes. This scheme illustrates the possible factors that induce generation of reactive oxygen species (ROS) in the ovary and the impact of sustained high levels of ROS and the resulting oxidative stress on meiotic cell cycle arrest and apoptosis in follicular as well as ovulated oocytes.

could be involved in cell cycle arrest and apoptosis in oocytes. In addition, exogenous supplementation of H_2O_2 in the culture medium induces meiotic cell cycle arrest and apoptosis in rat oocytes cultured in vitro [10] (**Figure 1**).

ROS induce meiotic cell cycle arrest and apoptosis in oocytes through mitochondria-mediated pathway [33–36]. This notion is further supported by the observations that the increased levels of ROS modulate the Bax/Bcl-2 ratio in mitochondrial membrane and thereby the membrane potential [33, 35, 36]. Changes in the mitochondrial membrane potential trigger the release of cytochrome c into the cytoplasm of oocytes [33, 35, 36]. Cytochrome c binds to apoptotic protease activating factor 1, resulting in the activation of upstream and downstream caspases [10, 36, 47–50]. The activated caspase-3 cleaves key structural and regulatory proteins, leading to several biochemical and morphological changes associated with oocyte apoptosis [36, 47–50]. Thus, high levels of ROS-mediated cell cycle arrest and apoptosis could be interlinked.

5. SUMMARY

ROS act as signal molecules and modulate various cellular functions, including meiotic cell cycle resumption, arrest, and apoptosis in oocytes of several mammalian species. A moderate increase of ROS triggers meiotic resumption from diplotene- as well as M-II arrest, while levels of ROS beyond the physiological range could induce MPF destabilization, reduce survival factors, and trigger mitochondria-mediated oocyte apoptosis. Hence, ROS act as a double-edged sword in oocyte biology, which, on the one side, could be beneficial at moderate levels and, on the other side, detrimental when their levels go beyond the physiological range.

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