Oxidative stress and ovarian aging: from cellular mechanisms to diagnostics and treatment

Omar F. Ammar 1,2, Claudia Massarotti 3,4, Mina Mincheva 5, Kashish Sharma 6, George Liperis 7,8, Sonia Herraiz 9, Aida Rodriguez-Nuevo 10, Filippo Zambelli 11, Bettina P. Mihalas 12, and Juan J. Fraire-Zamora 11

1IVF Department, Ar-Razzi Hospital, Ramadi, Iraq
2Department of Obstetrics and Gynaecology, College of Medicine, University of Anbar, Ramadi, Iraq
3IRCCS Ospedale Policlinico San Martino, Genova, Italy
4DINOGMI Department, University of Genova, Genova, Italy
5Independent Researcher, London, UK
6HealthPlus Fertility Centre, HealthPlus Network of Specialty Centers, Abu Dhabi, United Arab Emirates
7Westmead Fertility Centre, Institute of Reproductive Medicine, University of Sydney, Westmead, NSW, Australia
8Embryorigin Fertility Centre, Larnaca, Cyprus
9IVIRMA Global Research Alliance, IVI Foundation-IIS la Fe, Valencia, Spain
10Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain
11EUGIN Group, R&D, Barcelona, Spain
12The Oocyte Biology Research Unit, Discipline of Women’s Health, School of Clinical Medicine, Faculty of Medicine and Health, The University of NSW Sydney, Randwick, NSW, Australia

*Correspondence address. Eugin Group, R&D, C/Balmes 236, 08006, Barcelona, Spain. E-mail: jfraire@eugin.es  https://orcid.org/0000-0003-2870-0140

GRAPHICAL ABSTRACT

The December ESHRE Journal Club discussion focused on a study by Smits et al. (2023), the cellular and molecular implications of oxidative stress on oocyte aging, the factors that impair oocyte quality besides age, and the potential therapeutic strategies to mitigate oxidative stress and mitochondrial dysfunction in oocytes of advanced maternal age. AMA, advanced maternal age; GV, germinal vesicle oocyte; MI, metaphase I oocyte; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

Keywords: oxidative stress / ovarian aging / ovarian metabolomics / advanced maternal age / oocyte developmental competence
Introduction

Oxidative stress, a key factor in the aging process, has been increasingly recognized as playing a critical role in ovarian aging (Yan et al., 2022). The accumulation of reactive oxygen species (ROS) leads to oxidative stress by impinging damage in the delicate microenvironment of the ovaries, resulting in cellular and molecular damage to both somatic and germ cells that ultimately contribute to a decline in ovarian function (Sasaki et al., 2019). At the molecular and cellular level, it has been highlighted that oxidative stress has a detrimental effect on DNA integrity and mitochondrial function by affecting the development of healthy oocytes, accelerating the depletion of ovarian follicles and impacting female reproductive potential by reducing oocyte quality (Cimadomo et al., 2018; van der Reest et al., 2021; Martin et al., 2022; Yan et al., 2022). Furthermore, ROS overproduction in the ovaries has been linked to the pathophysiology of conditions such as premature ovarian failure (Timoteo-Ferreira et al., 2021). For these reasons, understanding the mechanisms by which oxidative stress contributes to ovarian aging is crucial in developing potential interventions and treatments to mitigate its impact on female fertility.

A recent study conducted by Smits et al. (2023), and published in Human Reproduction, adds to the growing body of evidence that human ovarian aging and the age-related decline in female fertility are caused by oxidative stress and mitochondrial dysfunction in oocytes. Using immunohistochemistry and metabolomics, the study found evidence of oxidative damage in oocytes of advanced maternal age, even at the primordial follicle stage. This study emphasized changes in protein oxidation, lipid peroxidation, and abundance in aged oocytes as well as active mitochondrial metabolism in primordial oocytes suggesting that oxidative stress may play an early role in the decline of oocyte quality and fertility. In the December edition of ESHRE Journal Club, the findings of this study were discussed, focusing on several topics: first, the cellular and molecular implications of the work by Smits et al. on oxidative stress and oocyte aging; second, factors that impair oocyte quality beyond age; and third, the potential therapeutic strategies to mitigate oxidative stress and mitochondrial dysfunction in oocytes of advanced maternal age.

Cellular and molecular pathways affected by oxidative stress and aging

At the cellular level, Smits et al. explored mitochondrial activity in fixed human ovarian samples. The authors examined the phosphorylation status of the enzyme pyruvate dehydrogenase (PDH), the entry point of pyruvate into the tricarboxylic acid cycle (TCA) within mitochondria as a proxy for oxidative phosphorylation (Patel and Roche, 1990; Kato et al., 2008). Although there was a trend toward age-related lower PDH phosphorylation (hence a lower oxidative phosphorylation activity), the authors did not find a statistically significant difference. Nevertheless, it is noteworthy to point out that the authors’ data show a 5-fold lower PDH activation in primordial follicles, suggesting a lower mitochondrial activity in earlier stages of oocyte development. This finding agrees with a recent study showing that human oocytes maintain a low mitochondrial metabolism in early developmental stages (Rodriguez-Nuevo et al., 2022). Despite these low levels of mitochondrial activity, Smits et al. hypothesized that ROS-induced cellular damage could potentially start to accumulate in primordial follicles. Next, the authors showed that primordial follicles present markers of protein oxidation and lipid peroxidation (i.e. oxidative damage), which increase with maternal age. Intriguingly, primordial follicles did not show an age-related increase of DNA oxidation markers, according to the authors. However, a cautionary statement should be made regarding their immunostaining images and interpretation. For instance, as the authors suggest in their discussion, primordial follicles are sensitive to apoptosis in response to DNA damage (Kerr et al., 2012), thus follicles with excessive DNA oxidation could have undergone apoptosis and not be present in the samples analyzed, masking an increased age-related DNA oxidation pattern. Another thing to point out is the difficulty to interpret the immunostaining images since they lack information on the age of the primordial follicle and the dynamic range of the oxidative damage markers (i.e. how these markers compare in cells with oxidative damage and/or upon antioxidant treatment). It must be acknowledged that measuring such a dynamic process as oxidative phosphorylation using fixed samples represents a challenge that can impact on the statistical and biological significance of the data; this is why dynamic range and age-related controls, as well as proper report on the units of variation (standard deviation or standard error of the mean) should be carefully reported. Overall, further evidence is necessary to address the effect of basal levels of ROS on the physiology of primordial follicles, specifically in DNA oxidation, and how these levels are regulated by antioxidant systems in early developmental stages (Kala et al., 2016; Sies and Jones, 2020).

After concluding that ROS-induced damage in lipids and proteins occurred in aged primordial follicles, Smits et al. explored whether cellular metabolism was impaired in aged oocytes. For this purpose, the authors used targeted metabolomics and lipidomics in germinal vesicle (GV) and metaphase I (MI) oocytes from women undergoing ovarian stimulation for IVF treatment. It is important to make the distinction that, while primordial follicles can remain in this early stage for decades, GV and MI oocytes have undergone morphological and endocrinological changes during follicular recruitment and are in the process of resuming stage I of meiosis (Baerwald et al., 2012). Thus, GVs and MIs oocytes are good physiological models for exploring the effect of ROS-induced damage during aging and the clinical implications of this damage in meiotically immature oocytes. Importantly, Smits et al. provide broad and novel metabolomics/lipidomics data in human oocytes, which is a remarkable achievement. Their analysis unveils interesting data patterns of the impact of age and oxidative stress on maturing oocytes, especially in the MI phase.

For instance, the authors found that age is clearly impacting the glutathione metabolism pathway. Glutathione disulfide or oxidogluthathione (GSSG) is the precursor of reduced glutathione (GSH), a cellular protective antioxidant. The ratio of GSH to GSSG within cells is often used as a marker of cellular toxicity, since oxidative stress results in the formation of GSSG and, thus, a lower GSH:GSSG ratio (Locigno and Castronovo, 2001; Schafer and Buettner, 2001; Zitka et al., 2012). Smits et al. reported a decrease in GSH abundance and an apparent increase in GSSG (lower GSH:GSSG ratio) in MI oocytes of advanced maternal age, confirming increased oxidative stress. Moreover, the authors observed a clear decrease in phospholipid abundance with increasing age in both GVVs and MIs (suggesting vulnerability of these molecules to peroxidation) and an accumulation of TCA cycle substrates (i.e. metabolites associated with glycolysis), while TCA cycle intermediates did not change, indicating a disruption in the processing of TCA substrates in oocytes of advanced maternal age due to oxidative stress. Altogether, the work by Smits et al. indicates that metabolites associated with mitochondrial...
function are altered with age, and although it is difficult to determine whether mitochondrial dysfunction is the cause or the consequence of oxidative damage, their results already spark new hypotheses about the cellular pathways that can be tackled to rescue biological processes affected by maternal age in maturing oocytes.

**Confounding factors affecting the metabolomics/lipidomics analysis**

Maternal age can directly affect mitochondrial integrity, oocyte quality, and developmental potential (Cimadomo et al., 2018; Krisher, 2018; Khan et al., 2023). However, since mitochondria are key players of cellular metabolism, there are a myriad of different factors that can affect them besides a woman’s age. Smits et al. excluded samples from female patients with malignancies, polycystic ovarian syndrome (PCOS), being HIV or hepatitis B/C positive or undergoing preventive resection of the ovary due to being a BRCA mutation carrier. Indeed, it has been pointed out that oxidative stress can offset oocyte maturation and contribute to reproductive dysfunction in patients with PCOS (Awonuga et al., 2023) and that BRCA carriers associate with both oxidative stress and defects in DNA repair that affect oocyte endowment and development (Lin et al., 2017, Turan and Öktay, 2020). Nevertheless, there are other factors that were not considered by Smits et al., which may increase oxidative stress and affect the ovarian environment, resulting in a reduced ovarian reserve and infertility. For instance, lifestyle factors such as heavy cigarette smoking have been associated with an increase in the risk of early menopause (Whitcomb et al., 2018). In an animal model, chronic stress has been shown to induce meiotic arrest failure and a decline in the ovarian reserve of females (Jiang et al., 2023). On the other hand, certain diseases have been shown to correlate with genomic and proteomic changes that reflect cellular stress (Keiding et al., 2017, Turan and Öktay, 2020). Although this would be an advantageous strategy, there is no solid evidence supporting its use in humans and the treatment would only supply antioxidants or metabolites found in low abundance in aged oocytes (e.g. NAD⁺, monophosphate end products, or ATP), but this in vitro supplementation would not reverse existing DNA damage from oxidative stress in meiotically immature oocytes.

A more invasive technique involving autologous germline mitochondrial transfer (AUGMENT) to aged oocytes using ICSI did not show an improved prognosis in a randomized controlled trial assessing blastocyst euploidy rate or cumulative live birth rate (Labarta et al., 2019), indicating that autologous mitochondrial transfer does not improve oocyte quality. The maternal spindle transfer (MST) technique involves the removal of the spindle–chromosome complex from an oocyte of a recipient and its transfer into an enucleated oocyte from a donor (Cree and Loi, 2015). MST has been proposed as a solution to prevent the transmission of mitochondrial DNA diseases into the progeny and it has shown promising results in overcoming repeated fertilization failure in patients (Costa-Borges et al., 2023). Rinaudo and Coutifaris (2023) have proposed that MST could theoretically be applied to advanced maternal age patients to overcome poor oocyte quality. However, safety concerns have been raised about the use of this technique (Baylis, 2013; Siristatidis et al., 2022; Rinaudo and Coutifaris, 2023), thus, further research and validation are needed to justify its use in patients of advanced maternal age.

**Potential therapeutic strategies to mitigate mitochondrial dysfunction and oxidative stress in oocytes from women of advanced reproductive age**

The targeted metabolomic/lipidomic approach used by Smits et al. provides a starting point to explore the design of strategies to prevent or ameliorate oxidative stress damage and its negative effects on female reproductive potential. An obvious initial consideration is to decide at which level to act. One potential option would be to mitigate mitochondrial dysfunction at the systemic level (i.e. directly targeting primordial follicles in the ovary). So far, the effects of antioxidant treatments have been addressed in the reproductive fitness of animal models (Bertoldo et al., 2020; Katz-Jaffe et al., 2020; Lane et al., 2021). However, when trying to translate these findings to human application, it is not clear whether the benefits of antioxidant therapies would show an actual advantage due to the complexity of treatments, uncertainty in the measured outcomes and heterogeneity of the strategies (Tesarik, 2021). A similar criticism stands for ovarian reactivation using platelet-rich plasma treatments (Serdarogullari et al., 2024). Recently supplementation with the polyamine spermidine has been proposed as a promising therapeutic strategy to recover oocyte quality through enhancement of mitochondrial function in aged animal models (Zhang et al., 2023). These results still need safety and effectiveness trials in humans.

An alternative to systemic treatment would be to mitigate the detrimental effects of oxidative stress in vitro, in meiotically immature oocytes (GVs and MIs) from women who have undergone ovarian stimulation. This strategy has been explored in different animal models by supplementing antioxidants in oocyte culture media (Rakhra et al., 2022). Although this would be an advantageous strategy, there is no solid evidence supporting its use in humans and the treatment would only supply antioxidants or metabolites found in low abundance in aged oocytes (e.g. NAD⁺, monophosphate end products, or ATP), but this in vitro supplementation would not revert existing DNA damage from oxidative stress in meiotically immature oocytes.

**Conclusions**

Overall, the targeted ‘omics’ method used by Smits et al. provides an extensive dataset of metabolites and lipids of meiotically immature oocytes and their changes in abundance depending on maternal age. This unique dataset offers the opportunity to explore the molecular and cellular dynamics that are affected by oxidative stress and elaborate hypothesis-driven studies to design effective therapeutic strategies to improve oocyte quality. To date, quantifying oxidative stress in patients is not an easy task. Finding clinically relevant biomarkers to quantify oxidative stress will provide tools to counsel and manage infertility related to oxidative stress. Much more work is needed to identify the
direction for the development of treatments for mitochondrial dysfunctions and mitigate infertility related to oxidative stress in oocytes. However, it must be stressed that scientific rigor, technical excellence, and academic discussions should be at the center of this approach. Modern methods should be applied to understand the dynamic nature of oocyte aging and to be able to implement efficient treatments in the clinic.

Data availability
No datasets were generated or analyzed in the current manuscript.

Acknowledgements
The authors would like to thank all the participants of ESHRE Journal Club on Twitter for their contribution to this discussion.

Authors’ roles
O.F.A., C.M., M.M., K.S., G.L., and J.J.F.Z. conceptualized and moderated the discussion. O.F.A. organized and led the discussion. S.H., A.R.N., F.Z., and B.P.M. contributed intellectually to the discussion as experts. O.F.A prepared the graphical abstract. All authors provided outlines for the manuscript. J.J.F.Z. drafted the manuscript. All authors provided critical revision to the graphical abstract and manuscript and approved the final version.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest
All authors declared no conflict of interest.

References


Siristatidis C, Mantzavinos T, Vlahos N. Maternal spindle transfer for mitochondrial disease: lessons to be learnt before extending the method to other conditions? Hum Fertil (Camb) 2022;25:838–847.


