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REVIEW PAPER

Oxidative homeostasis in follicular fluid and reproductive outcomes – from bench to bedside

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Abstract

Free radicals and oxidant molecules are part of our organism in a stable balance. However, when addressing female infertility, questions about their role in oocyte quality arise.

This review outlines the major alterations of redox homeostasis in the follicular fluid through pathophysiological conditions in female reproduction and its potential effect on IVF outcome.

A review of the literature was accurately performed. Manuscripts investigating follicular fluid biomarkers, especially related to oxidant molecules, were screened and used in this review. Studies assessing the follicular reactive species were found and screened. Moreover, studies assessing the IVF outcomes related to biomarkers were considered.

The results are provided in an analytical pathway. The study of biomarkers confirms the shift to enhanced oxidizing modification of macromolecules and antioxidative consumption in the follicular fluid of women undergoing IVF treatment. A lack of congruency in methods appears to be marked in the design of scientific studies. However, it is not clear whether redox disbalance has a disruptive effect on the oocyte competence or whether it plays a role in the oocyte maturation process.

Red-ox balance plays a questionable role in IVF outcomes. Possible further insights may consider the antioxidant role of adjuvants during controlled ovarian stimulation cycles.

Key words: reactive-oxygen species; follicular fluid; oxidant; in vitro fertilization; assisted reproductive techniques.

Introduction

Cell metabolism continuously generates reactive oxygen and nitrogen species (ROS, RNS) and their intermediaries. In addition to exploring the negative effect, numerous studies investigated their physiological role as part of the signal transduction within a cell. Exposing the cell to high levels of antioxidants could neutralize the effect of specific signalling proteins in the signalling network, causing a more harmful than beneficial effect [1].

The adverse effect of these highly reactive molecules is well studied, and it is prone to electron acceptance from the oxidizing macromolecules. The most reactive cell compounds eligible for oxidation are proteins, lipids, and DNA. The clearance of free radicals is highly regulated with the antioxidative cell compounds and enzymes preventing the cell from oxidative damage. Environmental factors or pathophysiological processes can generate excessive amounts of ROS/RNS, overwhelming the antioxidant system and altering

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redox homeostasis. This state is often called oxidative stress. Therefore, redox homeostasis represents the balance between prooxidant molecules and effective antioxidant response [2].

The large network of the antioxidant system is generally categorized as (I) enzymatic and (II) non-enzymatic antioxidants. Their primary role is a direct reaction with oxidants, preventing the oxidation of the third party, e.g. DNA, lipids, or proteins. Enzymes included in free radical scavenging are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and the family of paraoxonases and peroxiredoxin I–IV. Non-enzymatic antioxidants can be of endogenous or exogenous origin. Endogenous antioxidants are uric acid, lipoic acid, bilirubin, glutathione, and melatonin, while vitamin A, C, E, flavonoids, and carotenoids are from exogenous sources [2–4].

Highly reactive with a short lifespan, ROS/RNS are difficult to accurately analyse. To determine the alterations in the redox balance, a wide variety of assays are available for quantification of the antioxidant compounds or oxidation end products.

DNA oxidation products are well studied, through the determination of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxodG) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), as reliable markers for measuring the effect of oxidative damage to DNA.

The extent of lipid peroxidation is often analysed by measuring polyunsaturated fatty acids (PUFA), oxidized LDL cholesterol (oxLDL), trans-4-hydroxy-2-nonenal (4-HNE), malondialdehyde (MDA), and isoprostanes [3]. Thiobarbituric acid-reactive substances assay (TBARS) is the most widely used method for MDA determination.

There are numerous different types of protein oxidative modification, including the oxidation of sulphur-containing amino acids, aromatic amino acids, formation of carbonyls, nitration, glycoxidation, and lipoxidation [4]. The determination of stable carbonyl groups is the most commonly used method to assess protein oxidation.

In the field of female reproduction, free radicals are recognized as part of intracellular signalling in oocyte maturation, embryo development, and pregnancy. Numerous studies have investigated the relationship of oxidative stress biomarkers in a variety of clinical conditions e.g., endometriosis, polycystic ovary syndrome (PCOS), unexplained infertility (UI), and menopause. Assisted reproduction techniques (ART) encompass fertility treatments with a variety of hormone therapy. The procedure enables the simultaneous growth of several follicles within the ovary. The aim is to achieve an optimal number of mature oocytes, suitable for IVF (in vitro fertilization) procedures, consequently achieving pregnancy. Redox alterations in follicular fluid (FF) do not necessarily follow changes in the circulation. Systemic oxidative stress is the net result of the metabolism of all organ systems. In contrast, the follicular redox state represents the microenvironment of the oocyte within the growing follicle. For this reason, we performed a review of available evidence about redox homeostasis detected in the follicular fluid and its potential correlation with IVF outcomes. Specifically, pathophysiological considerations were drawn regarding the influence of reactive oxygen species and possible oocyte quality.

Material and methods

This review aimed to investigate the major alterations of redox homeostasis in the follicular fluid through pathophysiological conditions in female reproduction and its potential effect on IVF outcome.

Study selection process

The studies included in this review have been identified through research of electronic databases: ScienceDirect, MEDLINE, Scopus, Embase, the Cochrane Library, Clinicaltrials.gov, EU Clinical Trials Register, and the World Health Organization International Clinical Trials Registry until 30 November 2021. In each database, the following key words were searched: assisted reproductive technology AND follicular fluid, medically assisted reproduction AND oxidative, ART AND redox homeostasis. A manual search of the reference lists of the included studies and review articles was successively performed to detect any other potential papers. We searched for published and unpublished studies from the aforementioned electronic databases.

Specifically, we included only original articles and considered review papers as support to find other possible related articles.

Study selection and data extraction

Three authors (GB, IZ, and MSG) independently screened the titles and abstracts of the papers obtained by the above-mentioned search strategy. The text of each potentially relevant study was considered for inclusion in each section of this review independently by 2 authors (ASL and BB). They also independently extracted relevant qualitative data from the included studies. Another author (MM) independently reviewed the selection process. The results were compared, and any disagreements were discussed and resolved by consensus.

Results

The studies analysed and considered in this review showed a large variety in methodological approaches and main outcomes. For this reason, we adopted a nar-

rative review of results and discussion, dividing the results into subsections and paragraphs.

Follicular fluid and redox homeostasis

The functional integrity of cumulus cells and FF ensure oocyte quality and conditions to obtain pregnancy. The FF consists of secretory products of granulosa and theca cells and plasma exudate. The main compounds are steroid hormones, metabolites including ROS, and antioxidants, polysaccharides, and proteins. Great potential lies in the quantitative proteomics as a powerful tool to identify biologically important proteins and proteomic patterns associated with oocyte quality. Follicular proteome analysis revealed the involvement of proteins in the cellular metabolism, communication, immune responses, and cellular process, which mostly included coagulation factors and inflammatory-associated proteins. Matrix metalloproteinase-related proteins, IGF (insulin-like growth factor)-related proteins, anti-apoptotic proteins, and other growth factors are identified as an important part of the signalling network required for follicular growth and maturation [5–7]. Another field in development is a complex lipidome analysis that enables identification of cellular lipids in the FF. Lipids play a complex role in the cell. They are the main structural component in the cell membrane, an important energy source, mediators in the cell signalling pathways, and precursors for steroid hormones and prostaglandins [8]. Dynamic changes of sex steroids affect ovarian folliculogenesis including cell proliferation, apoptosis, and angiogenesis within the follicle [9]. It has been suggested that alterations of follicular compounds can impact oocyte quality and embryo development. Particular focus is given to the investigation of components related to oxidative stress against maturing oocytes, embryo quality, and achieving pregnancy.

Polycystic ovary syndrome and obesity

Polycystic ovary syndrome (PCOS) is a complex disorder with clinical heterogeneity in all phenotypes. The main features are polycystic ovaries, oligo- or anovulation, hyperandrogenism, irregular menstrual cycles, acne, and obesity. Polycystic ovary under controlled ovarian hyperstimulation produces a larger cohort of follicles, often with impaired oocyte quality. Most studies noted increased concentrations of MDA and decreased levels of SOD, thiol groups, and total antioxidant capacity (TAC) [1, 10–13]. These findings support excessive ROS generation in the follicular environment, which may lead to enhanced antioxidant consumption. PCOS is characterized by increased visceral adipose tissue. Obesity is also associated with redox alterations, affecting metabolic, vascular, and particularly inflammatory pathways. Most studies focused their research on oxidative stress related to obesity in PCOS.

Comparing oxidative stress compounds within BMI categories, higher MDA, ox-LDL, and decreased TAC is found in PCOS obese women [14–16]. Interestingly, a few studies did not find a significant difference in TAC and MDA concentrations between PCOS or obesity and the control group [17, 18]. These inconsistent reports lead to unclear conclusions.

Several studies reported alterations in redox state and obesity itself. Overweight or obese women without PCOS show higher levels of lipid hydroperoxides and lower TAC and PON1 activity than normal-weight women. High-density lipoprotein (HDL) serves as an antioxidant, reducing oxLDL. High-density lipoprotein functional properties measured through antioxidant activity, show higher ability to be oxidized in overweight or obese women [15]. Increased catalase and GPx activity noted in obese women could be explained as response to overproduction of hydrogen peroxide [14, 16]. In brief, PCOS patients show pronounced oxidative stress in FF compared to non-PCOS patients. Table 1 presents the list of studies and their basic characteristics related to PCOS and obesity.

Studies with better stratification of PCOS patients according to their phenotype are needed for better assessment of redox homeostasis. There is a relationship between excessive fat accumulation and oxidative stress, but this association is not fully understood.

Endometriosis

Endometriosis is an oestrogen-dependent pelvic inflammatory disease with unclear aetiology [19]. The redox alterations in the follicular microenvironment are the most studied in patients with endometriosis. High concentrations of 8-OHdG are associated with the enhanced occurrence of oxidative damage of DNA in endometriosis [20]. Enhanced lipid peroxidation is detected through high concentrations of MDA in the follicular compartment, but inconsistencies in study results demand more evidence to confirm this hypothesis [11, 19, 20]. In turn, the line activation from the thiol-redox system is recognized through the association of thioredoxin with increased inflammatory cytokines, and reduced levels of GPX, GR, and glutathione [19, 20]. Lower concentrations of vitamin E are expected together with high MDA concentrations, but they are not confirmed in all studies [11, 21–23]. The source of variation could be the lack of data regarding dietary intake. Significantly reduced concentrations of vitamin C are confirmed in patients with endometriosis compared to other infertile women [20, 22, 24]. Vitamin C is subjected to excessive consumption by regenerating the reduced state of vitamin E or scavenging the free radicals. SOD is a ubiquitous copper- and zinc-dependent enzyme for direct

Table 1. The main characteristics of studies investigating oxidative stress biomarkers in polycystic ovary syndrome and obese women

Study	Design		Results		
	Participants	GnRH-protocols	Oxidants	Antioxidants	Measured outcome
Artimani *et al.*, 2018	PCOS vs. controls	Agonist	MDA, TOS	TAC, thiol groups	Oocyte number/quality, embryo number/quality, fertilization rate
Chattopadhayay *et al.*, 2009	PCOS vs. controls	Agonist	ROS, MDA	TAC	Oocyte number/quality, embryo quality, fertilizati on rate, pregnancy rates, miscarriage rate
Liu *et al.*, 2020	PCOS vs. controls	Agonist	MDA, TOS	TAC, GSH, SOD	Embryo quality
Tola *et al.*, 2018	PCOS vs. controls	Antagonist	Disulphide bond	Thiol groups	Oocyte number/quality, embryo number/quality, fertilization rate, implantation rate, pregnancy rate
Seleem *et al.*, 2014	PCOS vs. controls	Agonist	–	SOD	Oocyte number, embryo number/quality, fertilization rate, pregnancy rate
Nasiri *et al.*, 2014	PCOS with or without obesity	Agonist	MDA	TAC	–
Bacchetti *et al.*, 2019	Normal weight vs. obese women	Agonist	lipid hydroperoxides	TAC, CoQ10, PON1, HDL oxidation rate	Oocyte number/quality, embryo quality, fertilization rate
Bausenwein *et al.*, 2009	PCOS vs. non-PCOS within BMI categories	Agonist	oxLDL	SOD, catalase, GPx, GR,	Pregnancy rate
Yuxel *et al.*, 2017	Normal responders within BMI categories	Agonist	MDA	Thiol groups	Oocyte number/quality, fertilization rate
Alviggi *et al.*, 2016	PCOS: women with or without supplements	Antagonist	8-isoprostane	GPx catalase, SOD, TAC	Oocyte number/quality
Fatemi *et al.*, 2017	PCOS: vitamin E/D3 treated vs. untreated	Agonist	MDA	TAC	Oocyte number/quality, embryo quality, fertilization rate, implantation rate pregnancy rate
Piomboni *et al.*, 2014	PCOS: DCI or metformin treated vs. untreated	Antagonist	–	Thiol groups	Oocyte number/quality,fertilization rate

BMI – body mass index, CoQ10 – coenzyme Q10, DCI – D-chiro-inositol, GnRH – gonadotropin-releasing hormone, GPx – glutathione peroxidase, GR – glutathione reductase, GSH – glutathione, HDL – high-density lipoprotein, MDA – malondialdehyde, PCOS – polycystic ovary syndrome, PON1 – paraoxonase-1, SOD – superoxide dismutase, TAC – total antioxidant capacity, TOS – total oxidant status. Underlined text represents significant changes related to oxidative stress.

neutralization of ROS/RNS to more stable products: H_2O and O_2. A few studies reported a significant decrease in SOD activity [20, 22]. Patients with endometriosis also suffer from deficiency of trace elements (Se, Cu, and Zn), which could directly affect the activity of SOD [22]. Other reports show the lack of evidence in diminished enzyme activity [21, 25]. Excessive consumption of antioxidative compounds is also recognized through decreased TAC [11, 20, 22, 26]. Determining the trace elements along with recording the diet intake would contribute to better understanding of redox alterations. The basic characteristics and significant differences between studies investigating redox alterations in the endometriosis are listed in Table 2.

ART outcome

Art outcomes are strongly related to various factors, affecting both ovaries and uterus [27]. There is little agreement between oxidative stress compounds and IVF outcomes. The latter is more difficult to interpret because the studies use different criteria, e.g. the number of mature oocytes, embryo quality, implantation rate, clinical pregnancy rate, or live births. Young women with low ovarian response have lower activity of GPx, GR, and GST, compared with oocyte donors and high responders. The lower enzymatic antioxidant activity follows higher concentrations of lipid peroxides, MDA, and 4-hydroxyalkenals [28]. Thioredoxin has significantly higher concentrations in oocyte-containing follicles from normal responders in comparison to poor responders [29]. A moderate negative association is noticed between vitamin C and TRX concentrations regarding the number of mature oocytes [24, 25]. Thioredoxin is upregulated by oestrogen, oxidative stress, and growth factors. These changes may not simply reflect the present status of follicles, but they certainly have an impact on the growth and maturation of follicles.

A lower fertilization rate is related with higher concentrations of 8-OHdG and 4-HNE, lower GSH activity, and higher activity of SOD and catalase [29, 30]. Increased antioxidative properties of HDL are associated with decreased chance of successful fertilization. The significant difference between follicular and serum HDL proteome may be responsible for enhanced antioxidative potential of follicular HDL [31]. These observations suggest the need for enhanced antioxidant protection to overcome damaging effects on the oocyte with deficient fertilization potential.

Assessment of embryo quality is often used to find the potential relationship with biomarkers in FF that originate from corresponding oocyte. High concentrations of ROS, MDA, and 8-OHdG obtained from women with different factors of infertility might have an adverse effect on embryo quality [30, 32–34]. When assessing hydrogen peroxide in FF of the sibling follicles,

Table 2. The main characteristics of studies investigating oxidative stress biomarkers in women with endometriosis

Study	Design		Results		
	Participants	GnRH-protocols	Oxidants	Antioxidants	Outcome
Campos-Petean *et al.*, 2008	Endometriosis vs. controls	Agonist	MDA	Vitamin E	–
Choi, *et al.*, 2015	Endometriosis vs. controls	Agonist or antagonist	MDA	SOD, GSH, GPx, TRX, TBP2, PRX4	Oocyte number/quality, embryo quality, fertilization rate, implantation rate pregnancy rate
Da Broi *et al.*,2016	Endometriosis vs. controls	Agonist	Total peroxide, MDA, AOPP	GSH, SOD, TAC, vitamin E, 8-OHdG	Pregnancy, live birth
Goud *et al.*, 2014	Endometriosis vs. controls	Antagonist	NOx	–	Oocyte number/quality, embryo quality, implantation rate, pregnancy rate
Nasiri *et al.*, 2016	Endometriosis vs. controls	Agonist	MDA	TAC	–
Singh *et al*, 2013	Endometriosis vs. controls	Agonist	ROS, NOx, MDA	SOD, catalase, GPx, GR, vitamins A, C and E, TAC	Oocyte number/quality, embryo quality, fertilization rate, pregnancy rates, miscarriage rate
Prieto *et al.*, 2012	Endometriosis vs. controls	Not reported	MDA	Vitamin C, vitamin E, SOD	Oocyte number/quality, embryo quality, fertilization rate, implantation rate, miscarriage rate
Pekel *et al.*, 2015	Infertile women vs. controls	Not reported	MDA	SOD, TAC,	Oocyte number, embryo quality, fertilization rate
Nishihara *et al.*, 2018	Infertile women	Agonist or antagonist	8-OHdG	TAC, GSH, vitamin C	Embryo quality, blastocyst rates, fertilization rate, pregnancy rate
Lu *et al.*, 2018	Endometriosis vs. controls with or without vitamin C	Agonist	ROS, MDA	Vitamin C, SOD, TAC	Oocyte number/quality, embryo quality, fertilization rate, implantation rate pregnancy rate

AOPP – advanced oxidation protein products, GnRH – gonadotropin-releasing hormone, GPx – glutathione peroxidase, GR – glutathione reductase, GSH –glutathione, MDA – malondialdehyde, NOx – nitric oxide metabolites, PRX4 – peroxiredoxin-4, SOD –superoxide dismutase, TAC – total antioxidant capacity, TRX – thioredoxin, TBP2-TRX – binding protein, 8-OHdG – 8-hydroxy-2'-deoxyguanosine
Underlined text represents significant changes related to oxidative stress.

the highest concentrations are observed from follicles following poor embryo quality, and the lowest concentrations are found in empty follicles [34]. Women with idiopathic infertility and greater GPx activity are related to high embryo quality [35].

To evaluate the pregnancy rate, several studies assessed the balance between oxidizing macromolecules and disposable antioxidants. Nitrate overproduction is attributed to women who did not achieve pregnancy [36]. Women who achieved pregnancy presented lower ROS, MDA, protein carbonyls, GR, SOD, and GST and higher TAC, GPX, and tocopherol [24, 35, 37–41]. Thus, enhanced lipid and protein oxidation are present among women with an unfavourable outcome. Da Broi *et al*. proposed follicular 8-OHdG as a predictive marker for clinical pregnancy in patients with endometriosis [21]. The lack of defined concentrations of prooxidant and antioxidant biomarkers in physiological conditions complicates interpretation of these findings. Table 3 represents the overview of the studies that investigated the significance of oxidative stress on ART outcomes.

Supplementation with antioxidants

A limited number of studies focused on the therapeutic potential of antioxidants on IVF outcomes. Varieties of antioxidative supplements were tested to potentially overcome the imbalance in redox homeostasis of PCOS women, who are prone to infertility due to lack of ovulation [42]. Patients who take supplements based on myoinositol, folic acid, and active antioxidants (glutathione, selenium, vitamins C and E, and zinc) have increased GPx activity in FF and significantly increased numbers of MII oocytes [43]. Interestingly, Vitamin D3 and E supplements, as the main defence mechanism against lipid peroxidation, do not affect follicular MDA and TAC. A positive impact is noticed through higher pregnancy and implantation rates compared to the placebo group [44]. D-chiro-inositol and metformin induce a significantly higher level of free-SH groups in FF compared to untreated PCOS patients. Apart from the protective effect on follicular proteins, treated women recovered good quality oocytes [45]. Melatonin expresses the antioxidative effect irrespective of infertility diagnosis, acting via receptors or directly on free radicals or facilitating other endogenous antioxidants. Several studies reported a positive association of higher melatonin concentrations and positive IVF outcomes, referring to the number of mature oocytes, fertilization rate or embryo quality. Subsequently, higher ROS, MDA, and 8-OHdG, and decreased TAC are associated with lower melatonin concentrations [46–48]. In contrast, Fernando *et al*. found no difference in IVF outcome regarding melatonin supplements [49]. However, significant differences coexist in the study population, dose, and duration of melatonin intake. Treatment with vitamin E

Table 3. The main characteristics of studies investigating association between oxidative stress biomarkers and ART outcomes

Study	Design			Results	
	Participants	GnRH-protocols	Oxidants	Antioxidants	Outcome
Nuñez-Calonge *et al.*, 2016	Low vs. high responders vs. controls	Agonist or antAgonist	Lipid hydroperoxides, NOx	GPx, GR, GST	Oocyte number/quality, embryo quality, fertilization rate, pregnancy rate
Kishi *et al.*, 2016	Infertile women: normal vs. poor responders	Agonist	-	TRX	Presence of oocyte, pregnancy rate
Prieto *et al.*, 2012	Endometriosis vs. controls	Not reported	MDA	Vitamin C, vitamin E, SOD	Oocyte number/quality, embryo quality, fertilization rate, implantation rate, miscarriage rate
Choi *et al.*, 2015	Endometriosis vs. controls	Agonist or antAgonist	MDA	SOD, GSH, GPX, TRX, TBP2, PRX4	Oocyte number/quality, embryo quality, fertilization rate, implantation rate pregnancy rate
Nishihara *et al.*, 2018	Infertile women	Agonist or antAgonist	8-OHdG	TAC, GSH, vitamin C	Embryo quality, blastocyst rates, fertilization rate, pregnancy rate
Nagy *et al.*, 2019	Infertile women	Modified natural cycle	–	HDL oxidation rate, PON1, apoA-I, apoA-IV vitamin E, S1P	Oocyte: number/quality, embryo quality, fertilization rate, pregnancy rate
Revelli *et al.*, 2017	Male infertility	Agonist	4-HNE	SOD, catalase	Oocyte quality, embryo quality, fertilization rate
Das *et al.*, 2006	Tubal factor infertility	Agonist	ROS, MDA	–	Oocyte quality, embryo quality, fertilization rate
Elizur *et al.*, 2014	Sibling follicles from infertile women	Agonist or antagonist	Hydrogen peroxide	–	Oocyte number, embryo quality
Jana *et al.*, 2010	Infertile women	Agonist	ROS, MDA	TAC	Oocyte quality, embryo quality, fertilization rate
Olszak-Wąsik *et al.*, 2019	Idiopathic infertility	Antagonist	TOS, MDA, lipofuscin	GPx, GR, GST, SOD, thiol groups, catalase, TAC	Embryo quality, pregnancy success
Goud *et al.*, 2014	Endometriosis vs. controls	Antagonist	NOx	–	Oocyte number/quality, embryo quality, implantation rate, pregnancy rate
Bedaiwy *et al.* 2012	Infertile women	Agonist	ROS	TAC	Oocyte number/quality, embryo quality, pregnancy rate
Borowiecka *et al.*, 2012	All causes of infertility	Agonist	TBARS, PC	Thiol groups	Pregnancy rate
Oral *et al.*, 2006	Infertile women	Agonist	MDA	–	Pregnancy success
Ashraf *et al.*, 2020	Unexplained infertility	Agonist	–	Vitamin E	Oocyte quality, embryo quality, pregnancy success
Kumar *et al.*, 2018	Infertile women	Not reported	MDA	SOD, GSH, thiol groups, vitamin C, GST, GR	Pregnancy rate

Apo – apolipoprotein, GPx – glutathione peroxidase, GR – glutathione reductase, GSH – glutathione, GST – glutathione S- transferase, HDL – high density lipoprotein, MDA – malondialdehyde, NOx – nitric oxide metabolites, PON1 – paraoxonase-1, PRX4 – peroxiredoxin, S1P – sphingosine 1 phosphate, SOD – superoxide dismutase, TAC – total antioxidant capacity, TRX – thioredoxin, TBP2-TRX – binding protein, 8-OHdG – 8-hydroxy-2′-deoxyguanosine, 4-HNE – 4-hydroxynonenal

Underlined text represents significant changes related to oxidative stress.

or a combination of both melatonin and vitamin E significantly reduces hexanoyl-lysine adduct from lipid peroxidation. Women under micronutrient and mineral supplementation have increased TAC and protein free-SH group and decreased MDA [50, 51]. Thus, the antioxidant changes compensate for oxidative stress, yielding fewer oocytes with impaired quality.

Ovarian stimulation

Treatment protocols in ovarian stimulation include GnRH antagonists or agonists [52, 53]. GnRH antagonists have several advantages over GnRH agonists such as a reduction in the treatment duration and the risk of ovarian hyperstimulation syndrome. Moreover, possible adjuvant therapies have recently been proposed [54]. A few studies compared redox homeostasis between protocols, drawing inconclusive results. Celik *et al*. noted higher concentrations of nitric oxide and SOD activity in the GnRH antagonist group, while Mathyk *et al*. found poor but significant differences in TAC [55, 56]. More studies are needed to highlight the effect of GnRH analogues on redox homeostasis in FF.

A limited number of studies compared possible alterations of FF redox balance between the natural cycle and ovarian stimulation. Reduced antioxidant properties are noted through lower PON1 paraoxonase activity, TAC, and tocopherol. Polyunsaturated fatty acids contain double bonds susceptible to oxidation in controlled ovarian stimulation, leading to a lower proportion of PUFAs and higher content of saturated fatty acids than those from a natural cycle. Among oxidative modifications of proteins, only N^{ε}-(carboxyethyl) lysine (CEL) shows higher concentrations in COH patients compared to a natural cycle [57, 58]. Oxidative damage induced by ROS could be selective, depending on amino acid composition and their vulnerability to oxidation. Exogenous gonadotropin therapy initiates intensive growth of multiple follicles creating the source of ROS overproduction [52]. Based on some studies, the microenvironment in the natural cycle differs from the microenvironment of several maturing follicles in ovarian stimulation.

Discussion

Limitations

There are several limitations to our review, because some of the research provides contradictory results. There are several possible reasons for variations in the study results. First, there is a lack of uniformity in the study design, so a larger number of prospective studies with proper patient stratification are needed to evaluate reliable conclusions. Secondly, the lack of data on dietary intake could seriously affect the homogeneity of the study group. Thirdly, because preanalytical steps are an important part in the study design, inappropriate collection and storage conditions could influence data interpretation due to oxidation ex-vivo. A comparison of the study results is difficult due to the variety of selected biomarkers and applied assays. For example, numerous studies preferred to assess overall antioxidative defence with TAC assay but consequently failed to detect the activation of specific antioxidative mechanisms. Another assay frequently used in the literature is the TBARS assay for MDA determination. The limited analytical specificity of TBARS assay with detection of other peroxidation products, rather than malondialdehyde alone, is often overlooked in the interpretation of study results. The last decade has showed improvements in the field of chromatography and mass spectrometry. These powerful technologies have become the preferred analytical technique, based on their specificity and sensitivity. Lastly, more studies with assessment of redox balance in natural cycles are needed to facilitate the effect of hormone treatment on the follicular microenvironment.

Conclusions

An extensive overview of biomarkers included in redox homeostasis confirms undoubtedly the shift to enhanced oxidizing modification of macromolecules and antioxidative consumption in follicular fluid of women undergoing IVF treatment. Still, it is not clear whether a redox disbalance has a disruptive effect on the oocyte competence or whether it plays a role in the oocyte maturation process. Considering the large number of studies addressing the importance of IVF outcomes for a couple's quality of life and health [59, 60], this study further promotes the knowledge of possible intervention sites for ART procedures.

Disclosure

The authors report no conflict of interest.

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