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REVIEW

Endoplasmic reticulum stress: a key regulator of the follicular microenvironment in the ovary

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ABSTRACT: Intra-ovarian local factors regulate the follicular microenvironment in coordination with gonadotrophins, thus playing a crucial role in ovarian physiology as well as pathological states such as polycystic ovary syndrome (PCOS). One recently recognized local factor is endoplasmic reticulum (ER) stress, which involves the accumulation of unfolded or misfolded proteins in the ER related to various physiological and pathological conditions that increase the demand for protein folding or attenuate the protein-folding capacity of the organelle. ER stress results in activation of several signal transduction cascades, collectively termed the unfolded protein response (UPR), which affect a wide variety of cellular functions. Recent studies have revealed diverse roles of ER stress in physiological and pathological conditions in the ovary. In this review, we summarize the most current knowledge of the regulatory roles of ER stress in the ovary, in the context of reproduction. The physiological roles of ER stress and the UPR in the ovary remain largely undetermined. On the contrary, activation of ER stress is known to impair follicular and oocyte health in various pathological conditions; moreover, ER stress also contributes to the pathogenesis of several ovarian diseases, including PCOS. Finally, we discuss the potential of ER stress as a novel therapeutic target. Inhibition of ER stress or UPR activation, by treatment with existing chemical chaperones, lifestyle intervention, or the development of small molecules that target the UPR, represents a promising therapeutic strategy.

Key words: endometriosis / endoplasmic reticulum stress / follicular microenvironment / granulosa cell / inflammation / oocyte / ovary / oxidative stress / polycystic ovary syndrome / unfolded protein response

Introduction

The follicular microenvironment undergoes dynamic changes during growth and maturation, ovulation, and formation of the corpus luteum. Gonadotrophins and intra-ovarian local factors contribute to regulation of the follicular microenvironment in a spatially and temporally well-coordinated manner. Recent work has shown that intra-ovarian local factors play crucial roles in ovarian physiology, as well as in pathological conditions such as polycystic ovary syndrome (PCOS) (Dumesic *et al.*, 2015a). On the contrary, recent research has revealed that endoplasmic reticulum (ER) stress, a newly recognized local factor, is an important determinant in the pathogenesis of various diseases and also plays important roles in the maintenance of physiological processes (Rutkowski and Kaufman, 2007; Walter and Ron, 2011; Hetz *et al.*, 2020). ER stress, which involves the accumulation of unfolded or misfolded proteins in the ER, is caused by various physiological and pathological conditions that increase the demand for protein folding or attenuate the protein-folding capacity in the ER. ER stress results in the activation of several signal transduction cascades, collectively termed the unfolded protein response (UPR), which affect a wide variety of cellular functions. ER stress and the UPR play critical roles in various human diseases, including diabetes, neurodegeneration, cancer, inflammatory conditions, and fibrosis, as well as in the maintenance of physiological events associated with organ function and development (Rutkowski and Kaufman, 2007; Hetz *et al.*, 2019). In this review, we present a summary of the most current knowledge of the regulatory role of ER stress in both physiological and pathological conditions in the ovary, in the context of reproduction; ER stress in malignancy is outside the scope of this review. Additionally, we present perspectives on ongoing research about ER stress in the ovary, including future directions and therapeutic applications.

ER stress

The ER is the organelle responsible for folding and assembly of secretory proteins. An imbalance between protein-folding load and capacity in the ER causes the accumulation of unfolded or misfolded proteins, a cellular state referred to as ER stress. ER stress is induced by various physiological and pathological processes, including oxidative stress, inflammation, high secretory demand, loss of calcium homeostasis, altered lipid and glucose homeostasis, pathogens, pharmacological agents, and the expression of disease-related mutant proteins (Hasnain *et al.*, 2012; Bettigole and Glimcher, 2015; Han and Kaufman, 2016; Urra *et al.*, 2016; Hetz and Saxena, 2017; Choi and Song, 2019; Hetz *et al.*, 2019; Karna *et al.*, 2020; Rocha *et al.*, 2020). ER stress activates three sensor proteins, inositol-requiring enzyme 1 (IRE1), double-stranded RNA-activated protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6), which represent the three branches of the UPR (Walter and Ron, 2011) (Fig. 1). In principle, the UPR first seeks to restore homeostasis and keep the cell alive via three main reactions: attenuation of translation to decrease the protein synthetic load; activation of synthesis of ER chaperones to increase protein-folding capacity; and induction of ER-associated degradation (ERAD) factors to remove irreparably misfolded proteins. However, if the ER stress cannot be resolved, the UPR switches to the induction of programmed cell death.

The three branches of the UPR overlap functionally and are tightly regulated in terms of timing and response amplitude. Under ER stress, the sensor proteins IRE1, PERK and ATF6 activate the three branches of the UPR, as follows (Walter and Ron, 2011; Hetz *et al.*, 2019, 2020) (Fig. 1). IRE1 dimerizes and trans-autophosphorylates in response to ER stress, resulting in activation of its endoribonuclease domain. Activated IRE1 cleaves *X-box-binding protein 1 (XBP1)* mRNA, resulting in the production of spliced XBP1 (XBP1s), a transcription factor that upregulates genes involved in ERAD and protein folding; the latter class includes chaperones such as heat shock protein family

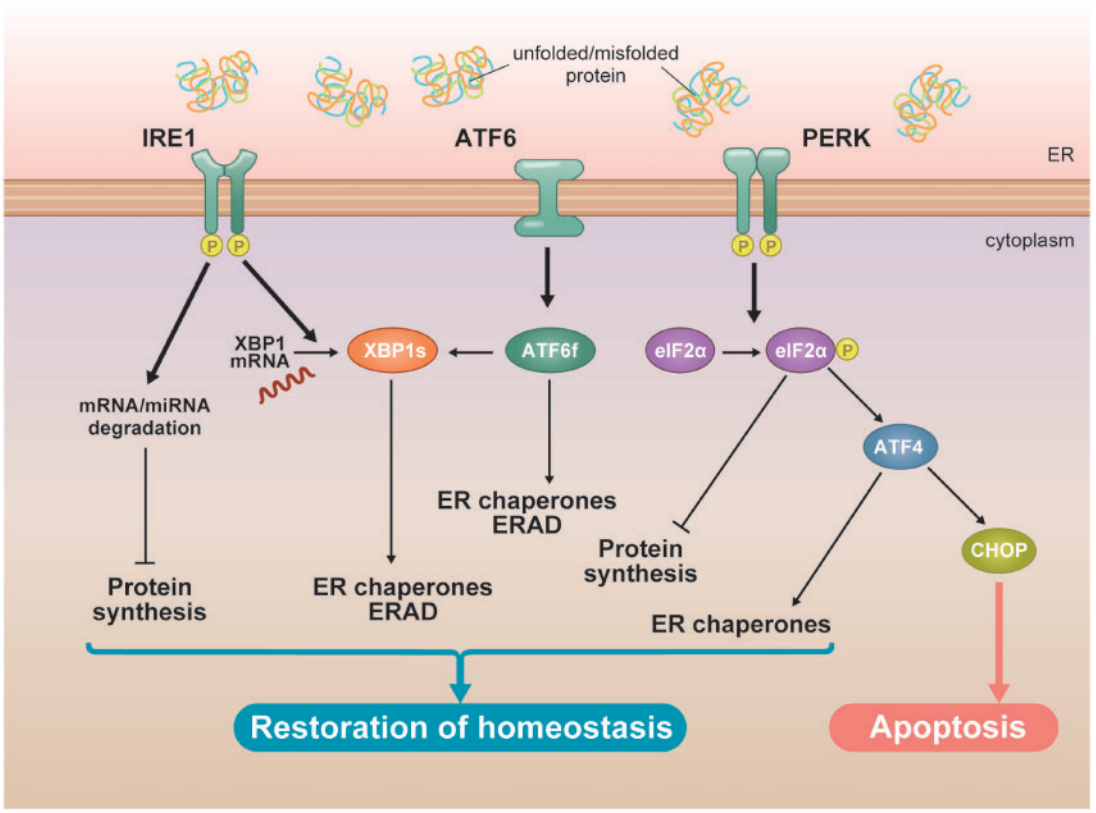

Figure 1. Endoplasmic reticulum stress and the unfolded protein response pathways. Endoplasmic reticulum (ER) stress, which involves the accumulation of unfolded or misfolded protein in the ER, results in the activation of several signal transduction cascades, collectively termed the unfolded protein response (UPR). ER stress activates the three sensor proteins, inositol-requiring enzyme 1 (IRE1), double-stranded RNA-activated protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6), which represent the three branches of the UPR. In principle, UPR first seeks to restore homeostasis and keep the cell alive via three main reactions: attenuation of translation to decrease the protein synthetic load; activation of synthesis of ER chaperones to increase protein-folding capacity; and induction of ER-associated-degradation (ERAD) factors to remove irreparably misfolded proteins. However, if the ER stress cannot be resolved, the UPR switches to induction of programmed cell death. The three branches of the UPR overlap functionally and are tightly regulated in terms of timing and response amplitude. CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; P, phosphorylation; XBP1, X-box-binding protein 1; XBP1s, spliced XBP1.

A (Hsp70) member 5 (HSPA5), also known as glucose-regulated protein 78 (GRP 78) or BiP. Activated IRE1 also degrades microRNAs and mRNAs other than *Xbp1* and, thereby reducing protein synthesis and decreasing the protein-folding load of the ER. Similar to IRE1, PERK oligomerizes upon sensing ER stress and autophosphorylates; activated PERK then phosphorylates eukaryotic initiation factor 2α (eIF2α), inactivating it and thereby inhibiting mRNA translation, eventually attenuating the protein-folding load of ER. However, inactivation of eIF2α results in preferential translation of certain mRNAs, including *ATF4*. ATF4 is a transcription factor that plays both protective and proapoptotic roles: it activates transcription of UPR target genes encoding ER chaperones, but under chronic ER stress it also upregulates the proapoptotic transcription factor C/EBP homologous protein (CHOP). ATF6 is cleaved upon activation, releasing an N-terminal cytosolic fragment, ATF6f, which acts as a transcription factor. ATF6f induces the expression of UPR target genes involved in protein folding, ERAD, and modulation of *XBP1* mRNA levels.

Activation of ER stress in the ovary

Activation of ER stress is determined either by activation of ER sensor proteins or by increased expression of UPR factors (Fig. 1). To determine the activation of sensors, a protein assay is used to detect active forms of three sensors, phosphorylated IRE1 (phospho-IRE1), phospho-PERK, and ATF6f. To determine the expression of UPR factors, an mRNA and/or protein assay is used to detect the expression of molecules in the three UPR branches, such as XBP1s, ATF6, phospho-eIF2α, ATF4, and CHOP, or a representative ER chaperone HSPA5. Table I shows a summary of representative genes/proteins involved in ER stress/UPR detected in the ovary. The findings in which activation of ER stress was examined only in whole ovary, without identifying specific ovarian cell types, are not included in this table. Most of the papers reported activation of ER stress in granulosa cells (GCs), oocytes/embryos, and cumulus-oocyte complexes (COCs); only one paper (Guerrero-Netro *et al.*, 2017) showed it in theca cells.

ER stress in physiology of the ovary

ER stress is activated in GCs of growing follicles, as well as in oocytes and pre-implantation embryos, as evidenced by activation of ER stress sensor proteins and expression of UPR factors. Activation of ER stress in mouse GCs of growing follicles is dependent on follicular stage; specifically, ER stress is activated in GCs of follicles in the later stages of development (large secondary, antral, and pre-ovulatory), but not in those of primary and small secondary follicles (Harada *et al.*, 2015). ER stress in oocytes and pre-implantation embryos has been less carefully examined; however, ATF6 is observed in mouse oocytes in all stages of growing follicles (Xiong *et al.*, 2017), and XBP1s is abundantly expressed in pig germinal vesicle stage oocytes and four-cell, morula, and blastocyst stage embryos (Zhang *et al.*, 2012b).

The role of ER stress in somatic cells during normal follicular growth and maturation, as well as in oocytes and embryos during oocyte maturation and embryo development, remains largely undetermined; the current knowledge is summarized in Table II. ER stress may modulate the roles of gonadotrophins in GCs; ER stress decreases FSH-stimulated estradiol production in mouse GCs, whereas FSH ameliorates ER stress activation, and HSPA5 is involved in regulation of LH receptor expression in rat GCs (Kogure *et al.*, 2013; Babayev *et al.*, 2016). In addition, the fertilization capacity of human oocytes is positively correlated with the expression level of XBP1s in surrounding cumulus cells (CCs) (Harada *et al.*, 2015). Moderate levels of ER stress, resulting in UPR activation in GCs and/or CCs, might contribute to oocyte maturation.

ER stress may play a role in follicular atresia during normal follicular selection. ER stress is activated in GCs of goat atretic follicles, and various UPR factors are more highly expressed in GCs of atretic follicles than in those of healthy follicles (Lin *et al.*, 2012). In addition, pharmacological activation of ER stress induces apoptosis of GCs in multiple species *in vitro* (Lin *et al.*, 2012; Wu *et al.*, 2012; Azhary *et al.*, 2019). Taken together with the observation that follicular atresia is initiated or caused by apoptosis of GCs, it is suggested that ER stress in GCs plays a role in atresia during follicular selection.

Maintenance and regression of the corpus luteum may also be regulated by ER stress. Examination of the corpus luteum during its natural history in both mouse and cow revealed that the three ER stress sensors are activated during its functional stage, whereas proapoptotic UPR factors, including CHOP, are highly expressed during its regression stage (Park *et al.*, 2013; 2014).

Expression of UPR factors increases in mouse ovaries with advanced reproductive age, concomitant with reduced expression of genes that protect against ER stress in oocytes (Kim *et al.*, 2018; Zhang *et al.*, 2019). Controlled ovarian stimulation upregulates the expression of HSPA5 in bovine oocytes, only in animals of advanced reproductive age (Cree *et al.*, 2015). Together with the findings that *in-vitro* treatment with ER stress inhibitors improves oocyte maturation and embryo development by decreasing apoptosis in multiple species (Kim *et al.*, 2012; Zhang *et al.*, 2012a; Khatun *et al.*, 2020a,b), these observations suggest that activation of ER stress in oocytes with age can decrease oocyte quality. In addition to oocytes, activity of ER stress is also affected by aging in GCs. Advanced glycation end products (AGEs) accumulate in GCs of women at late reproductive ages (Stensen *et al.*, 2014). AGEs are produced by the Maillard reaction, in which the carbonyl groups of carbohydrates react non-enzymatically with the primary amino groups of proteins; the resultant compounds bind to the receptor for AGEs (RAGE) and activate downstream signaling. AGEs accumulate in several tissues during normal aging, as well as under various pathological conditions (Unoki and Yamagishi, 2008). Treatment with AGEs upregulates expression of ATF4 in cultured human GCs, resulting in secretion of the inflammatory cytokines interleukin (IL)-6 and IL-8. In human follicular fluid (FF) and CCs harvested at IVF, the concentration of functional AGEs in FF and *ATF4* mRNA expression in CCs are significantly elevated in follicles containing oocytes that develop into embryos with poorer morphology. These findings suggest that AGE accumulation in follicles with age decreases oocyte competence by triggering inflammation via activation of ER stress in the follicular microenvironment (Takahashi *et al.*, 2019). In addition to the effects of ER stress on oocyte quality, it needs to be elucidated whether ER stress contributes to changes in ovarian stroma during reproductive aging, such as fibrosis and inflammation

Table I Representative genes/proteins involved in endoplasmic reticulum stress/unfolded protein response detected in the ovary.

Sites of activation	Genes/proteins	Species
GCs	HSPA5	Cows
	ATF4, ATF6, CHOP, HSPA5, p-IRE1	Goats
	HSPA5	Horses
	ATF4, ATF6, CHOP, HSPA5, p-eIF2α, p-IRE1, p-PERK, XBP1s	Humans
	ATF4, ATF6, CHOP, HSPA5, p-eIF2α, p-IRE1	Mice
	HSPA5, p-eIF2α	Rats
CCs	ATF4, ATF6, CHOP, HSPA5, XBP1s	Humans
TCs	ATF4, p-eIF2α	Cows
Oocytes	HSPA5	Cows
	p-PERK, XBP1s	Mice
	ATF4, ATF6, CHOP, HSPA5, p-eIF2α, XBP1s	Pigs
Embryos	ATF4, ATF6, CHOP, HSPA5, p-IRE1, XBP1s	Cows
	ATF4, CHOP, HSPA5, p-IRE1, p-PERK, XBP1s	Mice
	XBP1s	Pigs
COCs	ATF4, CHOP, HSPA5, p-IRE1, p-PERK	Cows
	ATF4, ATF6 , CHOP, HSPA5, p-IRE1, XBP1s	Mice
	ATF4, ATF6, CHOP, HSPA5, XBP1s	Pigs
Corpus luteum	ATF4, ATF6, HSPA5, p-eIF2α, p-IRE1, XBP1s	Cows
	ATF4, ATF6, CHOP, HSPA5, p-eIF2α, p-IRE1, XBP1s	Mice

ATF, activating transcription factor; CCs, cumulus cells; CHOP, C/EBP homologous protein; COCs, cumulus-oocyte complexes; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; GCs, granulosa cells; HSPA5, heat shock protein family A (Hsp70) member 5; IRE1, inositol-requiring enzyme 1; p-, phospho-; PERK, double-stranded RNA-activated protein kinase-like ER kinase; TCs, theca cells; UPR, unfolded protein response; XBP1s, spliced X-box-binding protein 1

Table II Physiological roles of ER stress in the ovary.

Sites of activation	Findings	Species	Ref.
GCs	Inhibits FSH-stimulated estradiol production	Mice	Babayev *et al.* (2016)
	Regulates LHR expression	Rats	Kogure *et al.* (2013)
	Positive correlation between oocyte fertilization capacity and XBP1s expression in CCs	Humans	Harada *et al.* (2015)
	Induces follicular atresia	Goats	Lin *et al.* (2012)
	Decreases oocyte developmental competence during reproductive aging	Humans	Takahashi *et al.* (2019)
Oocytes	Decreases oocyte quality during reproductive aging	Cows, mice	Cree *et al.* (2015) and Zhang *et al.* (2019)
Corpus luteum	Regulates maintenance and regression of corpus luteum	Cows, mice	Park *et al.* (2013, 2014)

LHR, LH receptor.

(Briley SM *et al.*, 2016; Amargant *et al.*, 2020; Zhang *et al.*, 2020), given that ER stress is closely related to the production of proinflammatory and profibrotic cytokines in GCs (Takahashi *et al.*, 2017b, 2019).

ER stress in pathological conditions of the ovary

Recent progress in this field has revealed the involvement of ER stress in various pathological conditions of the ovary (Table III). Previous studies focused mainly on the proapoptotic role of ER stress, whereas recent work has examined the various roles of the UPR in association with other local factors that constitute the follicular microenvironment. Collectively, these studies have elucidated the critical roles of ER stress as a regulator of the follicular microenvironment, suggesting novel therapeutic strategies that target ER stress. Figure 2 shows simple schemes for ER stress in the pathogenesis of various conditions, namely pharmacological insults, obesity, PCOS, ovarian hyperstimulation syndrome (OHSS), and endometrioma. These schemes are based on the current knowledge of activators of ER stress and resultant functional changes determined in the pathogenesis of each condition. Accordingly, it is

Table III Roles of ER stress in pathological conditions of the ovary.

Pathologies	Sites of activation	Findings	Species	Ref.
Pharmacological insults	GCs	↑cisplatin-induced apoptosis of GCs and follicular atresia	Mice	Wu *et al.* (2018)
	Oocytes	↑doxorubicin-induced oocyte apoptosis	Mice	Bar-Joseph *et al.* (2010)
	GCs, TCs	↑apoptosis of GCs and TCs and follicular atresia induced by endocrine disruptors (e.g. cadmium)	Cows, Humans, mice, rats	Wang *et al.* (2016), Guerrero-Netro *et al.* 2017), Wan *et al.* (2018), Chen *et al.* (2019a), Liu *et al.* (2019), and Yang *et al.* (2019)
	Oocytes, Embryos	↑cryopreservation-induced damage of oocytes and embryos	Cows, mice	Zhao *et al.* (2015), Yang *et al.* (2018), and Khatun *et al.* (2020a)
Obesity	GCs	↑ apoptosis of GCs and follicular growth arrest	Mice	Wu *et al.* (2010, 2017) and Chen *et al.* (2019b)
	COCs, Embryos	↑ apoptosis of COCs, ↓embryo development	Mice	Wu *et al.* (2010, 2012, 2015), and Yang *et al.* (2012)
	GCs	↓production of estradiol and progesterone in GCs	Goats, mice	Yang *et al.* (2017), Chen *et al.* (2019b), and Hua *et al.* (2020)
	GCs	↓hCG-stimulated progesterone production in GCs	Humans, mice	Takahashi *et al.* (2017a)
PCOS	GCs	↑secretion of TGF-β1 from GCs and interstitial fibrosis of the ovary	Humans, mice	Takahashi *et al.* (2017b)
	GCs	↑testosterone-induced apoptosis of GCs and follicular atresia	Humans, mice	Azhary *et al.* (2019)
	GCs	↑testosterone-induced accumulation of AGEs in GCs	Humans, mice	Azhary *et al.* (2020)
	COCs	↑testosterone-induced cumulus expansion	Mice	Jin *et al.* (2020)
OHSS	CCs, GCs	↑hCG-stimulated VEGF production in GCs and vascular permeability	Humans, rats	Takahashi *et al.* (2016)
Endometrioma	GCs	↑oxidative stress-induced apoptosis and cellular senescence of GCs	Humans	Kunitomi *et al.* (2020) and Lin *et al.* (2020)

AGEs, advanced glycation end products; OHSS, ovarian hyperstimulation syndrome; PCOS, polycystic ovary syndrome; TGF, transforming growth factor; VEGF, vascular endothelial growth factor

plausible that local factors other than those shown as activators of ER stress may also activate ER stress in each condition (e.g., ER stress is activated in obese individuals by high levels of free fatty acids as shown in Fig. 2b but oxidative stress and/or inflammation determined in the follicular microenvironment in obese individuals may also contribute to its activation). It also remains to be elucidated whether functional changes induced by ER stress determined in one pathology also contribute to pathogenesis of other conditions (e.g., whether the upregulation of vascular endothelial growth factor (VEGF) production in GCs by ER stress shown in OHSS also contributes to the pathogenesis of PCOS).

Pharmacological insults

Chemically induced ovarian damage can result in activation of ER stress (Fig. 2a). Administration of anticancer agent cisplatin to mice activates ER stress, mainly in GCs of secondary to antral follicles. Concomitant administration of an ER stress inhibitor ameliorates the cisplatin-induced loss of healthy follicles and the increase in the number of atretic follicles (Wu *et al.*, 2018). Another anticancer agent, doxorubicin, activates ER stress in mouse oocytes and thereby induces apoptosis (Bar-Joseph *et al.*, 2010). Cadmium, an environmental estrogen derived mainly from cigarette smoke, also activates ER stress in mouse and human GCs, and induces apoptosis of these cells, resulting in a decrease in antral follicles and an increase in atretic follicles (Wan *et al.*, 2018; Liu *et al.*, 2019; Yang *et al.*, 2019); the same effects are also observed in rat and mouse GCs and bovine theca cells following exposure to other endocrine disruptors (Wang *et al.*, 2016; Guerrero-Netro *et al.* 2017; Chen *et al.*, 2019a). Furthermore, cryopreservation of mouse and bovine oocytes and embryos, which involves exposure to vitrification solution and drastic changes in temperature, activates ER stress and induces apoptosis. Treatment with ER stress inhibitors before or during vitrification ameliorates the adverse effects of cryopreservation and improves viability and developmental competence of vitrified/warmed oocytes and embryos (Zhao *et al.*, 2015; Yang *et al.*, 2018; Khatun *et al.*, 2020a).

Obesity

Obesity in women impairs reproduction by affecting ovulatory function, as well as by decreasing oocyte quality and the ovarian response to hormonal stimulation (Practice Committee of the American Society for Reproductive Medicine, 2015). Obesity or intake of a high-fat diet (HFD) is associated with elevated concentrations of triglycerides and

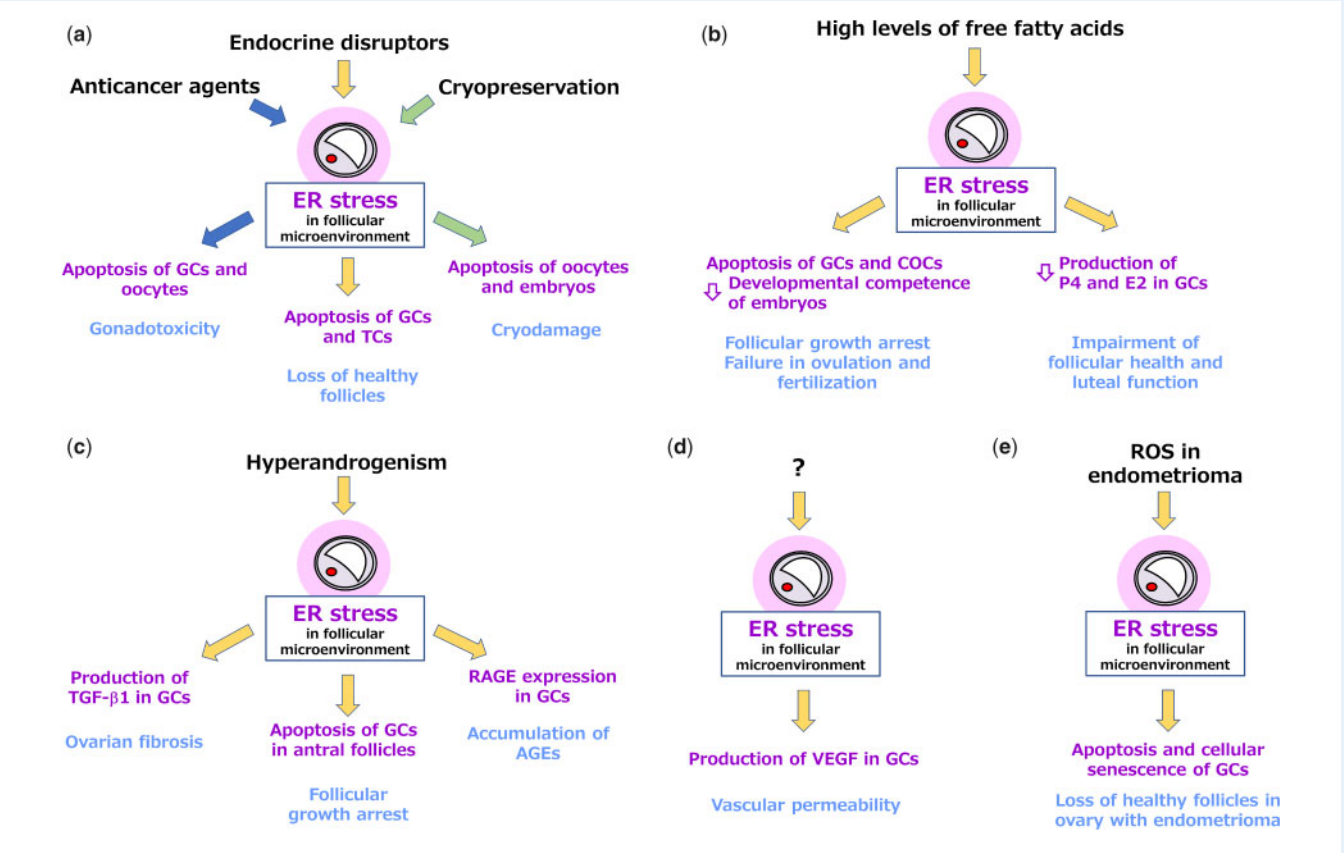

Figure 2. ER stress in the pathogenesis of various conditions. (**a**) Pharmacological insults. Chemically induced ovarian damage can result in activation of ER stress. Administration of the anti-cancer agents cisplatin and doxorubicin activates ER stress in granulosa cells (GCs) and oocytes, respectively, and induces apoptosis, which causes the gonadotoxicity of these agents. Endocrine disruptors, including cadmium, also activates ER stress in GCs and theca cells (TCs) and induces apoptosis of these cells, resulting in loss of healthy follicles. Furthermore, cryopreservation of oocytes and embryos activates ER stress and induces apoptosis, which causes cryodamage. Arrows in the same color indicate cause and result of activated ER stress in the same condition. (**b**) Obesity. High levels of free fatty acids in the follicular microenvironment of obese individuals activate ER stress in GCs, cumulus-oocyte complexes (COCs), and embryos. Activated ER stress induces apoptosis of GCs and COCs and impairs developmental competence of embryos that cause follicular growth arrest and failure in ovulation and fertilization. Activated ER stress also affects steroidogenesis by decreasing production of progesterone (P4) and estradiol (E2) in GCs; thus impairing follicular health and luteal function. (**c**) Polycystic ovary syndrome (PCOS). Local hyperandrogenism in the follicular microenvironment of PCOS activates ER stress. Activated ER stress in GCs increases production of the profibrotic cytokine transforming growth factor-β1 (TGF-β1) in GCs, thereby contributing to ovarian interstitial fibrosis. ER stress also induces apoptosis of GCs in antral follicles that causes follicular growth arrest. Furthermore, activated ER stress mediates testosterone-induced expression of receptor for advanced glycation end products (RAGE) in GCs and the resultant accumulation of advanced glycation end products (AGEs) that affect various cellular processes. (**d**) Ovarian hyperstimulation syndrome (OHSS). Cumulus cells (CCs) from patients who subsequently develop OHSS are under greater ER stress, although the activator of ER stress is not determined. Activated ER stress upregulates hCG-induced vascular endothelial growth factor (VEGF) production in GCs and increases vascular permeability, causing development of OHSS. (**e**) Endometrioma. Reactive oxygen species (ROS) present in endometrioma activates ER stress in GCs in ovaries affected by endometrioma. Activated ER stress mediates oxidative stress-induced apoptosis and cellular senescence of GCs, contributing to ovarian dysfunction in patients with endometrioma.

free fatty acids in FF in human and horse (Wu *et al.*, 2010; Sessions-Bresnahan *et al.*, 2016). High levels of free fatty acids and the resultant production of lipid peroxides cause lipotoxicity, which involves impairment of ER function (Borradaile *et al.*, 2006).

Lipotoxicity causes activation of ER stress, affecting the viability of GCs, COCs, and oocytes (Fig. 2b). ER stress is activated in GCs of obese humans and horses, and in the GCs and COCs of mice with obesity caused by HFD intake or overeating of standard chow (Wu *et al.*, 2010, 2015; Sessions-Bresnahan *et al.*, 2016; Takahashi *et al.*, 2017a). Obese mice show growth arrest of follicles at an early stage, follicular atresia, and lower rates of ovulation and fertilization; in addition, these animals have higher rates of apoptosis in GCs and COCs, and their embryos are less developmentally competent (Wu *et al.*, 2010, 2015, 2017). Activity of ER stress in COCs reflects the lipid content of the surrounding FF, as shown by the observation that treatment of mouse COCs with lipid-rich FF harvested from obese women activates ER stress in COCs (Yang *et al.*, 2012). *In-vitro* treatment of mouse COCs with pharmacological ER stress inducers or

palmitic acid, a major fatty acid in FF, activates ER stress, induces apoptosis, and impairs embryo development; these effects are reversed by co-treatment with an ER stress inhibitor (Wu *et al.*, 2012). Treatment of obese mice with ER stress inhibitors improves ovulation rate and the developmental competence of embryos (Wu *et al.*, 2015). *In-vitro* treatment with palmitic acid also activates ER stress in mouse GCs and induces apoptosis of these cells; again, this effect is reversed by co-treatment with ER stress inhibitors (Chen *et al.*, 2019b).

Obesity or intake of HFD not only impairs viability of GCs, but also affects steroidogenesis in these cells (Fig. 2b). Even in normally cycling women, elevated BMI is associated with a shorter luteal phase and lower progesterone levels (Santoro *et al.*, 2004). Indeed, activated ER stress impairs steroidogenesis of GCs, in addition to induction of apoptosis. Activation of ER stress decreases secretion of estradiol and progesterone from mouse and goat GCs *in vitro* (Yang *et al.*, 2017; Chen *et al.*, 2019b; Hua *et al.*, 2020), and abrogates upregulation of serum progesterone levels in response to hCG in mice (Takahashi *et al.*, 2017a). In human GCs, activation of ER stress decreases the hCG-stimulated secretion of progesterone. ER stress inhibits hCG-stimulated expression and enzyme activity of genes related to progesterone biosynthesis, steroidogenic acute regulatory protein, and 3β-hydroxysteroid dehydrogenase. Activation of ER stress attenuates the hCG-induced increase in the phosphorylation of protein kinase A substrates and extracellular signal-regulated kinase 1/2, without affecting hCG-stimulated activation of adenylate cyclase (Takahashi *et al.*, 2017a).

Polycystic ovary syndrome

PCOS is the most common endocrine disorder among reproductive-age women, affecting 6–20% of this population (Escobar-Morreale, 2018). Although the pathophysiology remains unclear, recent studies have shown that intra-ovarian factors play crucial roles in the pathogenesis of PCOS (Dumesic *et al.*, 2015b). ER stress is activated in GCs of both PCOS patients and immature female mice induced to develop PCOS by continuous administration of androgen, dehydroepiandrosterone (DHEA) or dihydrotestosterone (Takahashi *et al.*, 2017a; Jin *et al.*, 2020). Local hyperandrogenism in the follicular microenvironment of PCOS, regardless of serum testosterone levels, is an activator of ER stress in human and mouse GCs (Azhary *et al.*, 2019; Jin *et al.*, 2020) (Fig. 2c). Activated ER stress in GCs contributes to the pathogenesis of PCOS in several ways. Treatment of human GCs maintained *in vitro* with pharmacological ER stress inducers stimulates the expression of pro-fibrotic growth factors, including transforming growth factor (TGF)-β1, in these cells (Takahashi *et al.*, 2017b). ER stress also mediates testosterone-induced apoptosis of cultured human GCs via induction of the proapoptotic factor death receptor 5 (Azhary *et al.*, 2019). Furthermore, activated ER stress mediates testosterone-induced expression of RAGE and the resultant accumulation of AGEs in human GCs (Azhary *et al.*, 2020); it was recently recognized that AGEs accumulate in GCs of PCOS patients and contribute to its pathology (Diamanti-Kandarakis *et al.*, 2007; Merhi *et al.*, 2019). By mediating testosterone-induced AGE accumulation in GCs, ER stress may bridge hormonal and metabolic abnormalities in the pathology of PCOS, i.e., local hyperandrogenism and insulin resistance. ER stress also mediates the testosterone-stimulated mouse cumulus cell expansion *in vitro* (Jin *et al.*, 2020). The roles of activated ER stress in the pathogenesis of PCOS have been further confirmed by *in-vivo* experiments with a DHEA-induced PCOS mouse model. Treatment of PCOS model mice with ER stress inhibitors decreases interstitial fibrosis and collagen deposition in the ovary, apoptosis of GCs in antral follicles, and accumulation of AGEs in GCs, accompanied by a reduction in local ER stress in GCs (Takahashi *et al.*, 2017b; Azhary *et al.*, 2019, 2020). Intriguingly, treatment with ER stress inhibitors partially improves the reproductive phenotype of PCOS; in particular, it improves the estrous cycle and decreases the number of atretic antral follicles (Azhary *et al.*, 2020).

Critically, these findings show that ER stress directly contributes to the pathogenesis of PCOS. In addition to affecting the viability of GCs (and thus follicular and oocyte health, as proven by previous studies), activated ER stress induces interstitial fibrosis, follicular atresia, and accumulation of AGEs, thereby contributing to the pathogenesis of PCOS (Fig. 2c).

Ovarian hyperstimulation syndrome

OHSS is another disorder in which ER stress in GCs plays a critical role in its pathogenesis (Takahashi *et al.*, 2016) (Fig. 2d). OHSS is a major complication of infertility treatment that typically affects patients undergoing controlled ovarian stimulation with gonadotrophins followed by hCG administration. Excess production of VEGF has been implicated in its pathogenesis (Gómez *et al.*, 2010). CCs from patients who subsequently develop OHSS are under greater ER stress, with a positive correlation between the levels of XBP1s and VEGF (Takahashi *et al.*, 2016). ER stress upregulates hCG-induced VEGF production in human cultured GCs. Treatment of OHSS model rats with an ER stress inhibitor suppresses the increase in vascular permeability and prevents development of OHSS by decreasing VEGF production in GCs (Takahashi *et al.*, 2016).

Endometrioma

Endometrioma exerts detrimental effects on ovarian physiology and compromises follicular health (de Ziegler *et al.*, 2010). The proportion of atretic follicles in the ovarian cortex is elevated in ovaries with endometrioma, which is associated with high levels of apoptosis in GCs (Kitajima *et al.*, 2014; Sanchez *et al.*, 2014). On the contrary, GCs in ovaries affected by endometrioma are under high oxidative stress owing to the highly diffusible character of reactive oxygen species present in endometrioma (Seino *et al.*, 2002). ER stress is activated in human GCs in ovaries affected by endometrioma, as evidenced by the fact that UPR factors and activated ER stress sensors are present at higher levels than in GCs from disease-free ovaries (Kunitomi *et al.*, 2020; Lin *et al.*, 2020). In cultured human GCs, oxidative stress activates ER stress and induces apoptosis and cellular senescence; these effects are ameliorated by pretreatment with an ER stress inhibitor (Kunitomi *et al.*, 2020; Lin *et al.*, 2020). ER stress may contribute to ovarian dysfunction in patients with endometrioma by promoting oxidative stress-induced damage in GCs (Fig. 2e).

ER stress as a novel therapeutic target

The expansion of our knowledge about ER stress in various pathological conditions in the ovary has identified ER stress as a novel

therapeutic target. Hence, inhibition of ER stress and UPR induction in the ovary represents a promising therapeutic strategy that would be independent of hormonal therapies. Pharmacological approaches could proceed according to two strategies as listed in Table IV: by attenuating the protein misfolding that causes ER stress and by targeting specific UPR factors (Fig. 3).

Chemical chaperones are a group of low-molecular-mass compounds that stabilize folding proteins and buffer abnormal protein aggregation, thereby decreasing ER stress and improving ER function (Hetz *et al.*, 2013); these compounds have been used to decrease protein misfolding. Two chemical chaperones have been proven safe for clinical use in humans: tauroursodeoxycholic acid (TUDCA) and 4-phenylbutyrate (4-PBA), which have been used to treat liver diseases and urea cycle disorders, respectively, and recent research revealed their function as chemical chaperones. The *in-vivo* effectiveness of these chemical chaperones has been shown in several ER stress-related diseases in rodent models and human patients. Treatment with TUDCA or 4-PBA improves glucose tolerance in patients with insulin resistance or obesity (Kars *et al.*, 2010; Xiao *et al.*, 2011). 4-PBA also exerts a neuroprotective effect by alleviating local ER stress in a rodent model of brain ischemia–perfusion (Qi *et al.*, 2004). In pathological conditions in the ovary, administration of TUDCA improves the reproductive phenotype of PCOS and prevents development of OHSS in rodent models of each disease (Takahashi *et al.*, 2016; Azhary *et al.*, 2020).

The generation of small molecules that target the UPR has advanced rapidly, although none have yet been approved for clinical use. Most of these molecules target PERK signaling and function either as PERK inhibitors or eIF2α phosphatase inhibitors. GSK2606414 and GSK2656157, or their newly developed analogs AMG52 and AMG44, inhibit PERK phosphorylation and its resultant activation. These compounds exert inhibitory effects on tumor growth in several xenograft models, and neuroprotective effects in several animal models of neurodegenerative disease, including prion disease and Parkinson's disease (Hetz *et al.*, 2019). Salubrinal and the newly developed Sephin1, which is more selective, inactivates eIF2α phosphatase complexes, thereby increasing the levels of eIF2α phosphorylation, decreasing translation rates, activating downstream ATF4 signaling, and restoring ER homeostasis. Interestingly, unlike chronic ER stress or chemically induced stress, these eIF2α phosphatase inhibitors do not prompt the transition to apoptotic signaling, a downstream effect of ATF4. eIF2α phosphatase inhibitors are neuroprotective and confer functional improvement in multiple neurodegenerative disease models associated with abnormal protein aggregation and ER stress, including multiple sclerosis and amyotrophic lateral sclerosis (Hetz *et al.*, 2013, 2019). The effect of salubrinal was also shown in obesity-associated ovarian dysfunction. Treatment of obese mice with salubrinal reverses ovulatory dysfunction in these mice, and also alleviates poor oocyte developmental competence and skewed fetal growth in these animals, concomitant with restoration of oocyte quality and the mitochondrial DNA content of fetal tissue (Wu *et al.*, 2015). Inhibitors of phospho-eIF2α-mediated translational repression and ATF4 induction, including ISRIB, as well as molecules targeting other branches of UPR signaling, including IRE1 inhibitors (Fig. 3), have also emerged as potent candidates (Hetz *et al.*, 2019; Grandjean and Wiseman, 2020).

Pharmacological targeting is not the only way to manipulate ER stress. Given that ER stress is activated in the GCs of obese women and mice with obesity caused by HFD intake or overeating of standard chow (Wu *et al.*, 2010, 2015; Takahashi *et al.*, 2017b), lifestyle intervention might be an effective way to decrease ER stress in the ovary. Furthermore, ER stress is closely intertwined with other local factors, including oxidative stress, AGEs, and inflammation. Indeed, oxidative stress induces apoptosis of GCs via activation of ER stress, and several lines of evidence have shown that *in-vivo* and *in-vitro* supplementation with the antioxidant melatonin decreases ER stress in GCs and oocytes (Park *et al.*, 2018; Chen *et al.*, 2019b; Kunitomi *et al.*, 2020; Lin *et al.*, 2020). AGEs increase production of inflammatory cytokines in GCs, an effect that is mediated by activation of ATF4 (Takahashi *et al.*, 2019). AGEs form endogenously or are absorbed exogenously

Table IV Representative agents targeting ER stress/UPR.

Category	Agents	Mode of action
Chemical chaperones	TUDCA	Decreases protein misfolding
	4-PBA	Decreases protein misfolding
Targeting PERK signaling	GSK2606414	Inhibits activation of PERK
	GSK2656157	Inhibits activation of PERK
	AMG52/AMG44	Inhibits activation of PERK
	Salubrinal	Inactivates eIF2α phosphatase complexes, thereby increasing the levels of eIF2α phosphorylation
	Sephin1	Inactivates eIF2α phosphatase complexes, thereby increasing the levels of eIF2α phosphorylation
	ISRIB	Inhibits phospho-eIF2α-mediated translational repression and ATF4 induction
	2BAct	Inhibits phospho-eIF2α-mediated translational repression and ATF4 induction
Targeting IRE1 signaling	STF-083010	Inhibits IRE1α RNase, thereby inhibits degradation/splicing of mRNA/miRNA
	MKC3946	Inhibits IRE1α RNase, thereby inhibits degradation/splicing of mRNA/miRNA
	KIRA6/KIRA8	Inhibits IRE1α RNase, thereby inhibits degradation/splicing of mRNA/miRNA
Targeting ATF6 signaling	Compound 147	Activates ATF6

ATF6, activating transcription factor 6; eIF2α, eukaryotic initiation factor 2α; 4-PBA, 4-phenylbutyrate; TUDCA, tauroursodeoxycholic acid.

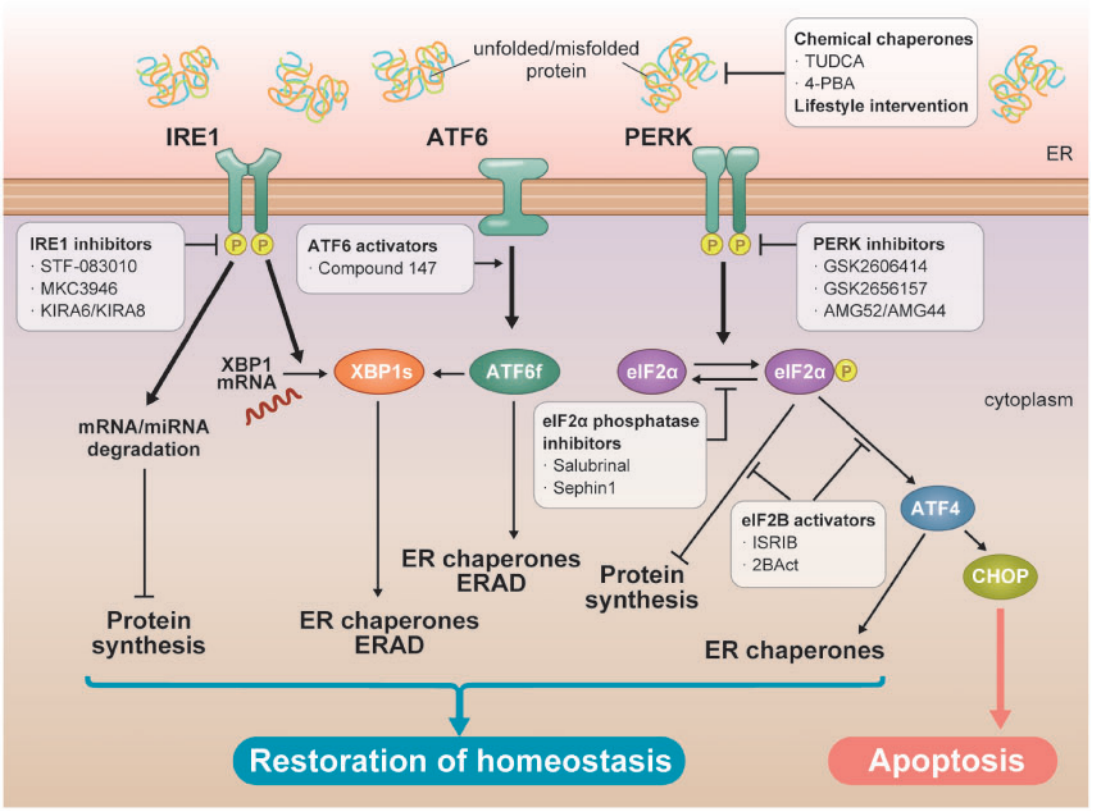

Figure 3. Potential interventions and therapeutic targets in ER stress and the UPR pathways. Potential approaches could proceed according to two strategies: by attenuating the protein misfolding that causes ER stress, and by targeting specific UPR factors. Chemical chaperones decrease protein misfolding; two molecules in clinical use, tauroursodeoxycholic acid (TUDCA) and 4-phenylbutyrate (4-PBA), were proven to function as chemical chaperones. Lifestyle modification, including weight loss, changes in dietary habits, and supplement intake, may also decrease ER stress either directly or indirectly. For the latter strategy, no small molecules that target the UPR have yet been approved for clinical use. Most of these molecules target PERK signaling, including PERK inhibitors, eIF2α phosphatase inhibitors, and eIF2B activators that inhibit downstream signaling of phosho-eIF2α. Molecules targeting other branches of UPR signaling, such as IRE1 inhibitors and ATF6 activators, are also under development.

by smoking or intake of a high-fat and/or high-protein diet, especially when the food is cooked at high temperature with low moisture (Garg and Merhi, 2015). Thus, lifestyle interventions, including supplement intake, which alleviate oxidative stress and/or AGEs could exert a beneficial effect by relieving ER stress. Lifestyle intervention targeting ER stress, either directly or indirectly, represents a potentially valuable mode of preconception care.

Conclusion

Recent research progress has revealed the critical role of activated ER stress in various pathological conditions in the ovary. Activated ER stress impairs follicular and oocyte health following exposure to various chemicals, including anticancer agents, as well as in obese women and patients with endometrioma. Intriguingly, activated ER stress not only hinders follicular growth and maturation, ovulation, and the production of high-quality oocytes, but also contributes to the pathogenesis of ovarian pathologies, including PCOS and OHSS. Future studies are necessary to determine the detailed molecular mechanisms by which the three UPR signaling pathways regulate ovarian function, as well as the interactions of these three pathways. In addition, research is also needed to elucidate the types of cells which undergo functional changes as a result of ER stress and contribute to the pathogenesis of each pathological condition, given that the ovary consists of various types of cells and UPR signaling induced by ER stress may depend on the nature of stimuli and cell types affected.

The physiological roles of ER stress and UPR remain largely undetermined. It was shown that ER stress/UPR is activated in GCs and oocytes of healthy follicles, as well as in embryos (Zhang *et al.*, 2012b, Harada *et al.*, 2015, Xiong *et al.*, 2017). Furthermore, the fertilization capacity of human oocytes is positively correlated with the expression level of XBP1s in surrounding CCs (Harada *et al.*, 2015). Accordingly, it is speculated that certain moderate levels of ER stress might be necessary for follicular, oocyte, and embryo development. Indeed, recent studies have uncovered the critical role of UPR in various physiological events. For example, ER stress and the UPR are prerequisites in shaping intestinal tissue homeostasis and immunity by playing a pivotal role

in the development, differentiation, activation, and cytokine secretion of immune cells (Coleman and Haller, 2019). Given that the primary role of the UPR is to maintain cellular homeostasis and that growing follicles, especially in later stages, undergo dynamic local environmental change, including a progressive increase in follicular size with massive proliferation of GCs and induction of vascular networks surrounding follicles, it is plausible that activated ER stress and the UPR play a role in normal follicular growth and maturation. Future research addressing the roles of ER stress and the UPR in normal follicular growth and maturation, as well as in oocyte maturation and embryo development, will open the way to understanding the regulatory machinery of normal ovarian function.

From a translational point of view, it is necessary to evaluate the effectiveness of chemical chaperones, such as TUDCA or 4-PBA, in humans. Obesity-related ovarian dysfunction, PCOS, and OHSS will be appropriate targets for testing the therapeutic effects of these chemical chaperones, which have already been shown to be effective in animal models. It will also be interesting to examine the effects of combined inhibition of ER stress and other local factors. ER stress and local factors, such as oxidative stress and AGEs, exacerbate each other, creating a vicious cycle that causes the follicular microenvironment to deteriorate. The combination of a chemical chaperone and antioxidant, both of which are safe in humans, could exert additive or even synergistic effects. Concomitantly, it is clearly necessary to investigate the *in-vitro* and *in-vivo* effectiveness of existing small molecules targeting specific UPR factors, as well as new molecules developed in the future. It would be particularly important to show *in-vivo* efficacy, as well as potential adverse effects, of candidate molecules using disease models in addition to *in-vitro* efficacy in culture systems, given the heterogeneity and temporal changes in composition of the cells in the ovary.

Data availability

No new data were generated or analyzed in support of this research.

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Authors' roles

M.H.: contribution to conception and design of the work; drafting of the article; and final approval of the version to be published. N.T.: contribution to conception and design of the work; critical revision of the intellectual content of the work; and final approval of the version to be published. J.M.K.A.: contribution to conception and design of the work; critical revision of the intellectual content of the work; and final approval of the version to be published. C.K.: contribution to conception and design of the work; critical revision of the intellectual content of the work; and final approval of the version to be published. T.F.: supervision of the work; critical revision of the intellectual content of the work; and final approval of the version to be published. Y.O.: contribution to conception and design of the work; critical revision of the intellectual content of the work; and final approval of the version to be published.

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Conflict of interest

The authors declare no conflict of interest.

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