Cellular stress responses in oocytes: molecular changes and clinical implications

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Running title: HSPs in metabolically compromised oocytes

1 **Abstract**

2 **Introduction:** The oocyte may be exposed to several sources of stress during its growth and maturation,
3 which may lead to reduced fertility. Unfolded protein responses (UPRs) play a central role to maintain
4 cell survival and repair. Transcription of heat shock proteins (HSPs) is a key element to facilitate re-
5 establishment of cellular homeostasis. Unlike somatic cells, cellular mechanisms by which oocytes can
6 sense and respond to stress are not well described.

7 **Focus:** In this chapter, we provide an overview about the impact of cellular stress, particularly due to
8 lipotoxicity, oxidative stress and heat stress on oocyte developmental competence. Next, we focus on
9 the expression of HSPs in oocytes and their potential role in UPRs in oocytes and embryos. This is based
10 on a comprehensive shot-gun proteomic analysis of mature bovine oocytes performed in our laboratory,
11 as well as a literature review. The topic is discussed in the light of our understanding of similar
12 mechanisms in other cell types and the limited transcriptional activity in oocytes.

13 **Highlights:** We show evidence that oocytes contain most HSPs in a relative abundance similar to that
14 found in cumulus cells. However, induced changes in HSP expression are limited, which is most likely to
15 be due to transcriptomic inactivity. Nevertheless, oocytes appear to utilize HSPs in an intrinsic cell-stress
16 pro-survival machinery that may temporarily extend the cellular lifespan under low/moderate stress
17 conditions, supporting further embryo development. However, this may not be sufficient to completely
18 eliminate stress and repair damage, which leads to persistent long lasting effects on embryo survival and
19 quality.

20 **Conclusions:** More fundamental research is needed both at the transcriptomic and proteomic levels to
21 further understand cell stress response mechanisms in oocytes and early developing embryos, their
22 critical interactions and their long term effects. Strategies to provide targeted external support to
23 prevent or reduce cell stress levels during oocyte maturation or early embryo development under
24 maternal metabolic stress conditions should be developed to maximize the odds of producing good
25 quality embryos and guarantee optimal viability.

26 **Keywords:** Cell stress, oocytes, embryos, ER, mitochondria, unfolded protein response, heat shock
27 proteins.

28 **Abbreviations**

29 ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; COCs, cumulus oocyte
30 complexes; eIF2α, eukaryotic translation initiation factor 2 alpha ; ER, endoplasmic reticulum; ERAD, ER-
31 associated degradation; FFAs, free fatty acids; FSH, follicle stimulating hormone; HS, heat stress; HSPs,
32 heat shock proteins; IRE1, Inositol-requiring enzyme 1; IVF, *in vitro* fertilization; MMP, mitochondrial
33 inner membrane potential; NEB, negative energy balance; OPU, ovum pick-up; OS, oxidative stress; PA,
34 Palmitic acid; PERK, protein kinase RNA-like endoplasmic reticulum kinase; ROS, reactive oxygen species;
35 SCNT, somatic cell nuclear transfer; UPRs, unfolded protein responses; UPR^{er}, UPRs induced by the ER;
36 UPR^{mt}, UPRs induced by the mitochondria; XBP1, X-box binding protein 1.

37

38 Introduction

39 The vulnerable oocyte and the increasing prevalence of infertility

40 Oocyte quality is a key factor that determines female fertility. Acquisition of oocyte
41 developmental competence is a cumulative process that takes place in the ovarian follicle
42 during oocyte growth and maturation (Fulka, et al. 1998, Watson 2007). This involves a
43 sequence of complex cytoplasmic and molecular changes that are essential to make the oocyte
44 fertilizable, ultimately leading to a viable offspring. Any perturbation in the micro-environment
45 of the oocyte within the ovarian follicle has a potential impact on oocyte quality and
46 developmental competence (Krisher 2013, Mermillod, et al. 2008), which puts fertility at risk.

47 Female infertility is a global public health issue. According to recent WHO reports, over 10% of
48 women in reproductive age are infertile or sub-fertile, and the prevalence of infertility could be
49 as high as one in every four couples in some developing countries (Mascarenhas, et al. 2012).
50 Reduced fertility is a multifactorial problem, however, more and more research clearly indicate that
51 reduced oocyte quality is a major factor (for review see Leroy, et al. (2008), and Wu, et al. (2011).
52 For example, in humans, maternal metabolic syndrome and the concomitant metabolic
53 disorders associated with western-type diets, obesity and type-2 diabetes are main causes of
54 subfertility and are strongly linked with reduced oocyte quality. Even when clinical assisted

55 reproductive services are provided, poor oocyte quality results in disappointing IVF success
56 rates (Pandey, et al. 2010).

57 Disappointing fertility results are not only relevant in a human clinical settings. In livestock,
58 fertility results determine the farmer's income, management efficiency and environmental
59 impact. Reproductive failure in pig and cow farming is now recognized as a main burden and
60 has serious economic consequences. Metabolic stress due to e.g. negative energy balance (NEB)
61 status, has been strongly correlated with disappointing fertility outcome in modern dairy industry
62 worldwide (Berry, et al. 2016). Excessive fat mobilization in NEB cows elevates blood free fatty
63 acid (FFA) concentrations, which result in a state of inflammation and lipotoxicity in several
64 tissues and organs including the ovary. This lipotoxicity has a direct negative impact on the
65 oocyte. The dairy cow model showed to be an interesting and ethically acceptable model to
66 learn more about the impact of lipotoxicity on oocyte and embryo quality (Leroy, et al. 2015).
67 Like in dairy cows, metabolic disturbances in women, associated with a significantly
68 upregulated lipolysis due to reduced insulin sensitivity, obesity and chronic stress, has been
69 associated with disappointing fertility (Gambineri, et al. 2019).

70 The resulting hyperlipidemia and elevated lipotoxic concentrations of FFAs in blood are
71 reflected in the ovarian follicular fluid and thus can have a direct negative impact on oocyte
72 quality (Leroy, et al. 2005, Valckx, et al. 2015). In women, high FFA concentrations in follicular
73 fluid were associated with a reduction in nuclear maturation and subsequent embryo cleavage
74 rates (Jungheim, et al. 2011). In IVF cycles, oocytes are routinely collected by ovum pick-up
75 (OPU) after hormonal stimulation (i.e. after maturation) and thus oocyte quality might be
76 already metabolically compromised if the mother suffers from a metabolic stress condition. The
77 phenomenon of impaired developmental capacity of oocytes exposed to a lipotoxic
78 microenvironment has been investigated in a series of studies in our laboratory and by other
79 research groups. Exposing oocytes *in vitro* during long-term (13 days) murine follicle cultures
80 (Valckx, et al. 2014) or short-term (24h) bovine oocyte maturation (Valckx, et al. 2015, Van
81 Hoeck, et al. 2014, Van Hoeck, et al. 2011) to patho-physiologically relevant concentrations of
82 FFA or follicular fluid collected from obese women (Yang, et al. 2012) resulted in a lower
83 percentage of good quality embryos after IVF. In these studies, embryo development to the

84 blastocyst stage was significantly decreased and higher embryo fragmentation rates were
85 observed. In addition, surviving blastocysts had higher apoptotic cell indices, lower cell
86 numbers, altered energy and amino acid metabolism, and exhibited different methylation and
87 transcription patterns compared to blastocysts derived from healthy oocytes (Desmet, et al.
88 2020, Van Hoeck, et al. 2013, Van Hoeck, et al. 2015). This can lead to high incidence of early
89 embryonic mortality and low pregnancy rates.

90 In addition, with the increasing importance of global warming, heat stress (HS) has also been
91 documented to have adverse effects on oocyte quality and subsequent embryonic
92 development(Yin, et al. 2019). This is due to the direct impact of heat at the level of the oocyte
93 or due to indirect stress-induced reduction in dry matter intake and the concomitant
94 (exacerbation of) negative energy balance status (Abdelatty, et al. 2018). It has been shown
95 that exposure of oocytes to HS either *in vivo* or *in vitro* impairs follicular development, reduces
96 cumulus cell expansion and oocyte quality with lower maturation and polar body extrusion
97 rates, and hampers early embryo development (Al-Katanani, et al. 2002, Yin, et al. 2019). Heat
98 stress also resulted in a lower embryo yield and quality following hormonal stimulation in cows
99 (Benyei, et al. 2003).

100 Although the patterns of development of the pathogenesis of reduced fertility under metabolic
101 and heat stress conditions may vary, it is commonly accepted that elevated levels of reactive
102 oxygen species (ROS) and reduced mitochondrial functions are key underlying causes at the
103 cellular level (Long, et al. 2015). This appears to be also true for oocytes. Oocytes and their
104 surrounding cumulus cells derived from obese mouse models or *in vitro*-exposed to
105 pathophysiological concentrations of FFAs displayed altered mitochondrial inner membrane
106 potential (MMP) [Fig. 1] and elevated ROS levels (Igosheva, et al. 2010, Marei, et al. 2012).
107 Similarly, the impact of HS on oocyte quality and early embryo development has been shown to
108 be mainly mediated through oxidative stress (OS), mitochondrial dysfunctions, and apoptosis in
109 oocytes (Li, et al. 2015, Tseng, et al. 2006) and in cumulus cells (Rispoli, et al. 2013, Shaeib, et al.
110 2016).

111 Cells can sense and respond to increased intracellular stress (e.g. due to lipotoxicity, oxidative
112 damage or direct effect of heat). These responses are mainly initiated in the endoplasmic
113 reticulum (ER) (Ozcan, et al. 2004, Zhang and Kaufman 2008) and the mitochondria (Runkel, et
114 al. 2014) due to the accumulation of misfolded or unfolded proteins. Hence, they are called
115 unfolded protein responses (UPRs). A mild degree of stress stimulates transcription of nuclear-
116 encoded chaperones (mainly heat shock proteins (HSPs)) to facilitate repair mechanisms and
117 re-establish cellular homeostasis, as described hereafter, whereas high stress levels activate
118 programmed cell death (apoptosis) to eliminate damaged cells.

119 Cellular stress responses have been mainly investigated and described in somatic cells. Our
120 understanding of the mechanisms by which HSPs influence oocytes and embryo developmental
121 capacity under different pathophysiological conditions is still emerging. Recent data from
122 studies investigating the impact of metabolic or heat stress on oocyte and embryo quality and
123 the underlying molecular changes provide a better insight and will be the focus of this chapter.

124 **Heat shock proteins and cell stress responses**

125 Heat shock proteins (HSPs) are universally expressed across most cell types and within virtually
126 all organisms. They are known to act as molecular chaperons and are involved in several cellular
127 functions related to protein homeostasis (proteostasis) such as facilitating protein and peptide
128 transport, stabilization of nascent proteins, inhibition of protein aggregation, and the mediation
129 of protein complex formation (Becker and Craig 1994). Thus, HSPs are basically responsible for
130 facilitating the biogenesis, stabilization, folding, trafficking, and degradation of proteins within
131 the cell (Nixon, et al. 2017).

132 Members and families of HSPs are classified mainly according to their molecular weight, which
133 varies from 10 kDa (HSP10) to over 100 kDa (e.g. HSP110). Recently, a new nomenclature has
134 been proposed (Kampinga, et al. 2009) where HSPs are divided into the following families:
135 HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40), and HSPB (small HSP), as well as
136 the chaperonin families HSPD (HSP60), HSPE (HSP10) and CCT (TRiC). Members of the HSPA
137 family are most abundant, with at least 13 gene products characterized in the human proteome
138 (Radons 2016).

139 The core structural elements of most HSP classes display a considerable degree of conservation,
140 and their expression and molecular functions are interconnected and complementary (Bukau,
141 et al. 2006). For example, DNAJ (HSP40) family are known to play a key regulatory role in
142 modulating HSP70 activity, directing substrates to the protein and focusing this family to
143 discrete subcellular compartments (Kampinga and Craig 2010). The HSP40–HSP70 partnership
144 can subsequently engage HSP90s, to assist in further folding or protein degradation pathways
145 (Nixon, et al. 2017). This illustrates the importance of estimating, not only the function of
146 individual HSPs, but also their network with other HSPs and regulatory molecules that interact
147 within the proteostasis pathways.

148 Importantly, in addition to the ability of HSPs to enhance the recovery of stressed cells,
149 constitutively expressed HSPs and their chaperoning function appear to be also essential under
150 physiological conditions for the house-keeping roles and for protection against stress (reviewed
151 in Kampinga and Craig 2010, Radons 2016). These maintenance functions include transport of
152 proteins between cellular compartments, folding and refolding of newly synthetized proteins,
153 degradation of unstable and misfolded proteins, prevention and dissolution of protein
154 aggregates, and control of regulatory proteins.

155 **Expression and abundance of HSP in oocyte and their surrounding somatic cells.**

156 Cell stress responses in oocytes and early developing embryos are expected to be different
157 from those described in somatic cells. This is because growing oocytes are arrested at the
158 prophase of the first meiotic division and are known to be transcriptionally inactive particularly
159 during the final stages of growth and maturation and until embryo-genome activation (Funaya
160 and Aoki 2017). During this phase of transcriptional silence, the oocyte relies on stored mRNA
161 transcribed earlier during oogenesis (Peaston, et al. 2010). It has been recently shown that the
162 surrounding cumulus cells may also deliver cargos containing mRNA molecules through the
163 trans-zonal projections which may also contribute to the oocyte's mRNA reserve (Macaulay, et
164 al. 2016). As mentioned above, UPRs involve stimulation of *de novo* expression of nuclear-
165 encoded factors, which may not be feasible at late stages of oocyte maturation and shortly
166 after fertilization due to the transcriptional inactivity. For this reason, studying cell stress

167 responses in oocytes and early embryos can be challenging and may be particularly misleading
168 if investigated at the transcriptomic level. More importantly, investigating these mechanisms in
169 whole cumulus-oocyte complexes (COCs) can be even more misleading due to the marked
170 evident differences between the two cell types. Nevertheless, the transcriptional inactivity *per*
171 *se* does not rule out the potential ability of oocytes to respond to cell stress by adaptive
172 changes in translation and protein synthesis.

173 A comprehensive study performed in our laboratory was the first to look into the molecular
174 changes that occur in metabolically compromised oocytes at the proteomic level as compared
175 to their surrounding layers of somatic (cumulus) cells (Marei, et al. 2019c). We used Tandem
176 Mass Tag (TMT)-labelling and shotgun mass spectrometry-based analysis to screen the
177 proteomic profile of cumulus cells and the enclosed denuded oocytes isolated from bovine
178 COCs matured in the presence or absence of pathophysiological, lipotoxic concentration of FFA
179 (Palmitic acid, PA). We could identify 1703 and 1185 proteins in cumulus cells and oocytes,
180 respectively, 679 of which were common. The database of this analysis offers a unique insight
181 into the relative abundance of these proteins, their functional annotations, and how they are
182 differentially regulated in response to PA-induced metabolic and oxidative stress (Marei, et al.
183 2019c). Several members of HSP families could be detected in cumulus cells as well as in the
184 oocytes. The relative abundance of these proteins in oocytes and cumulus cells is shown in
185 Table 1.

186 The unique insight from the data displayed in Table 1 suggests that the oocyte exhibit a very
187 similar expression pattern of most HSP family members at the protein level compared to
188 cumulus cells. In addition, the illustrated relative abundance indicates that few specific HSPs
189 are particularly highly expressed suggesting that they may play an important role in cellular
190 homeostasis in oocytes. These specific HSPs are namely related to the HSP70 (HSPA5 and
191 HSPA8) and HSP90 families (HSP90AA1 and HSP90B1). These molecules are crucial for UPR
192 signaling induced in the ER (UPR^{er}). In addition, few HSPs related to mitochondrial functions
193 (e.g. HSPD1 and HSPE1) could also be detected in the oocytes. This chapter will further expand
194 on ER and mitochondrial stress responses in oocytes, and the involvement of HSPs.

195 **Table 1.** The relative abundance (RA) of members of different HSP families that were identified
196 in a shotgun proteomic analysis of bovine denuded oocytes and cumulus cells isolated after *in*
197 *vitro* maturation of intact COCs.

ID	Description	Localization*	RA in Oocytes**	RA in Cumulus cell**
HSPH1	Heat shock protein 105 kDa	C;N	2,84	0,59
HSP90A	Heat shock protein 90kDa alpha	C;N	2,65	0,97
HSP90AA1	heat shock protein 90 alpha family class A member 1	C;X;m;N	25,86	9,10
HSP90AB1	Heat Shock Protein 90 Alpha Family Class B Member 1	C;X;m;M;N	2,45	0,00
HSP90B1	Heat Shock Protein 90 Beta Family Member 1	C;ER;m;N;L	10,26	23,18
CDC37	Hsp90 co-chaperone Cdc37	C	0,43	0,12
AHSA1	AHA1, activator of heat shock 90kDa protein ATPase homolog 1	C	0,37	0,27
HSPA1A	Heat shock 70kDa protein 1A	C	0,34	0,48
HSPA1B	Heat shock 70 kDa protein 1B	C;S	5,27	1,24
HSPA1L	Heat shock 70 kDa protein 1-like	C	0,05	0,00
HSPA2	Heat shock 70kDa protein 1A	C	2,94	0,16
HSPA4	Uncharacterized protein	C	1,86	0,95
HSPA4L	Uncharacterized protein	C	1,29	0,00
HSPA5	78 kDa glucose-regulated protein	C;ER;L	15,80	27,29
HSPA6	Uncharacterized protein	C	0,01	0,00
HSPA8	Heat shock cognate 71 kDa protein	C;D;m;N	10,62	7,88
HSPA8	HSPA8 protein (Fragment)	C;D;m;N	0,02	0,00
ST13	Heat shock 70kD protein binding protein	C	0,36	0,97
HSPB1	Heat shock protein beta-1	C;m;N	1,58	14,78
HSPBP1	HSPA (Heat shock 70kDa) binding protein, cochaperone 1	C	0,07	0,00
HSPD1	60 kDa heat shock protein, mitochondrial	C;D;m;M;L	7,05	5,10
HSPE1	10 kDa heat shock protein, mitochondrial	C;m;M;L	1,82	1,74
HSPA9	Stress-70 protein, mitochondrial	M;N	3,99	3,71
TRAP1	Heat shock protein 75 kDa, mitochondrial	m;M;L	1,44	0,51
DNAJA3	DnaJ (Hsp40) homolog, subfamily A, member 3	C;M;N;L	0,24	0,00

198

199 *Cellular localization of the identified HSPs is shown: C, cytoplasm; D, endosomes; ER, endoplasmic
200 reticulum; L, organelle lumen; M, mitochondria; m, membranes; N, nucleus; S, cytoskeleton; X,
201 extracellular.
202 **Relative abundance (RA) is calculated as a percentage of the total relative abundance of the detected
203 HSPs (i.e. not from the total proteins) in oocytes and cumulus cells respectively.
204

205 **Heat shock proteins, UPRs and ER stress in oocytes**

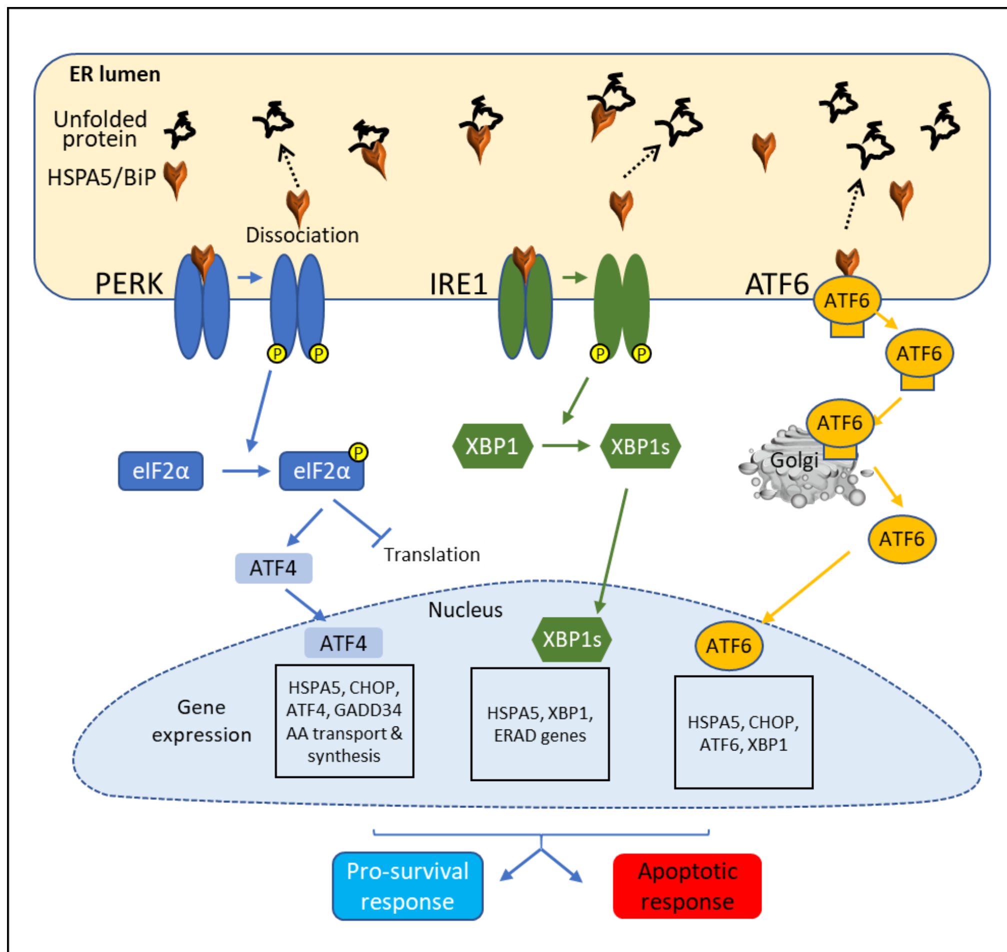
206 *Mechanisms of UPR^{er}*

207 The ER is a major intracellular organelle mainly responsible for protein synthesis and is also
208 involved in lipid synthesis and metabolism, and in cellular Ca^{2+} homeostasis (Ozcan, et al. 2004).
209 Oocyte growth and maturation and early embryo development require active *de novo* protein
210 synthesis. Synthesis of soluble and membrane proteins by translation of maternal mRNA, or
211 embryonic mRNA after embryo gene activation, is essential for several cytoplasmic and
212 molecular changes that occur during oocyte development, and for embryonic cell replication
213 and differentiation (Schultz and Wassarman 1977). Newly synthesized proteins must be
214 properly folded in the ER to acquire their functional 3D conformation (Lin, et al. 2019).

215 ER functions and protein folding processes are sensitive to several pathophysiological
216 conditions such as lipotoxicity, OS, HS as well as glucose deprivation, and aberrant Ca^{2+}
217 regulation (Adachi, et al. 2009, Pagliassotti, et al. 2016). Increased stress level results in failure
218 of protein folding or protein misfolding, which are then retained in the ER to be either repaired
219 or degraded by the ER-associated degradation (ERAD) machinery (Nakatsukasa, et al. 2008).
220 Accumulation of unfolded or misfolded proteins within the ER induces the ER stress responses,
221 which constitute an important quality control adaptive mechanism that allow the cell to sense
222 and respond to stress (Huang, et al. 2017, Lin, et al. 2019).

223 Unfolded protein responses induced by ER stress are mainly cytoprotective and aim at re-
224 establishment of cellular homeostasis by attenuation of protein synthesis (translation) to
225 reduce the folding load within the ER, and by activation of transcription of chaperone proteins
226 and folding catalysts to enhance the folding capacity (Trusina and Tang 2010). These cyto-
227 protective UPRs are usually efficient under transient or mild-degree of stress. Prolonged
228 exposure or high levels of stress, in contrast, stimulate cyto-destructive UPRs which induce
229 apoptosis to eliminate the cells that cannot be repaired (Pagliassotti, et al. 2016). Although
230 these mechanisms are vital to maintain normal tissue and body functions, excessive ER-stress
231 induced cell death has been shown to cause diseases such as diabetes mellitus (Brozzi and
232 Eizirik 2016).

233 The mechanism by which misfolded proteins stimulate ER stress response signaling cascade is
234 initially mediated by three ER transmembrane sensors, namely PERK, IRE1α and ATF6, and by
235 their interaction with the Heat Shock 70 kDa Protein 5 (HSPA5). The subsequent downstream
236 signaling transduction is initiated by destabilization and dissociation of HSPA5 from these
237 transmembrane sensors to bind the accumulated unfolded or misfolded proteins in the ER
238 lumen (Lin, et al. 2019) (Fig.1).



239

240 **Figure 1.** Unfolded protein responses induced by the endoplasmic reticulum (ER) in response to
241 accumulation of misfolded proteins in its lumen. Dissociation of HSPA5 from the ER
242 transmembrane sensors PERK, IRE1 and ATF6 results in activation of downstream signaling that
243 stimulate the expression of nuclear encoded chaperons. According to the level of stress these
244 mechanisms can lead to re-establishment of cellular homeostasis and cell survival or induction
245 of programmed cell death (apoptosis).

246 Upon activation of UPR^{er}, the dissociation of HSPA5 (BiP) from the transmembrane sensor PERK
247 to bind misfolded proteins triggers autophosphorylation of PERK, which then causes
248 phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2α). Activation of
249 eIF2α subsequently activates ATF4 (activating transcription factor 4) which stimulates
250 expression of nuclear encoded chaperons. Dissociation of HSPA5 from IRE1 and ATF6 similarly
251 activates XBP1 and ATF6, respectively, which in-turn upregulate nuclear expression of HSPs and
252 chaperons, including HSPA5 itself (Gardner, et al. 2013).

253 *Constitutive role of HSPs and UPR^{er} in oocytes*

254 ER-induced UPRs have been shown to play important physiological roles in reproductive
255 functions (for review see Huang, et al. (2017)). HSPs have been shown to be essential to protect
256 the gametes and embryos from damage during gametogenesis and embryogenesis (Neuer, et
257 al. 2000). HSPs, especially those related to the 70kDa family were found to be among the first
258 genes expressed at the zygotic genome activation (Christians, et al. 1995), and their protective
259 role during mammalian embryo development is well described (Christians, et al. 2003). In this
260 chapter we specifically focus on the described role of ER stress and UPRs at the oocyte level. As
261 shown in Table 1, we have identified several members of different HSP families in mature
262 bovine oocytes at the proteomic level.

263 The Heat Shock 70 kDa Protein 5 (HSPA5), which was found to be highly abundant in cumulus
264 cells and in oocytes, is a key molecule in ER stress responses. HSPA5 is one of the most
265 abundant members of the HSP70 (HSPA) family and is located in the lumen of the ER. HSPA5 is
266 also known as Binding immunoglobulin Protein (BiP) or Glucose-Regulated Protein, 78kD (GRP-
267 78). HSPA5/BiP binds newly synthesized proteins as they are translocated into the ER, and
268 maintains them in a state competent for subsequent folding and oligomerization (Gething
269 1999, Ng, et al. 1992).

270 Apparently, the activation of ER-induced UPR mechanisms plays important physiological role
271 during ovarian follicle growth and maturation. HSPA5 as well as other UPR^{er} markers: ATF4,
272 ATF6, and XBP1 have been shown to be expressed in immature and mature oocytes during all
273 stages of preimplantation embryo development in mice, pigs and cows (Yang, et al. 2012, Yoon,
274 et al. 2014, Zhang, et al. 2012a, Zhang, et al. 2012b). It has been recently shown that HSPA5
275 expression is upregulated in granulosa and cumulus cells during mice antral-follicle
276 development together with the activation of other ER-stress sensors (IRE1 and PERK) (Harada,
277 et al. 2015). This could be required to cope with the high rate of *de novo* protein synthesis,
278 which is vital during follicular development for the acquisitions of oocyte developmental
279 competence. Knock-out of UPR-associated genes such as HSPA5 (*Grp78-/- mice*) resulted in cell
280 proliferation defects and a massive increase in apoptosis in the ICM and failure of peri-
281 implantation embryo development (Luo, et al. 2006). Moreover, the expression level of XBP1
282 was found to be doubled in human cumulus cells enclosing oocytes that were successfully

283 fertilization compared to those that were unfertilized following ICSI (Harada, et al. 2015).
284 Relative high expression of HSPAs in oocytes was linked with better adaptation of cattle breeds
285 to environmental stress and to the production of good quality oocytes during ovum pick-up
286 programs (Souza-Cacares, et al. 2019). Altogether, this illustrates the important constitutive
287 role of UPR^{er} for oocyte quality.

288 *The role of HSPs and UPR^{er} under stress conditions*

289 Systemic effects of maternal metabolic alterations due to e.g. obesity are strongly connected to
290 increased levels of ER-stress in many cell types. The ER membrane has low cholesterol content
291 and is composed mainly of unsaturated phosphatidylcholine (Higgins 1981, Koh, et al. 2018).
292 This unique lipid composition is important to maintain a high degree of membrane fluidity,
293 which is essential for the ER functions. High fat diet feeding and obesity have been shown to
294 increase FFA concentrations in blood and ovarian follicular fluid (Ferrannini, et al. 2007, Valckx,
295 et al. 2012). These elevated FFA concentrations are predominantly saturated (e.g. composed of
296 high PA concentrations). PA is known to be a pathophysiological inducer of ER stress in different
297 types of cells and tissues (Haywood and Yammani 2016). *In vitro* exposure of murine (Yang, et
298 al. 2012) and bovine (Valckx, et al. 2015) COCs to follicular fluid from obese individuals or to
299 equivalent FFA concentrations has been shown to increase lipid droplet accumulation in the
300 enclosed oocytes. This may lead to hardening of cell membranes and intracellular membranous
301 structures including ER by increasing phosphatidyl-choline: phosphatidyl-ethanolamine ratio,
302 and can directly impact ER functions for protein synthesis, lipid metabolism, and signal
303 transduction in the affected oocytes. High levels of ER-stress have been associated with
304 inhibition of granulosa cell response to FSH *in vitro* and *in vivo* (Babayev, et al. 2016) and may
305 thus have a negative impact on follicle development and steroidogenesis. In addition, ER stress
306 and the associated UPRs were evident in metabolically-compromised oocytes collected from
307 obese mouse models (Robker, et al. 2011, Wu, et al. 2011, Yang, et al. 2012). This effect was
308 mimicked by direct exposure of bovine COCs to a lipotoxic concentrations of FFAs *in vitro* during
309 maturation (Marei, et al. 2017, Marei, et al. 2019c, Sutton-McDowall, et al. 2016).

310 Interestingly, functional analysis of the proteomic profile of oocytes from PA-exposed bovine
311 COCs (Marei, et al. 2019c) showed that the enclosed oocytes undergo activation of the EIF2

312 signaling, which is a feature of PERK-ATF4 mediated ER stress known to inhibit protein synthesis
313 (Fig. 1). Importantly, although the reduction in protein synthesis is an attempt to reduce protein
314 folding load in the ER and re-establishment of cellular homeostasis, this may have long term
315 consequences on oocyte developmental competence in surviving oocytes and embryos, due to
316 the highly dynamic nature of development.

317 ATF4 was found to be particularly upregulated in granulosa cells and COCs collected from HFD-
318 induced obese mice, and was associated with lower fertilization rates (Wu, et al. 2010). ATF4 is
319 a key UPR-mediated gene expression, however it is difficult to interpret the significance of its
320 upregulation in metabolically stressed-COCs. Firstly, upregulated gene expression of ATF4 in the
321 affected COCs is most likely to be in cumulus cells rather than in oocytes. Secondly, ATF4 can
322 activate both cell survival and cell death mechanisms depending on the degree and duration of
323 stress exposure. Under mild stress, ATF4 induces cell survival by stimulating mRNA expression
324 of genes involved in stress responses, protein secretion and amino acid metabolism (Harding, et
325 al. 2003). Interestingly, we observed that in PA-exposed COCs, pathways related to the
326 degradation of Phenylalanine, Isoleucine, Tryptophan, Threonine, and the biosynthesis of
327 Tyrosine were among the top canonical pathways reported in the enclosed oocytes based on
328 the changes in their proteomic profile (Marei et al 2019). This indicates that the downstream
329 effect of ATF4 is somehow active in the affected oocytes. Embryos produced by IVF of FFA-
330 exposed bovine COCs also exhibited long-term effects on amino acid turnover detected at the
331 blastocyst stage (Van Hoeck, et al. 2011).

332 On the other hand, dissociation of ATF6, another ER transmembrane sensor, from HSPA5
333 results in cleavage, activation and translocation of ATF6 to the nucleus where it is known to
334 induce transcriptional activation of ER-stress-response genes, particularly HSPA5, XBP1 and
335 CHOP (Fig. 1). The expression of *HSPA5* is thus expected to be significantly increased under
336 stress conditions and is therefore extensively used as a master marker for detecting the
337 induction of ER stress (Lee 2005). COCs from mice fed a high-fat diet had increased mRNA
338 expression of *HSPA5* (Wu, et al. 2010). We have also shown that HSPA5 protein was
339 significantly high in bovine COCs (as a whole unit) exposed to elevated concentrations of FFAs
340 and was linked with reduced developmental competence (Marei, et al. 2017). However

341 surprisingly, we found that the protein abundance of HSPA5 itself was significantly reduced
342 (fold change = 0.65, adjusted *P* value < 0.05) in oocytes in PA-exposed COCs compared to
343 controls (Marei, et al. 2019c). As mentioned above, this may reflect HSPA5 utilization for
344 misfolded protein transport and degradation in the absence of active HSPA5 transcription
345 leading to depletion of this important chaperon in the affected oocytes and in the subsequent
346 stages of early embryo development.

347 The fact that expression of some HSPs may actually decrease (and not increase) in oocytes
348 under stress conditions have also been reported in other studies. Yin, et al. (2019) examined
349 gene expression of *HSP70*, *HSP90α, HSP90*β*, HSP40, HSP40, HSPA4, HSPA4L, HSPH1* and *HSF1* in
350 porcine COCs subjected to HS at 41.5°C for 24h. All of these mRNAs were found to be
351 significantly downregulated in oocytes and upregulated in cumulus cells. The stage of oocyte
352 growth was also suggested to play a role. For instance, after 1h exposure to HS, *HSP70* was
353 reported to increase in growing porcine oocytes but decrease in the fully grown ones (Lanska,
354 et al. 2006), which could be related to the different transcriptomic activity at these stages. In
355 contrast, Pennarossa et al. (2012) found that *HSP70, HSP40, HSPH1, HSPA4, HSPA4L, HSF1*, and
356 *HFS2* genes (but not HSP90A and HSP90B which were also constitutively expressed) were
357 upregulated in porcine oocytes upon exposure to HS (41.5°C for 1 h) but surprisingly were not
358 affected in the surrounding CCs (Pennarossa, et al. 2012). In studies showing a reduced HSP
359 mRNA expression or increase HSP protein expression, this might be due to increased translation
360 of those HSPs and thus depletion of the corresponding mRNA reserve. In contrast, the increase
361 in HSP mRNA expression in oocytes reported in some studies is more difficult to explain, but
362 may be due to transfer of HSP mRNA molecules from cumulus cells to oocytes while transzonal
363 projections are still intact (Macaulay, et al. 2016).

364 What is even more perplexing is the conflicting responses of cumulus cells themselves to
365 different types of metabolic stress in different studies. While some studies show increased HSP
366 mRNA expression in cumulus cells under stress conditions e.g. as shown by Yin, et al. (2019), we
367 found that cumulus cells in PA-exposed COCs exhibited a significant reduction in protein
368 expression of HSP90B1, HSPA9, HSPB1, and HSPE1, as well as several other pro-survival proteins
369 involved in ER UPRs such as CALR, P4HB, PDIA6 and CANX (Marei, et al. 2019c). This was

370 associated with increased apoptosis in cumulus cells and was considered to be a sign of ER-
371 stress-induced cell death mechanisms mediated by "regulated IRE1α-dependent decay". This
372 mechanism degrades mRNA encoding for crucial pro-survival proteins such as ER chaperons and
373 is induced under high levels of ER stress (Hollien and Weissman 2006, Moore and Hollien 2015).

374 It is also important to notice that the effect of metabolic stress or HS is not homogenous across
375 all cumulus cells of the same COC. Using specific fluorescence markers, we have demonstrated
376 that cumulus cells exhibiting different levels of ROS, mitochondrial MMP, and caspase-3
377 expression (apoptosis marker) co-exist in a heterogenous pattern within the same metabolically
378 compromised COCs (Leroy, et al. 2005, Marei, et al. 2019c). Therefore, the overall expression of
379 HSPs either at the transcriptomic or proteomic level in cumulus cells will depend on the
380 proportion of cells experiencing low level of stress (and upregulated chaperon expression) and
381 those already at an apoptotic stage (exhibiting regulated IRE1α-dependent decay).

382 Focusing back at HSP induced responses in stressed oocytes, and in contrast to the expression
383 of HSPA5, we found that PA-exposed oocytes exhibited significant upregulation in protein
384 expression of HSPA8 (1.52X increase, adjusted *P* value <0.05). HSPA8 is a chaperon located in
385 the ooplasm, nucleus and endosomes and plays an important role in regulation of protein
386 import and stability, chaperon-mediated protein transport and refolding, chaperon-mediated
387 autophagy, and chaperone cofactor-dependent protein refolding. Interestingly, STIP1 (stress
388 induced phosphoprotein 1) which acts as a co-chaperone for HSPA8 and for HSP90AA1 was also
389 upregulated (1.26X) in PA-exposed oocytes (Marei, et al. 2019c). As shown in table 1,
390 HSP90AA1 is constitutively highly abundant in the oocytes but was not upregulated in response
391 to PA-exposure (Marei, et al. 2019c). Other upregulated factors involved in chaperon-mediated
392 protein folding were detected: FKBP4 (FK506 binding protein 4, 1.28X), CCT8 (chaperonin
393 containing TCP1 subunit 8, 1.36X), and NASP (nuclear autoantigenic sperm protein, 1.52X), as
394 well as those involved in ER-associated ubiquitin-dependent protein catabolic processes:
395 BCAP31 (B-cell receptor associated protein 31, 1.64X). These changes clearly illustrate the
396 capacity of the oocyte proper to sense and respond to the metabolic stress at protein level. Of
397 course this is dependent on the oocyte's mRNA reserves.

398 Interestingly, HSPA8, which was specifically upregulated, is also known to be involved in
399 positive regulation of mRNA splicing via spliceosomes. mRNA splicing is a process by which pre-
400 mRNA transcripts are transformed into a mature mRNA and could be needed to activate the
401 maternal pre-mRNA reserve for translation. We could also detect more evidence of proteomic
402 changes that support RNA activation, binding and stabilization, and RNA transport: including
403 the increased Poly(A) binding protein PABPC4 (1.34X) and Nucleoprotein 35 (1.51X), as well as
404 regulation of protein translation via the increased ribosomal proteins RPL27A (1.45X) (Marei, et
405 al. 2019c). This illustrates the ability of the oocytes to strategically use their RNA reserve for
406 adaptive mechanisms under stress conditions.

407 As mentioned above, ER stress does not only occur in response to lipotoxicity, HS and oxidative
408 stress. Several other physicochemical alterations may result in ER stress such as osmotic stress,
409 abnormal pH, and chemical exposure (Brozzi and Eizirik 2016)). There are several ER stress
410 inducing compounds that have been characterized and are widely used in research. Chemical
411 stimulation of ER stress by e.g. Tunicamycin (which inhibits N-glycosylation needed for protein
412 folding) has been shown to negatively affect oocyte maturation and resulted in marked
413 increase in embryonic arrest at the 2-cell stage and reduced blastocyst rates due to increased
414 apoptosis in mice, pigs and cattle (for a review see Nixon, et al. (2017)). It appears that the
415 impact of such experimental chemical induction of ER stress may go beyond the expected stress
416 levels induced by pathophysiological perturbations and induce apoptosis in the oocytes and
417 higher rates of apoptosis during subsequent embryo development.

418 **HSPs, UPRs, and mitochondrial stress in oocytes**

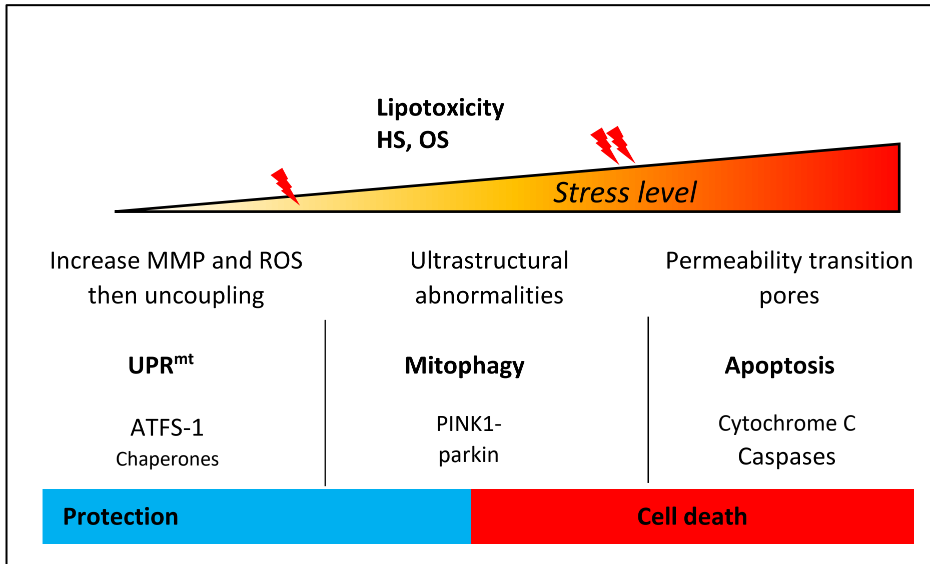
419 As mentioned above, several studies have shown that that metabolic stress associated with high fat
420 diet (HFD)-induced obesity causes altered mitochondrial functions in oocytes (Igosheva, et al. 2010,
421 Luzzo, et al. 2012, Mitchell, et al. 2009). Mitochondrial dysfunction clearly plays a central role in
422 the pathogenesis of reduced oocyte quality and subfertility. Oocytes from obese mice exhibit
423 altered mitochondrial inner membrane potential (MMP) and reduced ATP production, as well
424 as increased mitochondrial ultrastructural abnormalities, altered mitochondrial biogenesis and
425 mtDNA copy numbers, compared to mice fed control diet (Igosheva, et al. 2010, Wu, et al.

426 2010, Wu, et al. 2011, Wu, et al. 2015). Similarly, *in vitro* studies have shown evidence of
427 mitochondrial dysfunction (altered MMP and elevated ROS levels) when bovine oocytes are
428 exposed during final oocyte maturation to elevated patho-physiological concentrations of FFAs
429 (Marei, et al. 2017, Marei, et al. 2019b, Marei, et al. 2019c, Van Hoeck, et al. 2011).

430 Mitochondria are the main source of energy (ATP) through oxidative phosphorylation, and they also
431 regulate Ca^{2+} homeostasis and cellular metabolism. After fertilization, sperm mitochondrial
432 mitophagy occurs and embryo development entirely depends on the mitochondria inherited from
433 the oocyte. Therefore, altered mitochondrial activity and ATP availability in stressed oocytes are
434 directly responsible for a cascade of defects associated with infertility in maternal metabolic
435 disorders including chromosomal aneuploidy (Zhang, et al. 2006), failure of fertilization, increased
436 embryo cell fragmentation, and developmental delay or arrest (McPherson, et al. 2014).

437 Importantly, it is not all about energy. Similar to the ER, mitochondria are recognized as cellular
438 sensors for stress, which is currently an area of intense research. These mechanisms are usually
439 overlooked and not clearly defined in the pathogenesis of reduced oocyte quality and embryo
440 developmental failure associated with metabolic stress. As mentioned above, oxidative damage
441 and misfolding of proteins results in ER stress which stimulates UPR^{er}. Mitochondria also
442 incorporate *de novo* synthesized proteins in the complexes of the electron transport chain in the
443 inner mitochondrial membrane. Similar UPR responses occur in the mitochondria (UPR^{mt}) which
444 constitute the molecular mitochondrial quality control machinery (Runkel, et al. 2014)**.** Elevated
445 ROS levels beyond a certain threshold will lead to mitochondrial uncoupling. This uncoupling
446 coincides with dissipation of MMP, which is suggested to be an actively regulated process in
447 response to stress as an attempt to reduce ROS production and maintain cell survival (Brand 2000).
448 Dissipation of MMP not only results in low ATP production, but it also elicits stress signals.
449 Depending on the degree of stress, a series of sequential responses are induced [Fig. 2].

450



451 **Figure 2.** Mitochondrial quality control mechanisms in response to stress. According to stress levels
452 mitochondria can stimulate protective chaperones via unfolded protein response (UPR^{mt}), undergo
453 mitophagy, or induce apoptosis. HS, Heat stress; OS, oxidative stress.

454 The transcription factor ATFS-1 is postulated as a key regulator of the initial step in UPR^{mt} (Nargund,
455 et al. 2012). Low MMP reduces ATFS-1 transport to the mitochondria for its proteolytic inactivation.
456 ATFS-1 is then translocated to the nucleus where it induces expression of nuclear-encoded
457 mitochondrial chaperones (mitochondrial heat shock protein 70 (mtHSP70), HSP60 and HSP10) and
458 proteases. These molecular chaperons are translocated back to the mitochondrial matrix to
459 alleviate stress-induced damage and inhibit induction of apoptosis. This has been correlated with
460 cellular life-span extension. Higher stress levels may induce ultrastructural damage in the
461 mitochondria and stimulate organellar mitochondrial quality control mechanisms which requires
462 activation of mitochondrial biogenesis. This involves fission and fusion of mitochondria to allow
463 repair or exclusion of damaged parts of mitochondria by PINK1-parkin mediated mitophagy. For
464 stress levels that cannot be tolerated, mitochondria will initiate apoptotic pathways for removal of
465 the damaged cell (Runkel, et al. 2014). This occurs by the induction of mitochondrial membrane
466 permeability transition pores and the release of cytochrome-C, which in-turn activates caspases.
467 This will ultimately end in the induction of apoptosis (Hirsch, et al. 1997). We have shown evidence
468 of mitochondrial uncoupling occurs in oocytes matured under metabolic stress conditions (Marei,
469 et al. 2017) or shortly after their fertilization (Marei, et al. 2019b). However, importantly, embryos

470 lack the machinery for mitochondrial biogenesis until blastocyst stage (St John, et al. 2010) and are
471 unable to eliminate damaged mitochondria due to the inability to activate mitophagy (Boudoures,
472 et al. 2017). This means that if mitochondrial molecular UPR fails, thresholds to induce apoptosis
473 can be abruptly reached.

474 As stated above, mitochondrial heat shock proteins (HSPD1/HSP60, HSPE1/HSP10, as well as
475 HSPA9 and TRAP1 where all detected at abundant levels in both oocytes and cumulus cells at
476 the end of IVM of bovine COCs. HSPD1 (HSP60), which was more abundant, is known to be
477 involved in re-folding of proteins and formation of oligomeric protein complexes in the
478 mitochondria (Boilard, et al. 2004). Both HSPD1 (FC = 1.22, P = 0.065) and HSPE1 (FC = 1.62, P =
479 0.09) tended to increase in oocytes after PA-exposure compared to the control oocytes,
480 suggesting that UPR^{mt} mechanisms may also be actively involved in stress responses in oocytes
481 (Marei, et al. 2019c). Again the level of protein expression of these markers could be limited by
482 the transcriptional inactivity.

483 **Consequences of UPR^{er} and UPR^{mt} in oocytes on further embryo development**

484 The most logical question now is whether these limited transcription-free translation-based
485 UPR^{er} and UPR^{mt} signaling in oocytes are sufficient (or efficient) for the re-establishment of
486 protein homeostasis and cell repair. It is evident that oocytes matured under metabolic stress
487 or HS conditions have reduced developmental capacity and undergo embryonic arrest during
488 cleavage leading to reduced blastocyst rates (Krisher 2013, Valckx, et al. 2014, Van Hoeck, et al.
489 2011). In some cases, oocytes collected from obese women can be fertilized *in vitro* and
490 undergo relatively normal early embryo development, sometimes with slightly lower cleavage
491 rates or higher fragmentation rates, but finally producing morphologically acceptable
492 transferable embryos (Zander-Fox, et al. 2012). However, the resultant embryos achieve
493 significantly lower pregnancy rates after transfer and have a higher risk for preterm birth
494 compared to embryos from metabolically healthy patients (Zander-Fox, et al. 2012). The same
495 observations have been recorded in bovine *in vitro* studies, where many COCs exposed to
496 elevated levels of FFAs during IVM had acceptable cleavage rates (Marei, et al. 2019b). Despite
497 the arrest of some of the affected embryos during culture the reduction in blastocyst rate is

498 usually limited to a 10% difference compared with the control group, resulting in a relatively
499 moderate yield of blastocyst production that are morphologically normal and transferable
500 ((Marei, et al. 2017, Marei, et al. 2019b, Marei, et al. 2019c, Van Hoeck, et al. 2011).
501 Importantly, more in-depth investigation of the quality of these surviving blastocysts has shown
502 that they exhibit high levels of apoptotic cell index, altered cellular metabolism, and altered
503 transcriptomic and epigenetic patterns despite fertilization and culture in FFA-free standard
504 conditions (Desmet, et al. 2020, Marei, et al. 2017, Marei, et al. 2019b, Marei, et al. 2019c, Van
505 Hoeck, et al. 2015, Van Hoeck, et al. 2011).

506 Interestingly, we have also noticed that blastocysts produced under FFA-free standard culture
507 conditions but originating from PA-exposed COCs (24h exposure) exhibited upregulated mRNA
508 expression of both ATF6 and HSPA5 (but not ATF4) indicating persistent ER stress until day 8 of
509 development. Nevertheless, these surviving blastocysts exhibited a 6X increase in the
510 proportion of apoptotic cells compared with control embryos (Marei, et al. 2019b). In another
511 study, we investigated post-hatching development of PA-derived morphologically normal
512 surviving blastocysts by transferring them at Day 7.7 post-fertilization to uterine horns of
513 healthy cows and flushing them back at Day 14 (Desmet, et al. 2020). We found that PA-derived
514 embryos had retarded post-hatching growth and were less elongated, displayed altered cellular
515 metabolism, and had altered transcriptome profile particularly in pathways related to redox-
516 regulating mechanisms, apoptosis, cellular growth, interaction and differentiation, energy
517 metabolism and epigenetic mechanisms, compared to control embryos (Desmet, et al. 2020).
518 Collectively, it is evident that embryos produced from metabolically stressed oocytes might be
519 partially able to maintain their survival early during development. However, the underlying sub-
520 lethal stress levels appear to have persistent long-term effects that can be detected at the
521 blastocyst stage or even later despite further development in stress-free conditions.

522 When COCs are matured *in vivo* or *in vitro* under metabolic stress conditions, cumulus cells are
523 exposed first and are relatively more affected compared to the enclosed oocytes. Many of the
524 exposed cumulus cells undergo apoptosis. In the same time, cumulus cells protect oocyte
525 quality against oxidative stress and against lipotoxic effects in their microenvironment by
526 internalizing and neutralizing FFAs (Aardema, et al. 2013, Shaeib, et al. 2016). Our proteomic

527 data clearly show that despite the evident oxidative stress, altered mitochondrial activity, and
528 signs of UPR^{er} and UPR^{mt} , PA-exposed oocytes exhibit several anti-apoptotic changes such as
529 increased relative abundance of mitochondrial antioxidative proteins (particularly,
530 Peroxiredoxin 3 (PRDX3), NRF2-mediated oxidative stress response, activation of p70S6K- 14-3-
531 3 signaling (Marei, et al. 2019c), all of which are known to be pro-survival mechanisms (Amin, et
532 al. 2014, Chang, et al. 2004, Lim, et al. 2013). In addition, PA-exposed oocytes had upregulated
533 SLC24A5, a negative regulator of apoptosis (Landes and Martinou 2011) as well as upregulated
534 NLRP5 (NLR family pyrin domain containing 5) which plays an essential role for zygotes to
535 progress beyond the first embryonic cell division (Marei, et al. 2019c). This illustrates the
536 intrinsic ability of the oocytes to survive certain level and duration of stress. Oocytes may not
537 reach thresholds of intracellular stress that are enough to induce apoptosis, however, as
538 described above, intracellular stress appear to stay persistently high during fertilization and
539 further embryo development even in FFA-free culture conditions. This results in persistent UPR
540 signaling, which is apparently then not sufficient to combat the damage or prevent its further
541 aggravation, leading to failure of embryo development at some stage.

542 In addition to the carryover of stress induced during oocyte maturation, embryos may be exposed
543 to several additional sources of stress during fertilization and early development that may
544 aggravate the damage and accelerate embryonic loss. For example, due to mitochondrial
545 dysfunction and altered cellular metabolism in embryos derived from stressed-oocytes,
546 accumulation of increased concentrations of lactate (due to Warburg metabolism), or ammonia
547 (from amino-acid degradation) may progressively change pH and osmolality in the
548 microenvironment (Cagnone and Sirard 2016) creating additional secondary factors that may
549 have primary effects due to physicochemical stress. In fact, embryos appear to tolerate a range
550 of sub-optimal physico-chemical conditions such as reduced osmolality during IVC without
551 compromising their developmental capacity (Brinster and Troike 1979), however embryos
552 derived from metabolically-compromised oocytes may not have the same level of tolerance.

553 It is also worth mentioning that embryos produced after somatic cell nuclear transfer (SCNT)
554 were found to be more sensitive to tunicamycin-induced ER stress compared to *in vitro*
555 produced embryos (Babayev, et al. 2016). This was suggested to be caused by the electrofusion

556 step which is known to increase intracellular calcium ions and activate ER stress (Yin, et al.
557 2019)). SCNT-derived embryos exhibit increased *HSPA5*, *ATF6* and *XBP1* gene expression, yet
558 they have low developmental competence and quality (Lin, et al. 2019). Importantly, ICSI has
559 been also recently linked with increased levels of ER stress (Deng, et al. 2020). Both mRNA and
560 protein expression of GRP78 was found to be significantly higher in ICSI produced embryos
561 compared to IVF. ICSI embryos also showed higher apoptotic rates compared to IVF (Deng, et
562 al. 2020). Therefore, it is likely that a combination of these factors can amplify stress responses
563 to a threshold beyond which embryo development is arrested. For example, it was found that
564 human oocytes collected from follicles with high PA concentrations resulted in lower pregnancy
565 rates following ICSI compared to those from low PA-follicles (Mirabi, et al. 2017). ICSI as such
566 could be a contributing factor in this observation.

567 **Therapeutic applications linked with cellular stress in oocytes and embryos**

568 Fundamental understanding of the mechanisms by which oocytes and embryos handle cellular
569 stress and how this may subsequently affect developmental competence is important to
570 develop novel treatment strategies and identify molecular targets. This is particularly important
571 in human settings when preventive measures are not possible or when preconception care
572 strategies do not yield the expected results (Einarsson, et al. 2017, Sim, et al. 2014). Since it is
573 clear that the intrinsic UPR mechanisms in oocytes have limited capacity to protect the oocyte
574 and the growing embryo under cellular stress conditions, these treatment strategies should aim
575 at the prevention of metabolic stress, or at least at the elimination of additional or persistent
576 sources of stress during early embryo development and to optimally support oocyte and
577 embryonic vital metabolic activities.

578 There are several periconceptional care strategies such as weight loss, caloric restriction or
579 exercise proposed for obese women to correct their metabolic profile and oocyte quality (Sim,
580 et al. 2014). In dairy cattle production, strategies to control excessive change in body condition
581 score and excessive fat mobilization during the transition period have been shown to limit
582 metabolic stress and protect oocyte quality (Barletta, et al. 2017).

583 On the other hand, several *in vitro* treatments have been proven effective in improving oocyte
584 developmental competence under metabolic stress conditions. For example, the use of ER
585 stress inhibitor (salubrinal) was shown to alleviate FFA-induced defects on mitochondrial
586 activity, fertilization and development to the blastocysts stage (Wu et al 2012). Treatment of
587 ICSI embryos with tauroursodeoxycholic acid (TUDCA, another ER stress inhibitor) could also
588 alleviate ICSI effects on ER stress markers, enhance ICSI-embryo development and quality to
589 rates similar to IVF embryos (Deng, et al. 2020). On the other hand, mitochondria have also
590 been proposed as an important target for treatments aiming at enhancement of oocyte quality
591 and fertility under metabolic stress conditions (Giannubilo, et al. 2018, Legro 2019).
592 Mitochondrial targeted therapy have been shown to protect oocyte developmental
593 competence under elevated FFA concentrations *in vitro* (Marei, et al. 2019a). In this study, such
594 embryo treatment could eliminate persistent oxidative stress and rescue embryo development
595 when supplemented during *in vitro* culture of zygotes derived from metabolically compromised
596 oocytes (Marei, et al. 2019b). Recent technologies may additionally offer tools that may
597 prevent the buildup of physicochemical stress during culture by using micro-fluidic culture
598 systems for continuous renewal of culture medium.

599 **Conclusions**

600 Collectively, when studying the pathogenesis of reduced oocyte and embryo developmental
601 competence under maternal metabolic stress conditions and looking at the underlying cellular
602 stress responses, it is very difficult to interpret what is a cause and what is a consequence.
603 Nevertheless, there is enough evidence to show that oocytes and early developing embryos
604 have an intrinsic pro-survival machinery that may temporarily extend the cellular lifespan but
605 are apparently not enough to support embryo development on longer terms. External support
606 to prevent or reduce cell stress levels is thus inevitable to maximize the odds of producing good
607 quality embryos, guaranteeing optimal viability. The insights, concepts and speculations drawn
608 in this chapter about the role of HSPs and UPRs in determining oocyte developmental
609 competence are just the tip of the iceberg. More fundamental research is needed both at the
610 transcriptomic and proteomic levels to further elucidate these responses, and understand their
611 critical interactions and long term effects.

612 **Conflict of interest**

613 The authors have no conflict of interest to declare.

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