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Lipotoxicity: impact on oocyte quality and reproductive efficiency in mammals

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Abstract

Lipotoxicity is characterized by excessive saturated fatty acids in the blood, increasing storage in non-adipose cells, which leads to changes in the expression pattern of genes related to endoplasmic reticulum stress (e.g., ATF4, ATF6, CHOP, and GRP78), pro- and anti-apoptotic pathways (e.g., Bax and Bcl-2, and protein stability, including heat shock proteins, e.g., HSP70). A negative sub-cellular effect is usually an end result, which also occurs in the ovarian follicular population, affecting granulosa cells and cumulus-oocyte complexes (COCs), which leads to a decrease in oocyte quality and mitochondrial activity, and increased apoptosis. The addition of high doses of non-esterified fatty acids to oocyte *in vitro* maturation medium has been shown to slow the progression of meiosis, hampering oocyte maturation and subsequent *in vitro* embryo development. Due to its importance in the control of cellular lipid droplets and expression correlation with cytosolic lipid accumulation, the expression of the Plin 2 (Perilipin 2) protein is also highlighted. The aim of this review is to discuss some reproductive implications of dietary lipid supplementation in ruminant females, and the potential effects of lipotoxicity on oocyte quality and reproduction, and the main mechanisms involved in the expression of genes related to endoplasmic reticulum stress and cellular lipid accumulation.

Keywords: embryos, fatty acids, fertility, lipotoxicity, ooytes.

Introduction

Lipotoxicity is characterized by excessive plasma saturated fatty acids, increasing lipid storage in non-adipose cells, through a mechanism by which the fat intake can negatively influence reproductive tissues (Jungheim *et al*., 2010, 2011; Robker *et al*., 2011; Yang *et al*., 2012), affecting, for instance, granulosa cells and oocytes (Wu *et al*., 2010; Yang *et al*., 2012). These effects have been associated with a decrease in oocyte quality in cows (*in vitro*), rats (*in vivo*) and women (*in vivo*; Leroy *et al*., 2005; Wu *et al*., 2010; Yang *et al*., 2012). Such lipotoxicity effects can be associated with changes in the expression of various genes related to endoplasmic reticulum (ER) stress, such as activating transcription factors 4 and 6 (ATF4 and ATF6), glucose regulated protein 78 (Grp78; Yang *et al*., 2012), and the protein homologue of C/EBP (CHOP; Zinszner *et al*., 1998), as well as the pro- and anti-apoptotic genes, such as Bax (Bcl-2 asssociated protein X) and Bcl-2 (B cell lymphoma protein 2), respectively (Leroy *et al*., 2005), and genes associated with protein stability due to stress, such as the inducible 70-kDa heat shock protein (HSP70; Wu *et al*., 2010).

Studies have demonstrated that the addition of different concentrations of non-esterified fatty acids (NEFAs), such as linoleic acid (18:2), stearic acid (18:0), palmitic acid (16:0) and oleic acid (18:1) to the *in vitro* maturation (IVM) medium reduces oocyte maturation rates, with negative effects on subsequent embryo development (Marei *et al*., 2010; Van Hoeck *et al*., 2011). Moreover, the addition of NEFAs to the culture medium of granulosa cells reduces cell proliferation and increases apoptotic rates (Vanholder *et al*., 2005). It is believed that such negative effects due to exposure to high concentrations of fatty acids on oocyte quality are caused by the changes in the structure of the mitochondrial membrane and the ER. In certain situations, reactive oxygen species (ROS) can be formed by the enzymatic action of lipooxygenase, leading to lipid peroxidation of organelles such as the mitochondria, which contain large quantities of polyunsaturated fatty acids (PUFA) that could turn into peroxides. In the mitochondria, ROS can be toxic and harmful to the development of the oocyte, which can worsen in hyperlipidemia (Marei *et al*., 2012). Moreover, ROS hampers ER function in the folding and secretion of proteins, triggering a mechanism known as unfolded protein response (UPR), which lowers translation rates and activates a degradation system associated with the ER (Xu *et al*., 2005; Wu *et al*., 2011). Such reactions can compromise cell viability, and if reaching the gametes or embryos, can compromise fertility. This review aims to discuss some recent knowledge regarding the main reproductive changes caused by the dietary supplementation of lipids to mammalian females, with special regard to the effects of lipotoxicity on oocyte quality.

Lipotoxicity and reproductive response

The process of lipotoxicity is characterized by excessive circulation of long-chain saturated fatty acids,

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which are produced by adipocytes or obtained through the diet (Jungheim *et al*., 2012). When adipocytes are unable to store fatty acids, other types of cells store lipids (Jungheim *et al*., 2012). In fact, in a study where mice were fed a diet containing 22% fat, oocytes showed high lipid levels, before and after ovulation, in comparison with oocytes from mice fed a control balanced diet (Fig. 1), which led to alterations in mitochondrial activity and ER stress (Wu *et al*., 2010).

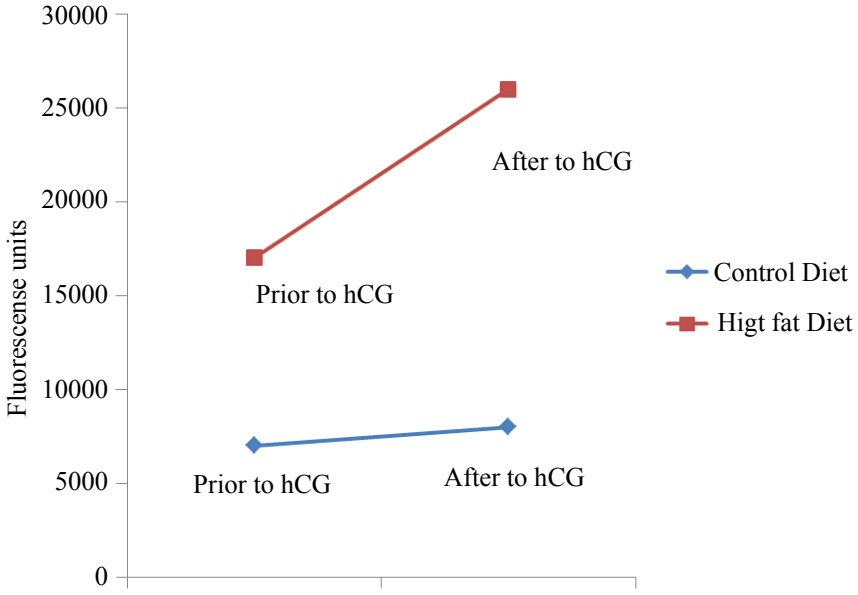

Figure 1. Increase in lipid concentration in COCs from female mice fed a fat rich diet, prior and after hCG treatment. Adapted from Wu *et al*. (2010).

The accumulation of intracellular lipids may lead to oxidative damage and formation of highly reactive cytotoxic lipid peroxides, harmful to intracellular organelles, particularly to the ER and mitochondria (Malhi and Gores, 2008; Li *et al*., 2011). Moreover, the increase in free fatty acids levels in the blood results in increased concentrations of free fatty acids in the follicular fluid, which can affect the morphology of the cumulus-oocyte complexes (COCs) and subsequent embryo quality (Leroy *et al*., 2005; Metwally *et al*., 2007; Jungheim *et al*., 2011).

Studies in rats have demonstrated that obesity induced by a fat-rich diet affects oocyte quality, reducing blastocyst rates and cell allocation following *in vitro* fertilization, with embryos showing a higher number of cells allocated to the trophectoderm and a slight reduction in the number of cells in the inner cell mass, thereby decreasing the percentage of cells destined to form the embryo (Minge *et al*., 2008). Such perturbations in cell allocation during blastocyst development may not be trivial, having a potential to compromise embryo and fetal development, also contributing to the embryo origin of diseases manifested later as an adult (Kwong *et al*., 2000). Such fact has also been shown in cattle, since the treatment of COCs with increasing doses of fatty acids during IVM compromised oocyte maturation and subsequent embryo development (Leroy *et al*., 2005; Aardema *et al*., 2011; Van Hoeck *et al*., 2011).

Physiological NEFA concentrations of stearic acid, palmitic acid, oleic acid are 25, 50 and 75 µm, respectively (Van Hoeck *et al*., 2011). But blastocyst rates in cattle can also be lowered when *in vitro*-produced embryos are exposed to stearic acid, palmitic acid, oleic acid in concentration of 75, 150, 200 µm, respectively (Van Hoeck *et al*., 2011). In cattle and human embryos, the first 3-4 cleavage rounds occur under the control of maternal mRNAs and proteins present in the oocyte, until the activation of the embryonic genome in humans and in cattle. In recent studies, a reduction in blastocyst rates indicated that exposure to NEFAs during oocyte development had a significant negative impact on the development of post-genome activation, as well as on the pattern of gene expression (Van Hoeck *et al*., 2011). In addition, high levels of NEFAs added to the bovine *in vitro* culture media were harmful to the function and development of granulosa cells (Vanholder *et al*., 2005). However, progesterone production by the cells was not affected, as a limiting step in the progesterone production rate by luteal cells is likely the transport cholesterol to the inner mitochondrial membrane (Diaz *et al*., 2002).

The supplementation of high concentrations of NEFA during the *in vitro* maturation of bovine oocytes significantly increased apoptosis rate in embryos, with the accumulation of higher levels of stearic acid in comparison with control embryos (Van Hoeck *et al*., 2011). Such difference can be related to the fact that oocytes have the

capacity to accumulate fatty acids, which can have variable levels and/or composition of stored lipids depending on the lipid supply (Kim *et al*., 2001).

Interestingly, the addition of NEFAs, such as stearic and palmitic acids to the follicular fluid of dairy cows during IVM slowed meiosis progression, which was expressed by a significantly greater number of oocytes held in metaphase I, along with a relatively low number of oocytes in metaphase II (Table 1; Leroy *et al*., 2005). Likewise, the addition of linoleic acid to the *in vitro* maturation medium for 24 h was shown to also hamper oocyte development (Marei *et al*., 2010), with a decrease in fully expanded COCs and low percentage of oocytes at the metaphase II stage.

Table 1. Effect of supplementation with stearic acid (C18:0) and palmitic acid (C16:0) to the *in vitro* maturation medium on oocyte maturation, compared to negative (IVM medium) and positive (IVM medium added with ethanol) controls.

Maturation rate (%)	Negative control	Positive control	Stearic acid	Negative control	Positive control (C18:0)	Palmitic acid (C16:0)
Metaphase I	9.2[a]	18.6[b*]	26.0[b*]	9.1[a]	12.5[a]	24.1[b]
Anapahse/Telophase	16.1[a]	11.6[a]	18.4[a]	15[a,b]	10.5[a]	19.9[b]
Metaphase I	74.8[a]	67.8[a]	54.0[b]	75.0[a]	77.1[a]	63.2[b]

[a,b]Data within a row marked with different superscripts differ significantly ($P < 0.05$). *P = 0.1. Adapted from Leroy *et al*. (2005)

Plin2 and accumulation of intracellular lipids

The major structural proteins present at the surface of intracellular lipid droplets are those belonging to the PAT protein family, named after perilipin (PLIN1), adipophilin (PLIN2), and tail-interacting protein of 47 kDa (TIP47). Perilipins are associated with the lipid droplets (LD) either during budding from the ER or derived from a soluble pool within the cytosol (Kimmel *et al*., 2010). Evidence suggests that some perilipin family members, such as Plin3 and Plin4, are associated with coat and very small nascent LDs (Wolins *et al*., 2005).

Perilinpin 2 (Plin2) is the main protein associated with lipid droplets in non-adipose tissues, important to the control of cellular lipid accumulation (Aminoff, 2012). Plin2 expression is highly correlated with cytosolic association of lipids (Brasaemle *et al*., 1997), being found in adipocytes during adipogenesis, involved in the storage of lipids in steroidogenic cells and other non-specialized cells (Bickel *et al*., 2009). Also, a protein related to the differentiation of lipid molecules found in all cells examined to date, named adipose differentiation-related protein (ADRP), has an N-terminal end highly similar to the perilipins, which may play a functional role or contain targeting signals in lipid storage, promoting the association of the proteins with lipid droplets in the cytosol (Brasaemle *et al*., 1997).

According to Aminoff (2012), the accumulation of lipids in non-adipose tissues results in cellular dysfunction, inflammation and eventually cell death. Thus, Plin2 protects cells from toxic lipid metabolites, by promoting the storage of neutral lipids into cytosolic droplets (Jungheim *et al*., 2012). Yang *et al*. (2012) evaluated the expression of Plin2 in mouse COCs *in vitro*-matured in lipid-rich follicular fluid, and observed higher expression of Plin2 and altered expression of ER stress marker genes, compared with COCs matured in lipid-poor follicular fluid or under *in vivo* conditions. Sastre *et al*. (2014) also evaluated the expression of Plin2 and Plin3 in oocyte maturation and early embryo development in dairy cows and observed that the expression of Plin2 was greater in *in vitro*-matured oocytes when compared with Plin3. Although Plin2 seems to be quickly degraded in the cytosol, Plin3 remains active for a longer time, being observed to be either soluble in the cytosol or associated with LD. A higher expression of Plin2 could reflect in the metabolic response of the oocyte to avoid total depletion of lipid stocks, considering the high energy demand during the first rounds of embryo cleavages.

Lipid diet effect on endoplasmic reticulum stress and expression of apoptotic genes

The ER is the main site for the synthesis and folding of proteins, serving also as a site for the biosynthesis of steroids, cholesterol and other lipids (Rao *et al*., 2001). The lumen of the ER is the entrance site of proteins designed for endo/exocytotic routes, and therefore has a unique environment for folding, assembly, formation of disulfide bridges and glycosylation (Bromati, 2009). Exposure of the ER to high levels of free fatty acids and lipid peroxides causes structural alterations and perturbs such functions (Diakogiannaki *et al*., 2008).

Studies have demonstrated that a fat-rich diet given to female mice causes an increase in lipid accumulation and induces the expression of ER stress marker genes, such as ATF4 and GRP78, decreasing mitochondrial activity and increasing apoptosis in COCs, which is associated with a reduction in fertilization rates under *in vivo* conditions (Wu *et al*.,

2010). Also, the expression of ATF4 was increased in rat granulosa cells in response to the high amount of fat in the diet. The expression of such gene was also significantly increased in the granulosa cells of obese women. Yang *et al*. (2012) confirmed this finding, observing a significant increase in expression of ER stress marker genes (ATF4, ATF6 and GRP78) in COCs matured in lipid-rich follicular fluid in comparison with COCs matured in lipid-poor follicular fluid.

However, under stress conditions, the ER is subjected to UPR (Malhi and Gores, 2008), such mechanism is initiated by the presence of large amounts of immature proteins in the ER and is characterized by the coordinated activation of multiple proteins that trigger the expression of gene coding for chaperones, enzymes and structural components of the ER, which can lead to ER activation, reduce the risk of errors in the assembly of the tertiary and quaternary structures of the proteins, thereby reducing the number of unfolded proteins in that organelle and allow cell survival (Rao *et al*., 2001; Xu *et al*., 2005).

When unfolded proteins accumulate in the ER the immediate response is the recruitment of resident chaperones from their binding sites in the ER membrane. The binding of chaperones to the cell occurs due to the presence of different transmembrane proteins, which act as signaling molecules of the reticular function throughout the cell. The main proteins of the ER membrane involved in the induction of UPR are IRE1 (inositol-requiring enzyme 1), PERK (RNA-activated protein kinase (PKR)-like ER kinase) and ATF6 (Shen *et al*., 2004). These transmembrane proteins have a cytosolic N-terminal domain and a C-terminal domain pointed towards the lumen of the ER, thereby constituting a connecting point between the two compartments. In the basal state, those three proteins are inhibited by the binding of the chaperone BiP (binding protein (Bertolotti *et al*., 2000). These transducers of ER stress are inactive when the chaperone GRP78/BiP binds to its luminal domains, thus impeding its aggregation. When there is an accumulation of proteins in the ER, the GRP78/BiP complex is recruited to inhibit the aggregation of accumulated proteins, dissociating them from PERK, IRE1α and ATF6. The last one, released from BiP, migrates to the Golgi apparatus, where the resident proteases S1P and S2P (site-1 protease and site-2 protease) cleave and release the transcription factor into the cytosol. The proteolytic cleavage of ATF6 directly induces the transcriptional activation of the genes encoding XBP-1 and chaperones (Ye *et al*., 2000; Seo *et al*., 2008). Released PERK and IRE1α homodimerize and undergo autophosphorylation, which activates their intrinsic kinase activities (Hussain and Ramaiah, 2007). Meanwhile, under such conditions, mRNAs coding for proteins functioning in the adaptation to stress gain a selective advantage, translation, such as the transcription factor of ATF4 (Harding *et al*., 2001).

A strict relation between PERK, CHOP and ATF4 exists, since chronic activation of PERK can increase the expression of CHOP (*C/EBP-homologous protein*) through ATF4 (Bromati, 2009). The increase in CHOP expression is associated with the translocation of the pro-apoptotic protein BAX from the cytosol to the mitochondria, reduction in the anti-apoptotic protein Bcl-2, and increase in proteins GADD34 (Growth Arrest and DNA Damage-Inducible Gene), DR-5 (Death Receptor 5), and TRB3 (Tribbles related protein 3; Yamaguchi and Wang, 2004; Ohoka *et al*., 2005; Pirot *et al*., 2007). The increased expression of CHOP, therefore, blocks cell cycle and causes cell death (Ron, 2002; Fig. 2).

It should be noted that HSPs stand out among the class of molecular chaperones, in which HSP70 is essential for the recovery of cells from stress, cell survival and normal cell functions (Guzhova and Margulis, 2006). However, no differences in HSP70 expression have been observed in animals fed either a high-fat diet or control diets (Wu *et al*., 2010). Nonetheless, GRP78 is the most abundant chaperone protein in the ER, serving as a master regulator of UPR and in detecting ER stress (Wang *et al*., 2009). The GRP78 protein has been considered essential for embryonic cell proliferation, protecting the inner cell mass from apoptosis during early embryo development (Luo *et al*., 2006). When evaluated in goats, the GRP78 gene was observed in granulosa cells of atretic follicles (Lin *et al*., 2012), with increased GRP78, CHOP, ATF6 and ATF4 levels in granulosa cells during follicular atresia. However, the biological significance of such findings still needs to be better defined.

When apoptosis is activated, the accumulation of intracellular lipids alters the expression of homologous proteins of the Bcl-2 family. This was demonstrated by Valckx *et al*. (2014), who demonstrated mouse granulosa cells to express Bax at high levels, also showing a high Bax/Bcl-2 ratio when cells were subjected to high NEFA concentrations (stearic acid at 112 mM; palmitic acid at 230 mM; and oleic acid at 210 mM) in culture. Apparently, excess lipids, when greater than the cell detoxification capacity, can lead to increased apoptotic rates, which still needs to be elucidated in *in vivo* or *in vitro* experiments with mammalian gametes and embryos.

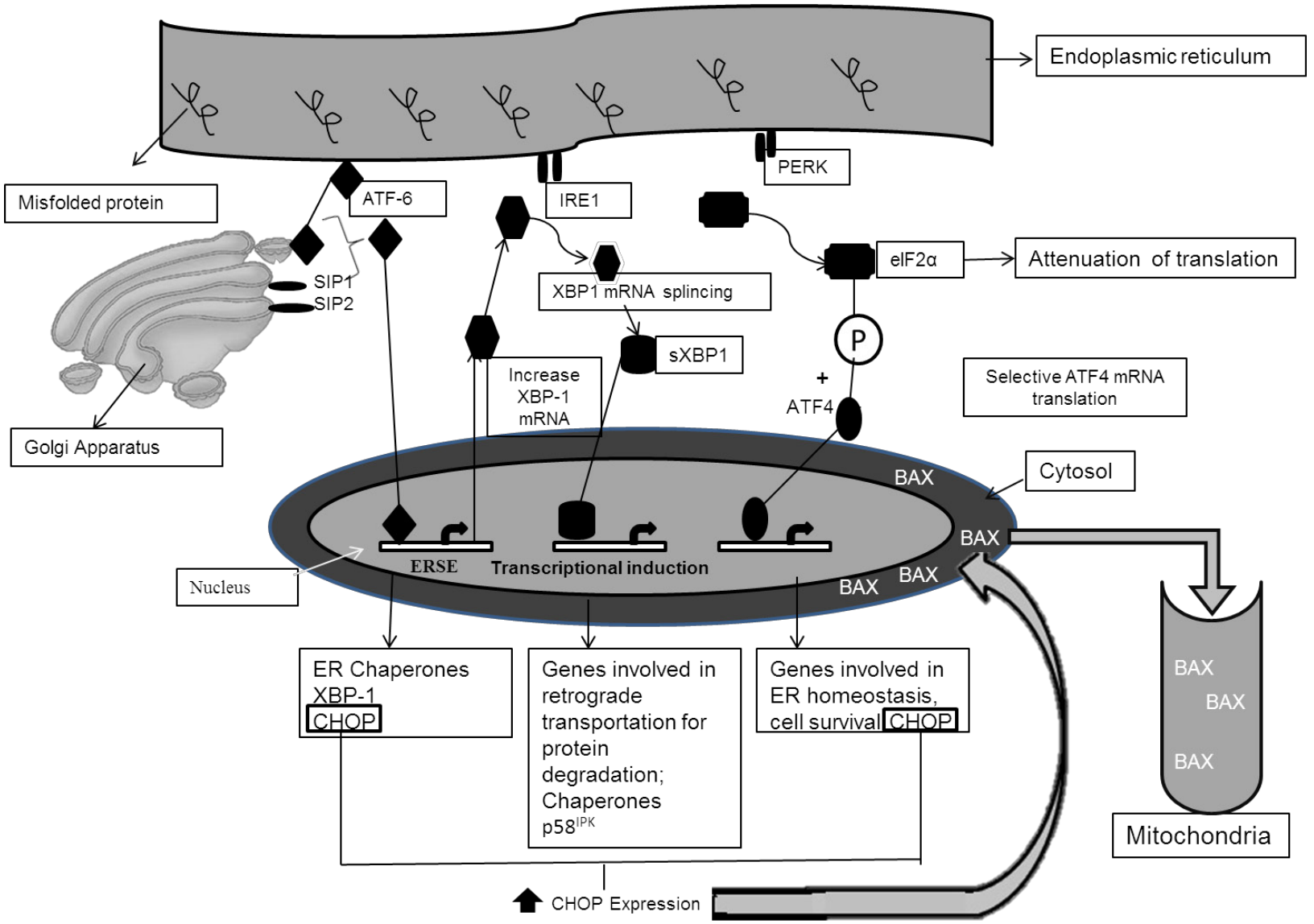

Figure 2. Events associated with endoplasmic reticulum (ER) stress and unfolded protein response (UPR). Adapted from Li *et al*. (2011).

Final considerations

Studies that relate the effect of hyperlipidemic diets with the COC quality and competence are still limited. However, available studies indicate that exposure to high lipid concentrations, either under *in vivo* or *in vitro* conditions, alters the expression pattern of different genes related to oocyte matured quality, including ATF4, ATF6, GRP78 and CHOP, BAX and Bcl-2, and HSP70.

It is clear, therefore, that for oocytes or embryos, high levels of free fatty acids in *in vivo* or *in vitro* conditions can have toxic effects that translate into alterations in the expression of important genes for direct or indirect lipid detoxification, and excess can lead to higher apoptotic rates and lower developmental competence, resulting in reduced fertility potential. Accordingly, new investigations into the molecular mechanisms involved in COC lipotoxicity should be carried out, aiming to increase developmental competence of oocytes and, consequently, enhancing reproductive efficiency.

References

Aardema H, Vos PLAM, Lolicato F, Roelen BAJ, Knijn HM, Vaandrager AB, Helms JB, Gadella BM. 2011. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. *Biol Reprod*, 85:62-69.

Aminoff A. 2012. Genetic and functional studies of MTTP and PLIN2 in relation to metabolic and cardiac dysfunction. Solna, Sweden: Karolinska Institutet. Thesis.

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. 2000. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nature Cell Biol,* 2:326-332.

Bickel PE, Tansey JT, Welte MA. 2009. PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. *Biochim Biophys Acta*, 1791:419-440.

Brasaemle DL, Barber T, Wolins NE,Serrero G, Blanchette-Mackie EJ, Londos C. 1997. Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. *J Lipid Res*, 38:2249-2263.

Bromati CR. 2009. Study of the expression of proteins involved in endoplasmic reticulum stress during the remodeling of maternal pancreatic islets during the perinatal period [in Portuguese]. São Paulo, Brazil: University of São Paulo. Thesis.

Diakogiannaki E, Welters, HJ, Morgan NG. 2008. Differential regulation of the endoplasmic reticulum stress response in pancreatic beta-cells exposed to long-chain saturated and monounsaturated fatty acids. *J Endocrinol*, 197:553-563.

Diaz FJ, Anderson LE, Wu YL, Rabot A, Tsai SJ, Wiltbank MC. 2002. Regulation of progesterone and prostaglandin F2alpha production in the CL. *Mol Cell Endocrinol,* 191:65-80.

Guzhova I, Margulis B. 2006. Hsp70 chaperone as a survival factor in cell pathology. *Int Rev Cytol*, 254:101-149.

Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD, Ron D. 2001. Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival. *Mol Cell*, 7:1153-1163.

Hussain SG, Ramaiah KVA. 2007. Endoplasmic reticulum : stress, signalling and apoptosis. *Curr Sci*, 93:1684-1696.

Jungheim ES, Schoeller, EL, Marquard, KL, Louden ED, Schaffer JE, Moley KH. 2010. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology*, 151:4039-4046.

Jungheim ES, Macones, GA, Odem RR, Patterson BW, Lanzendorf SE, Ratts VS, Moley KH. 2011. Associations between free fatty acids, cumulus oocyte complex morphology and ovarian function during in vitro fertilization. *Fertil Steril*, 95:1970-1974.

Jungheim ES, Travieso JL, Carson KR, Moley KH. 2012. Obesity and reproductive function. *Obstet Gynecol Clin North Am*, 39:479-493.

Kim JY, Kinoshita M, Ohnishi M, Fukui Y. 2001. Lipid and fatty acid analysis of fresh and frozen-thawed immature and in vitro matured bovine oocytes. *Reproduction*, 122:131-138.

Kimmel AR, Brasaemle DL, McAndrews-Hill M, Sztalryd C, Londos C. 2010. Adoption of perilipin as a unifying nomenclature for the mammalian PAT family of intracellular, lipid storage droplet proteins. *J Lipid Res*, 51:468-471

Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. 2000. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development*, 127:4195-4202.

Leroy JLMR, Vanholder T, Mateusen B, Christophe A, Opsomer G, Kruif ADe, Genicot G, Soom AV. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction*, 130:485-495.

Li X, Zhang K, Li Z. 2011.Unfolded protein response in cancer: the physician's perspective. *J Hematol Oncol Pharm*, 4:8.

Lin P, Yang Y, Li X, Chen F, Cui CC, Hu L, Li Q. Liu W, Jin Y. 2012. Endoplasmic reticulum stress is involved in granulosa cell apoptosis during follicular atresia in goat ovaries. *Mol Reprod Dev*, 79:423-432.

Luo S, Mao C, Lee B, Lee AS. 2006. GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. *Mol Cell Biol*, 26:5688-5697.

Malhi H, Gores GJ. 2008. Molecular mechanisms of

lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis*, 134:360-369.

Marei WF, Wathes DC, Fouladi-Nashta AA. 2010. Impact of linoleic acid on bovine oocyte maturation and embryo development. *Reproduction*, 139:979-988.

Marei WF, Wathes DC, Fouladi-Nashta AA. 2012. Differential effects of linoleic and alpha-linolenic fatty acids on spatial and temporal mitochondrial distribution and activity in bovine oocytes. *Reprod Fertil Dev*, 139:679-690.

Metwally M, Li TC, Ledger WL. 2007. The impact of obesity on female reproductive function. *Obesity Rev*, 8:515-523.

Minge CE, Bennett BD, Norman RJ, Robker RL. 2008. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. *Endocrinology*, 149:2646-2656.

Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H. 2005. TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. *EMBO J*, 24:1243-1255.

Pirot P, Ortis F, Cnop M, Ma Y, Hendershot LM, Eizirik DL, Cardozo AK. 2007. Transcriptional regulation of the endoplasmic reticulum stress gene chop in pancreatic insulin-producing cells. *Diabetes*, 56:1069-1677.

Rao RV, Hermel E, Castro-Obregon S, Del Rio G, Ellerby LM, Ellerby HM, Bredesen DE. 2001. Coupling endoplasmic reticulum stress to the cell death program. Mechanism of caspase activation. *J Biol Chem,* 276:33869-33874.

Robker RL, Wu LL, Yang X. 2011. Inflammatory pathways linking obesity and ovarian dysfunction. *J Reprod Immunol*, 88:142-148.

Ron D. 2002.Translational control in the endoplasmic reticulum stress response. *J Clin Invest*, 110:1383-1388.

Sastre D, Costa NN, Sá ALA, Conceição SDB, Chiaratti MR, Adona PR, Guemra S, Meirelles FV, Santos SSD, Sena L, Ohashi OM, Santos EJM, Miranda MS. 2014. Expression of PLIN2 and PLIN3 during oocyte maturation and early embryo development in cattle. *Theriogenology*, 81:326-331.

Seo HY, Kim YD, Lee KM, Min AK, Kim MK, Kim HS, Won KC, Park JY, Lee KU, Choi HS, Park KG, Lee IK. 2008. Endoplasmic reticulum stress-induced activation of activating transcription factor 6 decreases insulin gene expression via up-regulation of orphan nuclear receptor small heterodimer partner. *Endocrinology*, 149:3832-3841.

Shen Y, Schlessinger K, Zhu X, Meffre E, Quimby F, Levy D, Darnell JE. 2004. Essential role of STAT3 in postnatal survival and growth revealed by mice lacking STAT3 serine 727 phosphorylation. *Mol Cell Biol*, 24:407-419.

Valckx SD, Van Hoeck V, Arias-Alvarez M, Maillo V, Lopez-Cardona, AP, Gutierrez-Adan A, Berth M, Cortvrindt R, Leroy JLMR. 2014. Elevated non-esterified fatty acid concentrations during in vitro murine follicle growth alter follicular physiology and reduce oocyte developmental competence. *Fertil Steril*, 102:1769-1776.

Van Hoeck V, Sturmey RG, Bermejo-Alvarez P, Rizos D, Gutierrez-Adan A, Leese HJ, Bols PEJ, Leroy JLMR. 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. *Plos One*, 6:e23183.

Vanholder T, Leroy JL, MR, Van Soom A, Opsomer G, Maes D, Coryn MKA. 2005. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. *Anim Reprod Sci*, 87:33-44.

Wang M, Wey S, Zhang Y, Ye R, Lee AS. 2009. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. *Antioxid Redox Sig*n, 11:2307-2316.

Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A, Bickel PE. 2005. S3-12, adipophilin, and TIP47 package lipid in adipocytes. *J Biol Chem*, 280:19146-19155.

Wu LL, Dunning KR, Yang X, Russell DL, Lane M, Norman RJ, Robker RL. 2010. High-fat diet causes lipotoxicity responses in cumulus-oocyte complexes and decreased fertilization rates. *Endocrinology*, 151:5438-5445.

Wu LL, Norman RJ, Robker RL. 2011. The impact of obesity on oocytes: evidence for lipotoxicity mechanisms. *Reprod Fertil Dev*, 24:29-34.

Xu C, Bailly-Maitre B, Reed JC. 2005. Review series endoplasmic reticulum stress cell life and death decisions. *J Clin Invest*, 115:2656-2664.

Yamaguchi H, Wang HG. 2004. CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells. *J Biol Chem*, 279:45495-45502.

Yang X, Wu Ll, Chura Lr, Liang X, Lane M, Norman RJ, Robker RL. 2012. Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertil Steril,* 97:1438-1443.

Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, Goldstein JL. 2000. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell Biol,* 6:1355-1364.

Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D. 1998. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev*, 12:982-995.