Animal: An International Journal of Animal Bioscience

Is there a role for lipid modulators in assisted reproduction technologies (ART)?

--Manuscript Draft--

Manuscript Number:	ANIMAL-12-30651
Full Title:	Is there a role for lipid modulators in assisted reproduction technologies (ART)?
Short Title:	lipid modulators and ART
Article Type:	Review
Keywords:	oocyte, lipid droplets, assisted reproduction technologies, maturation, quality
Corresponding Author:	Rosa Maria Lino Neto Pereira, PhD Instituto Nacional de Investigação Agrária e Veterinária Vale de santarém, PORTUGAL
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Instituto Nacional de Investigação Agrária e Veterinária
Corresponding Author's Secondary Institution:	
First Author:	Rosa Maria Lino Neto Pereira, PhD
First Author Secondary Information:	
Order of Authors:	Rosa Maria Lino Neto Pereira, PhD
	Elsa Graça Prates, Biologist
	José Tirapicos Nunes, PhD
Order of Authors Secondary Information:	
Abstract:	Oocyte intracellular lipids are mainly stored in lipid droplets (LD) in order to provide energy for growth and development, but they are also important signalling molecules involved in the regulatory mechanisms of maturation and thence in oocyte quality. Recent studies show that LD are highly dynamic organelles changing their shape, volume and location within the ooplasm as well as their association to other organelles during the maturation process. However, high amounts of LD have been correlated with impaired oocyte developmental competence and oocyte/ embryo cryosurvival. This review presents a brief morphological and functional characterization of cumulus-oocyte complexes, the functional and dynamic unit playing a pivotal role in ART outcomes. Particular attention is given to the lipid-rich porcine oocyte, here rendered as an excellent model to understand the role of lipids and fatty acid metabolism during maturation and their implications on subsequent embryo development. Moreover the possibility of applying substances capable of modulating oocyte nuclear and cytoplasmic maturation, specifically oocyte or embryo lipid content and metabolism to improve ART success is further discussed. In addition, these principles might be applied in germplasm and embryo cryopreservation, in livestock production or in biomedical research.
Suggested Reviewers:	Tom McEvoy, PhD Professor, Scottish Agricultural College t.mcevoy@ab.sac.ac.uk Tom McEvoy is a senior researcher that during the past years has studied the role of fatty acids in oocytes and embryos as well as their implications in mammalian reproductive biotechnologies among other issues. His curriculum is excellent and we think that T. McEvoy could appreciate our revision work.
	Graça Ferreira Dias, PhD Professor, Universidade Técnica de Lisboa gmlfdias@fmv.utl.pt Graça Ferreira Dias is a senior researcher presenting a high experience in animal reproduction and physiological mechanisms. Graça has an extensive track of

	international publications including revision papers, in the scientific area of our manuscript
	Carlos Eugénio Plancha, PhD Professor, Faculdade de Medicina de Lisboa cplancha@fm.ul.pt Carlos Plancha is a senior researcher in the field of ART specifically in oocyte maturation mechanisms

Is there a role for lipid modulators in assisted reproduction technologies (ART)?

Running head: **lipid modulators and ART.**

E.G. Prates[1,2] J.T. Nunes[2], R.M. Pereira[1,3*]

[1]*INIAV Santarém, Quinta da Fonte Boa, 00-048 Vale de Santarém, Portugal.*

[2]*Universidade de Évora – Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), 7002-554 Évora, Portugal.*

[3]*Escola Universitária Vasco da Gama, Mosteiro de S. Jorge de Milréu, 3040-714 Coimbra, Portugal.*

*corresponding author: R.M. Pereira; *L-INIAV Santarém, Quinta da Fonte Boa, 2005-048 Vale de Santarém, Portugal*; e-mail: rosalnp@gmail.com

Abstract

Oocyte intracellular lipids are mainly stored in lipid droplets (LD) in order to provide energy for growth and development, but they are also important signalling molecules involved in the regulatory mechanisms of maturation and thence in oocyte quality. Recent studies show that LD are highly dynamic organelles changing their shape, volume and location within the ooplasm as well as their association to other organelles during the maturation process. However, high amounts of LD have been correlated with impaired oocyte developmental competence and oocyte/ embryo cryosurvival. This review presents a brief morphological and functional characterization of cumulus-oocyte complexes, the functional and dynamic unit playing a pivotal role in ART outcomes. Particular attention is given to the lipid-rich porcine oocyte, here rendered as an excellent model to understand the role of lipids and fatty acid

metabolism during maturation and their implications on subsequent embryo development. Moreover the possibility of applying substances capable of modulating oocyte nuclear and cytoplasmic maturation, specifically oocyte or embryo lipid content and metabolism to improve ART success is further discussed. In addition, these principles might be applied in germplasm and embryo cryopreservation, in livestock production or in biomedical research.

Keywords: oocyte, lipid droplets, assisted reproduction technologies, maturation, quality.

Implications

The effects of lipid modulators in ART are not clearly understood. High amounts of lipid droplets have been correlated with impaired oocyte/embryo developmental competence and cryosurvival. However, this large repository of endogenous lipids is vital for their energy production. Lipids are also important to form membranes or as intracellular messengers. Although modifications of intracellular lipids by chemical stimulation can improve oocyte or embryo quality and cryopreservation, some of the mechanisms by which lipolytic substances interfere with lipid metabolism may affect the metabolic *via* regulating their developmental potential. This review contributes to a better understanding of this intricate issue with repercussions in livestock production and biomedical research.

Introduction

Oocyte quality is a key limiting factor in female fertility (Gilchrist et al., 2008; Wang and Sun, 2007). The ovarian follicular microenvironment and maternal signals, mediated primarily through granulosa cells and cumulus cells (CC), are responsible for nurturing oocyte growth and its gradual acquisition of developmental competence (Gilchrist et al., 2008). *In vitro* maturation (IVM) of oocytes can generate mature oocytes which are capable of supporting preimplantation embryo development and full development to term (Gilchrist and Thompson, 2007). In livestock production, IVM oocytes can provide a large population of female germ cells (Hirao et al., 1994) that can be useful in breeding programmes and animal genetic cryo-conservation. However, the high intracellular lipid content of oocytes and embryos has been reported to impair cryopreservation, with particular relevance in porcine species (Seidel, 2006, Pereira and Marques, 2008). Different strategies can be used to manipulate oocyte or embryo lipid contents. Nevertheless, a role for lipids in energy production during preimplantation development as well as precursors in steroidogenic and eicosanoid pathways has been referred (Sturmey and Leese, 2003; Nuttinck et al., 2008; Lapa et al., 2011), suggesting that modifications in oocyte intracellular lipids should be carefully estimated. The high intracellular lipid content of porcine oocytes (Prates et al., 2012a, b) renders them an excellent model to understand the role of lipids and fatty acid metabolism during maturation and their implications on the decline of female fertility due to age and obesity (Ford and Tavendale, 2009; Purcell and Moley, 2011). Furthermore, mechanisms by which nuclear and cytoplasmic maturation can be synchronised might be related to metabolic pathways that are also involved in the mobilization of intracellular lipid reserves in the oocyte. This review will discuss current

knowledge of mechanisms regulating oocyte quality during maturation and highlight recent advances in modifying oocyte lipid metabolism and content to improve assisted reproduction technology (ART) outcome.

Morphological and functional characterization of cumulus-oocyte complexes

The cumulus-oocyte complex (COC) is composed of the feminine gamete and the surrounding CC (Figure 1) and is of primordial importance to the outcomes of ART. This complex forms a complete functional and dynamic unit playing a pivotal role in oocyte metabolism during maturation. Moreover the bidirectional exchanges between oocyte and contiguous CC are important for oocyte competence acquisition, CC expansion and early embryonic development (Sutton et al., 2003, Gilchrist and Thompson, 2007, Ouandaogo et al., 2011). The oocyte maturation process comprises two aspects, nuclear and cytoplasmic, both essential for normal fertilization and embryonic development, thus being highly coordinated (Hunter, 2000).

COC maturation

Oocyte nuclear maturation.

Oocyte competence to complete nuclear maturation is acquired at least in two steps: firstly, oocytes are able to reinitiate meiosis (Figure 2A), undergo germinal vesicle breakdown (GVBD) and progress to metaphase I; secondly, oocytes are competent to advance beyond metaphase I, enter anaphase and

proceed to metaphase II (MII) (Eppig, 1996). At the end of the maturation period, the meiotic spindle and chromosomal rearrangement at MII as well as the first polar body can be observed (Figure 2B). The location and length of meiotic spindle are crucial to assure an accurate chromosomal alignment and segregation during meiosis (Xia et al., 1997; Wang and Sun, 2007).

The oocyte has the largest diameter of any cell in female mammals (Ambruosi et al., 2009). Oocyte diameter and area measurements are often used to predict oocyte meiotic competence or maturity (Griffin et al., 2006; Prates et al., 2008). In vivo, oocytes reach their maturity within the antral stage of follicular development presenting different diameters according to species (mouse, hamster, pig and human) (Griffin et al., 2006). In porcine, full meiotic competence of the oocyte is reached in ovarian follicles with a diameter of 3 mm or more (Sun and Nagai, 2003). *In vitro*, porcine oocytes under 90 µm in diameter are unable to resume meiosis, while oocytes measuring 110-115 µm can complete the first meiotic division and acquire MII (Sun and Nagai, 2003; Prates et al., 2012a). Concurrently, the oocyte grows and its area may be an indicator of cytoplasmic maturation (Prates et al., 2012a). Furthermore, the size of periviteline space or thickness of zona pellucida are also related to the developmental competence of the oocyte for fertilization (Xia, 1997; Wang and Sun, 2007).

Oocyte cytoplasmic maturation.

Oocytes are complex cells comprising many organelles and compounds (Wang and Sun, 2007). The large repository of endogenous lipids as an energy source for oocyte and embryo development is mainly stored in the form of an organelle,

125 the lipid droplet (LD) (McEvoy et al., 2000; Romeck et al, 2010). LD is
126 composed of a core of neutral lipids enveloped by a phospholipid monolayer
127 containing a wide variety of proteins that can be embedded in both the
128 phospholipid monolayer and within the core (Walther and Farese, 2009). The
129 function of these cellular proteins in LD fractions is currently being studied. For
130 instance, perilipins that are located at the LD surface in adipocytes, but also in
131 steroidogenic tissues, were referred as having regulatory functions and as being
132 involved in LD lipolysis (Holm, 2003). These particular organelles can be formed
133 *de novo*, when cells are kept under delipidated conditions and re-fed with free
134 fatty acid (FA) or grow through a coalescence process of existing droplets,
135 mediated by SNARE proteins (Thiele and Spandl, 2008). However, regardless
136 of LD origin, they are highly dynamic, constantly changing shape, volume and
137 location (Watanabe et al., 2010). In particular, the porcine oocyte has been
138 identified as one of the most lipid-rich oocytes among domestic animals
139 (McEvoy et al., 2000), in which the cytoplasm is filled with LD. During IVM, a
140 considerable variation in LD areas, from 0.3 μm^2 to about 90 μm^2 (Figure 3),
141 was identified through maturation progression (Prates et al., 2012a).
142 Lipid droplets can be found in association with other organelles linked to cellular
143 metabolism such as mitochondrias, endoplasmic reticulum (ER), endossomes,
144 peroximes and cytoskeleton (Sturmey et al., 2006; Thiele and Spandl, 2008;
145 Walther and Farese, 2008; Ambruosi et al., 2009). During oocyte maturation,
146 the activity and organization of LD-mitochondria are particularly relevant, since
147 oxidative phosphorylation is the main pathway to supply ATP for cellular
148 activities (Sturmey and Leese, 2003; Wang and Sun, 2007). In mature porcine
149 oocytes two distribution patterns were identified for both, LD and mitochondria:

an even or homogenous distribution through the ooplasm and an uneven or heterogeneous allocation. The first distribution is more frequently observed (Cui et al., 2009). Moreover, the evidence of regions of 'co-localization' between LD and mitochondria and their relocalization during IVM of porcine oocytes was shown to be linked to intracellular oxygen gradients. Hence, Sturmey et al. (2006) observed that the peripheral mitochondrial clustering in porcine oocytes was correlated to higher oxygen availability in this region. In oocytes from other species, such as the equine, the aggregation of LD, which was related to nuclear maturation and cumulus expansion was also observed (Ambruosi et al., 2009). Moreover, the morphological changes observed in LD during oocyte maturation may reflect changes in the nature of lipids being stored in those droplets (Silva et al., 2011) but can also be a sign of abnormal maturation (Prates et al., 2012a)

In spite of several efforts, oocyte cytoplasmic maturation remains a key limiting step for ART. The reasons why fully grown oocytes are not capable of becoming viable embryos are still elusive, but incomplete cytoplasmic maturation and/or asynchrony between nuclear and cytoplasmic maturation are certainly among those critically responsible.

The surrounding investment of cumulus cells.

The simultaneous expansion of compact layers of CC surrounding the oocyte and deposition of mucoelastic material in the extracellular matrix is implicated in supporting of both nuclear maturation to the MII stage and cytoplasmic maturation (Gilchrist and Thompson, 2007; Ambruosi et al., 2009; Cui et al., 2009). Moreover, the communication between the oocyte and its surrounding

cells is essential for normal acquisition of oocyte developmental competence. However fully grown oocyte in vivo is associated with the loss of COC gap junctional communication (Thomas et al., 2004).

During porcine oocyte IVM, CC interfered with ooplasmic LD-mitochondria distributions, in which LD distribution in oocytes was more sensitive to CC absence than mitochondria (Cui et al., 2009), thus influencing oocyte morphological appearance. The presence of CC during IVM was also found to be effective in regulating the synthesis and concentration of important cytoplasmic factors such as glutathione (GSH) and Ca^{2+} (Hao et al., 2007). Denuded mature porcine oocytes present differences in Ca^{2+} homeostasis regarding those matured with CC. Indeed, the duration of Ca^{2+} rise was higher, although its amplitude was lower when compared to oocytes matured in the presence of CC: COC or CC added to culture medium (Cui et al., 2009).

Concomitantly with oocyte nuclear maturation, CC underwent a molecular maturation process. Moreover, oocytes actively control the COC microenvironment via oocyte-secreted factors that regulate fundamental aspects of CC function. In turn, the CC gene expression profile varies according to the stages of oocyte maturation and can be useful as predictors of oocyte quality (Gilchrist and Thompson, 2007, Ouandaogo et al., 2011). The beneficial effect of CC during oocyte growth to stimulate competence acquisition to further support embryonic development is therefore unequivocal.

Molecular mechanisms underlying oocyte maturation

The role of cAMP, PKA and PKC in meiotic progression.

The maturation of an oocyte is a multi-pathway process that includes the involvement of several mediator factors (Hurk and Zhao 2005). A critical signalling compound is the gonadotropin second messenger, cyclic AMP (cAMP), which is synthesized in the oocyte and in adjacent CC through the activation of the constitutively expressed transmembrane G-protein-coupled receptor (Racowsky, 1985; Hurk and Zhao, 2005; Marei et al., 2009). The newly synthesized cAMP stimulates the cAMP dependent protein kinase (PKA), which type I mediates the inhibitory action on oocyte GVBD, while type II regulates the meiosis-inducing pulse of cAMP occurring within CC following hormonal stimulation (Downs et al., 2002). Simultaneously to PKA stimulation, protein kinase C (PKC) can also be activated, delaying meiotic progression and enhancing cytoplasmic maturation in porcine and bovine oocytes (Sun and Nagai, 2003; Ali and Sirard, 2005). PKC also participates in cortical granules exocytosis during the fertilization process (Wu et al., 2010).

The regulation of cAMP level in the maturating oocyte transiently delays nuclear progression (Racowky, 1985; Xia et al., 2000), thus allowing simultaneous cytoplasm enrichment in nutritive substances to support embryonic development. Furthermore, the activity of AMP-phosphodiesterase (PDE) within oocytes hydrolyses cAMP to AMP, inactivating PKA protein, which in turn provides a positive stimulus for oocyte nuclear maturation inducing GVBD (Downs et al., 2002) and maturation progression.

Implications of mitogen-activated protein kinase (MAPK) and maturation promoting factor (MPF) on nuclear maturation.

Other important regulators of oocyte meiosis are MAPK and MPF (Sun and Nagai, 2003). The MAPK pathway is activated by AMP that is synthesized from cAMP or enters the oocyte from the exterior (Downs et al., 2002). The MAPK can be activated by growth factors or by gonadotropin stimuli in CC to induce GVBD. This is observed in the porcine species, for which sites of activation can be located both in the GV or in the cytoplasm (Seger and Krebs, 1995; Downs et al., 2002; Sun and Nagai, 2003; Hurk and Zhao, 2005). Two isoforms of MAPK proteins were identified (ERK1 and 2) as being involved in the regulation of cell cycle and microtubule dynamics during metaphase organization. Their activity enhances in G1 through S and G2/M phases (Seger and Krebs, 1995; Marei et al, 2009). Furthermore, MAPK is implicated: in retaining MII arrest, in regulating PDE action on cAMP degradation and in the maintenance of MPF activity (Downs et al., 2002; Hurk and Zhao, 2005).

The MPF is activated by cyclins and cyclin-dependent kinases following LH-surge. In the absence of the inhibitory influence of cAMP, MPF stimulates the oocyte to enter the M-phase and precede GVBD (Hurk and Zhao, 2005), thus being implicated in chromatin condensation, cytoskeletal reorganization, spindle formation and plasma membrane disassembly (Sun and Nagai, 2003). This complex protein is composed of cyclin B, the regulatory subunit, and p34cdc2 kinase, the catalytic subunit. The activity of MPF is regulated by cyclin B binding to Cdc2, and by phosphorylation of threonine 161, dephosphorylation of tyrosine 15 and threonine 14 (Hurk and Zhao, 2005, Zhang et al., 2010). During porcine oocyte maturation, this activity appears initially at GVBD, reaches a high level at metaphase I and disappears transiently at the time of first polar body emission. Then, MPF activity reappears at MII and remains at an elevated

level until fertilization (Dekel, 1995). Recent reports showed that the oscillatory activity of MPF during oocyte maturation depends on post-transcriptional regulation of cyclin B1 by cytoplasmic polyadenylation (Zhang et al., 2010). Moreover, a decreased expression of maternal cyclin B1 and Cdc2 was identified at mRNA or protein level in developmentally incompetent oocytes.

GSH, ATP and Ca^{2+} during cytoplasmic maturation.

In maturating oocyte cytoplasm, GSH is one of the most important factors synthesized, since it participates in the regulation of protein and DNA synthesis, in microtubule assembly, protection against oxidative damage and sperm nuclear decondensation and male pronuclear formation following fertilization (Hao et al., 2007). GSH can be formed from precursor amino acids added during IVM, such as cysteine (Cui et al., 2009) and cysteamine (Sutton et al., 2003).

Simultaneously to GSH utilization, alterations in ATP and Ca^{2+} levels have been related to developmental competence of porcine oocytes. The inhibition of GSH synthesis was shown to induce a decrease in the duration and amplitude of intracellular free Ca^{2+} rise as well as ATP depletion (Cui et al., 2009). These alterations in intracellular Ca^{2+} stores are crucial in the regulation of oocyte capacity to undergo activation. Intracellular Ca^{2+} release is highly increased during the progression of nuclear maturation. In fact, the Ca^{2+} signal is required for full activation of MPF. This activation stimulates the oocyte to enter the M-phase, thus overriding the negative effect of cAMP (Krisher, 2004; Hurk and Zhao, 2005). Repetitive Ca^{2+} transients are further generated during fertilization

to stimulate the emission of cortical granules released from smooth-membrane vesicles (Eppig, 1996; Kim et al., 1997; Krisher, 2004).

The role of lipids during oocyte maturation and initial embryo development.

Lipids are essential cellular compounds that play an important role as an energy source that is vital for proper mammalian oocyte maturation and succeeding embryo development (Romek et al., 2009). The most abundant intracellular lipids stored within porcine oocytes are triacylglycerols, representing approximately 46% (w/w) of total FA in the oocyte (Homa et al., 1986, McEvoy et al., 2000). These can be utilized in mitochondrial β-oxidation to produce energy during oocyte maturation (Sturmey and Leese, 2003, Sturmey et al., 2006). Furthermore, the enrichment in phospholipids and cholesterol during oocyte maturation is crucial to form membranes during rapid cell divisions after oocyte fertilization (McEvoy et al., 2000).

Second messengers reflect other phospholipid roles in cell function and, thereby, in oocyte maturation and embryo development. In porcine oocytes, phosphatidylinositol represents 6% of total phospholipids, being rich in arachidonic (20:4n-6) and stearic (18:0) acids and also in palmitic acid (16:0) (Homa et al., 1986). Hydrolysis of this membrane phospholipid yields two second messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. IP3 and its derivative (IP4) increase Ca^{2+} concentrations, while diacylglycerol stimulates PKC (McEvoy et al., 2000, Wu et al., 2006). The importance of Ca^{2+} and PKC in oocyte maturation and fertilization processes is already described above. Plasmalogens are another class of phospholipids identified in oocyte membranes (Homa et al., 1986, Prates et al., 2012b). Their composition differs

298 according to the oocyte maturation stage (immature and mature). Although
299 reference was made to plasmalogens controlling the dynamics of cellular
300 membranes (Nagan and Zoeller, 2001), their function during oocyte maturation
301 and embryo development remains unknown.
302 Analysis of FA composition of porcine oocytes during maturation showed that
303 16:0, followed by oleic (18:1 *c*9), 18:0 were the most abundant FA registered in
304 immature and mature oocytes, followed by n-6 polyunsaturated fatty acids
305 (PUFA), specifically linoleic (18:2n-6) and 20:4n-6, which may indicate that
306 oocytes are capable of synthesizing prostaglandins (PG) and leukotrienes
307 (Homa et al., 1986; McEvoy et al., 2000; Prates et al., 2012b). Prostaglandins,
308 namely PGE_2 can be critical mediators of CC expansion and of oocyte meiosis
309 resumption and progression (Marques et al., 1997, Nuttinck et al., 2008).
310 Moreover, FA profile of oocytes can change due to culture media or dietary lipid
311 supplementation and differences in oocyte FA composition were related to
312 oocyte developmental competence (Lapa et al., 2011, Warzych et al., 2011).
313 Although the role of lipids during oocyte maturation and initial embryo
314 development are currently under research, their primordial importance in female
315 gamete quality is unequivocal.
316
317 **Oocyte quality and recent evaluation techniques**
318
319 The use of morphological characteristics and metabolic predictors involved in
320 COC maturation can provide valuable information for the preselection of high-
321 quality oocytes to maximize embryonic developmental outcomes (Wang and
322 Sun, 2007). The huge number of LD as well as their distribution and association

to other organelles in the cytoplasm, eventually other cytoplasmic pigments, are responsible for the dark colour tone that characterizes the oocyte of some mammalian species, namely the porcine, bovine, equine or the minke whale (Fujihira et al., 2004; Genicot et al., 2005; Sturmey et al., 2006; Ambruosi et al., 2009). In addition, Cui et al. (2009) showed that the bright gray and uniform ooplasm in association with an intact plasma membrane were markers of better quality in porcine oocytes. During ageing of oocytes, mitochondrial distribution changed from diffuse to aggregate (Hao et al., 2007). Additionally, these authors referred that the shape of mitochondria changed from spherical to elongated and that LD became solidified concomitantly to modifications in oocyte homeostasis, namely in ATP content, Ca^{2+} rise and amplitude. These aged oocytes presented a dark colour and irregular ooplasm. Thus, these morphological characteristics can be used to predict oocyte quality. However, oocytes from different species may present different degrees of cytoplasmic transparency. Although a dark granulated ooplasm is a normal characteristic in the pig, is not so in humans, where dark colour and cytoplasmic inclusions are related to low oocyte quality and fertility failure (Xia, 1997; Wang and Sun, 2007).

The different amounts of emitted fluorescent light in bovine, porcine and murine oocytes were correlated with the lipid contents in these species (Genicot et al., 2005). Therefore, these microscopic techniques could be used to compare lipid contents of oocytes from different donors, sized follicles or cultured conditions. On the other hand, the gray mean value within the oocyte fat area was suggested as being another appropriate tool to evaluate the lipid content of a single porcine oocyte (Prates et al., 2012a). This can be a useful non invasive

technique, that upon record of oocyte morphology allows for its posterior use, namely for cryopreservation or fertilization. The possibility of posterior use is very important in humans or endangered species due to the limited number of available oocytes.

Other criteria besides morphological characterization, such as metabolic markers may be useful to evaluate oocyte quality and fertilization potential (Sturmey and Leese, 2003). *In vitro*, oocyte competence can be assessed by the brilliant cresyl blue, a dye that can be reduced by the glucose-6-phosphate, which is an enzyme that is characteristic of full grown and more competent oocytes (Antosik et al., 2010; Warzych et al., 2011). This test has been used in the porcine species to evaluate oocyte quality after modifications were made to maternal diet and thus in the FA profile of follicular fluid (Warzych et al., 2011). Interestingly, the FA profile in women changes in early mid-life and these changes have been implicated in the decline of fertility that commences before age 35 (Ford and Tavendal, 2009). Furthermore, the evaluation of other enzyme function, such as delta-9 and delta-5 desaturase, genetic expression monitorization by real-time PCR or gas chromatography analysis are among several other techniques that can be used to complete the information about oocyte quality that seems to be closely related to its lipid composition (Ford and Tavendal, 2009; Ouandaogo et al., 2011; Warzych et al., 2011, Prates et al., 2012a, b).

The manipulation of meiotic and cytoplasmic maturation

Meiotic inhibitors

In order to improve developmental outcomes of oocytes known to prematurely undergo nuclear maturation, pharmacological tools that elevate intracellular cAMP levels and transiently delay spontaneous GVBD, are frequently used (Laforest et al., 2005; Gilchrist and Thompson, 2007; Weng et al., 2007). These meiotic inhibitors are cAMP analogues, such as dibutyryl cAMP, adenylate cyclase activators like FSH and forskolin, PDE inhibitors as the non-specific inhibitor IBMX, the PDE type 4-specific inhibitor rolipram or the PDE type 3-specific inhibitors milrinone, cilostamide or Org9935 (Laforest et al., 2005; Gilchrist and Thompson, 2007). Their dose and effects are species dependent. For example, when compared to other species, porcine oocytes exhibit relatively high sensitivity to forskolin elevating cAMP levels (Racowsky, 1985). On the other hand, inhibitors of p34cdcc2/cyclin B kinase such as roscovitine, can also be used to delay nuclear maturation and thus promoting porcine oocyte competence acquisition (Coy et al., 2005)

Mechanisms of action of meiotic inhibitors.

The utilization of meiotic modulators (alone or simultaneously) has proven to induce an effective delay in GVBD and simultaneously an extension in oocyte-CC gap-junctional communication during the meiotic resumption phase, allowing for continued mRNA and protein accumulation within the ooplasm (Xia et al., 2000; Laforest et al., 2005; Gilchrist and Thompson, 2007). Different incubation times using the meiotic inhibiting agent are possible, after which the inhibitor is removed and the maturation process proceeds. Forskolin is a adenylate cyclase activator that also has lipolytic properties, interfering with LD

morphology and thus with oocyte cytoplasmic maturation (Fu et al., 2011; Prates et al., 2012a). In fact, the meiosis progression of porcine oocytes cultured with 10 µM of forskolin during half or the entire period of IVM was inhibited. Moreover their cytoplasmic morphology resembled that of oocytes in the immature stage, without the redistribution of LD (fat area) characteristic of mature ones (Prates et al., 2012a). Nevertheless, by decreasing supplementation time to the initial 2 h, oocyte meiotic competence was not affected, although forskolin still interfered with organelles dynamics and thus with cytoplasmic maturation.

Synchronization of nuclear and cytoplasmic maturation by meiotic inhibitors.

Asynchronous or incomplete oocyte maturation has been indicated as a common phenomenon in the porcine species that can predispose oocytes to multiple sperm penetration through the zona pellucida into the cytoplasm before the block to polyspermy (Hunter, 1990; Grupen et al., 1997; Laforest et al., 2005). Moreover, *in vitro* culture conditions during fertilization can be a cause for polyspermy (Funhashi and Romar, 2004; Amiñana et al., 2005). In vivo, this pathological situation leads to the formation of polyploidy embryos that usually die at a very early stage of development (Hunter, 1990). The changes in oviductal fluid composition, spermatozoa concentration, interval between mating and fertilization that includes the period of spermatozoa capacitation as well as the functional state of oocyte cortical granules can all account for polyspermy. Although extensive attempts have been made to reduce the penetration of porcine oocytes by more than a single spermatozoon, the high incidence of polyspermy remains a major obstacle in porcine ART (Hunter, 1990, Almiñana

et al. 2005). This problem is also present in aged female gametes of several species, including humans (Kikuchi et al., 2002; Hao et al., 2007, Wang and Sun, 2007). The utilization of meiotic inhibitors can improve oocyte competence and it can therefore partially resolve the problem of polyspermic fertilization. However, this is also related to *in vitro* culture conditions during IVF. Thus, further research is needed to improve the efficiency of IVF systems, especially in porcine, to produce good quality embryos.

Lipid modulators

Lipid modulators are substances that are capable of reducing and/or modifying intracellular lipid content of cells that have been successfully applied in ART. The *trans*-10, *cis*-12 conjugated linoleic acid (*t*10,*c*12 CLA) is one of these substances, being capable of interfering with lipid accumulation and metabolism in porcine adipose explants, as well as in bovine oocytes and embryos (José et al., 2008; Pereira et al., 2007; Lapa et al., 2011). Forskolin was also able to reduce lipid content in porcine oocytes and embryos through lipolysis stimulation (Men et al., 2006; Fu et al., 2011). In a recent experiment, different incubation times (44h, 22h and 2h) with *t*10,*c*12 CLA or forskolin interfered on oocyte lipid content showing that supplementation time affected the distribution and morphology of LD (Prates et al., 2012a). These authors reported that the fat area became lighter in the presence of *t*10,*c*12 CLA, while in forskolin treated oocytes the fat area was reduced and meiosis delayed beyond 2 h of supplementation. Moreover, as referred, the supplementation time of forskolin during IVM of porcine oocytes can impair fertilization since it delays meiotic

progression and oocyte growth (Prates et al., 2012a). Another lipid modulator that has been used to improve ART results is phenazine ethosulfate (PES). PES supplementation during embryo culture increased glucose metabolism and tended to increase the PPP flux of glucose, with a clear reduction in the accumulation of lipids (De La Torre-Sanchez et al., 2006).

Mechanisms of action of lipid modulators.

The use of adenylyl cyclase activators such as forskolin during porcine oocyte culture induces alterations in both, nuclear and cytoplasmic maturation, these latter being related to the reduction of LD containing area (Prates et al., 2012a). As referred to above, the cytoplasmic maturation in the developing oocyte implies LD movements that can induce LD coalescence and thus morphology modifications. Moreover, by stimulating lipolysis, intracellular LD content may also be modified due to shrinkage (Thiele and Spandl, 2008). Once a lipolytic substance, such as forskolin (figure 4), binds to the catalytic subunit of the adenylyl cyclase enzyme, the cAMP is hydrolysed from the available ATP in the cytoplasm and the cAMP level is increased, thus activating PKA protein (Xia et al., 2000; Holm, 2003). This protein phosphorylates endogenous lipases, as the hormone-sensitive lipase (HSL) and also perilipin protein located at the LD surface (Holm, 2003). Following phosphorilation, HSL is translocated to the cytoplasm where it binds to LD surface protein to induce fragmentation of large droplets in smaller ones, thus increasing accessible droplet surface and the degradation of its core (Holm, 2003; Thiele and Spandl, 2008). In the lipolysis of intracellular lipids, HSL catalyses triglycerides and diglycerides, while monoglyceride lipase is required to obtain complete hydrolysis of

monoglycerides (Holm, 2003; Men et al., 2006; Thiele and Spandl, 2008; Walther and Farese, 2008). The supplementation of porcine COC culture medium with forskolin, during the initial 2 h of IVM, influenced both oocyte and their CC FA and plasmalogens (measured by dimethylacetal, DMA) composition, although their total contents were not affected (Prates et al., 2012b). Depending on dose and exposure time, forskolin treatment may induce a higher modification in intracellular lipids (Fu et al., 2011; Prates et al., 2012a). Besides acylglycerols, HSL can hydrolyse cholesterol and steroid esters (Holm, 2003). The meiosis-activating sterols system was also referred to be stimulated by the MAKP pathway following AMP level rise due to cAMP hydrolysis (Sutton et al., 2003). Therefore, sterol metabolism might be affected by the utilization of lipolytic agents during oocyte maturation, such as adenylyl cyclase stimulators.

Through lipolysis, the generation of glycerol and the availability in free FA are increased. These metabolites may then be used for *de novo* synthesis of FA by re-esterification and phospholipids generation for membrane assembly during cell division of embryonic development (Men et al., 2006; Walther and Farese, 2008). Simultaneously, free FA may be used to produce energy through the β-oxidation pathway, to boost maturation progression as in porcine oocytes (Sturmey and Leese, 2003). On the other hand, *t*10,*c*12 CLA added during the culture of pig adipose tissue interfered with lipogenesis, decreasing the FA synthase activity and the rate of C-labelled glucose incorporation, but had no effect on non-esterified FA release (José et al., 2008). However, when porcine COC were matured with 100 μM *t*10,*c*12 CLA, this isomer was accumulated in both, oocyte and CC, changing their FA profiles, mostly in CC (Prates et al., 2012b). The *t*10,*c*12 CLA appears to affect the PKA signal transduction

498 pathway and thus, the cAMP cascade of reactions (figure 4) (Ashwell et al.,
499 2010). These authors showed that the expression of four genes from the PKA
500 transduction pathway were down regulated while four genes were up regulated
501 by *t*10,*c*12 CLA supplementation. On the other hand, an increase in lipolysis
502 and in cytosolic perilipin associated with smaller LD was identified in human
503 adipocytes cultured in the presence of this isomer (Chung et al., 2005). It is then
504 possible that PKA and MAPK/ERK pathways may be regulated by *t*10,*c*12 CLA,
505 thus interfering with LD lipolysis (figure 4). The analysis of FA and DMA
506 composition of porcine COC showed that independently from cell type, CLA
507 treatment reduced the proportions of several individual FA and plasmalogens
508 DMA-16:0, c9-16:1, 18:3n-6, and tended to reduce c7-16:1, c11-18:1 and
509 20:4n-6 (Prates et al., 2012b). Furthermore, it increased the concentration of
510 22:6n-3 in the maturation media at the end of the maturation period, suggesting
511 that this FA might have been elongated during maturation and exported, to
512 maintain oocyte PUFA content to avoid the generation of reactive oxygen
513 species (ROS). Indeed, CLA may follow different pathways once it is available
514 in the cell (Banni, 2002). CLA may then enter in mitochondrial β-oxidation to
515 produce energy for maturation proceeds or FA synthesis in ER and ER-Golgium
516 complexes (Sturmey and Leese, 2003; Thiele and Spandl, 2008). It is possible
517 that *t*10,*c*12 CLA might have entered the oocyte by pinocytosis and
518 accumulated in LD, thus interfering with its colouration under microscopic
519 observation and LD movements during maturation (Prates et al., 2012a). The
520 mechanisms of action of *t*10,*c*12 CLA on oocyte and embryos needs to be
521 further studied.

Alternatively, PES is another lipid modulator that stimulates different metabolic pathways. PES increases glucose metabolism through PPP during embryo culture (De La Torres-Sanchez et al., 2006). Hence, PES is a strong electron acceptor that readily oxidizes NADPH, thus decreasing NADPH required for the synthesis of numerous lipids, particularly long-chain fatty acids, and reducing intracellular lipid content of embryos (Seidel, 2006).

Lipid content reduction applied for cryopreservation of oocytes and embryos.

Considerable progress has been made in improving and simplifying oocyte and embryo cryopreservation procedures to be routinely used in transfer programs. In general, cryopreservation by slow freezing is a process where extracellular water crystallizes, resulting in osmotic gradient that draws water from the intracellular compartment until intracellular vitrification occurs (Pereira and Marques, 2008: Saragusty and Arav, 2011). On the other hand, in cryopreservation through vitrification, both intra and extracellular compartments vitrify after cellular dehydration has already occurred (Saragusty and Arav, 2011). These cryopreservation techniques have been improved to minimise damage and help oocytes and embryos of different developmental stages to regenerate by several strategies: using microsurgical manipulation, cytoskeletal relaxants (such as cytochalasin B or D), membrane and protein stabilisers, centrifugation, adjusting the concentration of cryoprotectors and/or reducing the cooling volume to a minimum (Esaki et al., 2004; Cuello et al., 2008; Gerelchimeg et al., 2009; Fu et al., 2011). Nevertheless, the success rate is still limited particularly in oocytes.

546 The plasma membrane of oocytes and embryos is the first cellular structure
547 whose integrity is affected by thermotropic phase transition (Nagashima, 1994;
548 Arav et al., 1996). During cooling, irreversible damage occurs shortly after
549 exposure to low, but not freezing temperatures just below 15 ºC (Nagashima,
550 1994). In oocytes, the greater cellular volume and higher cytoplasmic lipid
551 content increase chilling sensitivity when compared to embryo cells (Arav and
552 Zvi, 2008). Furthermore, the less submembranous actin microtubules present in
553 oocytes accounts for a less robust membrane and thus, cryopreservation can
554 cause disorganization of cytoskeleton and meiotic spindle, as well as
555 chromosome and DNA abnormalities (Fujihira et al., 2004; Arav and Zvi, 2008).
556 Changes in cellular chemical composition and LD association to other
557 organelles or to the cytoskeleton were also identified in GV oocytes and phase
558 separations in the plasma and/or internal membranes in embryos (Nagashima,
559 1994; Arav et al., 1996; Fujihira et al., 2004). Differences in LD colour tone of
560 fresh immature and vitrified-warmed porcine oocytes were identified: gray in
561 fresh and slightly dark in vitrified oocytes (Fujihira et al., 2004). However, LD
562 size or distribution was similar. On the contrary Isachenko et al. (2003) showed
563 that the two types of LD found in porcine oocytes, dark and 'gray', changed their
564 morphology during cooling into a spherical form with lucent streaks impairing
565 oocyte developmental competence.
566 As referred above, the high lipid content that has been related to an increased
567 sensitivity to chilling injury during cryopreservation is particularly important in
568 porcine species (Fujihira et al., 2004; Romek et al., 2009; Fu et al., 2011).
569 Changing the lipid content of porcine embryos by removing LD may have a
570 direct effect on embryo survival during chilling (Nagashima et al., 1994; Hara et

al., 2005). This process can be performed by mechanical delipidation through polarization of the cytoplasmic LD and subsequent physical removal of excess lipid, increasing the survival rates of cryopreserved embryos (Nagashima et al., 1994; Esaki et al., 2004; Pereira and Marques, 2008). As an alternative to such invasive techniques that can damage the cellular structure, it is possible to improve the success of cryopreservation of *in vitro* produced embryos by eliminating serum from the culture medium, or by inducing chemical delipidation through metabolic manipulation (Seidel 2006; Romeck et al., 2009). Indeed, it has been demonstrated that adding *t*10,*c*12 CLA to serum-containing media during *in vitro* culture of bovine embryos reduced lipid accumulation and significantly improved blastocyst survival following cryopreservation (Pereira et al., 2007; 2008). In oocytes, *t*10,*c*12 CLA was shown to interfere with lipid metabolism, both in bovine and porcine species, reducing lipid content in porcine species (Lapa et al., 2011; Prates et al., 2012a). Furthermore, by reducing the lipid content of porcine oocytes and embryos, forskolin was shown to increase cryosurvival following vitrification (Men et al., 2006; Fu et al., 2011). In the light of the above, other metabolic modulators, namely PES, can be used to reduce cytoplasmic lipid content improving cryosurvival of bovine embryos (Barceló and Seidel, 2007). However, the use of PES during *in vitro* culture had a limited effect on porcine blastocyst survival after vitrification. Nevertheless, PES increased the proportion of morula and blastocyst formation, reducing the index of DNA fragmentation and the cytoplasmic lipid content of cultured blastocysts (Gadja et al., 2011). Further studies are needed to broaden the knowledge of this promising approach to improve ART success.

Future Perspectives

The lipid content of porcine oocyte as well as the asynchrony between nuclear and cytoplasmic maturation renders it a good model in the field of oocyte biology research. Knowledge of the pathways and key molecules regulating these processes may highlight therapeutic possibilities to prevent the excessive accumulation or to modulate lipid composition in cytoplasmic droplets. In the future, lipid modulators may also be applied in germplasm cryopreservation to improve livestock production. Further studies are needed to clarify its potential to avoid disorders in lipid metabolism and its relation to infertility.

Acknowledgments

The “Fundação para a Ciência e Tecnologia” is greatly acknowledged for funding a grant for EG Prates (SFRH/BD/42359/2007). The authors are grateful to Ann Paula Barreiro for revising this manuscript.

References

Ali A and Sirard M 2005. Protein kinases influence bovine oocyte competence during short-term treatment with recombinant human follicle stimulating hormone. Reproduction 130, 303–310.

Almiñana C, Gil MA, Cuello C, Roca J, Vazquez JM, Rodriguez-Martinez H and Martinez EA 2005. Adjustments in IVF system for individual boars: Value of additives and time of sperm–oocyte co-incubation. Theriogenology 64, 1783–1796.

Ambruosi B, Lacalandra GM, Iorga AI, De Santis T, Murgnier S, Matarrese, Goudet G and Dell’Aquila ME 2009. Cytoplasmic lipid droplets and mitochondrial distribution in

621 equine oocytes: Implications on oocyte maturation, fertilization and developmental
622 competence after ICSI. Theriogenology 71, 1093-1104.
623 Amir A and Zvi R 2008. Do chilling injury and heat stress share the same mechanism
624 of injury in oocytes? Molecular Cellular Endocrinology 282, 150–152.
625 Antosik P, Kempisty B, Jackowska M, Bukowska D, Lianeri M, Brussow KP, Wozna M
626 and Jaskowski JM 2010. The morphology of porcine oocytes is associated with *zona*
627 *pellucida* glycoprotein 3 and integrin beta 2 protein levels. Veterinarni Medicina 55,
628 154–162.
629 Ashwell MS, Ceddia RP, House RL, Cassady JP, Eisen EJ, Eling TE, Collins JB,
630 Grissom SF and Odle J 2010. *Trans*-10, *cis*-12-conjugated linoleic acid alters hepatic
631 gene expression in a polygenic obese line of mice displaying hepatic lipidosis. Journal
632 of Nutritional Biochemistry 21, 848–855.
633 Barceló-Fimbres M and Seidel GE Jr 2007. Effects of Fetal Calf Serum, Phenazine
634 Ethosulfate and Either Glucose or Fructose During In Vitro Culture of Bovine Embryos
635 on Embryonic Development After Cryopreservation. Molecular Reproduction and
636 Development 74, 1395-1405.
637 Chung S, Brown JM, Sandberg MB and McIntosh M 2005. *Trans*-10,*cis*-12 CLA
638 increases adipocyte lipolysis and alters lipid droplet-associated proteins: role of mTOR
639 and ERK signaling. Journal of Lipid Research 46, 885–895.
640 Coy P, Romar R, Ruiz S, Cánovas S, Gadea J, Vázquez FG and Matás C 2005. Birth
641 of piglets after transferring of *in vitro*-produced embryos pre-matured with roscovitine.
642 Reproduction 129, 747–755.
643 Cuello C, Sanchez-Osorio J, Almiñana C, Gil MA, Perals ML, Lucas X, Roca J,
644 Vazquez and Martinez EA 2008. Effect of the cryoprotectant concentration on the *in*
645 *vitro* embryo development and cell proliferation of OPS-vitrified porcine blastocysts.
646 Cryobiology 56, 189-194.

647 Cui MS, Fan YP, Wu Y, Hao ZD, Liu S, Chen XJ and Zeng SM 2009. Porcine cumulus
648 cells influences ooplasmic mitochondria-lipid distributions, GSH-ATP contents and
649 calcium release pattern after electro-activation. Theriogenology 71, 412-421.
650 De La Torre-Sanchez JF, Gardner DK, Preis K, Gibbons J, George E and Seidel A
651 2006. Metabolic regulation of in-vitro-produced bovine embryos. II. Effects of
652 phenazine ethosulfate, sodium azide and 2,4-dinitrophenol during post-compaction
653 development on glucose metabolism and lipid accumulation. Reproduction, Fertility and
654 Development 18, 597–607.
655 Dekel N 1995. Molecular Control of Meiosis. Trends in Endocrinology and Metabolism,
656 6, 165-169.
657 Downs SM, Hudson ER and Hardie DG 2002. A Potential Role for AMP-Activated
658 Protein Kinase in Meiotic Induction in Mouse Oocytes. Developmental Biology 245,
659 200–212.
660 El Shourbagy SH, Spikings EC, Freitas M, St and John JC 2006. Mitochondria directly
661 influence fertilisation outcome in the pig. Reproduction 131, 233-245.
662 Eppig JJ 1996. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian
663 mammals. Reproduction, Fertility and Development 8, 485-9.
664 Esaki R, Ueda H, Kurome M, Hirakawa K, Tomii R, Yoshioka H, Ushijima H,
665 Kuwayama M and Nagashima H 2004. Cryopreservation of Porcine Embryos Derived
666 From In Vitro-Matured Oocytes. Biology of Reproduction 71, 432–437.
667 Ford JH and Tavendale R 2010. Analysis of fatty acids in early mid-life in fertile
668 women: implications for reproductive decline and other chronic health problems.
669 American Journal of Human Biology 22, 134–136.
670 Fu X, Wu G, Li G, Hou Y, Zhou G, Wang Y and Zhu S 2011. Positive effects of
671 Forskolin (stimulator of lipolysis) treatment on cryosurvival of *in vitro* matured porcine
672 oocytes. Theriogenology 75, 268–275.
673 Fujihira T, Kinoshita M, Sasaki M, Ohnishi M, Ishikawa H, Ohsumi S and Fukui Y 2004.
674 Comparative Studies on Lipid Analysis and Ultrastructure in Porcine and Southern

675 Minke Whale (*Balaenoptera Bonaerensis*) Oocytes. Journal of Reproduction and
676 Development 50, 525-532.
677 Funahashi H and Romar R 2004. Reduction of the incidence of polyspermic
678 penetration into porcine oocytes by pretreatment of fresh spermatozoa with adenosine
679 and a transient co-incubation of the gametes with caffeine. Reproduction 128, 789–
680 800.
681 Gadja B, Bryla M and Smorag Z 2008. Effects of Protein Source, Vitamin E and
682 Phenazine Ethosulfate on Developmental Competence and Quality of Porcine Embryos
683 Cultured *In Vitro*. Folia biologica (Kraków) 56, 57-53.
684 Genicot G, Leroy JLMR, Van Soom A and Donnaya I 2005. The use of a fluorescent
685 dye, Nile red, to evaluate the lipid content of single mammalian oocytes.
686 Theriogenology 63, 1181–1194.
687 Gerelchimeg B, Li-Qing L, Zhong Z, Jiang-Tian T, Qing-Ran K, Jun S, Xue-Dong W
688 and Zhong-Hua L 2009. Effect of chilling on porcine germinal vesicle stage oocytes at
689 the subcellular level. Cryobiology 59, 54–58.
690 Gilchrist RB and Thompson JG 2007. Oocyte maturation: Emerging concepts and
691 technologies to improve developmental potential in vitro. Theriogenology 67, 6–15.
692 Gilchrist RB, Lane M and Thompson JG 2008. Oocyte-secreted factors: regulators of
693 cumulus cell function and oocyte quality. Human Reproduction Update 14, 159–177.
694 Griffin J, Emery BR, Huang I, Peterson CM and Carrell DT 2006. Comparative analysis
695 of follicle morphology and oocyte diameter in four mammalian species (mouse,
696 hamster, pig, and human). Journal of Experimental & Clinical Assisted Reproduction 3,
697 2, doi:10.1186/1743-1050-3-2.
698 Grupen CG, Nagashima H and Nottle MB 1997. Asynchronous meiotic progression in
699 porcine oocytes matured *in vitro*: a cause of polyspermic fertilization? Reproduction,
700 Fertility and Development 9, 187-191.
701 Hao ZD, Liu S, WY, Wan PC, Cui MS, Chen H and Zeng SM 2007. Abnormal changes
702 in mitochondria, lipid droplets, ATP and glutathione content, and Ca^{2+} release after

703 electro-activation contribute to poor developmental competence of porcine oocyte
704 during *in vitro* ageing. Reproduction, Fertility and Development 21, 323-332.
705 Hara K, Y Abe, N Kumada, N Aono, J Kobayashi, H Matsumoto, H Sasada and E Sato
706 2005. Extrusion and removal of lipid from the cytoplasm of porcine oocytes at the
707 germinal vesicle stage: Centrifugation under hypertonic conditions influences
708 vitrification. Cryobiology 50, 216–222.
709 Hirao Y, Nagai T, Kubo M, Miyano T, Miyake M and Kato S 1994. *In vitro* growth and
710 maturation of pig oocytes. Journal of Reproduction and Fertility 100, 333-339.
711 Holm C 2003. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis
712 Biochemical Society Transactions 31, 1120-1124.
713 Homa ST, Racowsky C and McGaughey RW 1986. Lipid analysis of immature pig
714 oocytes. Journal of Reproduction and Fertility 77, 425-434.
715 Hunter RHF 1990. Fertilization of pig eggs *in vivo* and *in vitro*. Journal of Reproduction
716 and Fertility Suppl 40, 211-226.
717 Hunter MG 2000. Oocyte maturation and ovum quality in pigs. Reviews of
718 Reproduction 5, 122-130.
719 Isachenko V, Isachenko E, Rahimi G, Mallmann P, Krivokhrchenko A and Nawroth F,
720 2003. Ultra-structure of intracellular lipid vesicles of porcine GV-oocytes after *in vitro*
721 fertilization and parthnogenetic activation. Anatomia, Histologia, Embryologia 32, 126-
722 128.
723 José AAFBV, Gama MAS and Lanna DDP 2008. Effects of *trans*-10, *cis*-12 conjugated
724 linoleic acid on gene expression and lipid metabolism of adipose tissue of growing pigs.
725 Genetics and Molecular Research 78, 284-294.
726 Kim N, Do JT, Song H, Koo DB, Kim JH, Lee HT and Chung KS 1997. Involvement of
727 adrenergic system on the cortical granule exocytosis and polyspermic penetration
728 during in vitro fertilization of porcine oocytes. Theriogenology 48, 1351-1360.
729 Krisher RL 2004. The effect of oocyte quality on development. Journal of Animal
730 Science 4, 14-23.

731 Laforest MF, Pouliot E, Gueguen L and Richard FJ 2005. Fundamental Significance of
732 Specific Phosphodiesterases in the Control of Spontaneous Meiotic Resumption in
733 Porcine Oocytes. Molecular Reproduction and Development 70, 361–372.
734 Lapa M, Marques CC, Alves SP, Vasques MI, Baptista MC, Carvalhais I, Silva Pereira
735 M, Horta AEM, Bessa RJB and Pereira RM 2011. Effect of trans-10,cis-12 conjugated
736 linoleic acid on Bovine Oocyte Competence and Fatty Acid Composition. Reproduction
737 in Domestic Animals 46, 904–910.
738 Marei WF, Wathes DC and Fouladi-Nasta AA 2009. The Effect of Linolenic Acid on
739 Bovine Oocyte Maturation and Development. Biology of Reproduction 81, 1064-1072.
740 Marques CM, Pereira RM, Vasques MI, Baptista MC and Horta AEM 1997. Role of
741 prostaglandins on bovine oocyte maturation *in vitro*. In: Portuguese Society of Animal
742 Reproduction (ed.) Proceedings of the 1st Iberian Congress on Animal Reproduction,
743 Estoril 3–6 July 1997, Vol. II, Portugal, pp. 142–149.
744 McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JS and Speak BK 2000. Fatty acid
745 composition of lipids in immature cattle, pig and sheep oocytes with intact zona
746 pellucida. Journal of Reproduction and Fertility 118, 163–170.
747 Men H, Agca Y, Riley LK and Critser JK 2006. Improved survival of vitrified porcine
748 embryos after partial delipation through chemically stimulated lipolysis and inhibition of
749 apoptosis. Theriogenology 66, 2008-2016.
750 Nagashima H, Kashiwazaki N, Ashman RJ, Grupen CG, Seamark RF and Nottle MB
751 1994. Removal of Cytoplasmic Lipid Enhances the Tolerance of Porcine Embryos to
752 Chilling. Biology of Reproduction 51, 618-622.
753 Nuttinck F, Guienne BM, Clément L, Reinaud P, Charpigny G and Grimard B 2008.
754 Expression of genes involved in prostaglandin E2 and progesterone production in
755 bovine cumulus–oocyte complexes during *in vitro* maturation and fertilization.
756 Reproduction 135, 593–603.

Ouandaogo ZG, Haouzi D, Assou S, Dechaud H, Kadoch IJ, De Vos J and Hamamah S 2011. Human cumulus cells molecular signature in relation to oocyte nuclear maturity stage. PLoS ONE 6: e27179.

Pereira RM and Marques CC 2008. Animal oocyte and embryo cryopreservation. Cell Tissue Banking 9, 267–277.

Pereira RM, Baptista MC, Vasques MI, Horta AEM, Portugal PV, Bessa RJB, Silva JC, Pereira MS and Marques CC 2007. Cryosurvival of bovine blastocysts is enhanced by culture with trans-10 cis-12 conjugated linoleic acid (10t,12c CLA). Animal Reproduction Science 98, 293–301.

Pereira RM, Carvalhais I, Pimenta J, Baptista MC, Vasques MI, Horta AEM, Santos IC, Marques MR, Reis A, Pereira MS and Marques CC 2008. Biopsied and vitrified bovine embryos viability is improved by trans-10 cis-12 conjugated linoleic acid supplementation during *in vitro* embryo culture. Animal Reproduction Science 106, 322-332.

Prates EG, Marques CC, Baptista MC, Vasques MI, Horta AEM and Pereira RM 2008. Effect of different culture protocols on porcine oocyte nuclear maturation and area. Reproduction in Domestic Animals 43, 83.

Prates EG, Marques CC, Baptista MC, Vasques MI, Carolino N, Horta AEM, Charneca R, Nunes JT, Pereira RM 2012a. Fat area and lipid droplets morphology of porcine oocytes during *in vitro* maturation with *trans*-10, *cis*-12 conjugated linoleic acid (*t*10,*c*12 CLA) and forskolin. Animal (accepted, manuscript number: ANIMAL-12-30079R1).

Prates EG, Alves S, Marques CC, Baptista MC, Horta AEM, Bessa RJB and Pereira RM 2012b. Fatty acid composition of porcine oocyte during maturation: effect of *trans*10, *cis*12 conjugated linoleic acid and forskolin. Animal (submitted, manuscript number: ANIMAL-S-12-00640).

Purcell SH and Moley KH 2011. The impact of obesity on egg quality. Journal of Assisted Reproduction and Genetics 28, 517-524.

784 Racowsky C 1985. Effect of forskolin on maintenance of meiotic arrest and stimulation
785 of cumulus expansion, progesterone and cyclic AMP production by pig oocyte-cumulus
786 complexes. Journal of Reproduction and Fertility 74, 9-21.
787 Romek M, Gajda B, Krzysztofowicz E and Smorag Z 2009. Lipid Content of Non-
788 Cultured and Cultured Pig Embryo. Reproduction in Domestic Animals 44, 24-32.
789 Saragusty J and Arav A 2011. Current progress in oocyte and embryo cryopreservation
790 by slow freezing and vitrification. Reproduction 141, 1–19.
791 Seidel G 2006. Modifying oocytes and embryos to improve their cryopreservation.
792 Theriogenology 65, 228-235.
793 Seger R and Krebs GE 1995. The MAPK signalling cascade. The FASEB Journal 9,
794 726-735.
795 Silva RC, Báo SN, Jivago JL and Lucci CM 2011. Ultrastructural characterization of
796 porcine oocytes and adjacent follicular cells during follicle development: Lipid
797 component evolution. Theriogenology 76, 1647-1657.
798 Sturmey RG and Leese HJ 2003. Energy metabolism in pig oocytes and early
799 embryos. Reproduction 126, 197–204.
800 Sturmey RG, O'Toole PJ and Leese HJ 2006. Fluorescence resonance energy transfer
801 analysis of mitochondrial:lipid association in the porcine oocyte. Reproduction 132,
802 829–837.
803 Sun Q-Y and Nagai T 2003. Molecular Mechanisms Underlying Pig Oocyte Maturation
804 and Fertilization. Journal of Reproduction and Development 49, 347-359.
805 Sutton ML, Gilchrist RB and Thompson JG 2003. Effects of in-vivo and in-vitro
806 environments on the metabolism of the cumulus-oocyte complex and its influence on
807 oocyte developmental capacity. Human Reproduction Update 9: 35-48.
808 Thiele C and Spandl J 2008. Cell biology of lipid droplets. Current Opinion in Cell
809 Biology 20, 378–385.

Thomas RE, Thompson JG, Armstrong DT and Gilchrist RB, 2004. Effect of Specific Phosphodiesterase Isoenzyme Inhibitors During *In Vitro* Maturation of Bovine Oocytes on Meiotic and Developmental Capacity. Biology of Reproduction 71, 1142–1149.

van der Hurk R, Zhao J 2005. Formation of mammalian oocytes and their growth, differentiation and maturation within ovarian follicles. Theriogenology 63, 1717–1751.

Walther TC and Farese Jr RV 2009. The life of lipid droplets. Biochimica et Biophysica Acta 1791, 459–466.

Wang Q and Sun Q-Y 2007. Evaluation of oocyte quality: morphological, cellular and molecular predictors. Reproduction, Fertility and Development 19, 1-12.

Warzych E, Cieslak A, Pawlak P, Renska N, Pers-Kamczyc E and Lechniak D 2011. Maternal nutrition affects the composition of follicular fluid and transcript content in gilt oocytes. Veterinarni Medicina 56, 156–167.

Watanabe T, Thayil A, Jesacher A, Grieve K, Debarre D, Wilson T, Booth M and Srinivas S 2010. Characterisation of the dynamic behevior of lipid droplets in the early mouse embryo using adaptive harmonic generation microscopy. BMC Cell Biology 11, 38.

Wu X, Zhang X, Li X, Cheng H, Kuai Y, Wang S and Guo Y 2006. Translocation of classical PKC and cortical granule exocytosis of human oocyte in germinal vesicle and metaphase II stage. Acta Pharmacologica Sinica 27, 1353 –1358.

Xia P 1997. Intracytoplasmatic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. Human Reproduction 12, 1750-1755.

Xia GL, Kikuchi K, Noguchi J and Izaike Y 2000. Short time priming of cumulus-oocyte complexes with FSH and forskolin in the presence of hypoxanthine stimulates cumulus cells to secrete a meiosis-activating substance. Theriogenology 53, 1807-1815.

Zhang DX, Cui XS, and Kim NH 2010. Molecular characterization and polyadenylation regulated expression of Cyclin B1 and Cdc2 in porcine oocytes and early parthenotes. Molecular Reproduction and Development 77, 38–50.

838 **Figure 1 –** Immature porcine cumulus-oocyte-complex (COC) observed under a
839 Nomarski microscopy.
840
841 **Figure 2** - Nuclear configuration of porcine oocytes prior to (A) and after (B) *in*
842 *vitro* maturation (GV, germinal vesicle: MII and PB, metaphase II with polar
843 body).
844
845 **Figure 3** – Immature porcine oocyte observed under a Nomarski microscopy,
846 with lipid droplets (LD) highlighted in white colour (LD and oocyte areas were
847 measured using Image J software).
848
849 **Figure 4** – A model for possible effects of forskolin and trans-10,cis-12
850 conjugated linoleic acid on regulation of oocyte maturation and metabolic
851 pathways: stimulation of cAMP intracellular levels and PKA or MAPK pathways;
852 other potential mechanisms for CLA action are also illustrated with dashed
853 arrows. PKA, protein kinase A; MAPK, mitogen protein kinase; AC, adenylyl
854 cyclase; HSL, hormone sensitive lipase; MGL, monoglyceride lipase; P, perilipin
855 protein; LD, lipid droplet; NEFA, non-esterified fatty acids. See text for details.
856

Figure

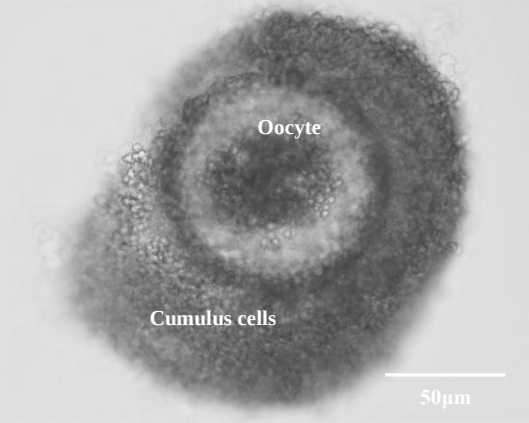

Figure 1

Figure

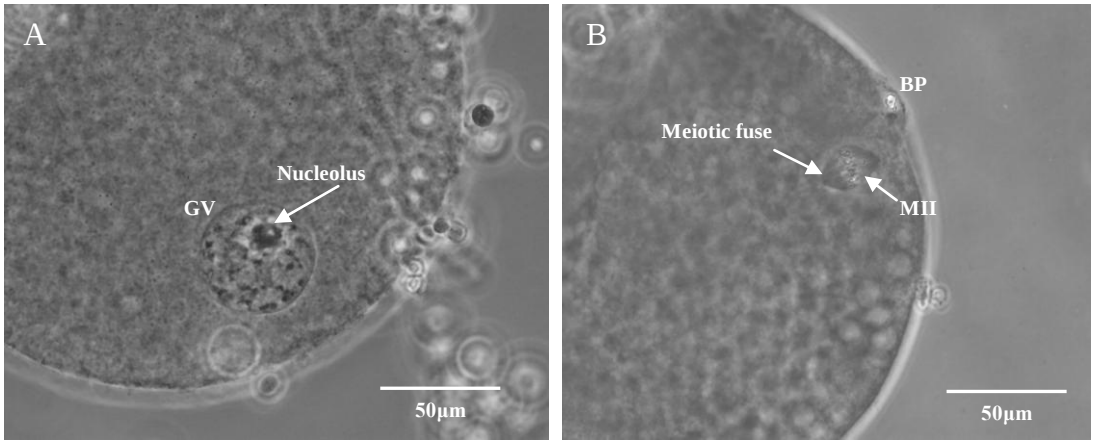

Figure 2

Figure

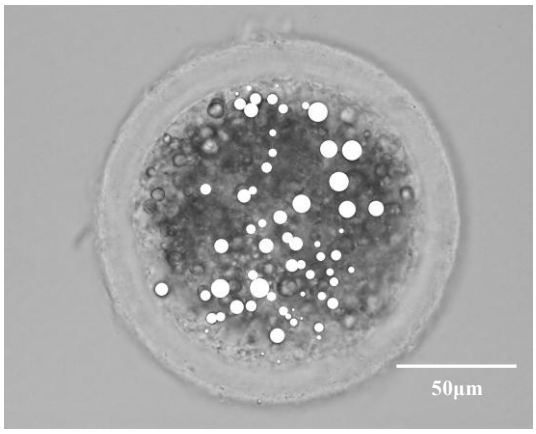

Figure 3

Figure

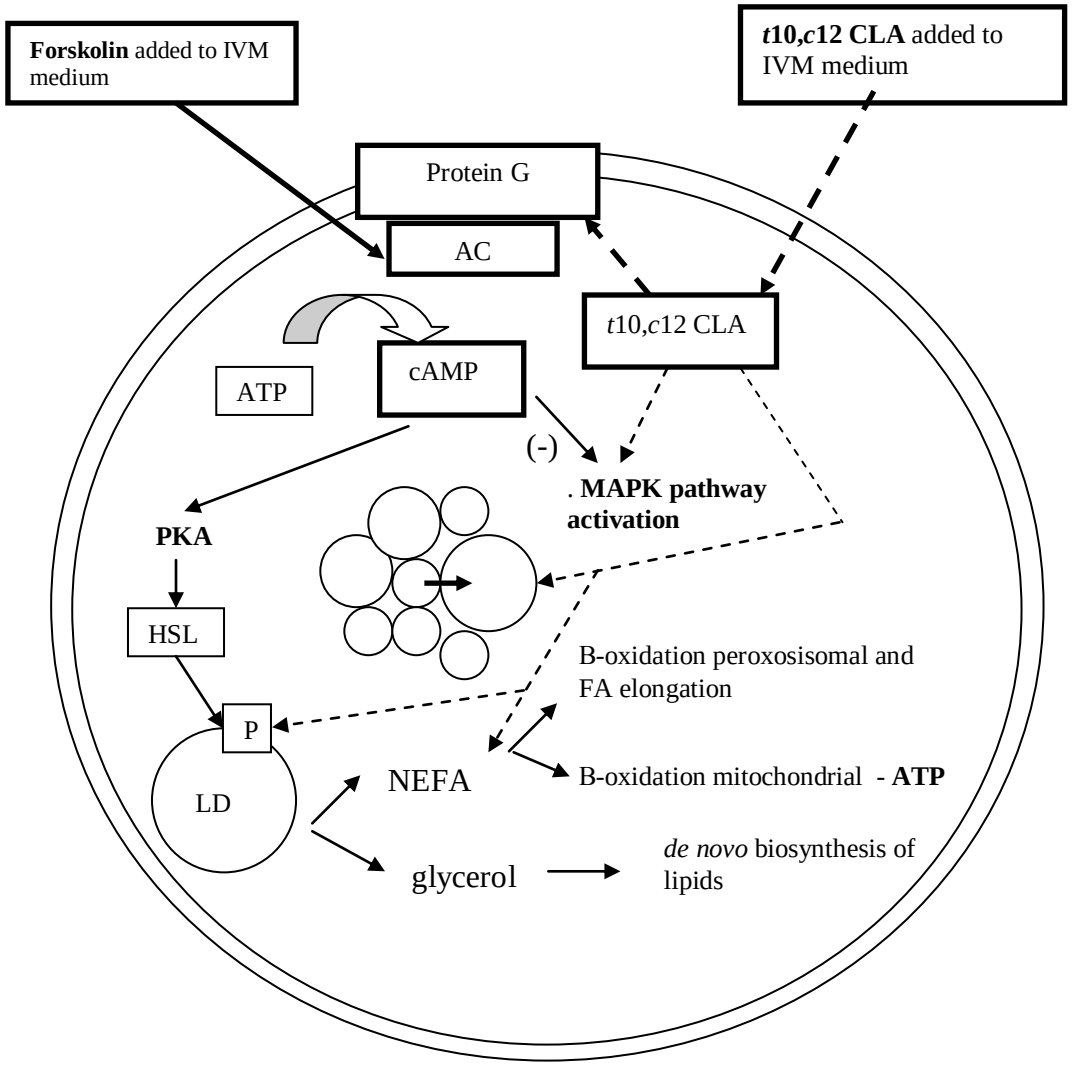

Figure 4