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MITOCHONDRIAL CONTRIBUTIONS TO OOCYTE AND EMBRYONIC QUALITY IN BOVINE

Contribución mitocondrial en la calidad del ovocito y embrión en bovinos

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RESUMEN

Las mitocondrias son orgánelos muy poderosos involucrado en varias funciones vitales en las células, manteniendo principalmente el equilibrio entre la síntesis de ATP y el estrés oxidativo durante el desarrollo embrionario temprano en mamíferos. Se ha demostrado que alteraciones de la función mitocondrial en el modelo bovino podría llegar a afectar la competencia de desarrollo de ovocitos y embriones. En esta revisión, analizamos las implicaciones del papel mitocondrial en el desarrollo temprano exitoso a través de estudios recientes sobre ovocitos y embriones bovinos.

Palabras clave: mitocondria, ovocito, embrión, bovino

ABSTRACT

Mitochondria are powerhouse organelles involved in several vital functions in cells, mainly maintaining balance between ATP synthesis and oxidative stress during early development in mammalian embryos. It has been shown that impaired mitochondrial function in bovine model might reach failure in oocyte and embryo developmental competence. In this review, we analyse implications of mitochondrial role on successful early development through recent studies on bovine oocytes and embryos.

Keywords: mitochondria, oocyte, embryo, bovine.

INTRODUCTION

Mitochondria are intracellular organelles that play an essential role as regulators in various signaling pathways and intracellular processes like calcium homeostasis, fatty acid oxidation and apoptosis. However, the main interest to study mitochondria is generally focussed on its potential to produce energy (ATP). Therefore, oxidative phosphorylation, a process that couples substrate oxidation to ATP synthesis, is the most well-known and studied metabolic pathway (Dumollard *et al.,* 2009).

Mitochondria are composed of two membranes (outer and inner), a dense matrix and include intermediate metabolic enzymes and multiple copies of a circular genome that encodes some intermembrane proteins and transfer RNAs (Frey and Mannella, 2000). Mitochondrial genome copy number has been studied as a potential marker of cellular viability and health and it was hypothesized that oocytes without sufficient mtDNA (Mitochondrial DNA) are not able to generate enough ATP to sustain early development (Ge *et al.* 2012; Babayev and Seli, 2015).

Mammalian oocytes and embryos have a highly regulated energy metabolism. Follicular cells provide energetic substrates to the oocyte during oogenesis through their transzonal projections, which are lost several hours following meiosis resumption caused by the LH surge or withdrawal of the gamete from its follicular environment (Scantland *et al.,* 2014; Macaulay *et al.,* 2015). Following fertilization, embryos uptake nutrients from their environment (oviductal and uterine epithelial secretions or culture medium). However, in many species, the mitochondria contingent of oocytes and of blastomeres of first embryonic cleaveages revert back to an immature form characterized by very limited cristae that is associated with a much reduced oxidative phosphorylation potential (Scantland *et al.,* 2014). In bovine, it was shown that glucose consumption is impaired due to an incapacity to perform glycolysis (Rieger and Loskutoff, 1994; Dumollard *et al.,* 2009). It is believed that low potential to produce ATP is palliated by the large numerical number of mitochondria within the oocyte (Chiaratti *et al.*, 2010). Nonetheless, mitochondrial ATP production has been associated with oocyte quality (Dumollard *et al.,* 2009) and it has been observed that impaired mitochondrial functions contribute to embryonic developmental arrest (Van Blerkom, 2011; Baldoceda *et al.,* 2014).

In consequence, the involvement of mitochondrial characteristics in association with reproductive success more precisely on oocyte quality and embryonic health has been reported in recent studies. However, the exact relationship between mitochondrial functions and developmental competence is still not well understood. In this review, we discuss the key roles of mitochondria during early development based on animal studies using the bovine model.

REVIEW

The mitochondrion is a unique organelle due to the presence of its own genome that is transcribed independently of the nuclear genome. Moreover, mitochondria are inherited only from the maternal side. Mitochondrial functions have been associated with developmental competence (Dummollard *et al.*, 2009; Van Blerkom, 2011) and culture conditions have been shown to impact mitochondrial functions (Plourde *et al.*, 2012). Since precise quantification of the number of mitochondria per oocyte or per blastomere is technically challenging to do, mtDNA copy number is most often used as an alternate quantification method although copy number is highly variable depending on the cell type, stage of development and species.

Nonetheless, mtDNA copy number have been proposed as a metric of oocyte quality (Chiaratti *et al.,* 2010; Wai *et al.,* 2010) although measurement is done through a destructive methodology. The current rationale is that reduction of mtDNA copy number is reflective of oocyte developmental competence caused by inadequate cytoplasmic maturation or mitochondrial biogenesis (Reynier *et al.,* 2001, Wai *et al.,* 2010). Chiaratti *et al.* (2010) reported that physical depletion of mtDNA in bovine embryos reversible up to the blastocyst stage, suggesting that low levels of mtDNA in mature oocytes can be sensed and adjusted upward in the preimplantation embryo. Although this important reduction in mtDNA copy numbers could be reflective of decreased mitochondrial activity that may in turn affect ATP production. Wai *et al.* (2010) concluded that mtDNA copy number may decrease but it still remains normal threshold support process for fertilization and normal embryonic development.

Interestingly, physiological interpretation of mtDNA copy number is opposite in the male gamete where it was reported that mature sperm cells which naturally contain an average of 100 mtDNA copies have a significantly increased amount on mtDNA in abnormal spermatozoa showing poor fertilization potential (Wai *et al.,* 2010). These findings are supported by the concept that high fertility rate of bulls is not related to higher mitochondrial activity. Indeed, Al Naib *et al.* (2011) reported that higher bull's sperm motility post-freezing are related to higher mitochondrial activity. However, blastocyst rate was no different compared to bulls with low motility post-freezing. They demonstrated that the fertilization power of spermatozoa is not related to mitochondrial activity.

The impact of the amount of mtDNA in sperm on the embryonic developmental potential is intriguing since at fertilization while both gametes are combined, mitochondrial inheritance is known to be strictly done from the maternal side. This well accepted dogma entails that early blastomeres and all adult cells are solely composed of the mtDNA originating from the oocyte (Song *et al.,* 2014). Although the spermatozoon can transfer its mitochondria inside the oocyte's cytoplasm at fertilization, these paternal mitochondria are eliminated during the early embryonic cell divisions (Chan and Schon, 2012). It has been reported that paternal mitochondria are naturally eliminated by proteolytic destruction (Chan and Schon, 2012; Sato and Sato, 2013). However, studies challenging the dogma have reported a possible mechanism that would allow paternal mtDNA to escape destruction when mitochondrial fusion occurs with maternal mitochondria (Cummins, 2000; Sato and Sato, 2013). On the other hand, this mixture of mitochondrial genomes could lead to abnormal development as demonstrated by the impacts of mitochondrial heteroplasmy (presence of several types of mtDNA in a cell by integration of mitochondria from different origins or derived from mtDNA mutations) (Cummins, 2000). In addition, the frequency of heteroplasmic mitochondria has been shown to be increased by the application of assisted reproductive technologies namely intra-cytoplasmic sperm injection (ICSI) (Shoubridge, 2000; *Sutovsky et al.,* 2004). In the context of human fertility where aging is the main factor negatively affecting oocyte quality, it has been proposed that injection of a mitochondrial extract within the oocyte cytoplasm could improve developmental rates (Cagnone *et al.,* 2016). This approach is still controversial and more validation is required before firm conclusions can be made.

After fertilization, mitochondria are redistributed in daughter cells to provide the necessary ATP for embryogenesis events (Stojkovic *et al.,* 2001; Tarazona *et al.,* 2006; Van Blerkom, 2011). During the first cell divisions, mtDNA copy numbers remain constant (Dumollard *et al.,* 2009; Chiaratti *et al.,* 2010) until the time around embryonic genome activation. During these first cell divisions, it has been suggested that embryos are sensitives to oxidative stress and may stop their development. Prior to genome activation, embryos arrest development by entering into senescence (Favetta *et al.* 2004). It is only following genome activation, which occurs at the 8-cell stage in bovine (Memili and First, 1998) that blastomeres begin to die by apoptosis (Tarazona *et al.,* 2006). It is also at this time, between 72 and 168 hours post fertilization, that mitochondrial activity shows a slight increase in activity as observed by oxygen consumption and ATP production at the morula stage (Tarazona *et al.,* 2006; Romek *et al.,* 2010). It is also at this stage of development that the mitochondrial contingent mature and adopt the traditional oblong shape (Crocco *et al.,* 2011) which is followed by a significant raise in mitochondrial activity (Tarazona et al., 2006) as well as an increase in mtDNA copy numbers (Chiaratti *et al.,* 2010; Wai *et al.,* 2010). This confirms that prior to genome activation, mitochondrial functions are limited (Tarazona *et al.,* 2006; Romek *et al.,* 2010) and that conversely, mitochondrial potential common to somatic cells is established between 8-16 cells and the morula stage in bovine embryos in bovine.

The timing of this burst in ATP production occurs concomitantly with the energy demanding events of compaction and formation of the blastocoel. During pre-hatching development most embryonic loss occur prior to embryonic genome activation and a second limiting step is the transition from morula to blastocyst. These events respectively fit with the period where cells harbour immature mitochondria and the time when they mature to increase their ATP production per organelle while reducing their overall numbers.

Impacts of culture media composition on mitochondrial functions has only been studied indirectly where in vitro conditions could lead to embryonic arrest which is also associated with increased free radicals and oxidative stress (Abe et al., 2002; Rizos et al., 2003). The adverse effects of media composition on the structure and function of mitochondria in relation with embryonic quality have been described at the ultrasstructure level (Crosier *et al.,* 2001; Fair *et al.,* 2001; Abe *et al.,* 2002) as well as from gene expression profiling (Rizos et al., 2003; Plourde *et al.,* 2012). Culture conditions, especially the presence of serum which contains growth factors and lipids have been found to impact embryonic developmental kinetics, morphology, developmental potential and capacity to survive cryopreservation (Abe *et al.,* 2002). Serum supplementation was found to increase the amount of intracellular lipid droplets which in turn decreases embryonic survival post-freezing (Abe *et al.,* 2002; Rizos *et al.,* 2003). This lipid accumulation could be reflective of impaired mitochondrial functions limiting the rate of lipid metabolism. In support of this, breed differences have been described where embryos from Jerseys naturally contain more lipid droplets of smaller size than Holstein counterparts (Baldoceda *et al.,* 2015a). This higher lipid content is known to negatively affect survival to cryopreservation (Abe *et al.,* 2002; Rizos *et al.,* 2003). The higher lipid content in Jersey embryos is also correlated lower mitochondrial activity (Baldoceda *et al.,* 2015a).

In order to reduce intracellular lipid content and improve freezing survival of the embryos, L-carnitine, which is co-factor transporting fatty-acids to the mitochondrial matrix for metabolism), was added to the embryo culture medium (Takahashi *et al.,* 2012; Phongnimitr *et al.,* 2013; Baldoceda *et al.,* 2015b). It was anticipated that this metabolic regulator could have the dual effects of reducing lipid content however that would do so would increase the level of the dangerous free radicals. Addition of L-carnitine to the bovine embryo culture medium significantly reduced lipid intracellular lipid content in embryos although the extent of response varied between individual embryos indicating that not all embryos could proceed to lipid catabolism with the same efficiency Furthermore, the impact of L-Carnitine was overall weaker and more variable in Jersey embryos (Baldoceda et al. (2015a) which were already found to have a reduced mitochondrial activity Also, Baldoceda et al. (2015b). This was also supported at the gene expression level where key genes involved in lipid management such as droplet size (perilipin-2) were differentially regulated in Jersey compared to Holstein. This further exemplified the influence of the genetic background on embryonic metabolism. So far, improvement of cryotolerance of Jersey embryos still requires further development and needs to account for the mitochondrial capacity that differ between genetic backgrounds.

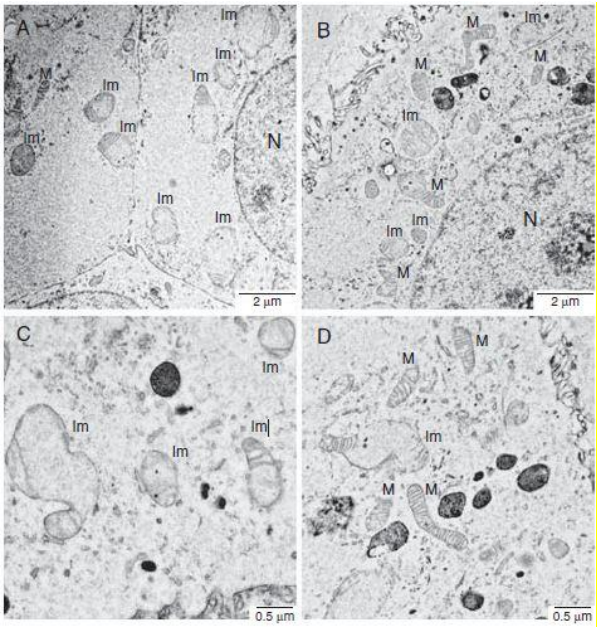

Figure 1. Electron micrographs showing morphological types of mitochondria in bovine embryos produced in vitro, (A and C) embryo cultured in a synthetic oviduct fluid (SOF) without added vitamin K2, (B and D) embryo cultured in a SOF with added vitamin K2. N, nucleus; M, mature mitochondria; Im, immature mitochondria (Baldoceda *et al.,* 2014).

Increasing lipid metabolism could also lead to an over-accumulation of free radicals that could be deleterious to embryo development. This was not observed as embryonic rates were not affected by the presence of L-carnitine (Baldoceda *et al.,* 2015b). In fact, management of free radicals during early development has been the subject of several studies. Most of them have been aiming at reducing the levels of free radicals which are increased in a high oxygen tension environment and known to be deleterious to development namely by causing lipid peroxidation and DNA damage (Rizos *et al.,* 2003). The addition of several

antioxidant molecules such as vitamins C and E were supplemented to culture media with limited beneficial impacts (Olson and Seidel, 2000, Sudano *et al.*, 2010) (Takahashi *et al.,* 2012; Phongnimitr *et al.,* 2013). This either indicates that embryonic cells have a high tolerance to free radicals or that impacts would be observed passed the stage of blastocyst which requires embryo transfer.

To help mitochondria carry out their functions, molecules aiding the electron transport chain of the oxidative phosphorylation pathway resulting in a more efficient oxygen use and ATP production were added to the culture media. One of the compound tested is Co-enzyme Q10 (CoQ10) which was found to have a beneficial impact on bovine embryonic rates (Stojkovic *et al.,* 1999). Supplementation of CoQ10 is now common practice in some human fertility clinics (Bentov *et al.,* 2014; Ben-Meir *et al.,* 2015). Alternatively, vitamin K2 which also aid the electron transport chain, was also tested. It was shown to significantly improve blastocysts rates and embryonic quality in vitro when added at the onset of embryonic genome activation at the 8-cell stage (Baldoceda *et al.* 2014; Figure 1). Taken together, these results show that mitochondrial functions are key in limiting embryonic development and thus, providing support to mitochondrial functions significantly improves developmental rates and quality.

CONCLUSSION

The literature clearly links mitochondrial activity with oocyte quality, developmental competence and embryonic quality. It has been demonstrated that mitochondrial function abnormalities are associated to developmental failure. In the last years, several research studies have attempted to improve mitochondrial functions in vitro. Our understanding of the natural level of mitochondrial functions during oogenesis, fertilization and early development is still fragmentary as well as being highly influenced by our understanding of mitochondrial functions in somatic cells. However, the cellular context of gametogenesis and embryogenesis is completely different from the one observed in somatic cells. Lessons could be learned from the general metabolism observed in stem cells.

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