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Facts around Mitochondrial Shape, Reorganization and Oocyte Maturation

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Abstract

Mitochondria are a membrane-enclosed organelles found in most eukaryotic cells. Mitochondria are sometimes described as "energy house" because they generate adenosine triphosphate (ATP), which is used as a source of chemical energy. In addition, they are involved in cell signaling, cellular differentiation, cell growth, cell cycle and cell death. However, the exact role of mitochondria during in vitro embryo production technology wasn't fully understood; especially the repositioning of active mitochondria during oocyte maturation, fertilization, and culturing. So, this study aimed to clarify the relationship between oocyte maturation and the repositioning of active mitochondria. It has been found and in contrast to previous reports that repositioning of active mitochondria isn't an utter sign to completion of oocyte maturation. In addition, oocyte mitochondria have fine crystal shape, other than, coarse particles in previous reports.

Keywords: Mitochondria, Oocyte, Maturation, Distribution,

Introduction

The complex events occurred during oocyte maturation depend not only on the correct dynamics of chromosome separation during nuclear maturation but also on the redistribution of cytoplasmic organelles and the storage of mRNA, proteins, and transcription factors needed for this process to occur (Ferreira *et al.*, 2009). Although the exact role of mitochondria during maturation, fertilization and embryonic development wasn't fully understood (Bavister and Squirrell, 2000); the ultra-structural analysis shown that mitochondria, ribosomes, endoplasmic reticulum, cortical granules and Golgi complex assumed different positions during the transition from the germinal vesicle stage to metaphase-II (Ferreira *et al.*, 2009).

Generally, the activation of metabolic pathways involved in protein synthesis and phosphorylation was indispensable for the oocyte cytoplasmic maturation (Ferreira *et al.*, 2009). Within this

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context, mitochondria played an extremely important role since they were key components of the metabolic machinery responsible for energy supply that was consumed during the maturation process (Krisher and Bavister, 1998 and Stojkovic *et al.,* 2001). It had been stated that mitochondria via their various biochemical pathways could generate ATP which used in proteins synthesis, and in turn, support the completion of subsequent maturation processes and embryo development (Reynier *et al.*, 2001; St John, 2002; Torner *et al.,* 2004; El Shourbagy *et al.,* 2006; May-Panloup *et al.,* 2007; Shoubridge and Wai, 2007 and Zeng *et al.,* 2007). ATP is necessary for several functions including motility, maintenance of cellular homeostasis and regulation of cell survival (Moyes *et al.*, 1998 and St John, 2002). Furthermore, the importance of ATP levels during *in vitro* maturation had been demonstrated in bovine oocytes where, high quality oocytes assessed by morphology usually contained significantly higher ATP levels and produced significantly high blastocyst rates after fertilization (Stojkovic *et al.,* 2001). Data in human and bovine oocytes suggested that the efficiency of mitochondrial respiration in oocytes was closely correlated with the rate of embryo development after fertilization (Stojkovic *et al.,* 2001 and Wilding *et al.,* 2001). In most instances, a mitochondrial contribution to developmental abnormality or embryo demise was more likely to be a metabolic deficit (Shoubridge and Wai, 2007).

For example, disproportionate mitochondrial segregation during cleavage in human (Van Blerkom *et al.,* 2000), and pig embryos (El Shourbagy *et al.,* 2006) was associated with arrested cytokinesis and lysis in the blastomere(s) (Van Blerkom and Davis, 2007). In these instances, developmental incompetence that could be related to mitochondria was considered in the context of the total bioenergetics capacity of the oocyte or the individual cells that comprise the early embryo (Van Blerkom and Davis, 2007). Moreover, recent evidences demonstrated that in addition to their bioenergetics activity, mitochondria had some regulatory functions that include participation in calcium homeostasis, signal transduction and oxygen sensing (Pozzan *et al.,* 2000; Bell *et al.,* 2005; Zimijewski *et al.,* 2005; Gutierrez *et al.,* 2006 and Quintero *et al.,* 2006). Moreover, the mitochondrial metabolic activity and reorganization became indispensable features of cytoplasmic maturation and resumption of meiosis (Hyttel et al., 1989; de Loos et al., 1989; Calarco, 1995; Cummins, 1998 and Steeves and Gardner, 1999); which affect subsequent development after fertilization (Van Blerkom and Runner, 1984; Van Blerkom, *et al.,* 1995; Calarco, 1995; Bavister, 2000 and El Shourbagy *et al.,* 2006).

During final pre-ovulatory maturation in pig oocytes it had been found that there was a relationship between meiotic progressions, cumulus cell expansion and mitochondrial redistribution and their oxidative activity (Sun *et al.,* 2001

and Torner *et al.,* 2004).The mitochondrial relocation to different areas of the cell was mediated by a cytoskeletal network of microtubules and microfilaments present in the cytoplasm that promote these movements and acted on chromosome segregation (Van Blerkom, 1991 and Ferreira *et al.*, 2009). It had been observed that the mitochondrial movement to areas of high energy and ions consumption was crucial for oocyte maturation, fertilization and early embryonic development (Van Blerkom and Runner, 1984; de Loos *et al.*, 1992; Calarco, 1995; Van Blerkom, *et al.*, 2000 and Thompson *et al.*, 2000).

Ultra structural analysis of the *in vitro* matured bovine oocytes shown that the mitochondria moved from more peripheral position to more dispersed pattern throughout the cytoplasm after 12-18h of culture (Hyttel *et al.*, 1986 and Bavister and Squirrell, 2000). This event was similar to what occurs *in vivo*, which involved a more peripheral distribution before the luteinizing hormone (LH) surge (a clustered cortical formation in the final stages of nuclear maturation) and a dispersed distribution after the extrusion of the polar body (approximately 19 h after the LH surge) (Kruip *et al.*, 1983 and Hyttel *et al.*, 1997). Furthermore, upon reaching metaphase-II the mitochondria in bovine oocytes together with lipid droplets might occupy a central position (Hyttel *et al.*, 1997). On contrast, Sathananthan (1997) and Sathananthan and Trounson (2000) reported that mitochondria in metaphase-I and metaphase-II human oocytes were numerous and evenly spread in the ooplasm, associating closely with vesicles or aggregates of tubular smooth endoplasmic reticulum. So, the distribution and location of ooplasmic mitochondria between species show subtle differences especially concerning timing and distribution patterns (Bavister and Squirrell, 2000). So, the comparison of mitochondrial function and physical location of the mitochondria are complicated (Bavister and Squirrell, 2000).

Material and methods

Oocyte collection and in vitro maturation (IVM)

Bovine ovaries were transported from the slaughter house to the laboratory in sterile Dulbecco's phosphate buffered saline (D-PBS) at 37˚C. They were washed in D-PBS and then in normal saline. Cumulus-oocyte complex (COCs) were aspirated from follicles ranging between 3-7 mm in diameter with an 18 gauge needle attached to a disposable 10 ml syringe within approximately 3-5h after animal slaughter. Evenly granulated oocytes surrounded with multi layered compact cumulus cells were selected for the experiments. Selected COCs were firstly washed three times in sterile D-PBS and then washed three times in IVM medium; IVM was performed in TCM-199 without phenol red (Gibco BRL, Grand Island, NY) supplemented with 0.025 ml porcine FSH (Denka pharmaceutical, Kawasaki, Japan) , 0.055 mg/ml sodium

pyruvate (Wako Pure Chemical Industries, Osaka, Japan), 100 IU/ml penicillin G potassium (Meiji Seika, Tokyo, Japan), 100 ìg/ml streptomycin (Meiji Seika) and 3mg/ml bovine serum albumin (BSA) (Sigma, St. Louis, MO, USA). The COCs were cultured in groups of 30-50/well using 10 µl IVM medium / oocyte in 4-well dishes (Nunclone™, Roskilde, Denmark) without mineral oil overlay for 22 h at 38.5°C in a humidified atmosphere of 5% CO_2 in air.

Evaluation of mitochondrial characteristics in bovine oocytes

Distribution of mitochondria in IVM oocytes were evaluated according to Torner *et al*. (2004) with some modifications. This method is based on the uptake of MitoTracker® Red CMXRos dye (Molecular Probes, Eugene, OR, Oregon, USA) which selectively stains live mitochondria. According to the manufacturer's description the accumulation of this stain depends on mitochondrial membrane potential and its retention after fixation. Matured oocytes were denuded by pipetting in TCM-199 medium, and then incubated with 200 nM MitoTracker® Red CMXRos (MitoTracker stock solution was diluted to the required concentration) for 30 min in D-PBS containing 5% BSA under culture conditions. The oocytes were washed three times (each time extended to 20-30 minutes) in pre-warmed D-PBS without BSA, and then fixed for one hour at 37 ˚C using 2% paraformaldehyde in D-PBS. After fixation the oocytes were washed twice in D-PBS (each time 10 minutes), mounted on slides under cover slips and examined immediately under room temperature in a dark room. The distribution of active mitochondria was investigated qualitatively using a laser-scanning confocal microscope (Nikon D-eclipse C1) equipped with an argon–krypton–helium/neon ion laser and a 543nm excitation barrier filter. To classify the distribution of active mitochondria, a cross-sectional image at the largest diameter of each oocyte was taken. Where, peripheral mitochondrial distribution was characterized by the location of active mitochondria beneath the plasma membrane, and diffuse distribution was characterized by the homogeneous location of mitochondria throughout the cytoplasm according to El-Raey *et al.* (2011).

In vitro fertilization

Frozen semen of Japanese Black bull (Hatsamaki and Kitaguni-Sakae) in 0.5 ml straw was thawed for 10-15 Sec in 37°C water bath according to Correa and Zavos (1996) and Rasul *et al.* (2001). Thawed semen was evacuated in 15ml tube then resuspended in 10ml of Caffeine-BO solution. Washing was applied twice by centrifugation for 5 minute at 600xg in each time the supernatant was discarded. Pelleted sperm resuspended again in a small volume of caffeine-BO solution according to Brackett and Oliphant (1975) and Nagai *et al.* (2009). The concentration of the spermatozoa in the

tube was adjusted to 30 x10^6 cells / ml; this adjusted sperm suspension concentration was then diluted two fold by IVF drop that consists of BO solution containing 20 mg/ml of crystallized BSA and 10 IU/ml heparin. After 22 hours of maturation, groups of 20~25 oocytes were washed three times in BO washing solution containing 5 mM caffeine, 10mg/ml of crystallized BSA and 5 IU/ml heparin and then transferred into 100µl IVF drop (BSA-BO) at a rate of 20~25 oocyte /100µl of media according to Takada *et al.* (2010), that contained sperm concentration 15x10^6 according to Abd-Allah (2009). In vitro fertilization was carried out for 6 hours at 38.5°C in humidified atmosphere of 5% of CO2 according to Geshi *et al.* (2000), using Falcon® Petri dishes 35x 10mm style. After insemination the oocytes were subjected to denudation using handmade pipette for several times. Careful attention should be paid to remove all attached cumulus cells, adhering sperms. Additionally, avoid oocyte plasma membrane damaging. Denuded oocytes washed two times in oocyte BO washing solution then two times in modified CR1aa culture medium.

Assessment of the fertilization rate

The oocyte fertilization rate was examined on the base of the pronuclei number found in the oocyte after fertilization. In brief, the previously matured oocytes were incubated with the sperms for 6 hours at 5 % CO_2 and 20 % O_2 in humid incubator; after that the oocytes were subjected to denudation with perfect handmade pipette. Then they were cultured for 10~12 hour in CR1aa medium. They were fixed for 24~48 h in ethanol: acetic acid (3:1 v/v), then stained with 1% Orcein dissolved in 45% acetic acid according to Marei *et al.* (2009). The oocytes were evaluated under a phase-contrast microscope (Nikon, Tokyo, Japan). Oocytes were evaluated for normal fertilization according to Martino *et al.* (1994), on the basis of oocyte that had set of male and female pronuclei in the ooplasm were considered to be fertilized normally.

In vitro culture of bovine embryo

From the first day after insemination till the end of the fourth day groups of 20 of denuded oocytes were cultured in 100µl (20/100µl) of modified Charles Ronsenkrans (BSA- CR1aa) medium under mineral paraffin oil at 38.5°C in humidified atmosphere of 5% CO_2 and 5% O_2 and 90% N_2 . From day 5 till day 9 the embryos were transferred to BSA-199 medium under mineral paraffin oil at 38.5°C in humidified atmosphere of 5% CO_2 and 5% O_2 and 90% N_2. After 24 and 48hours for starting embryo culturing early embryogenesis (cleavage rate or No. of blastomeres) was checked morphologically using Olympus SZx16 stereoscopic microscope. From day 6 to day 8 the embryo were examined morphologically for blastocyst formation according to Nagai *et al.* (2009).

Results

Is the mitochondrial distribution

considered an indicator for completion of oocyte nuclear maturation or for the oocyte developmental competence?

According to the previously mentioned review articles about mitochondrial distribution, many scientists tried to find an intimate link between the acquisition of nuclear maturation and mitochondrial distribution patterns. This isn't absolutely true. In other sense, mitochondrial dispersion to the center of oocyte cytoplasm isn't an utter sign for oocyte nuclear maturation. Where, by looking through image (1) and comparing between the different oocytes depending upon the maturation state and patterns of mitochondrial distribution, it

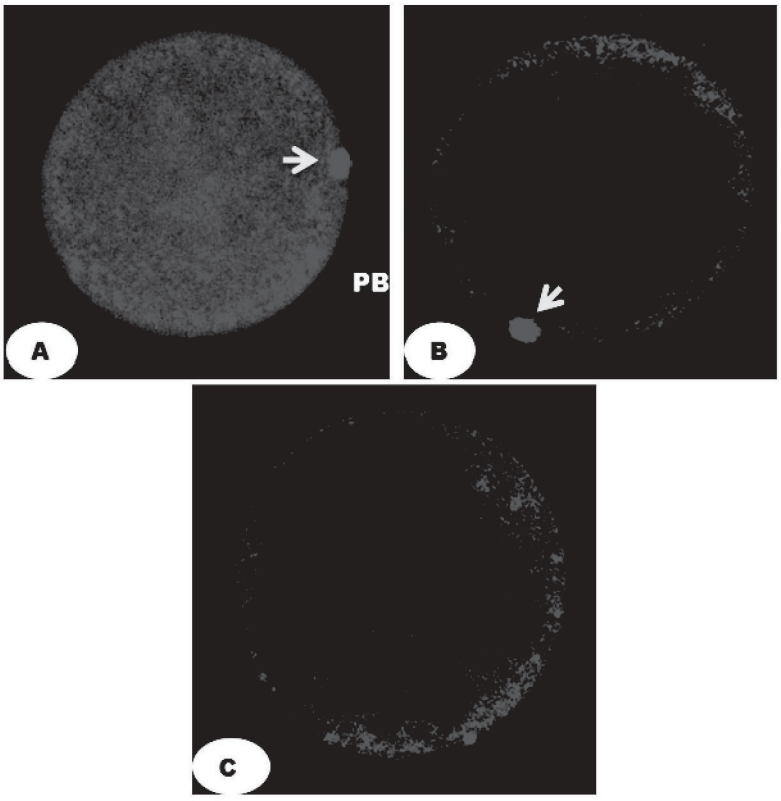

Image 1: Illustrating that mitochondria distribution wasn't an utter sign for the oocyte maturation. Image (1A) denotes mature oocyte with dispersed mitochondrial distribution pattern; yellow arrow indicates the 1st polar body. Image (1B) denotes mature oocyte with peripheral mitochondrial distribution pattern; yellow arrow indicates 1st polar body. Image (1C) denotes immature oocyte with peripheral mitochondrial distribution pattern. The images were taken by CLSM after staining with MitoTracker® Red CMXRos. Original magnification ×400.

was found that the oocyte (1A) showed the 1st polar body (mature) and dispersed pattern of mitochondrial distribution, while oocyte (1B) was mature but displayed peripheral pattern of mitochondrial distribution. Furthermore, the third oocyte (1C) didn't show the 1st polar body and had peripheral pattern mitochondrial relocalization. So, now it was clear that mitochondrial reorganization isn't an absolute sign for oocyte nuclear maturation completion, but broadly it may be an indispensable feature for the oocyte developmental competence.

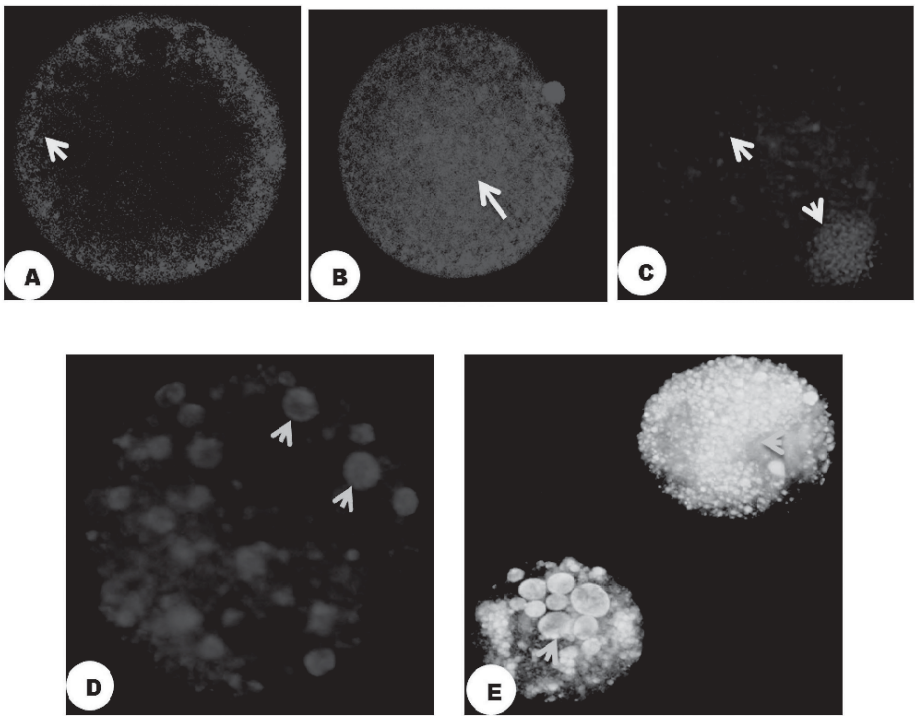

Image 2: denotes the patterns of mitochondrial aggregations in bovine oocytes: Image (2A) represent peripheral mitochondrial distribution, the arrow head denotes fine mitochondrial pixels (fine crystal). Image (2B) shows dispersed mitochondrial distribution; the arrow head denote fine mitochondrial pixels aggregation in the center of oocyte cytoplasm. Image (2C) illustrates dispersed mitochondrial distribution; lower arrow head denotes fine mitochondrial pixels aggregation around the nuclear materials. Image (2D and 2E, gray scale) denotes cluster pattern of mitochondrial aggregation as denoted by the arrows head. The images were taken by CLSM after staining with MitoTracker® Red CMXRos. Original magnification ×400.

Table (1,2): The relationship between different parameters of oocyte developmental competence and mitochondrial reorganization:

No. of Trails	Maturation (MT-II)	Uniform Distrusted Mitochondria	Peripherally Distrusted Mitochondria	Fully Expanded Cumulus Cells
1.	55.555	47.368	52.632	39.13
2.	58.333	40	60	42.5
3.	40.816	40	60	43.478
4.	52.174	43.6	56.4	27.5
Mean	51.72	42.74	57.26	38.15
±	±	±	±	±
S.E.	3.846	1.760	1.760	3.671

No. of Trails	In-vitro fertilization (2PN)	Zygote development after 24 after culturing	Zygote development after 48 after culturing	Blastocyst rate on day 8 after culturing
1.	71.428	61.765	64.706	26.471
2.	58.823	50	58.333	13.889
3.	60	55.882	55.882	35.294
4.	60	51.351	59.459	24.324
Mean	63.86	54.75	59.6	24.99
±	±	±	±	±
S.E.	2.877	2.655	1.86	4.397

Table (3): Correlation coefficient (r) for the different parameters that used as indictors for the oocytes developmental competence in relation to the mitochondrial distribution:

Item	Central mitochondrial distribution *(r)*	P value (P<0.05)	Peripheral mitochondrial distribution *(r)*	P value (P<0.05)
Oocyte maturation	0.368	0.631	-0.363	0.636
Cumulus cell expansion	-0.322	0.677	0.499	0.500
In vitro fertilization	0.928	0.071	-0.841	0.158
Maturation after 24h of development	0.747	0.252	-0.639	0.360
Maturation after 48h of development	0.958	0.042	-0.920	0.079
Blastocyst development after 8 days of culturing	0.096	0.903	-0.083	0.916

Patterns of mitochondrial aggregation in bovine oocyte after maturation in TCM-199 without phenol red

As shown in image (2) for the patterns of mitochondrial aggregation in bovine oocytes after 22h of maturation in TCM-199 without phenol red. Bovine mitochondria were aggregated in the form of small pixels of fluorescence (fine crystals) like narrow zone under oolema called as peripheral distribution (Image 2A), or distributed in the cytoplasm with more concentration in the center or around the nuclear material described as dispersed distribution (Image 2B and 2C, respectively). These patterns of mitochondrial distribution mainly to provide the energy required for each process of nuclear and cytoplasmic maturation.

Bigger or cluster shape pixels of fluorescent areas (Image 2D and 2E) had been improperly denoted by Torner *et al.* (2004) as pattern of mitochondrial aggregation. This pattern of mitochondrial aggregation that had been observed in the current work it was considered as a sign of default in the original technique used by Torner *et al.* (2004). This default occurred mainly due to slide freezing at 4-5˚C before examination that led to freezing of the fat droplet inside the oocytes. The mitochondria had a tendency to attach to the frozen fat droplets and smooth endoplasmic reticulum leading to this artifact.

- Four replications of each experiment were conducted.
- Percentages are based on the number of oocytes examined.
- After IVM, oocytes were classified according to the degree of cumulus expansion as not expanded, partially expanded (the outer layer of cells was loosened), or fully expanded (all cumulus cells were loosened).

From tables (1, 2 and 3) the current study revealed that there are strong correlation (r) between the degree of mitochondrial distribution (dispersed pattern) and IVF (normal fertilization), zygote development after 24 and 48h of development after culturing but not with the other parameters especially oocyte maturation.

Finally, the current work concludes that mitochondrial distribution isn't absolute sing for oocyte maturation completion (image 1) as stated in some previous reports, but might be indicator for its degree of developmental competence.

Discussion

Repositioning of active mitochondria during oocyte maturation or fertilization had been documented in several species (Bavister and Squirrell, 2000); such as bovine (Stojkovic *et al.*, 2001 and Tarazona *et al.*, 2006), goat (Velilla *et al.*, 2006), sheep (Smaili *et al.*, 2000), horse (Aguilar *et al.*, 2007; Torner *et al.*, 2007). Even though, the distribution and location of ooplasmic mitochondria between species show subtle differences especially concerning timing and distribution patterns (Bavister and

Squirrell, 2000). So, the comparison of mitochondrial function and physical location of the mitochondria are complicated (Bavister and Squirrell, 2000). During final pre-ovulatory maturation in pig oocytes it had been found that there was a strong relationship between meiotic progressions, cumulus cell expansion and mitochondrial redistribution and their oxidative activity (Sun *et al.,* 2001 and Torner *et al.*, 2004).The mitochondrial relocation to different areas of the cell was mediated by a cytoskeletal network of microtubules and microfilaments present in the cytoplasm, promoted these movements and acted on chromosome segregation (Van Blerkom, 1991; Ferreira *et al.*, 2009). It had been observed that the mitochondrial movement to areas of high energy and ions consumption was crucial for oocyte maturation, fertilization and early embryonic development (Van Blerkom and Runner, 1984; de Loos *et al.,* 1992; Calarco, 1995; Van Blerkom, *et al.*, 1995; Thompson *et al.*, 2000; Van Blerkom, *et al.,* 2000). Furthermore, upon reaching metaphase-II the mitochondria in bovine oocytes together with lipid droplets might occupy a central position (Hyttel *et al.,* 1997). In accordance to our results this wasn't absolutely true (Image1) where mature oocyte (M-II) may have centrally or peripherally located mitochondria. Moreover, our results may came in agreement with Sathananthan (1997) and Sathananthan and Trounson (2000) who reported that mitochondria in metaphase-I and metaphase-II human oocytes were numerous and evenly spread in the ooplasm, associating closely with vesicles or aggregates of tubular smooth endoplasmic reticulum. In other meaning isn't an utter sign for oocyte maturation. This difference might be attributed to the difference in the maturation system (Krisher, Bavister, 1998; Bavister, 2000; Bavister and Squirrell, 2000; Stojkovic *et al.,* 2001; Torner *et al.,* 2004), and oocyte quality. Where, in bovine oocytes, the major relocation of mitochondria occurred during *in vitro* maturation (IVM) was influenced by hormones and energy substrates in the maturation medium (Bavister, 2000). IVM conditions might cause incomplete movement of mitochondria to the inner cytoplasm and thus might affect the cytoplasmic maturation (Torner *et al.,* 2004).Where, the oocytes matured in culture medium containing glucose and amino acids the mitochondria became located primarily in the center of the oocyte, and this pattern was associated with more competence to form blastocysts following IVF; whereas, maturation of germinal vesicle stage oocytes in medium containing glucose and lactate led to poor embryo development after IVF as mitochondria remained in the cortex of the oocyte **(**Bavister and Squirrell, 2000**).** Additionally, confocal studies revealed a higher incidence of mitochondrial clustering in the cytoplasmic periphery of oocytes matured in standard (serum-containing) or chemically defined good medium,

whereas mitochondria of oocytes matured in chemically defined poor medium often appear homogeneously distributed **(**Krisher and Bavister, 1998; Stojkovic *et al.*, 2001**)**. Ultimately, culture conditions might disturb mitochondrial relocation and block embryo development **(**Bavister and Squirrell, 2000). Finally, the current work concludes that mitochondrial distribution isn't absolute sing for oocyte maturation completion, but might be for the degree of oocyte developmental competence.

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References

Abd-Allah SM. 2009: In vitro production of buffalo embryos from stepwise vitrified immature oocytes. Vet Ital **45**(3):425-429.

Aguilar J, Mas C, Reyley M, Ludueña R, Mouguelar H and Losinno L. 2007. Nuclear maturation and mitochondrial distribution in equine oocytes matured in vitro. Anim Reprod **4:** 88-97.

Bavister, B. and Squirrell J.M. 2000. Mitochondrial distribution and function in oocytes and early embryos. Hum Reprod 15 (Suppl. 2):189-198.

Bavister, B.D. 2000. Interactions between embryos and the culture milieu. Theriogenology, **53:**619-626.

Bell E, Brooke M, Emerling B and Navdeep S. 2005. Mitochondrial regulation of oxygen sensing. Mitochondrion, **5:**322-332.

Brackett, B.G. and Oliphant, G. 1975: Capacitation of rabbit spermatozoa in vitro. Biol Reprod, **12:** 260-274.

Brunet, S. and Maro, B. 2005: Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. Reprod **130:** 801-811.

Calarco, P.G. 1995. Polarization of mitochondria in the unfertilized mouse oocyte. Dev Genet, **16:** 36-43.

Correa, J.R. and Zavos, P.M. 1996: Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. Theriogenology, **46**(7):1225-1232.

Cummins J. 1998. Mitochondrial DNA in mammalian reproduction. Rev Reprod **3:**172-182.

de Loos F, Van Maurik, P., Van Beneden, T. and Kruip TAM. 1992. Structural aspects of bovine oocyte maturation in vitro. Mol Reprod Dev, **31:** 208-214.

de Loos FAM, van Vliet C, van Maurik P and Kruip ThAM. 1989. Morphology of immature bovine oocytes. Gamete Res **24:**197-204.

El Shourbagy SH, Spikings EC, Freitas M and St John JC. 2006. Mitochondria directly influence fertilisation outcome in the pig. Reprod, **131:**233-245.

El-Raey, M., Geshi, M., Somfai, T., Kaneda M., Hirako, M., Abdel-Ghaffar AE, Sosa GA, Abou El-Roos MEA and Nagai T. 2011. Evidence of melatonin synthesis in the cumulus oocyte complexes and its role in enhancing oocyte maturation in vitro in cattle. Mol Reprod and Deve, **78:** 250-262.

Ferreira EM, Vireque AA, Adona PR, Meirelles FV, Ferriani RA and Navarro PA. 2009. Cytoplasmic maturation of bovine oocytes: structural and

biochemical modifications and acquisition of developmental competence. Theriogenology, **71:** 836-848.

Geshi M, Takenouchi N, Yamauchi N and Nagai T. 2000: Effects of Sodium pyruvate in nonserum maturation medium on maturation, fertilization, and subsequent development of bovine oocytes with or without cumulus cells. Biol Reprod, **63:** 1730-1734.

Gutierrez, J., Ballinger, S., Darley-Usmar, V. and Landar, A. 2006. Free radicals, mitochondria, and oxidized lipids. The emerging role in signal transduction in vascular cells. Circ Res. **99:** 924-932.

Hyttel, P., Fair, T., Callesen, H. and Greve, T. 1997. Oocyte growth, capacitation and final maturation in cattle. Theriogenology, **47**(1):23-32.

Hyttel, P., Greve, T. and Callense, H. 1989. Ultra structural aspects of oocyte maturation and fertilization in cattle. J Reprod Fert Suppl, **38:** 35-47.

Hyttel, P., Xu KP, Smith, S. and Greve, T. 1986: Ultrastructure of in-vitro oocyte maturation in cattle. J Reprod Fertil **78:** 615 - 625.

Krisher, R.L. and Bavister, B.D. 1998. Responses of oocytes and embryos to the culture environment. Theriogenology, **49:**103-114.

Kruip, TAM, Cran, D.G., van Beneden TH and Dieleman SJ. 1983: Structural changes in bovine oocytes during final maturation in vivo. Gamete Res. **8:** 29-47.

Marei, W.F., Wathes, D.C. and Fouladi-Nashta A.A. 2009: The Effect of linolenic acid on bovine oocyte maturation and development. Biol Reprod, **81:** 1064 - 1072.

Martino A, Mogas T, Palmo MJ and Paramio MT. 1994: Meiotic competence of prepubertal goat oocytes. Theriogenology, **41:** 969-980.

May-Panloup, P., Chretien, M.F., Malthiery, Y. and Reynier, P. 2007. Mitochondrial DNA in the oocyte and the developing embryo. Curr Top Dev Biol, **77:**51-83.

Moyes, C.D., Battersby, B.J. and Leary, S.C. 1998. Regulation of muscle mitochondrial designs. J Exp Biol, **201:** 299-307.

Nagai, K., Sata, R., Takahashi, H., Okano, A., Kawashima, C., Miyamoto, A. and Geshi, M. 2009: Production of trophoblastic vesicles derived from day 7 and 8 blastocysts of in vitro origin and the effect of intrauterine transfer on the inter estrous intervals in Japanese black heifers. J. Reprod Dev **55**(4):454-459.

Pozzan, T., Magalhães, P. and Rizzuto, R. 2000. The comeback of mitochondria to calcium signalling. Cell Calcium **28**(5-6):279-283.

Quintero, M., Colombo, S., Godrey, A. and Moncada, S. 2006. Mitochondria as signaling organelles in the vascular endothelium. Proc Natl Acad Sci USA **103:**5379-5384.

Rasul, Z., Ahmad, N. and Anzar, M. 2001: Changes in motion characteristics, plasma membrane integrity and acrosome morphology during cryopreservation of buffalo spermatozoa. J. Androl, **22:**278-283.

Reynier, P., May-Panloup, P., Chretien, M.F., Morgan, C., Jean, M., Savagner, F., Barriere, P. and Malthiery, Y. 2001. Mitochondrial DNA content affects the fertilizability of human oocytes. Mol Hum Reprod, (5): 425-429.

Sathananthan, A.H. and Trounson, A.O. 2000. Mitochondrial morphology during pre-implantational human embryogenesis. Hum Reprod 15(Suppl. 2):148-159.

Sathananthan, A.H. 1997. Ultrastructure of the human egg. Hum.Cell; **10:**21-38.

Shoubridge, E. and Wai, T. 2007. Mitochondrial DNA and the mammalian oocyte. Curr Top Dev Biol., **77:** 87-111.

Smaili, S.S., Hsu, Y.T., Youle, R.J. and Russell, J.T. 2000. Mitochondria in Ca2 signalling and apoptosis. Bioenerg Biomembr, **32:**35-46.

St. John, J.C. 2002. The transmission of mitochondrial DNA following assisted reproductive techniques. Theriogenology, **57:**109-123.

Steeves, T. and Gardner, D.K. 1999. Metabolism of glucose, pyruvate, and glutamine during the maturation of oocytes derived from pre-pubertal and adult cows. Mol Reprod Dev., **54:** 92-101.

Stojkovic, M., Machado, S.A., Stojkovic, P., Zakhartchenko, V., Hutzler, P., Goncalves, P.B. and Wolf, E. 2001. Mitochondrial distribution and adenosine triphosphate content of bovine oocytes before and after in vitro maturation: correlation with morphological criteria and developmental capacity after in vitro fertilisation and culture. Biol Reprod **64:** 904-909.

Sun, Q.Y., Wu GM, Lai L, Park KW, Cabot R, Cheong HT, Day BN, Prather RS and Schatten H. 2001. Translocation of active mitochondria during pig oocyte maturation, fertilization and early development in vitro. Reprod, **122:**155-163.

Takada, L., Junior, A.M., Mingoti, G.Z., Balieiro JCC and Coelho LA. 2010: Melatonin in maturation media fails to improve oocyte maturation, embryo development rates and DNA damage of bovine embryos. Sci Agric. (Piracicaba, Braz.) **67**(4): 393-398.

Tarazona, A.M., Rodriguez, J.I., Restrepo LF and Olivera-Angel, M. 2006. Mitochondrial activity, distribution and segregation in bovine oocytes and in embryos produced in vitro. Reprod Domest Anim, **41:** 5-11.

Thompson, J.G., McNaughton, C., Gasparrini, B., McGowan, L.T. and Tervit, H.R. 2000. Effect of inhibitors and uncouplers of oxidative phosphorylation during compaction and blastulation of bovine embryos cultured in vitro. J Reprod Fertil, **118:**47-55.

Torner, H., Alm, H., Kanitz, W., Goellnitz, K., Becker, F., Poehland, R., Bruessow, K.P. and Tuchscherer, A. 2007. Effect of initial cumulus morphology on meiotic dynamic and status of mitochondria in horse oocytes during IVM. Reprod. Domest Anim, **42:** 176-183.

Torner, H., Brüssow, K.P., Alm, H., Ratky, J., Pçhland, R., Tuchscherer, A. and Kanitz, W. 2004. Mitochondrial aggregation patterns and activity in porcine oocytes and apoptosis in surrounding cumulus cells depends on the stage of pre-ovulatory maturation. Theriogenology, **61:** 1675-1689.

Van Blerkom, J. and Davis, P. 2007. Mitochondrial signaling and fertilization. Mol Hum Reprod, **13** (11): 759-770.

Van Blerkom, J. and Runner, M.N. 1984. Mitochondrial reorganization during resumption of arrested meiosis in the mouse oocyte. Am J Anat. **171:** 335-355.

Van Blerkom, J., Davis, P. and Alexander, S. 2000. Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization.ATP content and competence. Hum Reprod, **15:**2621-2633.

Van Blerkom, J., Davis, P. and Lee, J. 1995. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Hum Reprod, **10** (2): 415-424.

Van Blerkom, J. 1991. Microtubule mediation of cytoplasmic and nuclear maturation

during the early stages of resumed meiosis in cultured mouse oocytes. Proc Natl Acad Sci USA. **88:**5031-5035.

Velilla, E., Rodriguez-Gonzalez, E., Vidal, F., Izquierdo, D. and Paramio, M.T. 2006. Mitochondrial organization in prepubertal goat oocytes during in vitro maturation and fertilization. Mol Reprod Dev. **73** (5):617- 626.

Wilding, M., Dale, B., Marino, M., di Matteo, L., Alviggi, C., Pisaturo, M.L., Lombardi, L. and De Placido, G. 2001. Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. Hum Reprod **16:**909-917.

Zeng, H.T., Ren, Z., Yeung, W.S., Shu, Y.M., Xu YW, Zhuang GL and Liang XY. 2007. Low mitochondrial DNA and ATP contents contribute to the absence of birefringent spindle imaged with PolScope in in vitro matured human oocytes. Hum Reprod, **22**(6): 1681-1686.

Zimijewski, J., Landar., A., Watanabe., N., Dickinson, D., Noguchi, N. and Darley-Usmar, V. 2005. Cell signalling by oxidized lipids and the role of reactive oxygen species in the endothelium. Biochem Soc Trans **33:**1385-1389.