Open Access

Confocal fluorescence assessment of bioenergy/redox status of dromedary camel (Camelus dromedarius) oocytes before and after in vitro maturation

Roberto Russo1, Davide Monaco1, Marcello Rubessa2, Khalid A El-Bahrawy3, Ashraf El-Sayed4,6, Nicola A Martino1, Benedicte Beneult5, Francesca Ciannarella1, Maria E Dell'Aquila1, Giovanni M Lacalandra1 and Manuel Filioli Uranio1*

Abstract

Background: Reproductive biotechnologies in dromedary camel (Camelus dromedarius) are less developed than in other livestock species. The in vitro maturation (IVM) technology is a fundamental step for in vitro embryo production (IVP), and its optimization could represent a way to increase the success rate of IVP. The aim of the present study was to investigate the bioenergy/oxidative status of dromedary camel oocytes before and after IVM by confocal microscopy 3D imaging.

Methods: Oocytes were retrieved by slicing ovaries collected at local slaughterhouses. Recovered oocytes were examined before and after IVM culture for nuclear chromatin configuration and bioenergy/oxidative status, expressed as mitochondria (mt) distribution and activity, intracellular Reactive Oxygen Species (ROS) levels and distribution and mt/ROS colocalization.

Results: The mean recovery rate was 6 oocytes/ovary. After IVM, 61% of oocytes resumed meiosis and 36% reached the Metaphase II stage (MII). Oocyte bioenergy/redox confocal characterization revealed changes upon meiosis progression. Immature oocytes at the germinal vesicle (GV) stage were characterised by prevailing homogeneous mt distribution in small aggregates while MI and MII oocytes showed significantly higher rates of pericortical mt distribution organized in tubular networks (P < 0.05). Increased mt activity in MI (P < 0.001) and MII (P < 0.01) oocytes compared to GV stage oocytes was also observed. At any meiotic stage, homogeneous distribution of intracellular ROS was observed. Intracellular ROS levels also increased in MI (P < 0.01) and MII (P < 0.05) oocytes compared to GV stage oocytes. The mt/ROS colocalization signal increased in MI oocytes (P < 0.05).

Conclusions: This study provides indications that qualitative and quantitative indicators of bioenergy and oxidative status in dromedary camel oocytes are modified in relation with oocyte meiotic stage. These data may increase the knowledge of camel oocyte physiology, in order to enhance the efficiency of IVP procedures.

Keywords: Dromedary camel oocyte, in vitro maturation (IVM), Mitochondrial distribution pattern, Mitochondrial activity, Intracellular reactive oxygen species (ROS) levels, Mitochondria/ROS colocalization

Background

The dromedary camel (Camelus dromedarius) is a seasonal polyestrous breeder and reproductive activity is limited to the coolest month of the year [1]. Local environmental factors such as rainfall, external temperature, and nutrition, rather than photoperiod, are supposed to influence the moment of initiation of sexual activity and length of breeding season [2]. Like other camelids, dromedary camels are induced ovulators [3] but spontaneous ovulation sometimes occurs [4]. The breeding season occurs during winter (November/December to March/April), though there are conflicting reports about its beginning and length [5]. Outside the breeding season, mating activity ceases and the ovaries are inactive or have a few small follicles [5].

* Correspondence: m.filioliuranio@gmail.com
1 Section of Clinics and Animal Productions, Department of Emergency and Organ Transplantation (DETO), University of Bari Aldo Moro, Str Provv Casamassima, km 3, Bari Valenzano 70010, Italy
Full list of author information is available at the end of the article

© 2014 Russo et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
The interest in dromedary camel breeding is due to its role as a food resource in semi-arid areas and also to the commercial interest in the racing sector. The total number of camels in the world is reckoned to be around 25 million heads; though this number is probably underestimated, a steady increase has been recorded in the last 20 years [6]. In order to optimize breeding programs in this species, monitoring and increasing efficiency plans of the reproductive activity are desirable. To this end, application of assisted reproductive technologies (ARTs), such as artificial insemination, oocyte in vitro maturation (IVM) and fertilization, oocyte and embryo cryopreservation, embryo transfer and cloning could provide a substantial opportunity to improve reproductive efficiency and genetic performance and to better understand factors regulating reproductive activity in this species.

The successful development of ARTs relies on obtaining functionally competent oocytes which are a mandatory requisite for successful embryo production. IVM is a fundamental step in in vitro embryo production (IVP) and, in any species, its optimization could represent a way to increase the success rate of IVP. The regulation of oocyte maturation not only affects the proportion of oocytes capable of undergoing maturation, but also their subsequent fertilization and embryo development [7]. There is very little information on oocyte physiology in camelids, although the kinetics of oocyte nuclear maturation has been studied [8,9]. The complex process of cytoplasmic maturation has not been widely investigated in this species. To date, only one study reported offspring in camelids obtained by transfer of embryos produced by IVP [10]. These authors recovered oo-
cytes from abattoir-derived ovaries, cultured them for 30 h in TCM-199 medium supplemented with fetal calf serum (FCS), epidermal growth factor (EGF), follicle stimulating hormone (FSH), estradiol and cysteamine. After maturation, oocytes underwent in vitro fertilization with motile spermatozoa obtained by centrifugation of fresh semen on a Percoll discontinuous gradient and putative embryos were cultured in a semi-defined medium (mKSOMaa supplemented after 48 h with 10% FCS). The same authors reported 23% blastocyst formation rate and 13 normal dromedary camel offspring produced by IVP [10]. Other studies have been reported on IVM technology in dromedary camel oocytes [5,9,11-13]. These studies analysed the role of reproductive season and the effects of different culture media on in vitro oocyte meiotic development.

Increasing evidence shows the role of mitochondria (mt) as determinant for developmental competence of human and mammalian oocytes [14-16]. Regular mitochondrial bioenergetic activities within the oocyte include mt adenosine triphosphate (ATP) generation, roles in Ca\(^{2+}\) homeostasis, regulation of cytoplasmic redox state, and signal transduction [17,18]. It has been reported that oocyte competence is directly related to cytoplasmic bioenergetic capacity, and that ATP supply/demand imbalances, associated with suboptimal levels of mitochondrial ATP generation, may be a common cause of several developmental defects [15]. Mitochondrial dysfunctions or abnormalities may compromise developmental processes by inducing chromosomal segregation disorders, maturation and fertilization failures, or oocyte/embryo fragmentation resulting in mt-driven apoptosis [15,17]. Mitochondria are the major source of reactive oxygen species (ROS), which are produced during oxidative phosphorylation [19]. Under physiologic conditions, ROS are neutralized by an elaborate defence system [20]. If disequilibrium between ROS production and antioxidative capacity of the cell takes place, oxidative stress occurs [21]. Although the impact of oxidative stress on oocyte and reproductive function remains unclear, it has been shown that oxidative stress affects oocyte Ca\(^{2+}\) homeostasis, and thus also oocyte maturation and oocyte-sperm interaction [22].

To our knowledge, only one study has been reported to date on mt complement distribution and activity in dromedary camel oocytes as assessed by confocal microscopy [12]. These authors investigated mt distribution and activity, ATP, glutathione (GSH) content and calcium oscillation during IVM. They observed a highly polarized mt distribution pattern in MII oocytes compared to immature ones. In the same study, higher ATP content was observed in MII oocytes than in immature ones.

The aim of the present study was to investigate the bioenergy/oxidative status of dromedary camel oocytes in relation to their meiotic stage by simultaneous analysis of oocyte nuclear chromatin configuration and qualitative and quantitative aspects of bioenergy/oxidative status. A confocal laser scanning microscopy (CLSM)-based multiparametric assessment of oocyte bioenergy/redox status, expressed as mitochondrial distribution pattern, mitochondrial activity, intracellular ROS levels and localization and mitochondria/ROS colocalization, was performed. Increasing the knowledge of camel oocyte physiology could be useful to enhance the efficiency of IVM procedures, for a species in which reproductive biotechnologies would have wide application.

**Methods**

The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its subsequent amendments. All the procedures with animals were performed following good veterinary practice for animal welfare according to the Italian law (D.Lgs 116/92).

**Chemicals and drugs**

All chemicals for in vitro cultures and analyses were purchased from Sigma-Aldrich (Milan, Italy) unless otherwise indicated.
**Oocyte collection and maturation in vitro**

Oocyte collection and in vitro culture were performed in Egypt. Five replicates were performed during the early non-breeding season (May-June 2012 and 2013). Ovaries were collected at a local abattoir, transported to the laboratory in physiological saline solution at 30–35°C and washed once in 70% ethanol and twice with warm (37°C) phosphate buffered saline (PBS) containing 100 IU/mL penicillin G sodium and 100 μg/mL streptomycin. Cumulus-oocyte complexes (COCs) were recovered by slicing ovaries as previously reported [23]. Oocytes with uniform cytoplasm and multilayered cumulus cells were selected, washed twice in HEPES-TCM medium: TCM 199 supplemented with 25 mM HEPES, 2 mM sodium bicarbonate, 2 mM sodium pyruvate, 1 mM L-glutamine and 10% FCS and destined to either immediate evaluation or in vitro maturation (IVM) culture. The IVM culture was performed in TCM199 (Gibco 22340) supplemented with 15% FCS, 0.25 mg/mL pyruvic acid, 20 ng/mL EGF, 50 μg/mL gentamicin, 10 μg/mL FSH (Sigma-Aldrich, F2293), 10 μg/mL luteinizing hormone (LH; Sigma-Aldrich, L6420), 1 μg/mL estradiol and 0.3 mM cystine. Groups of 25 COCs were matured in 400 μl of IVM medium, covered with 400 μl of mineral oil, in four well culture plates (Nunc, Roskilde, Denmark). On the basis of previously reported studies, oocytes were cultured for 40–42 h at 38.5°C under 5% CO₂ in air with 95% relative humidity [12,24]. Oocytes were denuded by gentle pipetting in HEPES-TCM 199 with 80 IU of hyaluronidase and underwent epifluorescence and confocal microscopy evaluation.

**Oocyte mitochondria and ROS staining**

To investigate mitochondrial distribution and apparent energy status, oocytes were washed three times in PBS with 3% bovine serum albumin (BSA) and incubated for 30 min in the same medium containing 280 nM MitoTracker Orange CMTM Ros (Molecular Probes M-7510, Oregon, USA) at 38.5°C under 5% CO₂ [25,26]. The cell-permeant probe contains a thiol-reactive chloromethyl moiety. Once the MitoTracker probe accumulates in the mitochondria, it can react with accessible thiol groups on peptides and proteins to form an aldehyde-fixable conjugate. This cell-permeant probe is readily sequestered only by active mitochondria [27,28]. The organelle-specificity of the probe was assessed, as reported by Valentini et al. [29]. Control oocytes were imaged after incubation in MitoTracker Orange and further incubation for 5 min in the presence of 5 μM of the mt membrane potential (Delta Psi)-collapsing uncoupler carbonyl cyanide 3-chloro phenyl hydrazone (CCCP; Molecular Probes; Monza, Italy). This molecule inhibits mt respiratory activity, thus reducing fluorescence intensity. After incubation with mt probe, oocytes were washed three times in PBS with 0.3% BSA and incubated for 15 min in the same media containing 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (H₂DCF-DA) [30-32] in order to detect and localize intracellular sources of ROS. The principle underlying this procedure may be described briefly as follows: non-ionized H₂DCF-DA is membrane-permeant and is therefore able to diffuse readily into cells. Once within the cell, the acetate groups are hydrolysed by intracellular esterase activity forming 2′,7′-dichlorodihydrofluorescein (H₂DCF) which is polar and thus trapped within the cell. H₂DCF fluoresces when it is oxidized by H₂O₂ or lipid peroxides to yield 2′,7′-dichlorofluorescein (DCF). The level of DCF produced within the cells is related linearly to that of peroxides present and thus its fluorescent emission provides a measure of the peroxide levels [30]. After incubation, oocytes were washed three times in pre-warmed PBS without BSA and fixed overnight at 4°C with 2% paraformaldehyde solution in PBS.

**Nuclear chromatin evaluation of oocytes**

To evaluate nuclear chromatin, oocytes were stained with 2.5 μg/mL Hoechst 33258 in 3:1 (v/v) glycerol/PBS and mounted on microscope slides covered with cover slips, sealed with nail polish and kept at 4°C in the dark until observation. Oocytes were evaluated in relation to their meiotic stage under an epifluorescence microscope (Nikon Eclipse 600; Nikon Instruments, Amsterdam, The Netherlands; 400x magnification) equipped with B-2 A (346 nm excitation/460 nm emission) filter, as germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) with 1st polar body extruded. Oocytes with multi-polar spindle, irregular chromatin distribution or absence of chromatin were classified as degenerated and were excluded from further analysis.

**Assessment of oocyte mitochondrial distribution pattern and intracellular ROS localization**

For evaluation of mt distribution pattern, oocytes were selected among those having homogeneous cytoplasmic texture. Oocytes were observed at 600x magnification in oil immersion with Nikon C1/TE2000-U laser scanning confocal microscope (Nikon Instruments, Amsterdam, The Netherlands). A helium/neon laser ray at 543 nm and the G-2 A filter (551 nm exposure and 576 nm emission) were used to point out the MitoTracker Orange CMTM Ros. An argon-ion laser ray at 488 nm and the B-2 A filter (495 nm exposure and 519 nm emission) were used to point out the DCF. For each oocyte, scanning was conducted with 25 optical series from the top to the bottom of the oocyte with a step size of 0.45 μm to allow three-dimensional distribution analysis. General criteria for definition of oocyte mt patterns were adopted on the basis of previous studies in other species [25,26,29,32]. Homogeneous/even distribution of small mt aggregates (SA) throughout the cytoplasm was considered as indication of immature cytoplasmic condition,
whereas heterogeneous/uneven distribution of small and/or large aggregates within the cytoplasm indicated a metabolically active ooplasm. In particular, tubular mt networks spread throughout the cytoplasm (diffused tubular network, DTN) or their accumulation in the peripheral cytoplasm (pericortical tubular network, PCTN) or in the periphery and around the nucleus (perinuclear/ pericortical tubular network, PN/PCTN) were considered as sequential aspects of the developmental program of cytoplasmic maturation [33]. Oocytes showing irregular distribution of large mt clusters unrelated to the specific cell compartments were classified as abnormal and were excluded from further quantification analysis.

Quantification of MitoTracker orange CMTM Ros and DCF fluorescence intensity

In each individual oocyte, MitoTracker Orange CMTM Ros and DCF fluorescence intensities were measured at the equatorial plane, as in previous studies in human [34] and animal oocytes [25,26,29,32], with the aid of the EZ-C1 Gold Version 3.70 image analysis software platform for Nikon C1 (Nikon Instruments) confocal microscope. A circle of area = 100 in diameter (arbitrary value) was drawn to measure only the cytoplasmic area (512 by 512 pixels). The fluorescence intensity encountered within the programmed scan area was recorded and plotted against the conventional pixel unit scale (0–255). Fluorescence intensity was expressed as Arbitrary Densitometric Units (ADU). Parameters related to fluorescence intensity were maintained at constant values for all measurements. In detail, images were taken under fixed scanning conditions with respect to laser energy, signal detection (gain) and pinhole size.

Oocyte mitochondria/ROS colocalization analysis

Colocalization analysis of mitochondria and ROS was performed by using the EZ-C1 Gold Version 3.70 software. Degree of colocalization was reported as a Pearson’s correlation coefficient quantifying the overlap degree between MitoTracker Orange CMTM Ros and DCF fluorescence signals [26,35].

Statistics

Nuclear maturation rates and mt distribution patterns were compared by chi-square analysis. For confocal quantification analysis of mt activity and intracellular ROS levels, the least-square means of the dependent variable (MitoTracker Orange CMTM Ros and DCF fluorescence intensity) were calculated in examined samples and the statistical significance of the least-square means between treated and control groups was calculated by one-way ANOVA followed by Multiple Comparison Dunn’s method (SigmaPlot software). For mt/ROS colocalization, mean values of Pearson’s correlation coefficient were compared between treated and control groups by one-way ANOVA followed by Multiple Comparison Dunn’s method (SigmaPlot software). Differences with P < 0.05 were considered as being statistically significant.

Results

After retrieval, 23 oocytes were immediately stained for epifluorescence/confocal analysis. All of them were found at the GV stage and further analysed for bioenergy/oxidative status. One-hundred sixty-five oocytes underwent IVM and after denuding were stained and analyzed. After IVM, 41 oocytes were at the MI stage (25%) and 59 oocytes reached the MII stage (36%). All of them underwent confocal analysis. Data from 23 out of 23 GV stage oocytes, 27 out of 41 MI stage oocytes and 45 out of 59 MII stage oocytes, showing normal ooplasmic size and texture, are reported in Table 1 and Figure 1. Remaining oocytes, which were found at the GV stage (n = 34, 20%) or showing degenerated chromatin configurations (n = 31, 19%) after IVM culture, were excluded from confocal analysis.

Mitochondrial distribution pattern

Results of mt distribution pattern analysis are presented in Table 1. The rate of oocytes showing PCTN mt distribution pattern was significantly higher in oocytes cultured in vitro and reaching the MI and MII stages compared with uncultured GV stage oocytes (31% vs 9% for MII and GV; 33% vs 9% for MI and GV, respectively, P < 0.05). The rate of oocytes showing PN/PCTN also increased with maturation, though not statistically significant. Likewise, the rate of oocytes showing mt distribution in SA was significantly reduced both in MI and MII oocytes compared to GV oocytes (41% versus 78% for MI and GV, respectively, P < 0.02; 38% vs 78% for MII and GV, respectively, P < 0.01).

Table 1 Mitochondrial distribution pattern of dromedary camel oocytes before (*) and after IVM

| Nuclear chromatin configuration | N° oocytes | SA (%) | DTN (%) | PCTN (%) | PN/PCTN (%) | Abnormal (%) |
|-------------------------------|------------|--------|---------|----------|-------------|--------------|
| GV*                           | 23         | 18 (78)a| 2 (9)   | 2 (9)a   | 1 (4)       | 0            |
| M I                           | 27         | 11 (41)b| 2 (7)   | 9 (33)b  | 5 (19)      | 0            |
| M II                          | 45         | 17 (38c)| 8 (18)  | 14 (31f) | 4 (9)       | 2 (4)        |
Bioenergy/redox status analysis

Significant increases in mt activity in MI and MII oocyte groups compared to GV group (1060.92 ± 478.4 vs 659.06 ± 352.5 ADU for MI and GV, respectively; P < 0.001; 1030.01 ± 421.38 vs 659.06 ± 352.5 ADU for MII and GV, respectively; P < 0.01) were observed. The same, intracellular ROS levels significantly increased in MI and MII oocyte groups compared to GV group (1571.78 ± 527

Figure 1 Quantitative analysis of bioenergy/redox parameters in dromedary camel oocytes before and after in vitro maturation. Mean ± SD of mitochondrial (mt) activity (Panel A), intracellular ROS levels (Panel B) and mt-ROS colocalization (Panel C) of dromedary camel oocytes, as examined before and after in vitro maturation. Numbers of analyzed oocytes per group are indicated above the histograms. Mitochondrial activity and ROS levels are expressed as MitoTracker Orange CMTM Ros fluorescence intensity and 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA) fluorescence intensity in Arbitrary Densitometric Units (ADU). Mt/ROS colocalization is expressed as Pearson’s correlation coefficient of MitoTracker Orange CMTM Ros and DCF fluorescent labelling. One-way ANOVA: a,b: P < 0.001; a,c: P < 0.01; a,d: P < 0.05.
and 250 μM cysteamine. Wani and Wernery [9] reported that 81% of in vitro cultured oocytes reached the MII stage by using TCM-199 supplemented with 0.4% bovine serum albumin, 10 μg/mL FSH, 10 μg/mL LH, and 1 μg/mL estradiol.

Regular mt bioenergetic activities within the oocyte play a crucial role as determinants for developmental competence for human and mammalian oocytes [15,16]. During oocyte maturation and fertilization, nuclear changes are coordinated with movements of organelles, in particular mitochondria, and with biochemical changes in the cytoplasm to ensure normal embryo development [36]. Of the numerous cytoplasmic changes occurring during oocyte maturation, the positioning of mitochondria may be involved in concentrating ATP or calcium or in regulating pH in specific regions of the oocyte to support normal developmental processes. A mt distribution pattern polarized in the peri-spindle (perinuclear) and in the pericortical region of the oocyte has been regarded as one aspect of cytoplasmic maturation in different species, as reported in previous studies from our unit in equine, ovine, canine and human oocytes [25,26,29,32,34]. In the present study, qualitative analysis of mt distribution in dromedary camel oocytes, performed before or after in vitro maturation, revealed that mt distribution patterns within the oocytes were changed in relation to the meiotic stage. Oocytes at the GV stage showed a predominantly uniform mt distribution, organized in SA. In oocytes at the MI and MII stages, a reduction in oocytes showing SA mt distribution and an increase in oocytes with a heterogeneous PCTN mt distribution pattern were observed. These results are in line with those reported in previous studies. Using confocal analysis, Abdoon et al. [12] reported mt clustering in MI oocytes (referred as matured oocytes without PB in their study). These authors also reported highly polarized mt distribution pattern in the subcortical region of MII oocytes showing the first PB extruded. Using ultrastructural TEM analysis, Kafi et al. [8] reported arrangement of most mitochondria in the oocyte periphery at 24–36 hours IVM culture, which is demonstrated as being the time of MI to MII transition.

To our knowledge, this is the first study jointly evaluating the nuclear chromatin configuration and the bioenergy and redox status of dromedary camel oocytes, with a triple staining procedure and an epifluorescence/3D confocal evaluation of individual oocytes, in relation with meiotic progression. Moreover, the quantification study was performed by objective multiparametric analysis. MitoTracker Orange CMTM Ros fluorescence intensity analysis revealed an increase of mt activity in oocytes at MI and MII stage compared with those at GV stage. This finding indicated that the achievement of nuclear maturation was related to an increase in the number of actively respiring mitochondria within the ooplasm and/or to an increase in the number of active sites per each mitochondrion, as well as to a...
Figure 2 (See legend on next page.)
change in mt distribution. These data are in line with observations by Abdoon et al. [12], concerning increased ATP content in MII compared with GV oocytes. DCF fluorescence intensity also revealed an increase in intracellular ROS levels in oocytes at MI and MII compared to GV stage. This result could be explained in terms of increased oxidative phosphorylation which may be translated into an increase in ROS production.

Mitochondria/ROS colocalization, objectively expressed as Pearson’s correlation coefficient, was higher in oocytes at MI stage compared with GV stage oocytes. It also tended to remain at higher values in MII compared with GV stage oocytes, though not significantly different. Colocalization of ROS and actively respiring mitochondria was reported to be indicative of higher ATP turnover resulting from a more intense mt activity and thus indicative of healthy cell conditions, as demonstrated in hepatocytes [37]. Another study, in mouse oocytes, reported that the regions within the oocyte producing high levels of ROS colocalized with the active mitochondria, as visually assessed by using confocal microscopy, in the majority of in vivo MII ovulated oocytes [38]. Moreover, higher levels of mt/ROS colocalization were reported as a reliable marker of in vivo matured MII ovine oocytes [26].

Conclusions

In conclusion, this study provides simultaneous information on bioenergy and redox status of dromedary camel oocytes as related to their meiotic stage before and after in vitro maturation and contributes to knowledge of camel oocyte physiology, which may enhance the efficiency of IVM procedures and in vitro production of embryos in this species.
Competing interests
The authors declare that they have no competing interest.

Authors’ contributions
MED, DM and GML conceived the study. DM, MR, BB and FC performed dromedary camel oocyte collection, in vitro maturation and mt/ROS stainings. RAE and AES coordinate oocyte recoveries and oocyte collection. RR, BB and FC performed epifluorescence chromatin configuration analysis. RR, MFU and NAM performed confocal analysis and statistical evaluations. MED and GML performed confocal analysis and statistical evaluations. MFU, RR and MED wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements
This document has been produced with the financial assistance of the European Union: ENPI-CBC-MED “PROCAVED” Project: Promotion des systèmes camelins innovants et des filières locales pour une gestion durable des territoires saharienne; reference number IB/1.1/493. The contents of this document are the sole responsibility of “Section of Clinical and Animal Productions, Department of Emergency and Organ Transplantation - University of Bari Aldo Moro, Italy” and can under no circumstances be regarded as reflecting the position of the European Union. Authors are grateful to the Direction of CURP for the logistic support and help during the implementation of this work. Additional supports were received from CEGBA project, University of Bari Aldo Moro and ONEV MJUR PON3_00134 project n.254/RSC. 18/05/2011. The authors thank Anthony Green of the Department of Veterinary Medicine, University of Bari Aldo Moro for language revision and assistance.

Author details
1. Section of Clinical and Animal Productions, Department of Emergency and Organ Transplantation (DETO), University of Bari Aldo Moro, Str Prov Casamassima, km 3, Bari Valenzano 70010, Italy. 2. National Research Council (CNR), ISPAA, c/o Istituto tecnico agrario statale “E. De Celli”, Via Argine 1085, Naples Porticiello1047, Italy. 3. A. Lab., Maryour Research Station (MRS), Desert Research Center (DRC), Al-Nassiy St., Al Amryia, Alexandria, Egypt. 4. Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Giza 12613, Egypt. 5. SupAgro, Systemes d’Elevage Mediterraneens et Tropicaux, UMR_SELNET, Montpellier 34598, France. 6. Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

Received: 22 October 2013 Accepted: 12 February 2014
Published: 18 February 2014

References
1. Skidmore JA: Reproductive physiology in female Old world camels. Anim Reprod Sci 2011, 124:146–154.
2. Fatnassi M, Padalino B, Monaco O, Khochrani T, Lalacalanda GM, Hammadi M: Evaluation of sexual behavior of housed male camels (Camelus dromedarius) through female para-sex: correlation with climatic parameters. Trop Anim Health Prod 2014, 46:313–321.
3. Marie M, Anouassi A: Induction of luteal activity and progesterone secretion in the non-mated dromedary camels (Camelus dromedarius). J Reprod Fertil 1987, 80:183–192.
4. Nagy P, Juhasz J, Wernery U: Incidence of spontaneous ovulation and development of the corpus luteum in non-mated dromedary camels (Camelus dromedarius). Theriogenology 2005, 63:292–304.
5. Faye B, Bonnet P: Camel sciences and economy in the world: current situation and perspectives. In Proceeding of the 3rd Conference of the international Society of Camelid Research and Development. 29th Jan–1st Feb 2012, Muscat, Sultanate of Oman. Edited by Johnson EH., 2012:23–15.
6. Zeidan AEB, El-Hanany MA, Gabr SA, Tag El-Dien MA, Abd El-Rahman SA, Amer AM: In vitro maturation of camel oocytes as affected by different media during breeding and Non-breeding seasons. J Arie Sci 2011, 7:460–472.
7. Bavister BD, Rose-Hellekant TA, Pinyopummin T: Development of in vitro matured/in vitro fertilized bovine embryos into morulae and blastocysts in defined culture medium. Theriogenology 1992, 37:127–146.
8. Kafi M, Mesbah F, Nilil H, Khalil A: Chronological and ultrastructural changes in camel (Camelus dromedarius) oocytes during in vitro maturation. Theriogenology 2005, 63:2458–2470.
9. Wani NA, Wernery U: In vitro maturation of dromedary (Camelus dromedarius) oocytes: effect of different protein supplementations and epidermal growth factor. Reprod Dom Anim 2010, 45:189–193.
10. Wani NA, Wernery U: Effects of in vitro maturation and in vitro fertilized and in vitro cultured abbotar-derived oocytes. Theriogenology 2006, 65:1721–1736.
11. Nagy P, Skidmore JA, Juhasz J: Use of assisted reproduction for the improvement of milk production in dairy camels (Camelus dromedarius). Anim Reprod Sci 2013, 136:205–210.
12. Abdoon ASS, Kandil OM, Zeng SM, Cui M: Mitochondrial distribution, ATP-GSH contents, calcium (Ca2+) oscillation during in vitro maturation of dromedary camel oocytes. Theriogenology 2011, 76:1207–1214.
13. Thierer H, Anouassi A, Tibary A: Quality and developmental ability of dromedary (Camelus dromedarius) embryos obtained by IVM/IVF, in vivo matured/IVF or in vivo matured/fertilized oocytes. Reprod Dom Anim 2007, 42:263–270.
14. Eicheneb-Ritter U, Wieczorek M, Lüke S, Seidel T: Age related changes in mitochondrial function and new approaches to study redox regulation in mammalian oocytes in response to age or maturation conditions. Mitochondrion 2011, 11:763–796.
15. Van Blrickom J: Mitochondrial function in the human oocyte and embryo and their role in developmental competence. Mitochondrion 2011, 11:797–813.
16. Wang LY, Wang DH, Zou XY, Xu CM: Mitochondrial functions on oocytes and preimplantation embryos. J Zhejiang Univ Biophys 2009, 107:835–842.
17. Brooks PE, Yoon K, Rebooth JLM, Anders MW, Sheu SS, Calcium, ATP, and ROS: a mitochondrial love-hate triangle, Am J Physiol Cell Physiol 2004, 287:C317–C383.
18. Dumollard R, Carroll J, Duchen MR, Campbell K, Swann K: Mitochondrial function and redox state in mammalian embryos. Sem Cell Biol 2009, 20:346–353.
19. Cadenas E, Davies KJ: Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000, 29:223–230.
20. Winyard PG, Moody CJ, Jacob C: Oxidative activation of antioxidant defence. Trends Biochem Sci 2005, 30:453–461.
21. Finkel T, Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. Nature 2000, 408:239–247.
22. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shanan A, Gupta S: The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol 2012, 10:49.
23. Harmano S, Kuwayama M: In vitro fertilization and development of bovine oocytes recovered from the ovaries of individual donors: a comparison between the cutting and aspiration method. Theriogenology 1993, 39:703–712.
24. Wani NA, Nawshari MA: Kinetics of nuclear maturation and effect of holding ova at room temperature on in vitro maturation of camel (Camelus dromedarius) oocytes. Theriogenology 2005, 64:75–85.
25. Ambrosini LA, Lalacalanda GM, Iorga AI, De Santis T, Mugnier S, Mataresse R, Goudet G, Dell’Aquila ME: Cytoplasmic lipid droplets and mitochondrial distribution in equine oocytes: implications on oocyte maturation, fertilization and developmental competence after ICSI. Theriogenology 2009, 71:1093–1104.
26. Martino NA, Lalacalanda GM, Filalli Uranio M, Ambrosini B, Caira M, Silvestre F, Pizzi F, Desantis S, Accogli G, Dell’Aquila ME: Oocyte mitochondrial bioenergetic potential and oxidative stress: within-/between-subject, in vivo versus in vitro maturation, and age-related variations in a sheep model. Fertil Steril 2012, 97:720–728.
27. Poot M, Zhang YZ, Klamer JA, Wells KS, Jones LJ, Hanzel DK, Laugade AG, Singer VL, Haaglund RP: Analysis of mitochondrial morphology and function with novel fixable fluorescent stains. J Histochem Cytochem 1996, 44:1363–1372.
28. Torner H, Brüssow KP, Alm H, Rask M, Pohland T, Ruchschwer A, Kanzit W: Mitochondrial aggregation patterns and activity in porcine oocytes and apoptosis in surrounding cumulus cells depends on the stage of pre-ovulatory maturation. Theriogenology 2004, 61:1675–1689.
29. Valentini L, Iorga AI, De Santis T, Ambrosini B, Reynaud K, Chastain-Maillard S, Guaricci AC, Caira M, Dell’Aquila ME: Mitochondrial distribution patterns in canine oocytes as related to the reproductive cycle stage. Anim Reprod Sci 2010, 117:166–177.
30. Yang HW, Hwang KJ, Kwon HC, Kim HS, Choi K, Oh KS: Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. Hum Reprod 1998, 13:996–1002.
31. Kuznetsov AV, Kehrer I, Kozlov AV, Haller M, Redl H, Hermann M, Grimm M, Troppmair J. Mitochondrial ROS production under cellular stress: comparison of different detection methods. Anal Bioanal Chem 2011, 400:2383–2390.

32. Ambruosi B, Filioli Uranio M, Sardanelli AM, Pocar P, Martino NA, Paternoster MS, Amati F, Dell’Aquila ME. In vitro acute exposure to DEHP affects oocyte meiotic maturation, energy and oxidative stress parameters in a large animal model. PLoS One 2011, 6:e27452.

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

34. Dell’Aquila ME, Ambruosi B, De Santis T, Cho YS. Mitochondrial distribution and activity in human mature oocytes: GnRH agonist vs antagonist for pituitary downregulation. Fertil Steril 2009, 91:249–255.

35. Zinchuk V, Grossenbacher-Zinchuk O. Recent advances in quantitative colocalization analysis: focus on neuroscience. Prog Histochem Cytochem 2009, 44:125–172.

36. Van Blerkom J. Microtubule mediation of cytoplasmic and nuclear maturation during the early stages of resumed meiosis in cultured mouse oocytes. PNAS 1991, 88:5031–5035.

37. Raval J, Lyman S, Nitta T, Mohuczy D, Lemasters JJ, Kim JS, Behrs MS. Basal reactive oxygen species determine the susceptibility to apoptosis in cirrhotic hepatocytes. Free Radic Biol Med 2006, 41:1645–1654.

38. Wakefield SJ, Lane M, Schulz SJ, Hebart ML, Thompson JG, Mitchell M. Maternal supply of omega-3-polyunsaturated fatty acids alter mechanisms involved in oocyte and early embryo development in the mouse. Am J Physiol Endocrinol Metab 2008, 294:E425–E434.

doi:10.1186/1477-7827-12-16
Cite this article as: Russo et al. Confocal fluorescence assessment of bioenergy/redox status of dromedary camel (Camelus dromedarius) oocytes before and after in vitro maturation. Reproductive Biology and Endocrinology 2014 12:16.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit