PROCAMED

"Promotion des systèmes camelins innovants et des filières locales pour une gestion durable des territoires saharienne"

Summary report action 2.1

Location of the activity: Cairo, Egypt
Period of the activity reported: May-June 2012
Date of the report: August 2012

Coordination:

Prof. Lacalandra G.M. Department Of Animal Production Bari, Italy

Report delivered by: Dr. Davide Monaco

Action 2.1. “Amélioration de la productivité numérique par l’introduction de biotechnologie de la reproduction”.

- Reproductive Biotechnologies in camels are less developed than in other species: basic knowledge available on Oocytes maturation and Oocytes quality parameters are still far less in this species.

Genetic and Reproductive improvement take advantage by development and application of in vitro Oocyte maturation, cryopreservation of gametes and embryos and other associated technologies.

Cryopreservation of Gametes and Embryo may offer many advantages to commercial animal production. However, the establishment of suitable methods for Oocytes cryopreservation needs to be joined with evaluation of structural, functional, and energy/metabolic Oocyte quality parameters.

Purposes of this activity was to standardize a procedure for camel Oocytes maturation and to set Oocytes quality parameters for mature and immature dromedary camels Oocytes (Staining and evaluation of nucleus, mitochondria and Reactive Species of Oxygen: ROS).

The first part of the work (collection and staining of Oocytes and glass slide preparation) has been carried out from 13 May to 10 June 2012, in Egypt, at the Faculty of Agriculture Research Park in Cairo. The second part (evaluation) will be carried out from September to October 2012 at the Biotechnology Laboratories of Animal Production Department and of the Faculty of Biotechnologies in Bari, Italy.

In the first part of the activity, participants from different Institutes have been involved:

Dr. Asharaf Abdel Halim El-Sayed (Supervisor of Embryology lab., Cairo University, ***Egypt***), **Mohamed Magdy Hassan** (Animal Physiology Teaching Assistant at Department of Animal Production, Cairo University, ***Egypt***), **Mohammed Hassan Khalifa** (Lab Specialist at Cairo University Research Park, ***Egypt***), **Abd El-Hay Gaber Abu-Hessin** (Lab Specialist at Cairo University Research Park, ***Egypt***), **Omnia El-Sayed** (Lab Specialist at Cairo University Research Park, ***Egypt***), **Prof. Khalid Ahmed El- Baharawy** (Procamed Egyptian coordinator, Desert Research Center ***Egypt***), **Dr. Davide Monaco** (Philosophal Doctor on Camel Reproduction, Department of Animal Production, ***Italy***), **Dr. Marcello Rubessa** (Philosophal Doctor on Reproduction Biotechnologies, National Research Council, ***Italy***), **Mrs. Ciannarella Francesca** (Master Degree Student, Department of Animal Production, ***Italy***), **Mrs. Beneult Benedicte** (Agronomical Engineering Student, Supagro, ***France***),

Picture: Some components of the team involved in the ACTIVITY 2.1. in Cairo

From The Left: Mohamed Magdy Hassan(CURP), Ahmed Kamel (DRC), Davide Monaco (DPA), Marcello Rubessa (CNR), Francesca Ciannarella (DPA), Asharaf Abdel Halim El-Sayed (CURP), Mohammed Hassan Khalifa (CURP), Benedicte Beneult (Supagro), Omnia El-Sayed (CURP).

The following steps have been carried out.

Collection and shipment of the Ovaries

Ovaries were collected in different slaughterhouses and delivered to the Embryology lab within at least 4 hours in a thermos with normal saline solution

Washing of the Ovary

Ovaries were cleaned from other tissues (ovarian bursa, fallopian tubes, ligaments), washed with saline solution, quickly washed in 70% ethanol and finally, let in saline solution in a baker glass in a water bath at 32, 5 °C.

Pictures: Dr Rubessa, showing the procedure for washing the ovaries. Dr. Rubessa and Mrs Omnia El-Sayed during the maturation media preparation. Mrs Omnia El-Sayed and Francesca Ciannarella, Mrs. Benedicte Beneult with Francesca Ciannarella and Omnia El-Sayed

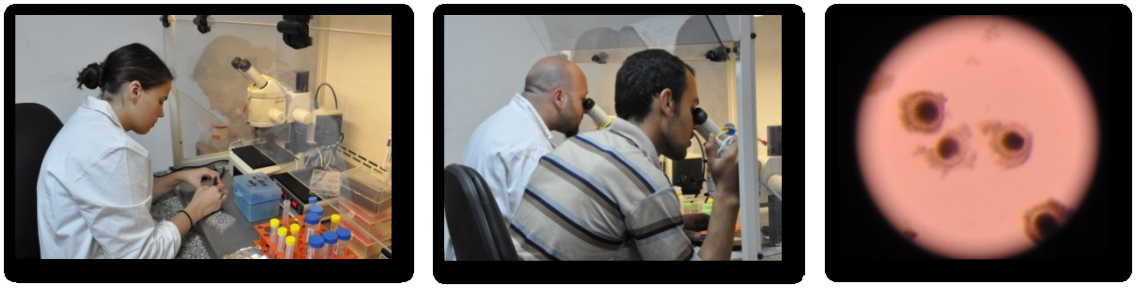
Oocyte collection

Oocytes were then recovered by the slicing method: each follicle was opened with a razor blade in a Petri dish with PBS. Once all follicles have been opened and washed the Petri dish has been examined under the stereomicroscope for the Oocytes observation and collection.

Pictures: Mrs Ciannarella performing the slicing of the ovaries with a razor, a detail of the slicing method and some of the dromedary camel ovaries after the slicing.

Oocytes Selection

Oocytes (A and B grade) were selected, moved from PBS and washed into a washing media before the maturation. Some immature Oocytes have been stained.



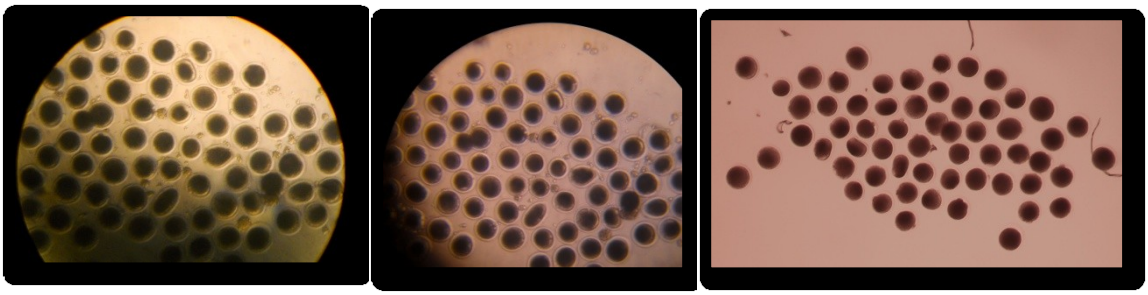
Pictures: Mrs Beneult preparing the maturation media and the 4 well plate for Oocytes maturation. Dr. Rubessa and Mohammed Khalifa at the laminar flow observing Oocytes. Dromedary camel Cumulus Oocyte Complesses (COC's: Oocytes plus Granulosa cells) under the stereo microscope.

Maturation

Oocytes were placed in a maturation media (in a 4 well nunc) and put in a CO2 incubator for 40-42 hours.

Evaluation

Preliminary maturation tests with different chemical compound have been tried in order to find the best media composition for the dromedary camel Oocytes maturation. After every maturation Oocytes have been morphologically evaluated for calculating the maturation rate percentage and before the staining. The evaluation process was performed by denuding (removing the Granulosa cells around the Oocytes with the enzyme ialuronidase) and by observing the polar body extrusion (sign of completion of metaphase II).

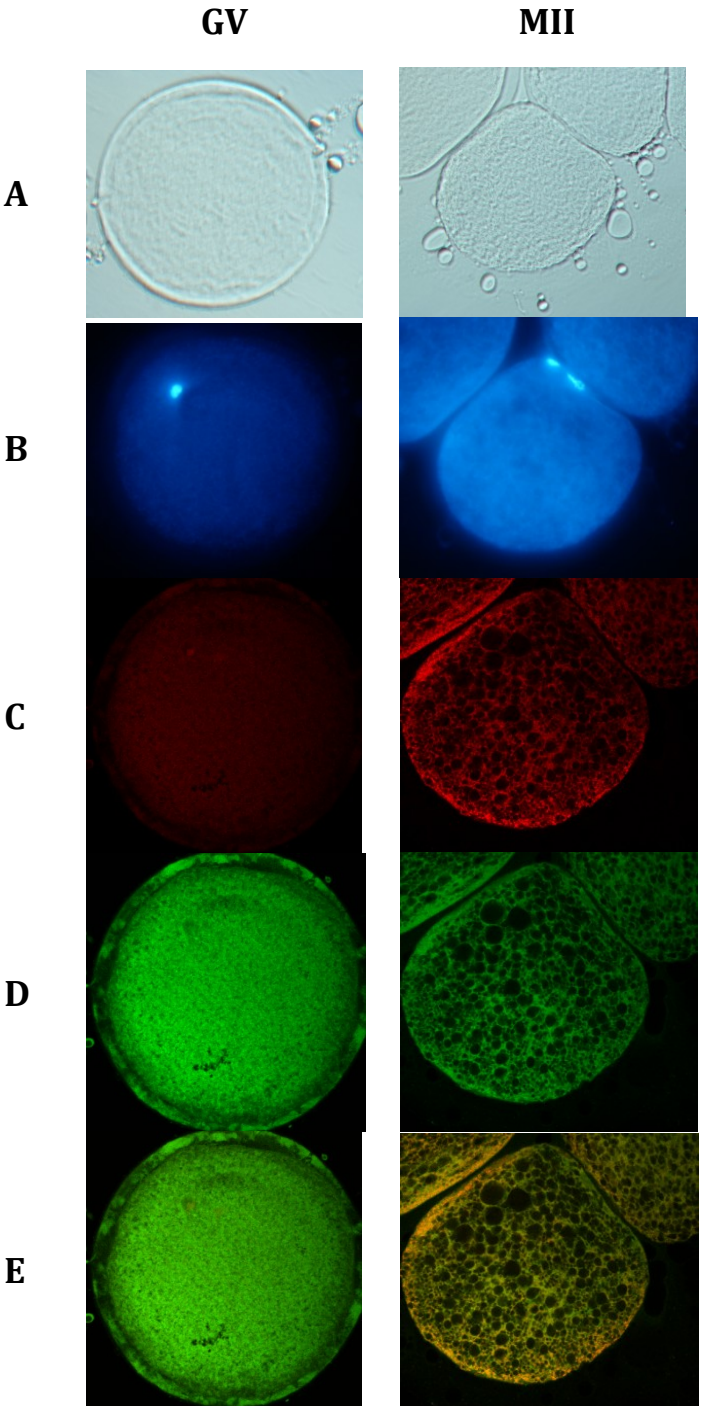


Pictures: Some Oocytes during initial evaluation of maturation. Picture 3 Mature denuded Oocytes: note that, in some of them, is possible to observe the extruded polar body.

Staining

Matured Oocytes were stained in order to assess nuclear maturation, mithocondria distribution and Reactive Oxigen Species (ROS) and fixed on glass slides for further evaluation at the fluorescence and confocal microscopes in Italy, **under the Supervision of Prof. Mrs. Dell'Aquila M.E..** The obtained data will be considered as standard, quality parameters, for evaluating effects of different agents (Chemicals, Freezing procedures ecc) on the structure and metabolism of the Dromedary camel Oocyte cells.

Table 1: Sample Image of Camel Oocytes after different staining and microscopes observation* (A: contrast, B: Fluorescent, C &D Confocal)



GV: Germinal Vescicle(Immature Oocyte)

MII: Mature Oocyte

A : Contraste microscope observation

B : Nuclear statut observation

C : Mitochondrial ditribution

D : ROS distribution

E : Mitonchondria and ROS colocalisation

*** Images collected by Prof. Dell'Aquila research staff, at the Biotechnologies and Animal Production Department Labs of University "Aldo Moro" of Bari, Italy.**

Es: Description and analysis of table 1

The initial analysis on the contrast microscope (A) is used to evaluate the Oocyte morphology and its proper fixation and location on the glass slide (in some Oocytes cytoplasmic breakage could be observed as well as other changes).

The fluorescent coloration (pic B) is used to evaluate the nuclear status (maturation). For the GV stage, only one chromatin spot is visible while for M II stage (matured Oocytes), two spots could be observed. These spots correspond to the nucleus of the cell and to the nucleus of the first polar body.

The analysis of mitochondria and intracellular ROS distribution (C &D) identifies some notable changes between the two stages of the Oocytes.

The mitochondrial fluorescence intensity in GV stage is lower compared to M II stage, and the overall distribution of mitochondria shows a different pattern between the two stages of the germinal cells.

In the GV stage, mitochondria and Intracellular ROS are organized in small aggregates and their arrangement within the cell is quite homogeneous. In M II stage instead, mitochondria and Intracellular ROS are organized in clusters in a a tubular network disposition and are located mainly at the peripheral space of the Oocyte.

Finally, the colocalisation (D: disposition of the mitochondria and ROS within the cell) is similar between GV and M II: Any difference concerning the distribution of the mitochondria and the Reactive Oxigen Species could be noted in the GV or in the MII Oocytes.

Conclusions Analysis of the stained Oocytes are still going on: detailed results of the whole Dromedary camel Oocytes analysis protocol are expected in the month of October and will be published in the thesis report of Mrs Beneult and Mrs Ciannarella: Engineer Student at the UMR- Systèmes d'Elevage Méditerranéens et Tropicaux (UMR_SELMET) SupAgro-INRA, (France), and Veterinary Student of University “Aldo Moro” of Bari (Italy), respectively.

Moreover: Some immature and mature Oocytes have also been taken out for the assessment of another investigation: a survey about quality genes expression. The work aim at investigating differences in the expression of some genes related to the quality in different Oocytes: A and B grade immature Oocytes, B and C grade Oocytes, as well as in mature Oocytes.