MOUSE INTER-STRAIN MITOCHONDRIA TRANSFER USING PIEZO MICROMANIPULATOR FACILITATED INJECTION

Ana Claudia Carstea[1*], A.T. Bogdan[2], Elena Daniela Ilie[3], Roxana Vintila[4]

[1] Clinica de Reproducere Umana Mures, Romania
[2] Academia Romana Bucuresti, Romania
[3] Statiunea de Cercetare Dezvoltare pentru Cresterea Bovinelor Arad, Romania
[4] Universitatea de Medicina si Farmacie „Victor Babes" Timisoara, Romania

Abstract

Cytoplasm donation by transferring a small amount (5-15%) of ooplasm to boost mitochondrial number and quality is increasing the energy output necessary to fuel the embryo through pre-implantation development. In this study we have investigated the possibility of transferring fluorescence- labelled mitochondria by ooplasm manipulation using a Piezo micromanipulator facilitated injection. We performed mouse inter-strain mitochondria transfer from C57BL/6 oocytes to CD1 zygots. The mitochondria were fluorescent stained with MitoTracker GreenFM and droplet of approximately 5% of the CD1 oocytes donor ooplasm, were injected into the CD1 zygotes. A control group was injected with only buffer to compare the effect of ooplasma and buffer injection.

The embryos mitochondrial heteroplasmy was detected using fluorescence microscopy and we have analyzed the effects of ooplasm injection on "in vitro" development of CD1 zygots. The cleavage, morula and blastocysts developmental rate (30±4.5%, 22±29.7%, 19±25.6%) was significant lower comparing with the control group (53±100%, 45±84.9%, 40±75.4%). The mitochondrial heteroplasmic embryos were associated with subtle, transient effects on preimplantational development. Our results indicate that cytoplasmic mitochondria donation by Piezo micromanipulator injection may be an efficient method for producing heteroplasmic animals, even exerting subtle effects on growth early in life.

Key words: mithocondria, micromanipulator, inter-strain, ooplasm

INTRODUCTION

Cytoplasmic transfer is an assisted reproductive technique that involves the infusion of ooplasm from a donor oocyte into a recipient oocyte or zygote of inferior developmental competence. Although this technique has shown some success for couples with recurrent in vitro fertilization failure, it results in mitochondrial heteroplasmy in the offspring, defined as the presence of two different mitochondrial genomes in the same individual. Because the long-term health consequences of mitochondrial heteroplasmy are unknown, there is a need for appropriate animal models to evaluate any physiological changes of dual mtDNA genotypes. This study was designed as a preliminary screen to create heteroplasmic mice by using a new method: Piezo micromanipulator facilitated injection. Piezo micromanipulation involves a simple and easily made injection pipette of very thin diameter and wall thickness. The piezo actuator (Prime-Tech, Japan) attaches to conventional micromanipulators and acts to mechanically advance the pipette tip through the zona pellucida using a piezoelectric effect [4]. The new created heteroplasmic strain could be future used for basic physiological functions for heteroplasmic effect.

Mitochondrial heteroplasmy it is an important topic in the reproductive sciences with the immediate application in as a fertility treatment by cytoplasmic transfer. This treatment was based on results of earlier animal experiments involving mouse embryos from strains that experience a developmental block.

*Corresponding author: clau_genetic@yahoo.com
The manuscript was received: 09.03.2012
Accepted for publication: 19.11.2012

There have been several concerns surrounding both the scientific methodology and ethics of performing this technique in the clinical setting [2], as the underlying mechanism and the long-term safety of cytoplasmic transfer remain unknown.

A number of ooplasm components are transferred from the donor oocyte to the recipient oocyte or zygote, including mRNA, proteins, ribosome, and other organelles, including mitochondria. The injection of multiple factors has, therefore, hampered the identification of the cytoplasmic factor responsible for improved developmental potential. We believe it is likely that mitochondria play a significant role in the embryonic rescue, as the injection of an enriched mitochondrial fraction, isolated from granulosa or embryonic stem cells, is capable of reducing the incidence of in vitro fragmentation of oocytes [1, 3].

MATERIAL AND METHODS

Unless indicated otherwise, all chemicals, media were purchased from Sigma 106 Chemical Co. St. Louis, MO, USA and plastics from Corning, Inc. Corning, NY, USA.

Reagents and culture media: CZB▪HEPES (CZB▪H), KSOM**,** PVP solution**,** Embryo-tested bovine testis hyaluronidase stock, Mineral oil, PMSG and hCG for superovulation.

Preparation of Oocytes

C57BL/6 strain female mice were superovulated by intraperitoneal injection of 5 IU of PMSG followed at 48 h with 5 IU of hCG. Oocytes were collected by rupturing the oviducts at 15–17 h after hCG injection. Oocytes were treated with 0.1% bovine testicular hyaluronidase in CZB-Hepes medium to dissociate cumulus cells.

Preparation of Zygots

For collection of zygotes, CD1 females were superovulated (as previous described) and mated with CD1 males. Zygotes were collected from the oviducts at 15–17 h after hCG injection.

Mitochondria staining

Before preceding the ooplasm isolation, the oocytes were incubated in KSOM medium containing 20 nM mitochondrion-specific vital dye MitoTracker green FM for 15-20 min at 37ºC (fig. 1).

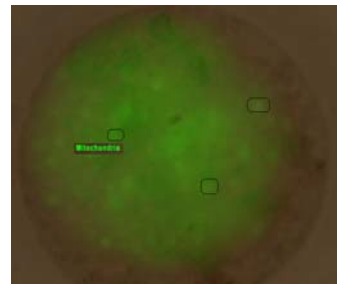

Fig. 1 Mitochondria staining in C57BL/6 oocytes

Mitochondrial injection

The zona pellucida of C57BL/6 oocyes was removed using Tyrode acid and ooplasmic droplets were isolated (fig. 2)

All micromanipulations were performed by using Narishige micromanipulation system installed on Olympus IX 71 microscope.

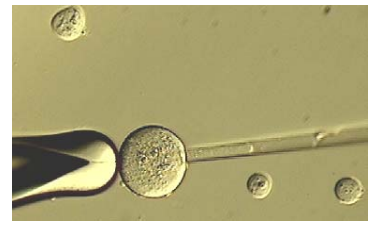

Fig. 2 Isolation of the ooplasm dropets

Zygotes were placed in CZB medium with cytoskeleton inhibitors for microsurgery (5µg/ml cytochalasin B); Droplet of approximately 5% of the donor ooplasm, were injected into the CD1 zygotes using a Piezo micromanipulator facilitated injection.

A control group was injected with only CZB-Hepes buffer to compare the effect of ooplasm and buffer injection.

Fig. 3 Intraooplasmatinc injection of foreigner ooplasma droplets

Fluorescence- labeled mitochondria were detected after injections using fluorescence microscopy with the FITC/GFP filter (excitation 520 nm, emission 450–490).

Surviving zygotes were incubated in KSOM medium for 3 days at 37ºC under 5% CO2 in air until the blastocyst stage, and the preimplantational development was scored.

RESULTS AND DISCUSSIONS

Our overall objective was to employ a mouse genetic model to evaluate the possibility of creating heteroplasmic mice by using a new method: Piezo micromanipulator facilitated injection.

Detection of embryos mitochondrial heteroplasmy

The active mitochondrial distribution in the heteroplasmic embryos was detected using a Nikon inverted microscope equipped with a fluorescent lamp (FITC/GFP filter, excitation 520 nm, emission 450–490).

The presence of the fluorescent labelled oocyte donor mitochondria was detected in all stages of the embryos development (fig. 4). The green staining intensities were determined in different regions of the embryos cytoplasm.

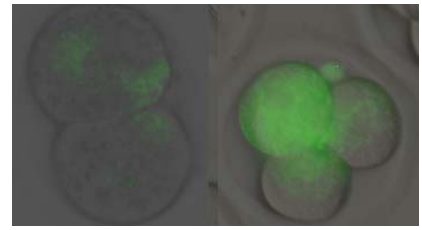

Fig. 4 Foreigner mitochondria presence in 2 and 4 cells stage heteroplasmic embryos

A group of seventy-four CD1 zygotes was injected with donor C57BL/6 ooplasmic droplets and a group of 53 was injected with CZB-H. We have analyzed the effects of ooplasm injection and we have compared the two groups “in vitro” development. From the resulted data presented in the table 1 we can observe that the cleavage, morula and blastocysts developmental rate (30±4.5%, 22±29.7%, 19±25.6%) in the heteroplasmic group was lower comparing with the control group (53±100%, 45±84.9%, 40±75.4%).

Table 1 Effects of ooplasm injection on “in vitro” development of CD1 zygotes

Injected	Total	Cleaved	4-8 cells	Morula	Blastocyst
Ooplasm	74	30 (4.5%)	28 (37.8%)	22 (29.7%)	19 (25.6%)
CZB-H Buffer	53	53 (100%)	51 (96.2%)	45 (84.9%)	40 (75.4%)

Comparisons of preimplantational growth rates of heteroplasmic and control embryos we concluded that the mitochondrial heteroplasmy was associated with subtle, transient effects on preimplantational development. These results can be related with the quality of the oocyte cytoplasm and whether the mechanical manipulation, is affecting the preimplantational development of the embryo.

Staining of the mitochondria using fluorescent MitoTracker green FM heteroplasmy indicate that donor mitochondria segregated random in the embryos recipient cytoplasm.

CONCLUSIONS

Mitochondrial heteroplasmy became an important topic in the reproductive sciences with the development of cytoplasmic transfer as a fertility treatment. This treatment is based on results of animal experiments; therefore there is a need for appropriate animal models to evaluate any physiological changes of dual mtDNA genotypes.

This pilot study was designed as a preliminary screen to create heteroplasmic mice by using a new method *Piezo* micromanipulator facilitated injection.

Our method combined with fluorescent mitochondria detection, may be an efficient way for producing heteroplasmic animals, even exerting subtle effects on growth early in life.

ACKNOWLEDGEMENTS

This work was co-financed from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 "Postdoctoral school for zoo-technical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by eco-san-genesis" and POSDRU/89/1.5/S/62371 "Postdoctoral School of Agriculture and Veterinary Medicine".

REFERENCES

[1]. Cean Ada, Alexandru T. Bogdan, Nicolae Păcală, Alexandra Ivan, Daniela E. Ilie, Mammalian Oocyte Cryopreservation - Review Scientific Papers: Animal Science and Biotechnologies, Timisoara, Vol 44 (1)- 2011, p 370-375, ISSN 1221-5287, E-ISSN 1841-9364

[2]. Hawes SM, Sapienza C, Latham KE. Ooplasmic donation in humans: the potential for epigenic modifications. Hum Reprod 2002; 17:850–852

[3]. Perez GI, Acton BM, Jurisicova A, Perkins GA, White A, Brown J, Trbovich AM, Kim MR, Fissore R, Xu J, Ahmady A, D'Estaing SG, et al. Genetic variance modifies apoptosis susceptibility in mature oocytes via alterations in DNA repair capacity and mitochondrial ultrastructure. Cell Death Differ 2007; 14:524–533.

[4]. Yoshida N, Perry AC. Piezo-actuated mouse intracytoplasmic sperm injection (ICSI). Nat Protoc 2007; 2:296–304.