Generalitat de Catalunya

Unión Europea

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BUENAS PRÁCTICAS
Operaciones Cofinanciadas

"Development of a new TRA Procedure: Meiotic Spindle Transfer".

Agency for Business Competitiveness (ACCIÓ)

Programa Operativo de Cataluña

Fondo Europeo de Desarrollo Regional

Año 2017

GOBIERNO DE ESPAÑA
MINISTERIO DE HACIENDA Y FUNCIÓN PÚBLICA
SECRETARÍA DE ESTADO DE PRESUPUESTOS Y GASTOS
DIRECCIÓN GENERAL DE FONDOS EUROPEOS

Submitted as Good Practice: the MST project "Development of a new TRA Procedure: Meiotic Spindle Transfer".

This project forms part of a line of action devoted to research and innovation processes involving SMEs (including systems of vouchers, processes, design, services and social innovation). More specifically, it responds to the call for proposals for Industrial Research and Experimental Development Clusters, aimed at cofinancing projects that focus on local and international industrial research and experimental development. The body responsible for managing this call for proposals is the Agency for Business Competitiveness (ACCIÓ) of the Government of Catalonia's Ministry of Business and Knowledge.

Developed by the firm of Embryotools S.L., the project is aimed at formulating an innovative strategy to prevent the transmission of mitochondrial diseases and problems of cytoplasmic origin without the need for women to give up the possibility of giving birth to biological children.

The project is aimed at addressing a need that is not covered at present. To date, no treatment has been developed that resolves the problem of the transmission of mitochondrial diseases, and women have to resort to oocyte donation processes. To this end, the project involves research into an innovative technique of oocyte enucleation in MII for the subsequent transfer of the meiotic spindle to another enucleated oocyte. In this way, an oocyte is obtained that will have maternal nuclear DNA (giving rise to a biological son) and donated cytoplasm (healthy). Work has been conducted with mouse oocytes to validate the effectiveness and clinical safety of the process at molecular level.

The research was carried out with the collaboration of the research group led by Dr Wells (Oxford University). The research group is a cutting-edge player in the field of preimplantation genetic diagnosis (PGD).

The project will enable sufficient evidence to be obtained to apply to the National Ethics Committee for authorisation to proceed to the human model.

The trial with humans will take place in a second stage in which strategic alliances could be formed with other international companies and applications to join European programmes could be made.

This is a clear example of an R&D project with innovations at world level that enables Embryotools to position as a pioneering biotechnology company in the research and development of techniques and products to improve assisted reproduction treatments in the Catalan health industry.

The MST project was cofinanced by the ERDF OP Catalonia 2014-2020 within the framework of the "local and international industrial research and experimental development clusters" line of support. The aim of this line of support, managed by ACCIÓ, is to provide incentives for the implementation of industrial research and experimental development activities in the sectors described in the RIS3 strategy for Catalonia.

The project has a total and eligible cost of 188,768.75 euros and ERDF support of 68,353.17 euros. The results obtained from it have already had a significant impact on the embryology

sector, and clinics, hospitals and research centres have expressed interest, particularly, in extending their knowledge in such a new field as Meiotic Spindle Transfer (MST). The project has also helped to position the company itself in this field.

This operation is presented as Good Practice because it meets the following criteria:

1. High level of dissemination among beneficiaries, potential beneficiaries and the general public

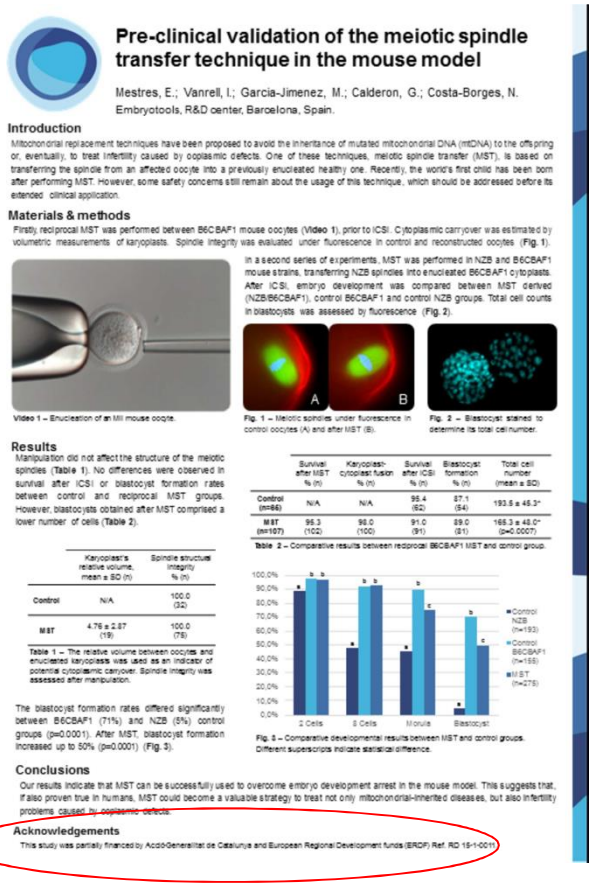
A series of actions were conducted to disseminate the project and its results, all of them complying to the specifications as regards the communication and identification of operations cofinanced by the ERDF OP 2014-2020. Moreover, the R&D activity carried out as part of the project was appropriately identified at the company's facilities:

More specifically, the dissemination actions were:

- Publicity included on the company's website. The company mentions the project on the "Research and Development" section of its website, noting the ERDF cofinancing and the support of the Government of Catalonia through ACCIÓ https://embryotools.com/es/research-and- development/

- Dissemination of project results at congresses and through international dissemination actions.

  - **ESHRE (European Society of Human Reproduction and Embryology) Congress,** July 2017, Geneva - Abstract and poster - approximately 10,000 attendees



Pre-clinical validation of the meiotic spindle transfer technique in the mouse model

Mestres, E.; Vanrell, I.; Garcia-Jimenez, M.; Calderon, G.; Costa-Borges, N.
Embryotools, R&D center, Barcelona, Spain.

Introduction

Mitochondrial replacement techniques have been proposed to avoid the inheritance of mutated mitochondrial DNA (mtDNA) to the offspring or, eventually, to treat infertility caused by ooplasmic defects. One of these techniques, meiotic spindle transfer (MST), is based on transferring the spindle from an affected oocyte into a previously enucleated healthy one. Recently, the world's first child has been born after performing MST. However, some safety concerns still remain about the usage of this technique, which should be addressed before its extended clinical application.

Materials & methods

Firstly, reciprocal MST was performed between B6CBAF1 mouse oocytes (**Video 1**), prior to ICSI. Cytoplasmic carryover was estimated by volumetric measurements of karyoplasts. Spindle integrity was evaluated under fluorescence in control and reconstructed oocytes (**Fig. 1**).

In a second series of experiments, MST was performed in NZB and B6CBAF1 mouse strains, transferring NZB spindles into enucleated B6CBAF1 cytoplasts. After ICSI, embryo development was compared between MST derived (NZB/B6CBAF1), control B6CBAF1 and control NZB groups. Total cell counts in blastocysts was assessed by fluorescence (**Fig. 2**).

Video 1 – Enucleation of an MII mouse oocyte.

Fig. 1 – Meiotic spindles under fluorescence in control oocytes (A) and after MST (B).

Fig. 2 – Blastocyst stained to determine its total cell number.

Results

Manipulation did not affect the structure of the meiotic spindles (**Table 1**). No differences were observed in survival after ICSI or blastocyst formation rates between control and reciprocal MST groups. However, blastocysts obtained after MST comprised a lower number of cells (**Table 2**).

	Karyoplast's relative volume, mean ± SD (n)	Spindle structural integrity % (n)
Control	N/A	100.0 (32)
MST	4.76 ± 2.87 (19)	100.0 (75)

Table 1 – The relative volume between oocytes and enucleated karyoplasts was used as an indicator of potential cytoplasmic carryover. Spindle integrity was assessed after manipulation.

	Survival after MST % (n)	Karyoplast-cytoplast fusion % (n)	Survival after ICSI % (n)	Blastocyst formation % (n)	Total cell number (mean ± SD)
Control (n=84)	N/A	N/A	96.4 (82)	87.1 (54)	193.5 ± 46.3[a]
MST (n=107)	96.3 (102)	98.0 (100)	91.0 (91)	89.0 (81)	166.3 ± 48.0[c] (p=0.0007)

Table 2 – Comparative results between reciprocal B6CBAF1 MST and control group.

The blastocyst formation rates differed significantly between B6CBAF1 (71%) and NZB (5%) control groups (p=0.0001). After MST, blastocyst formation increased up to 50% (p=0.0001) (**Fig. 3**).

Fig. 3 – Comparative developmental results between MST and control groups. Different superscripts indicate statistical difference.

Conclusions

Our results indicate that MST can be successfully used to overcome embryo development arrest in the mouse model. This suggests that, if also proven true in humans, MST could become a valuable strategy to treat not only mitochondrial-inherited diseases, but also infertility problems caused by ooplasmic defects.

Acknowledgements

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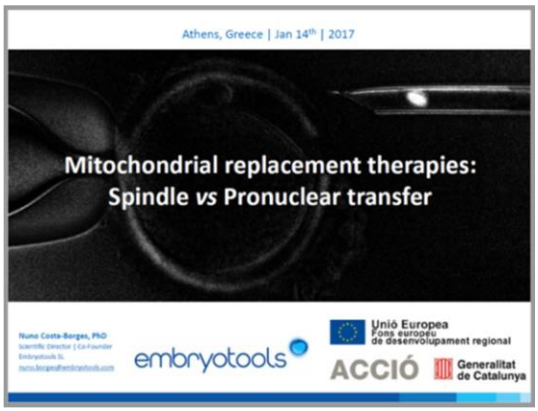
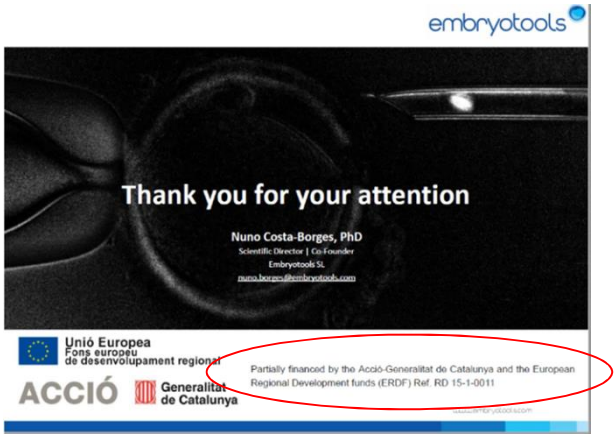
Poster at ESHRE – Dissemination of results indicating support received from the European Union through the ERDF and the management of ACCIÓ.

  - **ASRM (American Society for Reproduction Medicine) Congress**, October 2017, San Antonio, Texas (USA) - Abstract selected for presentation - approximately 10,000 attendees and competition with prize for best paper.

  - **ASEBIR (Association for the Study of Reproductive Biology) Congress**, November 2017, Madrid - Abstract selected for oral communication - approximately 1,000 attendees

  - **Dissemination at international event** (Athens, November 2107). Paper delivered by the researcher Dr Nuno Costa-Borges (co-founder of Embrytools)





- Presentation of the project at the kick-off session for projects approved at the 2016 call for proposals in the "R&D Clusters" line of financial support managed by ACCIÓ (the line of support under which the project was cofinanced through the ERDF as a result of the 2015 call for proposals).

  The company presented the project, its development and the results obtained as an example of a project subsidised through this support programme. The implementation of the project was shown as a successful case to the beneficiaries of the 2016 call for proposals with the aim of encouraging them to replicate the implementation model established by Embrytools.

- Press release and news of the success of the trial of a new technique to prevent the transmission of diseases were reported in several media (La Vanguardia, EFE, etc.)

2. Inclusion of innovative elements

The main objective of the project is the research and development of a new mechanical technique for the transfer of the maternal spindle in murine oocytes retained at the MII (Metaphase II) stage, as well as to evaluate the potential of the technique to become a new TRA (transference) procedure at in vitro clinical fertilisation laboratories.

At the start of the project, the first steps had already been done in research into the transfer of the nuclear genome using oocytes at MII, but there were still many limitations that needed to be addressed. The fact is that, currently, there is no cure for mitochondrial disorders and the treatments available merely alleviate the symptoms and delay its advance.

Moreover, when this project was first launched, no effective technology existed to perform the transfer of the chromosomal complex (meiotic-chromosome spindle) of oocytes retained at Metaphase II that could prevent the transmission of mutations in the mitochondrial DNA from mother to child.

The implementation of this project enabled the development of an innovative mechanical technique to transfer the maternal or meiotic spindle into murine oocytes retained at the MII (Meiosis II) stage in order to prevent inherited mitochondrial disorders, as well as to evaluate its potential as a new TRA procedure at clinical in vitro fertilisation laboratories. The development of this project lays the foundations for the leap to the human model and to provide a new TRA procedure, so that both women affected by mitochondrial diseases and those with problems of infertility caused by ovoplasmic defects will have the possibility of having their own healthy biological children, avoiding the need for them to join an oocyte donation programme.

3. Linkage between results obtained from the supported actions and the objectives established

The results of the MST project are perfectly aligned with the objectives established in the project. The activities and trials planned were duly conducted, and the results obtained led to useful conclusions. In some cases, impacts on innovation and knowledge (described in the previous section) have already been identified, corroborating the linkage between objectives and results.

4. Contribution to resolving a regional problem or weakness

This project and the results obtained from it lay the foundations for the provision of a new TRA treatment, both for women affected by mitochondrial diseases and those with problems of infertility caused by ovoplasmic defects, giving them the possibility of having their own healthy biological children and avoiding their need to join an oocyte donation programme.

5. Degree of coverage of the target population

Thanks to the approach taken and the nature of the project, the treatment developed can be offered at all hospitals and to all women in the circumstances described above, without exception. Similarly, the innovation of the treatment and its benefits have a direct impact, not only on women, but also on couples who plan for the birth of a biological son or daughter using this technique, and on society in general, as the technique prevents the transmission of certain hereditary diseases.

6. Compliance with horizontal principles (sustainable development, equality between men and women and the principle of non-discrimination) and environmental legislation

The project was implemented in compliance with Community, state and regional laws concerning gender policy and environmental sustainability.

The project focuses on developing a treatment specifically for women and couples, increasing their chances of conceiving biological children.

7. Synergies with other policies or instruments of public intervention

International public support programmes that could be applied to, based on the results of this project, have been identified.

As an example of the project's linkage to the HORIZON 2020 themes of interest, with regard to the Societal Challenges defined, the initiative is aligned with the challenge: "Health, demographic change and wellbeing". This challenge focuses on finding ways of keeping older people active, as well as improving our understanding of the causes and mechanisms underlying health, healthy ageing and disease. In this sense, the project developed by Embryotools amply meets the objectives as set out in the Horizon 2020 programme: it enhances scientific knowledge about how to address inheritable diseases caused by mitochondrial anomalies, offering alternatives to increase the viability of reproductively limited women becoming pregnant.

Another objective that the European programme establishes regarding this societal challenge is the improvement our ability to monitor health and to prevent, detect, treat and manage diseases.