

IMPROVEMENT OF ICSI OUTCOME USING SPERMATOZOA SELECTED BY THERMOTAXIS

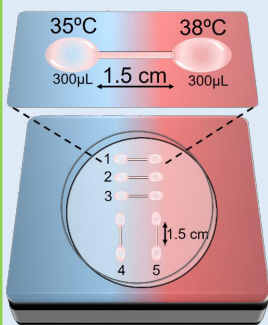
S. PÉREZ-CEREZALES, R. FERNÁNDEZ-GONZÁLEZ, A. CHACÓN DE CASTRO, B. RODRÍGUEZ ALONSO, M.J. SÁNCHEZ-CALABUIG, R. LAGUNA-BARRAZA, A. GUTIÉRREZ-ADÁN

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria; Departamento de Reproducción Animal, INIA, 28040 Madrid, Spain



INTRODUCTION: The intracytoplasmic sperm injection (ICSI) has been shown to report low efficiency as Assisted reproductive technology (ART). This could be attributed to the incompetence of the used spermatozoa because of an inefficient selection. One of the strategies suggested to improve this efficiency is by developing new methods for selecting the spermatozoa. Here we show that spermatozoa selected by their ability to migrate within a gradient of temperature by thermotaxis carry high integrity genetic material. Furthermore, we report that the use of mice spermatozoa selected by thermotaxis significantly increase the ICSI outcome.

MATERIAL AND METHODS:



1- Sperm selection by thermotaxis:

Mice and human spermatozoa were selected by their ability to migrate within a temperature gradient towards the warmer temperature in an *in vitro* system shown in Figure 1. As control, spermatozoa were also prepared by Swim-Up.

2- Sperm DNA integrity (comet assay):

DNA fragmentation was analysed in spermatozoa before and after preparation by Swim-up or thermotaxis.

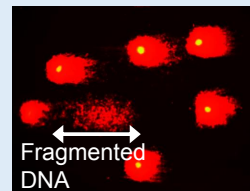


Figure 2: Comet assay.

Microphotography of spermatozoa subjected to neutral comet assay. The arrow borders the fraction of fragmented DNA of one spermatozoa. The technique allows to quantify in individual cells the degree of fragmented DNA.

3- ICSI with selected spermatozoa in mouse:



ICSI was performed with mouse spermatozoa separated by Swim-Up or by thermotaxis. The embryo development was followed *in vitro* and *in vivo*.

Figure 1: System for sperm selection by thermotaxis *in vitro*. Two drops of medium (300 µL) are connected by a capillary (1.5cm) fixed on a petri dish located on a metallic plate. Half of this plate is placed on a heater plate to create the temperature gradient as shown. For sperm selection, the spermatozoa are loaded in one of the capillary edges (the colder edge) and left to migrate for a specific time (Capillaries 1 to 3). Capillaries 4 and 5 are used for monitoring coincidental accumulation.

RESULTS:

Sperm thermotaxis and DNA damage

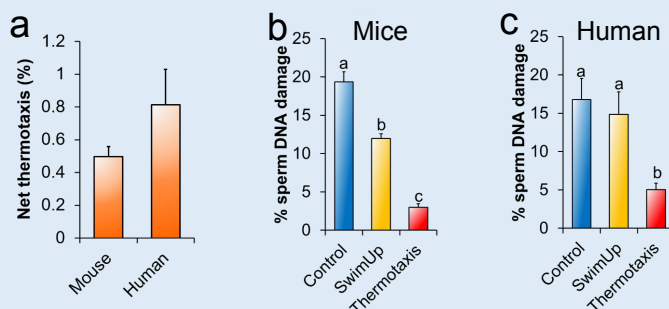


Figure 3: Sperm thermotaxis (a) and DNA damage of mouse (b), and human (c) spermatozoa. (a) The net thermotaxis is calculated as the percentage of spermatozoa from the total loaded in the cold compartment that migrated to the warm compartment after the subtraction of the coincidental accumulation (Figure 1). In average the number of spermatozoa recovered per separation was of 10786 ± 1721 (mouse, $n=14$) and 23462 ± 5322 (human, $n=8$) (average \pm SD). (b,c) percentage of fragmented DNA in each sperm sample. Results are represented as average \pm SEM. Different letters above columns indicate significant difference between the sperm samples (two way ANOVA, $P<0.05$), ($n=7$ and 5 for mouse and human respectively, ~ 50 spermatozoa per replicate).

Development of ICSI derived embryo in mouse

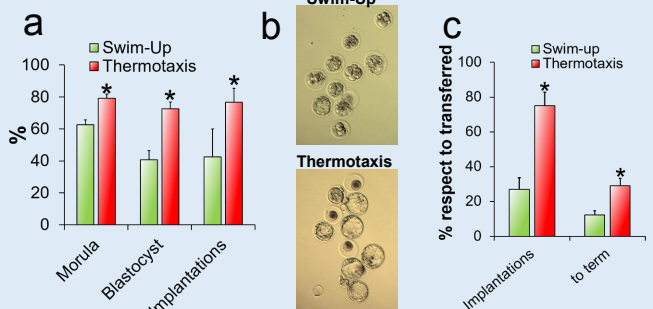


Figure 4: Development of ICSI derived embryos cultured *in vitro* to blastocyst stage (a and b) or transferred in two cell stage (b). (a) The living blastocysts were transferred and 11 days later implantation rate was determined ($n=8$, 122 and 126 *in vitro* cultured embryos). (b) representative image of *in vitro* cultured blastocysts at day 4 after ICSI. Notice the differences in morphology of blastocysts obtained with sperm separated by Swim-up or by thermotaxis. (c) 2 cell embryo were transferred and 15 days later implantation was analyzed ($n=10$, 97 and 130 embryos). A group of pregnant females were left to analyze pregnancy to term ($n=15$, 150 and 202 embryos). (a and c) (Student's t-test, $*P<0.05$)

CONCLUSIONS: Selection by thermotaxis of mouse and human spermatozoa resulted in an enrichment in spermatozoa with low fragmented DNA. In mouse, the use of this selected sperm fraction significantly improved the ICSI outcome by increasing the percentage of embryo reaching to blastocyst stage in *in vitro* culture as well as implantation and live births rates after 2-cell embryo transfer.

ACKNOWLEDGEMENTS: This work was funded by Grant AGL2012-39652-C02-01 and S. Pérez-Cerezales was supported by project FPD1-2013-18402 both of the Spanish Ministry of Economy and Competitiveness.