



Laser assisted IVF or modified ICSI each markedly improve the efficiency of IVF in C57BL/6J mice

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ABSTRACT

Assisted reproductive technologies (ART) are an essential component of a modern mouse husbandry program. Of all ARTs, in vitro fertilization (IVF) has, perhaps, the most potential because only a single male is necessary to provide a large number of pups. Unfortunately, in some inbred strains of mice, IVF is not highly efficient. Recently, we tried two modifications of IVF to see if we could increase the effectiveness. Specifically, we performed laser assisted IVF and a simplified version of intracytoplasmic sperm injection. All studies described herein utilized wild-type C57BL6/J oocytes and sperm. Laser assisted IVF was performed utilizing a xyclone ablator (model # 270384) to make two to three holes in the zona pellucida of eggs. Ablated oocytes were co-cultured with sperm in Human Tubal Fluid (HTF) for 5-hours, washed and cultured overnight in KSOM at 37°C in 5% CO₂. Modified ICSI was conducted utilizing simple, manual microinjection of sonicated sperm heads deposited within the oolemma with gentle, positive, micropipette, air pressure. A sperm microinjection micropipette was calibrated in-house for easy aspiration of sperm cells and entry past the zona and oolemma barriers while averting oocyte lysis. After injection of eggs with sperm in fluid holding medium (FHM), eggs were transferred to KSOM and incubated overnight at 37°C in 5% CO₂. Standard IVF was conducted as described for laser assisted IVF without ablating the zona prior to incubation with sperm. Fertilization rate for all procedures was scored by analyzing the amount of eggs that proceeded to the two-cell stage following overnight incubation. Both Laser assisted IVF and the simplified ICSI method produced significantly greater fertilization rates (55% and 50% respectively) compared to standard IVF (2%; P<0.0001). In conclusion, both laser assisted IVF and the modified ICSI technique are simple, rapid methods to increase the fertilization rate of IVF in inbred mice.

INTRODUCTION

In vitro fertilization in mice utilizing standard methods is prone to failure. A variety of factors may contribute to failure of IVF including the inability of sperm cells to cross the zona pellucida or oolemma. Intracytoplasmic sperm injection (ICSI) alleviates these problems but ICSI requires a variety of specialized equipment, can be difficult to perform, and can be time consuming particularly for individuals that are new to the technique. Here we test the ability of two alternative approaches to improve standard IVF without the need for the costly equipment and extensive training of standard ICSI. Modified ICSI, utilizing standard manual microinjection, as well as laser-assisted IVF were tested in our transgenic facility in order to evaluate fertility of oocytes as compared to conventional IVF. Our results indicate that manual ICSI or laser-assisted IVF improve fertilization when compared to standard IVF.

MATERIALS AND METHODS

1. In Vitro Fertilization of mouse oocytes using Laser Assisted IVF

Laser-assisted IVF was conducted in a 100 ul microdrop of Fluid Holding Medium (FHM) at room temperature. Two to three 10 micron holes were made in the zona pellucida of oocytes (approximately 100 can be done in 20 minutes) utilizing a xyclone laser system (model # 270384). Ablated oocytes were then washed in two, 200 ul microdrops of Human Tubal Fluid (HTF) and then incubated in a third, 200 ul microdrop with sperm for 5-hours. Following fertilization, oocytes were washed in three 50 ul microdrops of KSOM medium and cultured in a fourth 50 ul of KSOM overnight at 37 °C.



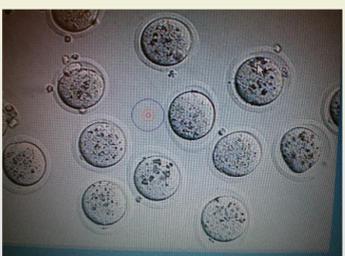
Laser Workstation



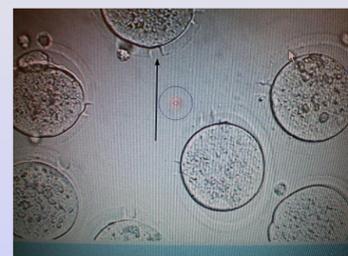
Sperm Cells



Oocytes with laser targeting apparatus



Oocytes with laser targeting apparatus



Laser-Ablated Zona Pellucida of Unfertilized Oocytes. Holes are highlighted with arrows



In Vitro Fertilization of mouse oocytes using manual ICSI

Manual ICSI was carried out in a 100 microliter drop of Fluid Holding Medium (FHM) at room temperature. Sonicated sperm was added to the microdrop. A sperm microinjection pipette was especially calibrated in order to ensure easy aspiration of sperm cells and entry past the zona and oolemma barriers without oocyte rupture (R6 glass: OD 1mm, ID .8mm; microinjection pipette: OD 8 microns, ID 5 microns; handle length, 6cm; taper length, 100 reticule units at 1.5 X de Fonbrune microforge angle, 40 microns tip travel distance on x axis, away from Y axis at 1.5 X de Fonbrune microforge). The micropipette was created with a short, sharp, sturdy tip and was siliconized overnight in a siliconizing jar placed in a culture hood. The sperm head was carefully deposited beneath the oolemma with gentle positive micropipette air pressure. Manually microinjected oocytes were cultured in 60 microliters of KSOM under embryo culture oil. Culture dishes were cultured overnight at 37°C for embryo morphology score.

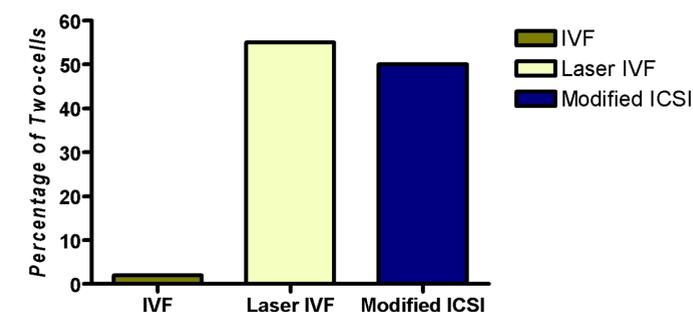


ICSI Workstation



Manual ICSI: -Sperm Microinjection

RESULTS



Both laser assisted IVF and the modified ICSI technique significantly (P<0.05) increased the percentage of B6 oocytes progressing to the 2-cell stage. 2% (2/114) of oocytes subjected to standard IVF progressed to the 2-cell stage. In contrast, 55% (70/128) of laser-assisted IVF oocytes and 50% (23/46) of oocytes subjected to the modified ICSI progressed to the two-cell stage.



Live B6 mice produced by laser assisted IVF are pictured next to their CD-1 recipient mother

CONCLUSIONS

- 1) Either laser-assisted IVF or the modified ICSI technique dramatically improve the efficiency of sperm recovery
- 2) Manual microinjection may be a faster less expensive option compared to conventional ICSI. Conventional ICSI requires a 3-microdrop microinjection method, the use of hazardous mercury and an expensive piezo device whereas the modified technique described here utilizes a standard microinjection approach with a single drop
- 3) Laser assisted IVF offers remarkable advantage over conventional IVF; zona ablation is computer-aided and rapid.

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