

# Laser Assisted IVF: A Simple Technique To Improve IVF In Mice Without Micromanipulation

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## Introduction

- Spermatozoa from the inbred mouse C57BL/6J strain provides a useful model to study male infertility, as they produce a poor fertilization rate with IVF.
- Piezo-ICSI is the preferred method for zygote production in this strain. However, ICSI:
  - Risks spindle disruption as the technique is performed at room temperature
  - Risks injecting genetically abnormal sperm & accidental transgenesis
  - Is a time-consuming procedure requiring micromanipulation
  - Bypasses the multiple steps of normal fertilisation
- Partial zona dissection (PZD) is a technique whereby holes are cut into the zona pellucida (ZP). Performing PZD on oocytes prior to insemination facilitates sperm contact with the oolemma.
- Previously UV laser PZD was used to facilitate IVF, but UV lasers were abandoned due to risks of cytotoxicity and mutagenesis.
- Newly developed infrared lasers eliminate concerns of UV lasers.
- PZD assisted IVF using infrared lasers such as the XYClone (Hamilton Thorne Biosciences, Beverly, USA) has yet to be assessed in the mouse.
- Our objective was to examine the efficiency of laser PZD assisted IVF using C57BL/6J spermatozoa and oocytes from the C57BL/6J and hybrid B6D2F1 strains.

## Materials and Methods

- Oocytes were allocated to either laser PZD assisted IVF or standard IVF.
- Groups of 20 denuded oocytes were transferred to 4 x 20 µl droplets of Mouse Vitro Cleave culture medium (Cook, Brisbane, Australia) in a tissue culture dish (Falcon 1006, Fahrenheit, UK), with 5 oocytes per droplet.
- The dish was placed on a heated stage set to maintain the oocytes at 37°C.
- Using a stage controller to move the dish, 3 holes were cut into the ZP using the XYClone 1480nm diode laser attached to a 40x objective.
  - Oocytes were then inseminated with 2 x 10<sup>6</sup> motile C57BL/6J spermatozoa/ml at 37°C and 5% CO<sub>2</sub> in air.
- Fertilization, blastocyst and hatching rates were assessed on day 4 of development.



## Results



**TABLE 1 - EFFECT OF LASER PZD ASSISTED IVF COMPARED TO CONVENTIONAL IVF FOR C57BL6J & B6D2F1 OOCYTES**

| TREATMENT           | CONVENTIONAL IVF |                          |               | LASER PZD ASSISTED IVF     |                            |                |
|---------------------|------------------|--------------------------|---------------|----------------------------|----------------------------|----------------|
|                     | C57BL6J          | B6D2F1                   | TOTAL         | C57BL6J                    | B6D2F1                     | TOTAL          |
| N                   | 71               | 61                       | 132           | 69                         | 65                         | 134            |
| 2-CELL              | 13<br>(18.3%)    | 1<br>(1.6%) <sup>a</sup> | 14<br>(10.6%) | 61<br>(88.4%) <sup>b</sup> | 60<br>(92.3%) <sup>b</sup> | 121<br>(90.3%) |
| BLASTOCYST          | 3<br>(4.2%)      | 0<br>(0.0%)              | 3<br>(2.3%)   | 28<br>(40.6%) <sup>c</sup> | 50<br>(76.9%) <sup>d</sup> | 78<br>(58.2%)  |
| HATCHING BLASTOCYST | 0<br>(0.0%)      | 0<br>(0.0%)              | 0<br>(0.0%)   | 3<br>(4.3%)                | 41<br>(63.1%) <sup>e</sup> | 44<br>(32.8%)  |

Values within rows with different superscripts (<sup>a,b,c,d,e</sup>) significantly differ (P<0.001)

- Up to 20 oocytes were lasered in less than 5min.
- Laser PZD assisted IVF significantly improved fertilization rates compared to zona intact oocytes (88.4% vs. 18.3% for C57BL/6J; 92.3% vs. 1.6% for B6D2F1).
- There were significant differences between inseminated B6D2F1 and C57BL/6J oocytes in development to the blastocyst (76.9% vs. 40.6%) and hatching blastocyst stage (63.1% vs. 4.3%).

## Conclusions

- Zona laser dissection of mouse oocytes significantly improved the fertilization rate following IVF.
- This simple swift technique avoided micromanipulation and problems associated with ICSI. Furthermore, the reduced operator time allowed numerous oocytes to be treated in a short period.
- Differences in embryonic development highlighted inbred and hybrid strain differences.

