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<b>Protocol Section:</b>	<b>Assisted IVF</b>	<b>Policy No:</b>	P-CMMR3-ICSI-00
<b>Protocol Subject:</b>	<b>Intracytoplasmic Sperm Injection (ICSI)</b>	<b>Effective Date:</b>	
		<b>Date Reviewed:</b>	6 January 2003
		<b>Date Revised:</b>	

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## 1.0 Animals

- 1.1 Oocyte donor female mice (a chosen strain, 4-6 weeks old or older)
- 1.2 Sperm donor Male mouse or frozen-thawed sperm
- 1.3 Recipient female mice (pseudopregnant CD1s)

## 2.0 Materials and Equipment

- 2.1 Reversed microscope with micromanipulation arms.
- 2.2 Piezo drill unit
- 2.3 Stereo microscope.
- 2.4 CO<sub>2</sub> incubator.
- 2.5 Disposable sterile plastic ware:
  - Filters, 0.22µm pore size
  - Pipettes
  - Petri dishes
- 2.6 Watchmakers forceps
- 2.7 Scissors
- 2.8 Mouth pipette.
- 2.9 70% ethanol.

## 3.0 Reagents

- 3.1 Superovulation hormones (Pregnant mare serum gonadotropin, PMSG and human chorionic gonadotropin, hCG).
- 3.2 MEM (GIBCO).
- 3.3 KSOM
- 3.4 Bovine Serum Album (BSA).
- 3.5 Fetal bovine serum (FBS).
- 3.6 Hepes and polyvinylpyrrolidone (PVP)
- 3.7 Hyaluronidase (Sigma)
- 3.8 Mineral oil

## 4.0 Methods

- 4.1 Holding and injection pipettes setup

The injection pipette and the holding pipette are directed at the focal plane and the positions are stored. The injection pipette is equipped with a Piezo drill unit. Sperm and oocytes are put onto the petri dish in several drops of medium cover with oil.

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#### 4.2 Sperm Preparation

Fresh collected or frozen-thawed sperm is prepared in accordance with the Mini Swim-Up process or dilute with 20mM HEPES buffered MEM + 10% FBS + 12% PVP. Magnified x20 times, a sperm cell is immobilized by means of a quick movement of the injection pipette across the tail, or by pressing the tail of the sperm cell against the bottom of the dish until it stops moving or by applying of Piezo Drill pulses. The sperm cell is aspirated, tail first into the injection pipette (inner diameter approx. 5–6  $\mu\text{m}$ ). Sperm tail can be cut by applying of Piezo Drill pulses.

#### 4.3 Oocyte preparation

Oocytes are collected from oviducts of superovulated mice and cumulus cells are removed by incubating oocytes in 0.1% hyaluronidase for 5-10 minutes. The operation media is 20mM HEPES buffered MEM + 10% FBS. The oocytes are brought into focus and held with the holding pipette (inner diameter approx. 10-15  $\mu\text{m}$ ). The oocyte is turned with the aid of the injection pipette until the polar body comes to rest either above (12 o'clock) or below (6 o'clock). In this position, the oocyte is held with the holding pipette.

#### 4.4 Sperm injection

The holding pipette and the oocyte, (magnification x40) are sharply focused. By moving the joystick slightly, the injection pipette is carefully pushed through the zona pellucida at 3 o'clock by applying Piezo Drill pulses. The sperm cell (or head) in the injection pipette is brought to the tip. The oocyte should be pricked in the middle and the oolema membrane is broken gently by applying one Piezo Drill pulse. The sperm cell is then injected. In order to introduce only one minimal volume of the medium or none at all, into the cytoplasm, the injection pipette is withdrawn gently after the head of the sperm cell has left the pipette tip and is no longer injected.

The injected oocyte is released from the holding pipette. If several oocytes are obtained, only 10 oocytes are injected as a rule. They are then placed into operating medium and the remaining oocytes are then injected.

If fertilization has occurred, the embryos are transferred into the oviducts of recipients shortly after microinjection or uterus after 3 days cultured in KSOM + 1mg/ml BSA.

### 5.0 Guidelines to customers

- 5.1 Alive animals are acceptable at the age of 3 weeks older. After that the younger the better.
- 5.2 Dead animals are acceptable only if the animals are dead within 12 hours and kept in 4 °C refrigerator.
- 5.3 Frozen semen is acceptable either stocked in – 80 °C or liquid nitrogen.
- 5.4 No contamination is accepted.
- 5.5 Call us for more information.

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## 6.0 References

- 6.1 Yasuyuki Kimura and Ryuzo Yanagimachi. Intracytoplasmic sperm injection in the mouse. *Biology of Reproduction*. 1995; 52: 709-720
- 6.2 Monika ASzczygiel, Hirokazu Kusakabe, Ryuzo Yanagimachi, and David G Whittingham. Intracytoplasmic sperm injection is more efficient than in vitro fertilization for generating mouse embryos from cryopreserved spermatozoa. *Biology of Reproduction*. 2002; 67: 1278-1284

**Issued by Lab Manager:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Approved by Facility Management:** \_\_\_\_\_ **Date:** \_\_\_\_\_