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Fact Sheet 29

# Sperm Micromanipulation

updated October 2011

**During fertilisation, spermatozoa have to penetrate the 'cloud' of cumulus cells surrounding the egg, then bind to and penetrate a shell-like structure, the zona pellucida, before reaching the egg cell itself, the oocyte. Finally, a single spermatozoon binds to the membrane of the oocyte, the oolemma, and fuses with the oocyte.**

**Because some cases of failure to fertilise during IVF were believed to be due to the inability of spermatozoa to penetrate the zona pellucida, in the mid-1980s scientists began trying to adapt techniques which were already being used in experimental work on laboratory and farm animals. Micromanipulation uses special instruments so that very small objects can be handled under the microscope. The various techniques used to micromanipulate spermatozoa into oocytes are sometimes referred to collectively as 'mechanically-assisted fertilisation'.**

Initially these instruments were simple mechanical devices but later models are hydraulic and/or electronic to allow even finer movement control. Due to the micron scale of movements being used (a micron is one thousandth of a millimetre, with a human hair being about 100 microns in diameter) special anti-vibration 'stable tables' are required to eliminate vibrations from footsteps, doors opening and closing, lifts operating nearby, or even traffic.

The tools used to actually handle the oocytes and spermatozoa are made from extremely fine glass capillary tubes that are heated, pulled, ground, etc, in the manufacturing facilities to make different instruments such as holding pipettes, (pointed glass needles), or injection pipettes of various designs. A single typical micromanipulation setup as used in many Australian IVF laboratories costs $85,000 (+ GST).

## Techniques Available

Several different techniques of mechanically-assisted fertilisation have been used by groups around the world, however all are used within the context of a normal IVF stimulation treatment cycle. The only differences are just exactly when the semen specimen is needed in relation to the oocyte retrieval, and the precise details of the actual insemination procedure. Before micromanipulation can be performed, the cumulus cells have to be removed by careful enzyme treatment. Today ICSI (intra-cytoplasmic sperm injection) is the commonly used technique worldwide.

### Zona drilling

The technique of zona drilling uses an acidic solution to make a hole in the zona pellucida so that poorly motile spermatozoa can swim through. Although this method showed great promise in studies using mouse oocytes, it does not work with human oocytes because they are too sensitive to acid. However, careful zona drilling of human embryos is a safe procedure and is the basis of assisted hatching.

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## Zona tearing/cracking

An example of zona tearing/cracking is where two tiny glass hooks are used to tear a hole in the zona pellucida. This approach is no longer used.

## Partial zona dissection (PZD)

PZD used a glass needle to cut a slit in the zona pellucida to allow poorly motile spermatozoa to swim through and reach the oocyte. This method is rarely used nowadays.

## Sub-Zonal Insertion (SUZI)

In 'SZI' or 'SUZI' glass pipettes were used to inject usually several spermatozoa through the zona pellucida into the perivitelline space. However, the spermatozoa still needed to be able to fuse with the actual membrane of the oocyte, and very few sperm from men with extremely poor sperm quality were able to do this. There was also the risk of more than one spermatozoon fertilising the oocyte; polyspermic embryos are genetically abnormal and cannot be transferred to the woman. Also, antibodies on the spermatozoa could block even this final step in the fertilisation process. SUZI has also been referred to as Direct Under-Zona Insemination (UZI or DUZI) and Micro-Insemination by Sperm Transfer (MIST).

SUZI has now been effectively replaced by ICSI throughout Australia owing to ICSI's higher fertilisation and pregnancy rates.

## Intra-Cytoplasmic Sperm Injection (ICSI)

ICSI was perfected by a group in Brussels (Belgium) and uses a very fine pipette to inject a single spermatozoon directly into the oocyte itself. While a spermatozoon that is at least 'twitching' must be taken for injection (this movement or motility being used as evidence that it is still alive), the spermatozoon must be immobilised before injection. Otherwise it will swim around inside the oocyte destroying its structure. As far as is known ICSI is unaffected by antibodies on the spermatozoa. One group in the United Kingdom also calls this method 'DISCO' (Direct Injection of Spermatozoa into the Cytoplasm of the Oocyte). However, it is now known as ICSI worldwide.

## Indications and Contra-Indications

Sperm microinjection (sometimes referred to as 'SMI') is used to treat couples in whom fertilisation has either failed at one or more previous IVF attempts, where fertilisation failure is anticipated due to a man's spermatozoa being of extremely poor quality, or where too few spermatozoa are available for traditional IVF (even using 'microdroplet insemination'). However, sperm microinjection is not indicated purely on the grounds of an abnormal semen analysis using criteria such as those published by the World Health Organisation (WHO), since their 'normal ranges' relate to the likelihood of conception in vivo. As a general guide, the following criteria may be considered indications for sperm microinjection:

- A finding of too few sperm for IVF following more than one semen analysis or a 'trial sperm wash'
- Extremely poor sperm motility (very few or no progressive spermatozoa) and/or morphology (e.g. less than five per cent normal forms using WHO criteria)
- Failed fertilisation on at least one occasion with very poor sperm quality
- Failed fertilisation on at least two occasions in spite of apparently satisfactory sperm quality (the usual criteria employed previously for SUZI)

In addition, antisperm antibodies present on all the motile spermatozoa might be an indication for ICSI, but it is a contra-indication for SUZI.

## Success Rates

Typical results of ICSI range from 60 to 75 per cent fertilisation rate (about five per cent of oocytes may be damaged during the microinjection procedure), with very few patients suffering zero fertilisation. Pregnancy rates defined as positive pregnancy tests are up to 30 per cent per embryo transfer, with early spontaneous abortions being only a little higher than in the general IVF population. Consequently, a clinical pregnancy rate of at least 20 per treatment cycle is a reasonable expectation – although some clinical circumstances may adversely affect this, such as the age of the female partner which reduces the chance of implantation even under natural conditions.

ICSI is also very effective when used in conjunction with sperm aspiration from the epididymis in men with obstructions or congenital absence of the vas deferens.

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Supported by an untied educational grant from:

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