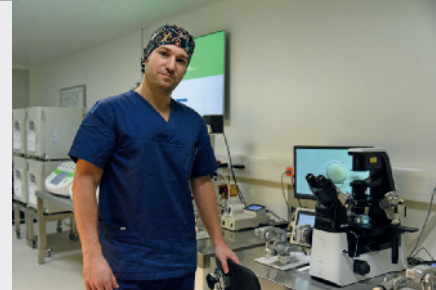




The optimal parameters for ICSI – perfect your ICSI with precise optics

Credits: Alejandro Montoya, OVA IVF Clinic Zurich



The current prevalence of infertility lasting for at least 12 months is estimated to be around 9% worldwide for couples aged 20-44 [1]. Two main fertilization techniques are used during the process: standard in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). ICSI accounts for 66% of the treatments worldwide and conventional IVF around 33%. ICSI fertilizes 50% to 80% of eggs [2], and the mean pregnancy rate per embryo transfer was 33% after ICSI in Europe in 2014. Although other factors might play a role in a successful pregnancy rate, maximizing ICSI success is the first step towards a successful pregnancy. At the OVA Clinic Zurich, fertilization rates for ICSI are as high as 80-90%. Here we describe how the optimal parameters and the most precise equipment can help to achieve the best ICSI.

APPLICATION

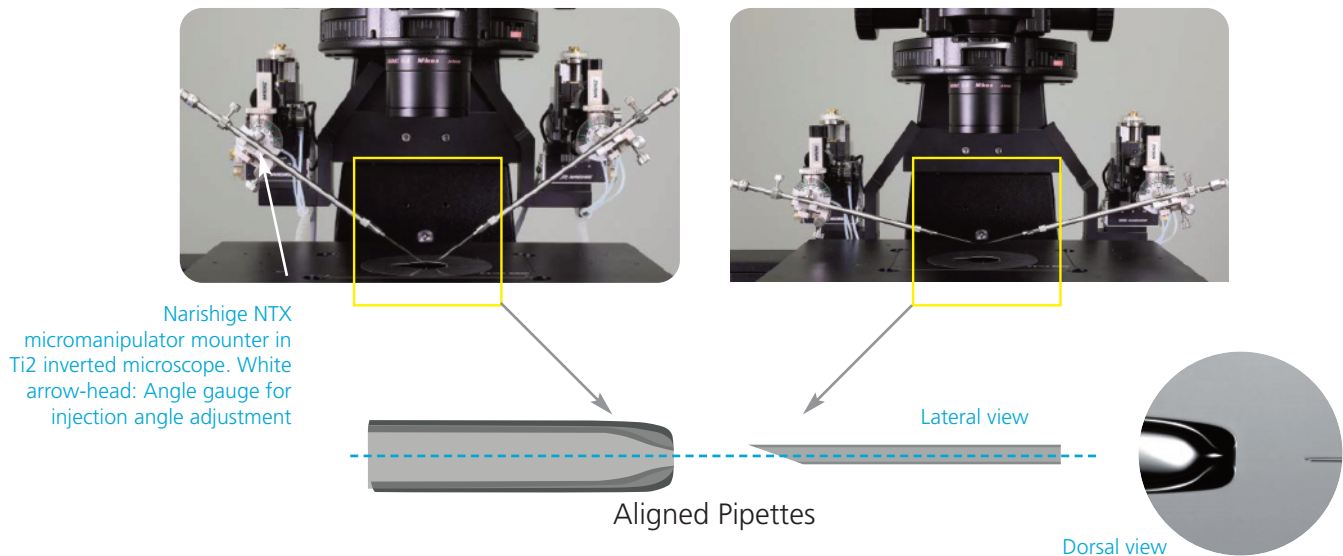
ICSI is the process within the assisted reproductive cycle that enables the fertilization of the gametes by direct injection of a single spermatozoon into a metaphase II oocyte with the aid of an inverted microscope and micromanipulators. Several steps are involved in the process and the most important parameters for this process to be successful are stability, precision and speed.

PROCESS

Pre-ICSI

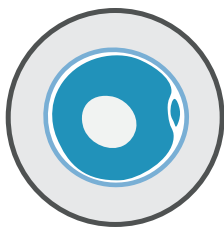
1-Equipment preparation:

Angle adjustment, pipette positioning and focusing using 100-200x magnification. The angle of injection is determined by the embryologist preference and by the pipette used; the OVA Clinic Zurich has found that 35-37 degrees is the most successful angle.

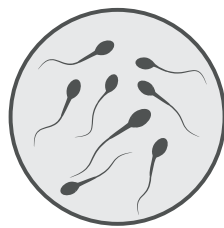


2- Gamete preparation:

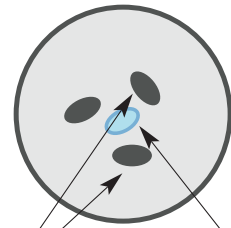
Oocyte decoration to retrieve zona pellucida (chemical or mechanical), selection and loading in ICSI dish in egg drops. Spermatozoa loading in PVP drops within the ICSI dish.



Oocyte selection and decoration



Andrology – analysis and selection of spermatozoa



EGG DROPS
10µl cleavage media

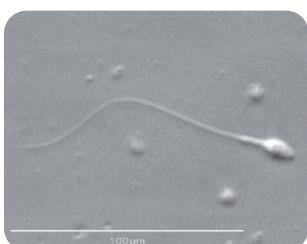
SPERM DROP
5µ PVP + 1-2µl of sperm prep

ICSI

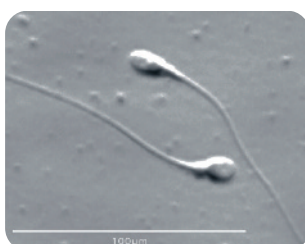
3- Sperm selection and hunting:

With the aid of an inverted microscope and at 400x magnification, the embryologist will search for the most suitable spermatozoon according to its head morphology and its movement speed and pattern. An ideal sperm is flat, has a disc shaped head 5.1 µm by 3.1 µm and a 50 µm long tail. As the sperm is amongst the smallest cell type in our body, clear and precise optics to resolve these details are pivotal. If the details cannot be determined quickly, the whole ICSI cycle could result in failure. After identification, the ICSI pipette is brought down to “gently” hit the selected spermatozoa in the neck to induce it, reduce its motility and load it, usually starting from the tail, into the micropipette.

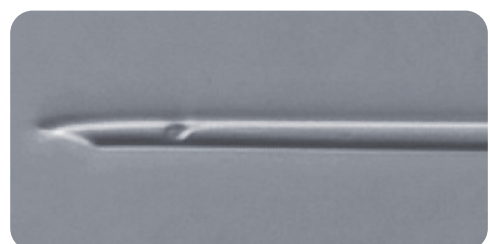
NAMC sperm



Emboss sperm



Hunted sperm



4- Oocyte immobilization and stabilization:

With the help of the holding pipette at 200x, the oocyte is positioned with the polar body at 6 or 12hrs and just the appropriate amount of suction is applied to gently hold its position in the field. Positioning the polar body in those axes enables a perpendicular puncture of the oocyte, minimizing the possibilities of spindle damage.

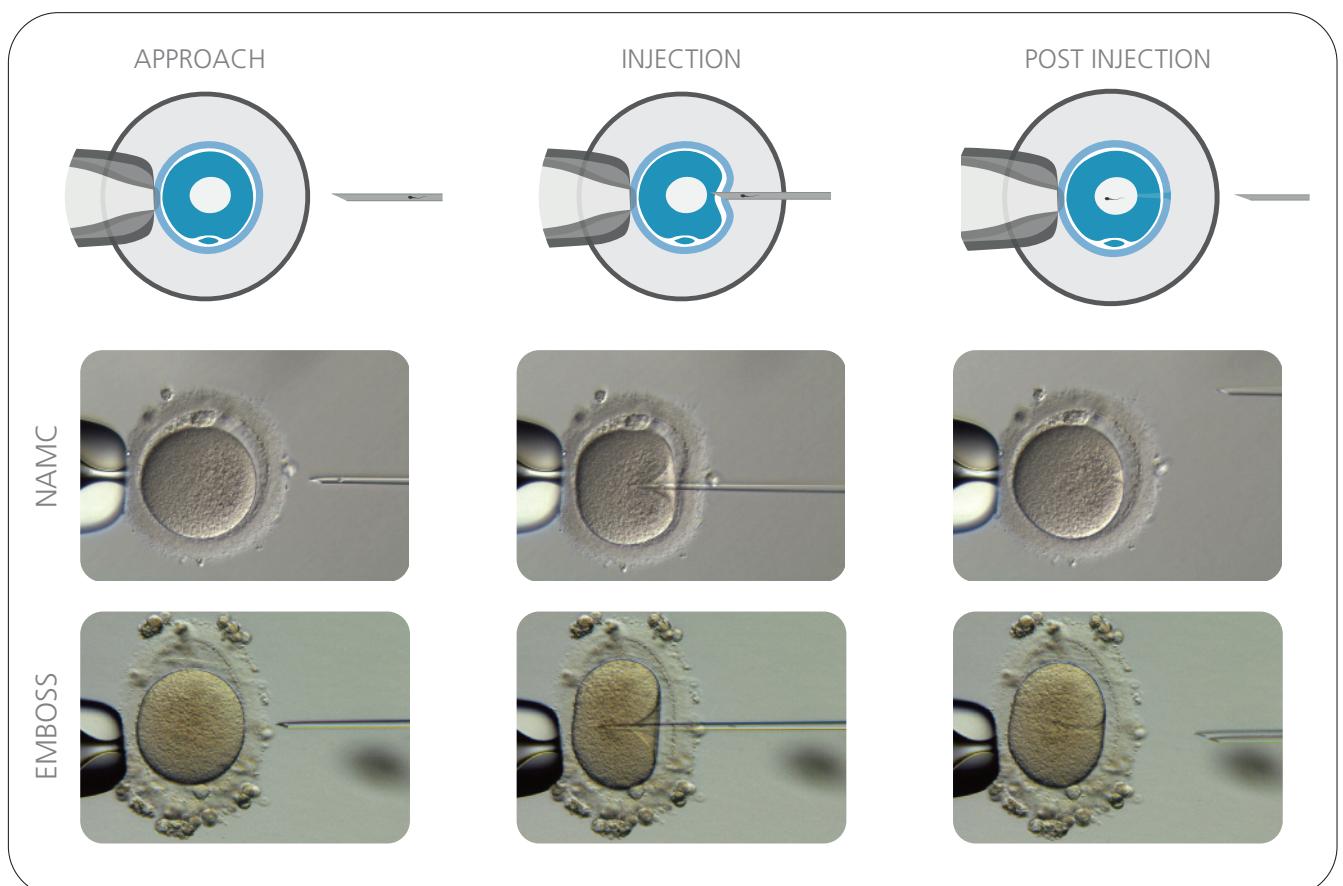


5- ICSI:

Excess media in the injection pipette is ejected to bring the spermatozoa down to the tip and align the oocyte and the injection point in the sharp focus plane before proceeding to the injection. After feeling the membrane perforate, a small amount of cytoplasm is aspirated into the pipette and reinjected along with the spermatozoa and the minimum amount of extra medium. To regulate the amount of aspiration and injection, precise and responsive microinjectors are required, but the ability of the embryologist to see the details of the sperm and fluid position is what will ultimately produce a successful ICSI.

Fertilized eggs are brought to incubation for embryo development and monitoring.

The whole ICSI process typically lasts between 15 and 20 minutes. That is the time where the gametes are subjected to the maximum stress outside of the incubator, therefore speed is pivotal to minimize this period. In order for speed to be maximized, the equipment should not become an impediment for the process but facilitate it.



References

1. ART facts sheet (ESHRE)
2. <https://www.reproductivefacts.org/news-and-publications/patient-fact-sheets-and-booklets/documents/fact-sheets-and-info-booklets/what-is-intracytoplasmic-sperm-injection-icsi/>

RELEVANT PARAMETERS

The stability of the conditions during ICSI is crucial to maintain stress-free gametes, especially the oocyte. Temperature is kept at 37 °C outside of incubators due to heated surfaces, including the thermal stage in the ICSI microscope.

Precision is enabled by the operator's experience but determined by the exactitude of the tools he uses: the inverted microscope's optics and the micromanipulators quality have a crucial role in determining a successful ICSI. Optics must be excellent to have a sharp, focused and contrasted image to perform a precise ICSI.

Loss of precision during ICSI is the most common cause of ICSI failure. Non-precise optics can have devastating effects: problems in selecting and hunting the sperm for ICSI as it might be hard to identify; or the wrong injection site by not having the pipettes aligned in the same plane, which would lead to excessive invagination and potential oocyte decay. If the optics do not render the maximum detail, the whole process could be unsuccessful. Non-precise microinjectors can lead to stress and potential loss of quality by holding the oocyte too strong; injecting excess media along with the spermatozoa will result in vacuolation and will prevent the development of a healthy embryo.

TECHNOLOGY

To achieve precision, ICSI is performed on an inverted microscope with the appropriate contrasting method. Nikon's Eclipse Ti (now the Ti2) and Ts2R inverted microscopes with the established Nikon Advanced Modulation Contrast (NAMC) and the newly developed Emboss Contrast for Ts2R, both enable sharp and clear images. NAMC gives more sense of depth, while Emboss provides a pseudo-relief sharper image in the plane of focus. With a fertilization success rate of 80-90%, Alejandro Montoya at OVA Zurich comments: "Both methods are sufficient for a successful ICSI. What will be the determinants are the experience and comfort and working conditions of the embryologist." Seeing the necessary information on the gametes and during the process will increase the operator's ability.

Emboss Contrast is a cost-effective optical technique which does not require costly optics. Utilizing just a bright-field objective lens and two contrast sliders, Emboss Contrast provides pseudo-three dimensional and glare-free images for thick specimens such as iPSC cells which would normally suffer from halos with conventional phase contrast methods. Additionally, Emboss Contrast is compatible with both glass and plastic culture chambers, making it a very versatile observation technique.

The Eclipse Ti, as well as its successor Ti2, has a large working surface and a wide operational space around the nosepiece and condenser, both of which enable comfortable operation. Between the sample positioning, sperm selection and ICSI, magnifications may vary. The frontal extra tube lens of 1.5x/2x enables the operator to go from 200x to 300x/400x without having to reach deep into the microscope nosepiece. This might result in a speed advantage in manual systems. Having a motorized condenser and/or nosepiece might be convenient for the operator, slightly reducing the time for ICSI, although it has not been shown to have an effect on the success of pregnancy. Ultimately, it is the quality of the gametes what determines proper embryo development. The Ti's successor, the Ti2, maintains that functionality and features less power consumption and more stability in the system.

The Ts2R is designed to place the highest priority on cost performance for clinical applications. Its compact design and small footprint ensure the microscope will fit in your current lab design, while the anti-vibration design and Emboss Contrast provide the embryologist with the most precise instrument for successful ICSIs. Its ergonomic design ensures the operator's comfort for a higher chance of success.

