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**Intracytoplasmic Morphologically
selected Sperm Injection (IMSI):**

a better start in life

Abstract:

Intracytoplasmic morphologically selected sperm injection (IMSI) is a high magnification light microscopy imaging method used increasingly in the field of in vitro fertilisation (IVF) to select sperm for ICSI (intracytoplasmic sperm injection). This technique has been shown to increase pregnancy rates and reduce abortion rates compared to routine ICSI-IVF (Berkovitz et al, 2006 and 2005; Bartoov et al 2003). This technical note describes IMSI using a Nikon inverted microscope equipped with Normarski DIC optics and digital imaging system to achieve magnifications in excess of 6000x.

Introduction:

ICSI is a procedure, most commonly used to overcome male infertility problems, in which a single sperm is injected directly into an oocyte in vitro to achieve fertilisation. The fertilised egg is then observed through a number of divisions before being transferred into the female reproductive tract. The success of ICSI may depend on several factors, such as the age of the oocyte, the skill of the ICSI practitioner and, importantly, the quality of the single sperm selected for oocyte fertilisation.

Sperm quality may be evaluated through a number of criteria such as maturity, sperm count, motility and morphology. Sperm morphology, especially of the head region containing the nucleus, can be used to select the best sperm for injection. It is known, for example, that sperm with severely abnormal head shape such as 'pin', 'amorphous', 'tapered', 'round' and 'multinucleated' reduce implantation and reproduction rates and should be rejected. These defects can be identified using microscope observation at relatively low magnification. However, even sperm that appear superficially normal at low magnifications may have subtle organelle defects in the sperm head or sperm surface that prevent fertilisation. These subtle changes require magnifications of at least 6000x for detection.

The IMSI method is based on motile sperm organellar morphology examination (MSOME) at magnifications in excess of 6000x to select sperm with morphologically normal nuclei. Use of this method for ICSI has resulted in higher pregnancy and delivery rates and lower abortion rates (Berkovitz et al, 2006 and 2005; Bartoov et al 2003). Here, we describe a complete imaging solution for IMSI using a Nikon inverted microscope equipped with Normarski optics, videozoom and digital imaging system.

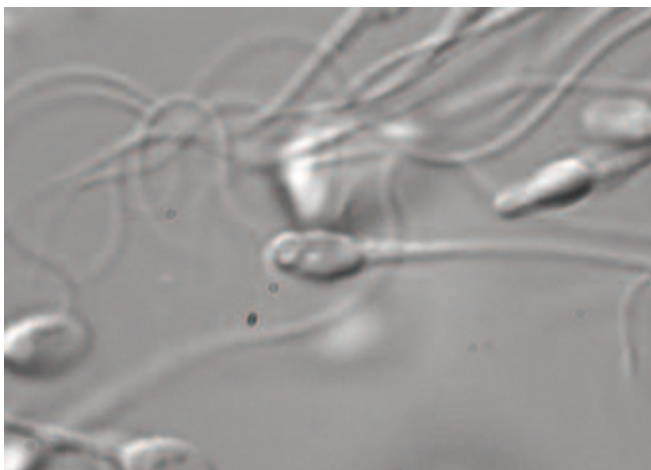


Figure 1: Sperm observed with 60x oil immersion objective

Technology:

The main objective in configuring a microscope for IMSI is to achieve magnifications of at least 6000x. This may be achieved using a Nikon inverted microscope equipped with high resolution Normarski optics (100x oil or 60x oil Plan Apo VC) enhanced by a videozoom and digital imaging. The final magnification of the system can be calculated using the following formula:

(Objective) x (magnification changer) x (videozoom) x (diagonal of image / camera CCD chip diagonal)

Depending on the screen size and live resolution, the magnification achieved is up to 10.145x (between 5.1x and 10.145x). While any Nikon research level inverted microscope can be used for IMSI, motorised functions offer additional advantages

Motorisation

The inverted Nikon microscope in combination with NIS-D software enables complete management of motorised functions, microscope parameters and camera settings. All parameters can be saved and retrieved as required. NIS software allows users to adjust the condenser, objective, DIC, light, optical path or camera parameters with just one click of the mouse. This not only reduces the risk of disturbing the sample during observation, but also reduces the need for any manipulation above the sample that could lead to sample contamination. An 'escape' function, in addition, provides an additional layer of security that allows users to override a motorised function when required.

A novel, ergonomically designed foot-operated switch system allows users to select microscope parameters without having to take their eyes away from the sample or their hands from micromanipulators. Easy and comfortable to use, the foot switch (from Kinesis corporation: www.kinesis.com) is linked to the NIS-D software and interpreter module via a macro (controlled with a keyboard shortcut).

Flexible imaging with DIC

The option of being able to switch to DIC observations reveals details not visible with other contrast methods and provides users with a high level of imaging flexibility. The examination of sperm, for example, normally uses 60x oil or 100x oil Plan Apo VC objectives. This is followed by the use of 20x or 40x objectives and Hoffman modulation contrast imaging for the ICSI procedure. This means users have to use two different Petri dishes – the first with a 0.17mm glass bottom to select sperm using Plan Apo VC oil objectives and a second standard dish (for non-oil objectives) for the ICSI procedure. By using DIC for ICSI, rather than Hoffman modulation contrast, injection can be carried out with 20x oil or 40x oil objectives using the same glass-bottom Petri dishes.

The 60x oil objective is ideal choice for sperm selection. As well as offering greater brightness and greater depth of focus than the 100x objective, the 60x objective also provides a wider field-of-view. This is particularly useful when observing rapidly moving sperm, which may easily move out of a limited field-of-view. Indeed, the field-of-view resulting from the combined effects of objective and camera can be an important parameter when examining sperm.

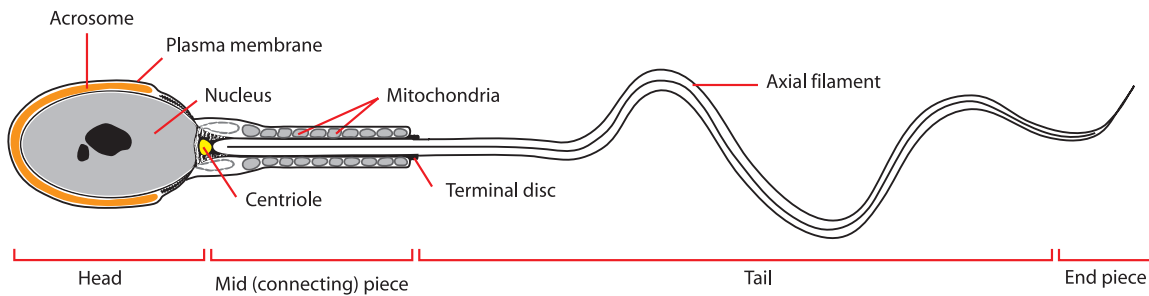


Figure 2: Regions of the normal sperm

Digital imaging camera and videozoom

The DSQi1Mc camera is the camera of choice for IMSI because of high sensitivity and fast capture rate to ‘freeze’ fast moving sperm. When using the 100x objective, the binning 2x2 function enables capture rates of 30 frames per sec. There are two options for videozoom, C-Mount Zooming Adapter Set (zooming range : 0.9 - 2.25x), or a fixed magnification 2.5x videozoom.

Application:

The aim in IMSI is to select the most morphologically normal sperm (with the greatest potential for successful IVF) for ICSI. During IMSI, sperm samples are contained within a glass-bottomed dish under sterile paraffin oil with 4µl of sperm medium containing polyvinyl pyrrolidone (to reduce sperm motility). High magnification in IMSI enables recognition of subtle defects in sperm organelles such as the acrosome, mitochondria, and nucleus (figure 2).

Acrosome abnormalities are a common cause of IVF failure as they prevent penetration of the egg by the sperm. This cap-like structure on the sperm head contains lytic enzymes that digest the outer surface of the egg (the zona pellucida) allowing sperm to inject haploid DNA into the oocyte. The post acrosomal lamina, in addition, is the area of the sperm first recognised by the egg during fertilisation. This lines up internally with areas of the nucleus not covered by the acrosome. Defects in this structure may affect sperm-egg recognition and compromise fertilisation. Another common defect is abnormal vacuolation. Nuclear chromatin, for example, is considered abnormal if it contains more than one vacuole. The shape of the nucleus, in addition, is a key factor in deciding whether the sperm is normal as it determines the shape of the sperm head.

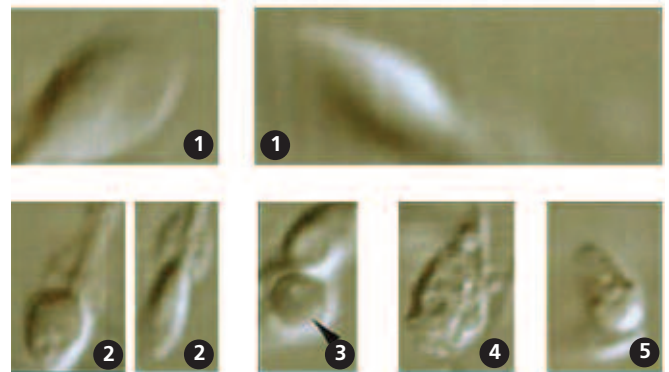


Figure 3: 1. Normal spermatozoa
2. Spermatozoa with excess residual cytoplasm
3. Microcephalic spermatozoa
4. Macrocephalic spermatozoa
5. Bent tail spermatozoa

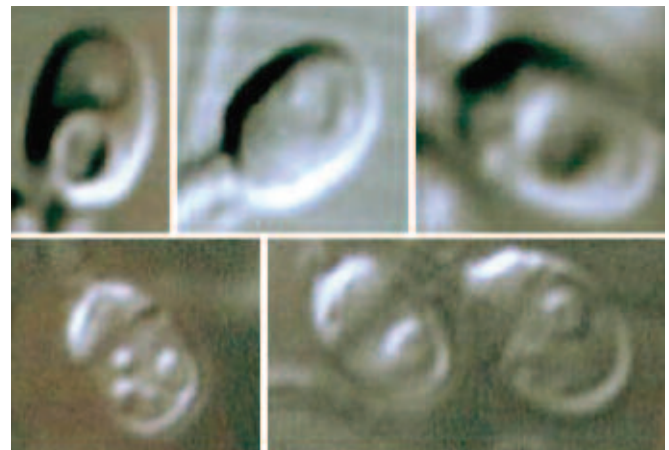


Figure 4: Abnormal sperm showing vacuolisation observed with 100x oil immersion objective

Conclusion:

High magnification in the IMSI method reveals critical details in sperm morphology, especially that of the nucleus, allowing selection of highest quality sperm for ICSI. Microinjection of sperm with a morphologically normal nucleus significantly improves IVF success.

High magnifications suitable for IMSI, together with motorised functions for ease of use, can be easily achieved using a Nikon inverted microscope equipped with Normarski optics, videozoom and digital imaging system.



Authors' Background

Sandrine Batard is Marketing Manager for Nikon Instruments France. She joined Nikon in 2006 after 10 years experience as a sales representative and microscopy product manager. Her current activities focus on the analysis of market changes and trends for product and market development.

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Christian Lainé is Regional Sales Manager in the biological field in France. He joined Nikon in 1991 after 6 years experience as a sales representative in microscopy and micromanipulators. He manages a team of five area managers and brings his skills and experience of the IVF market to the whole French team.

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 Bartoov B et al (2003) *Fertil Steril*;80(6):1413-9.

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- IMSI improves ICSI-IVF success
- IMSI required high magnifications in excess of 6000x
- IMSI can be carried out on inverted Nikon microscopes
- Motorised IMSI with a unique foot control option for hands-free, uninterrupted observations can be achieved on an inverted Nikon microscope

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 Nikon inverted microscopes go to:

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