

**High power light microscope
morphological examination
as a tool for single sperm selection
prior IVF-ICSI.**

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Dogma

Any sperm characteristics,
other than **viability**,
have no significant impact on
pregnancy outcome
in in-vitro fertilization using
ICSI

To **challenge** this dogma a quality analysis of a single sperm cell, selected for ICSI, should be build .

For this purpose, we developed a method of unstained real-time light microscopy high power **Motile Sperm Organellar Morphology Examination**

MSOME

The sperm organellar morphological characteristics, which were established by us 20 years ago, using:

1. SEM (external structure)
2. TEM (internal information)

were "translated" to MSOME criteria, using LM, equipped with Nomarski differential interference contrast optic (supplies both external and internal information).

Criteria for morphologically normal nucleus

Shape

Smooth, symmetric, and oval configuration

Average length $4.75 \pm 0.28 \mu\text{m}$

Average width $3.28 \pm 0.20 \mu\text{m}$

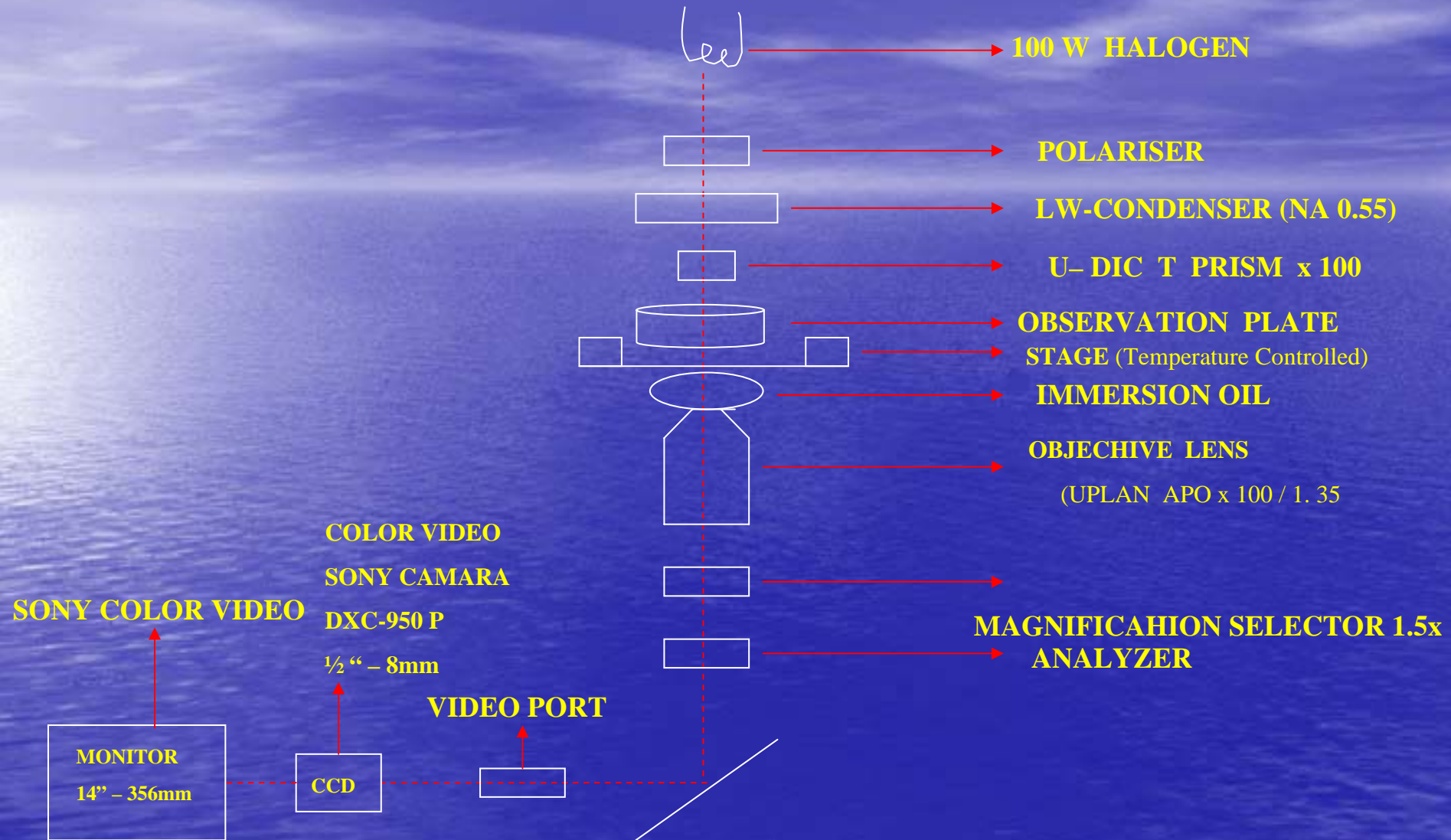
Content

No extrusion or invagination of the nuclear chromatin mass

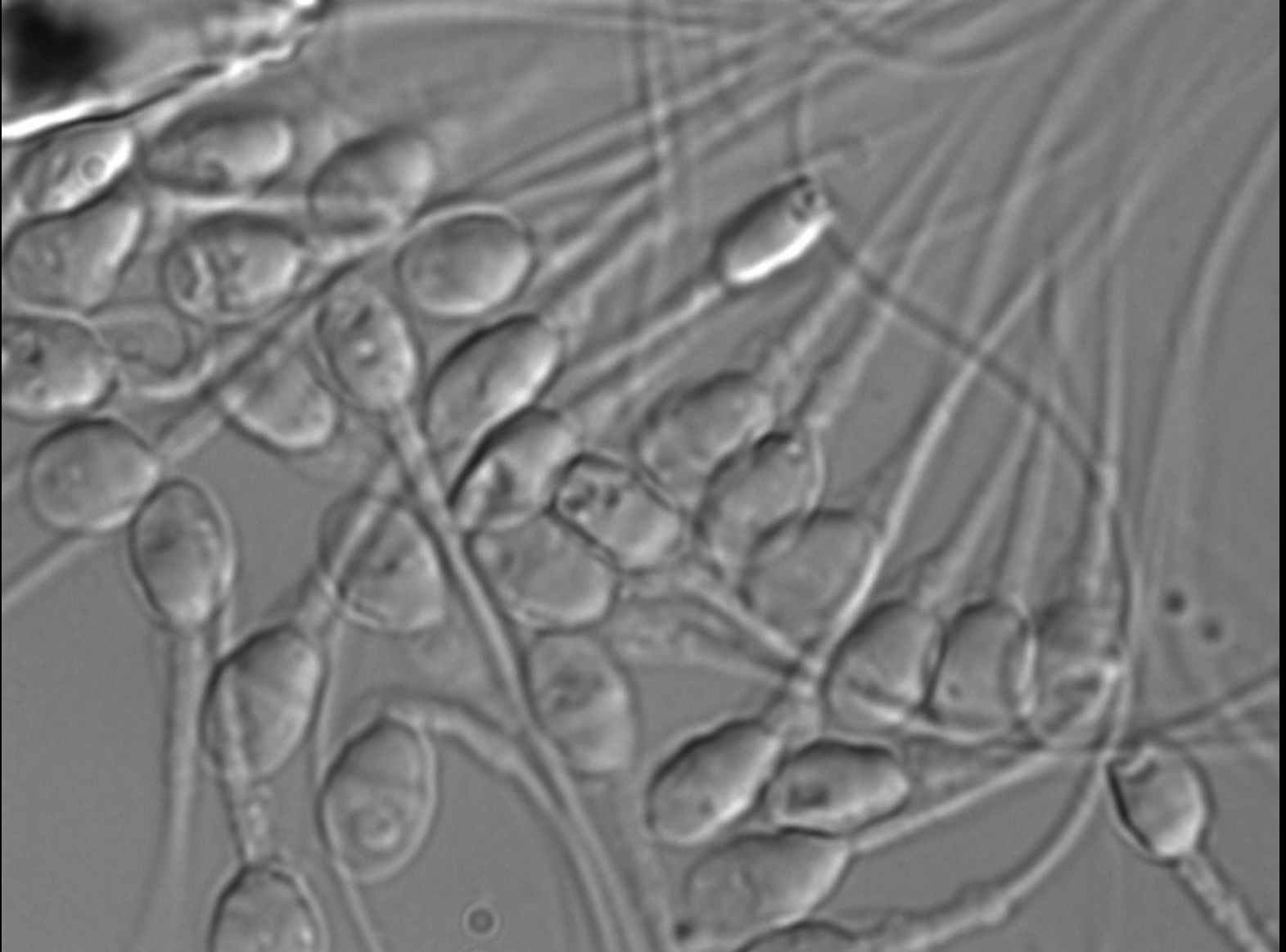
No vacuoles more than 4% of the normal nuclear area

Microscopic set-up for MSOME

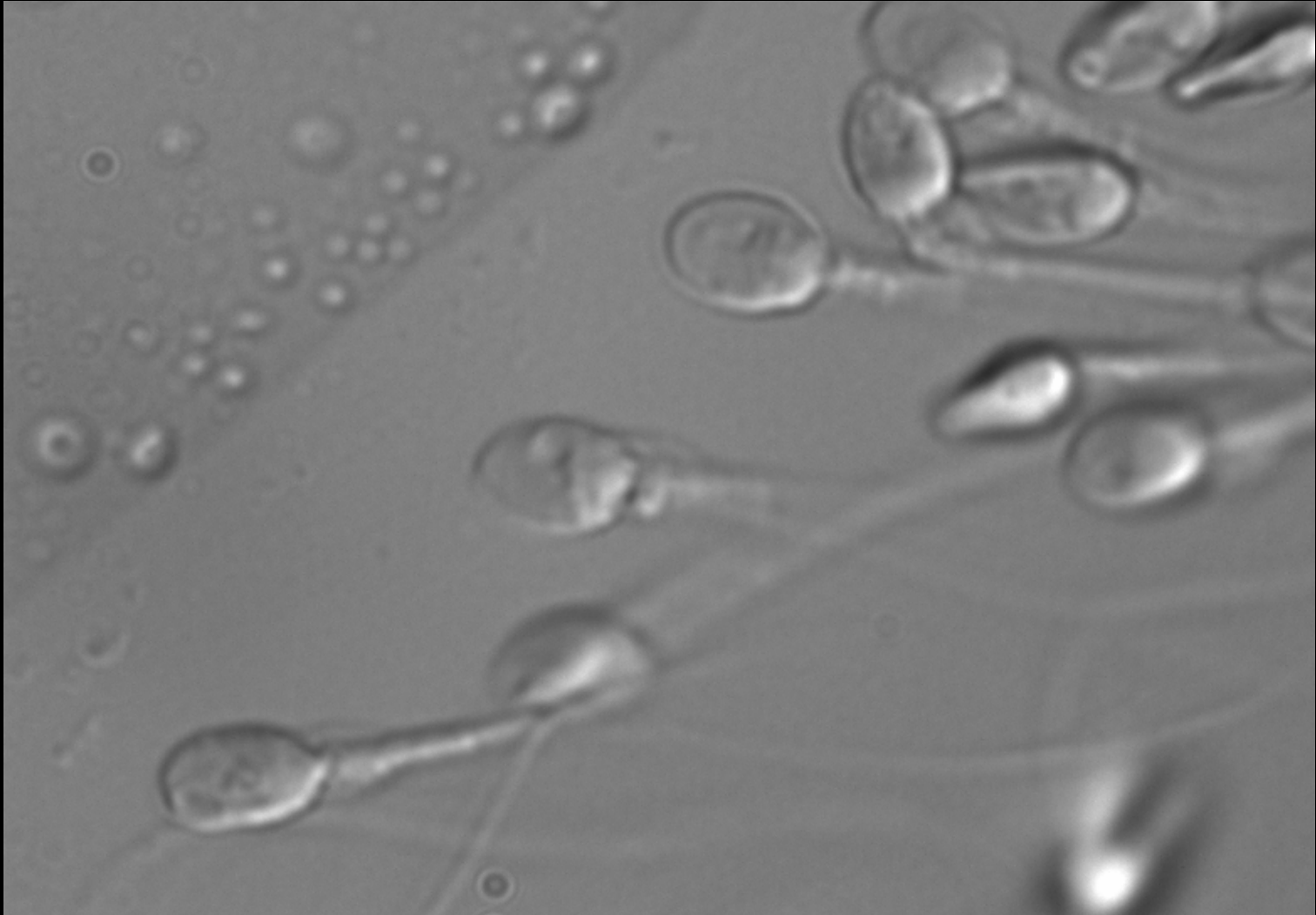
Olympus IX-70 Inverted + Differential Interference Contrast (DIC)



Spermatozoa in a bay

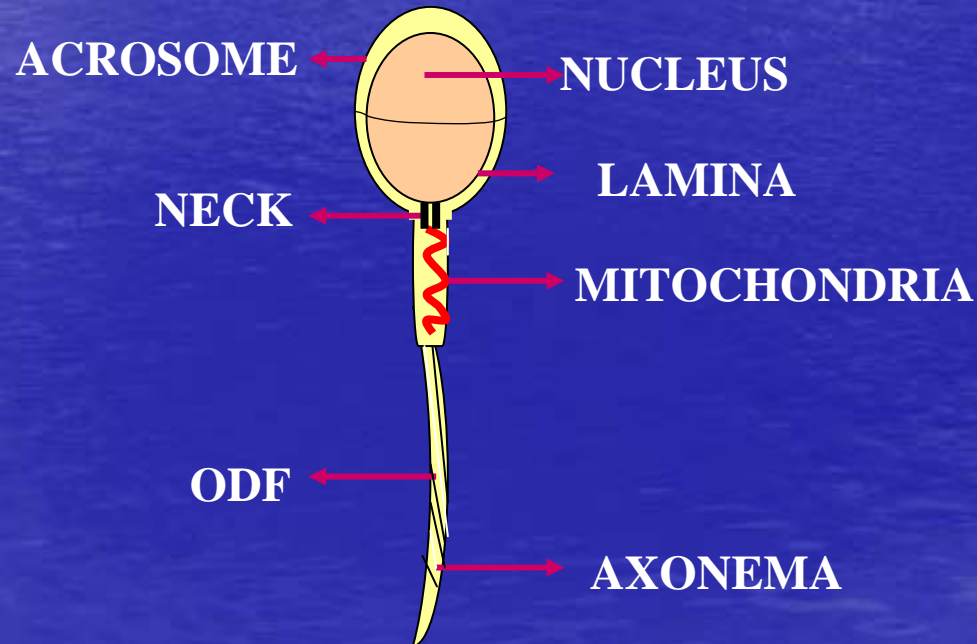


spermatozoa in a field



Aim 1:

To determine whether and which subtle sperm morphological characteristics, observed by MSOME, are related to the outcome of Intracytoplasmic Sperm Injection (ICSI).



Design:

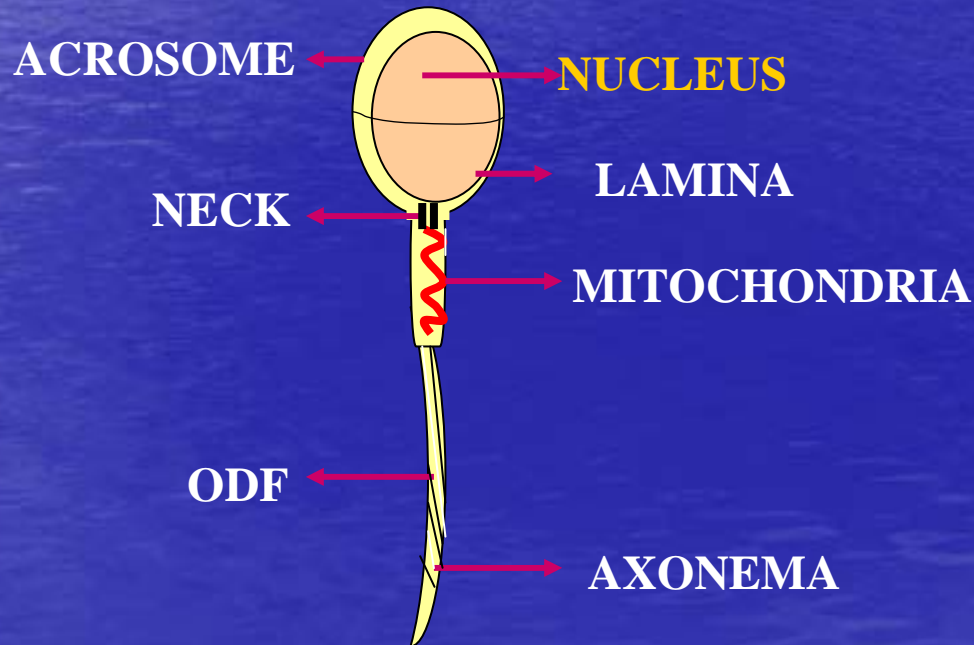
MSOME was applied to the leftover sperm fraction selected for microinjection in 100 random couples referred for ICSI treatment at 3 IVF centers

Results 1:

A total of 1074 oocytes was retrieved. Sperm injection was performed in 908 (84%) mature oocytes, fertilization occurred in 591 (65%) cases, 368 embryos were transferred and 28 (28%) pregnancies were achieved.

Results 2:

Out of the 7 sperm subcellular organelles observed, only the morphological normalcy of the sperm **NUCLEUS**, defined by **MSOME** had predictive value of **74%** for pregnancy occurrence.



Conclusion:

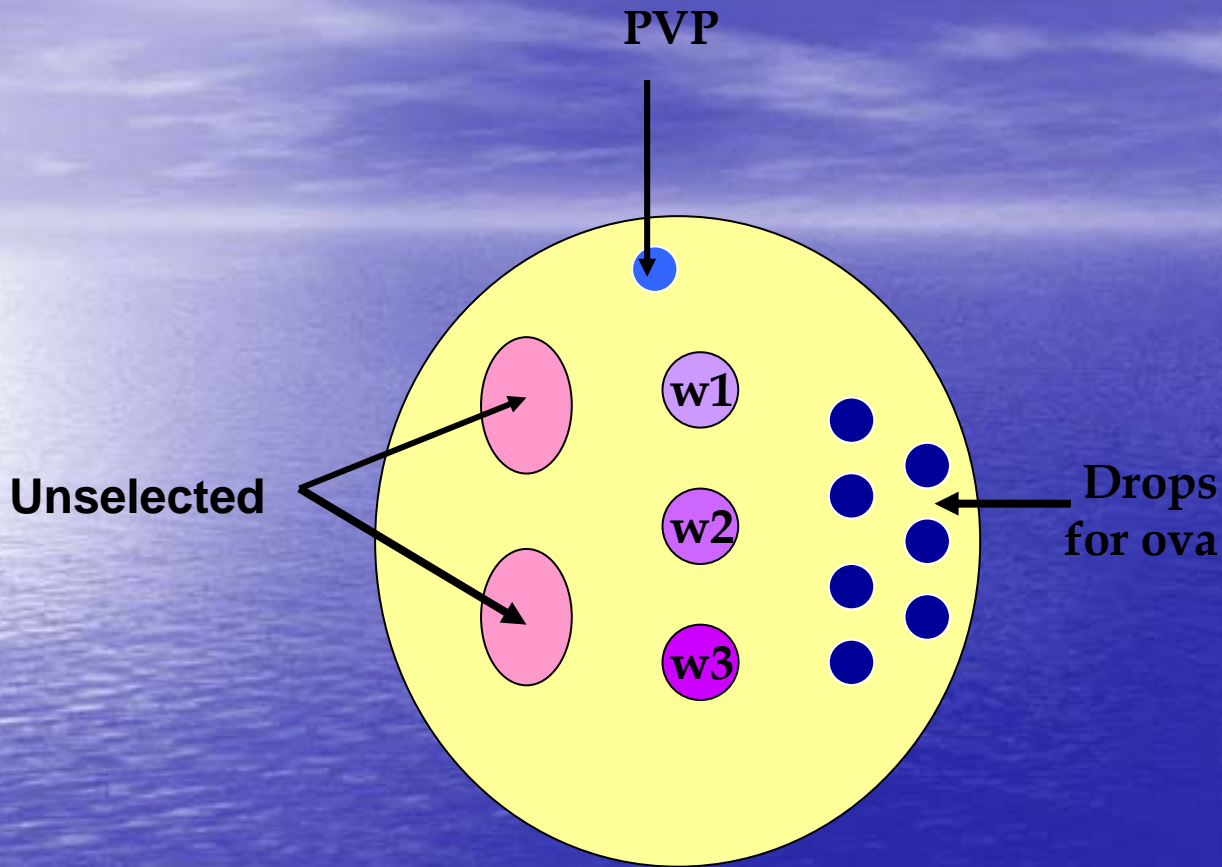
ICSI-associated pregnancy rate may be affected by **subtle** morphological malformations of the sperm **nucleus**, which may remain **undetected** by the embryologist during the **routine selection** procedure.

Aim 2:

To verify whether microinjection into retrieved oocytes of motile spermatozoa with morphologically **normal nuclei**, strictly defined by MSOME, improves the **IVF-IMSI pregnancy outcome**.



IMSI



W1- "Best" sperm

W2-w3- "Second best" sperm

Intracytoplasmic
Morphologically **S**elected
sperm **I**njection

IMSI

Design:

Matched prospective study

experimental group

n=50

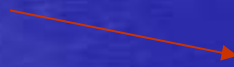
Failed ICSI



Failed ICSI



IMSI



control group

n=50

Failed ICSI



Failed ICSI



ICSI



=

IVF Outcome

Selection criteria:

- **Female partner younger than 37 years**
- **More than three retrieved M-II ova in the last ICSI cycle**

Written, informed consent was obtained from each couple.

Results:

- The percentage of top embryos was significantly **higher** in the **IMSI** group as compared with the ICSI one (45.2 ± 28.2 VS 31.0 ± 19.5 , $F = 18.0$, $P \leq 0.2$)
- Implantation and pregnancy rates after **IMSI** were significantly **higher**, and the abortion rate was significantly **lower**, compared to the current ICSI trial ($F = 18.0$, $P \leq 0.1$; $\chi^2 = 4.4$, $P \leq 0.2$ and $\chi^2 = 4.4$, $P \leq 0.5$).

Conclusion:

- Visible advantage of the modified IMSI trial over the conventional IVF/ICSI
- The fine morphological state of the sperm nucleus is an important factor in achieving pregnancy after ICSI

ICSI



IMSI

- Use of density gradient
- Low temperature
- Glass bottom dish
- Prolonged sperm manipulation
- Storage prior to microinjection

Aim 3:

To determine whether the increased pregnancy outcome was attributable to the preferred nuclear morphology of the selected spermatozoa, and not to the special sperm preparation technique modified by **IMSI**

IMSI

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graph TD; IMSI[IMSI] --> Best["best spermatozoa"]; IMSI --> SecondBest["second best spermatozoa"]
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"best spermatozoa"

"second best spermatozoa"

Design:

Comparison between two matched IMSI groups:

Positive: involving 38 ovum microinjection by spermatozoa with strictly defined morphologically **normal** nuclei only.

Negative: including 38 cycles, where no spermatozoa with intact nuclei were available for microinjection (“**second best**”).

Results 1:

Fertilization rate, percentage of top embryos and implantation rates were significantly **higher** in the **Positive group** than in the Negative one (F=16.3, $p \leq 0.01$; F=4.9, $p \leq 0.03$; and F=15.8, $p \leq 0.01$ respectively).

Results 2:

Pregnancy rate was significantly **higher**, and abortion rate significantly **lower**, in the **Positive group** compared with the Negative one (Chi Square=9.7, $p \leq 0.01$ and Chi Square=7.1, $p \leq 0.02$, respectively)

Conclusions:

- **Pregnancy outcome** by ICSI is, indeed, associated with morphological **nuclear normalcy of sperm**.
- Sperm with a morphologically abnormal nucleus usually have low fertility potential but some with certain nuclear abnormalities may still be able to produce pregnancy following ICSI.

**This conclusion leaded us
to investigate each typical
morphological category of
the sperm nucleus
separately**

Aim 4:

To determine whether the specific malformation of the sperm nuclear content

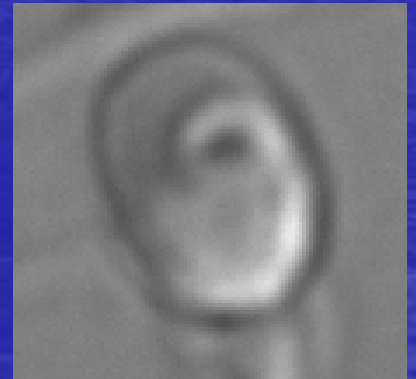
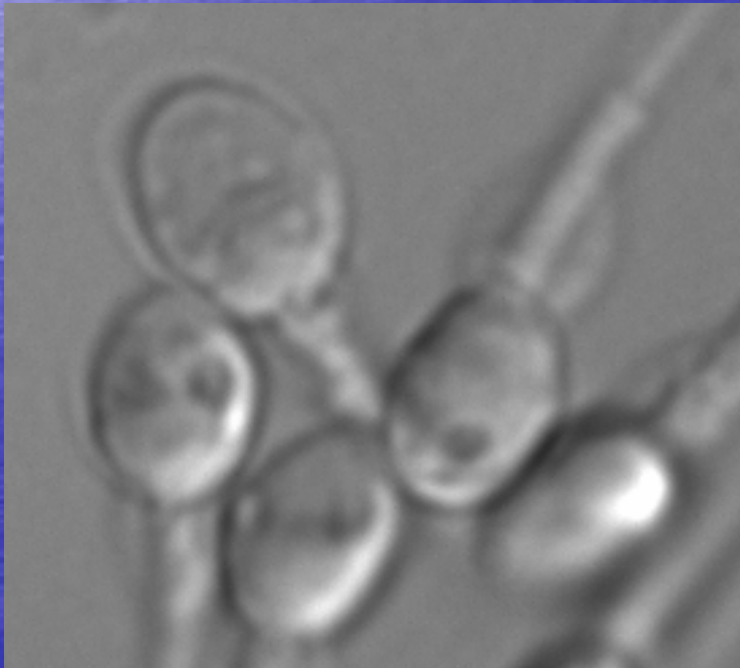
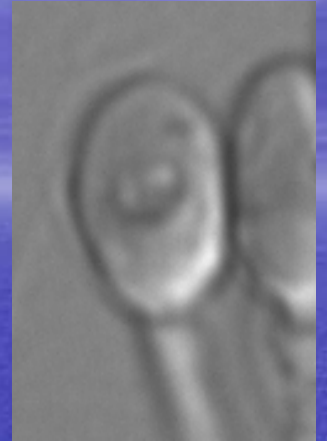
- **large vacuoles**- may affect ICSI outcome

Design:

Experimental group: 19 IVF-IMSI cycles, where only embryos obtained from microinjection of spermatozoa with **normal nuclear shape but large vacuoles** were transferred

Control group: 19 matched IVF-IMSI cycles where only embryos obtained from microinjection of spermatozoa with strictly defined morphologically **normal nuclear shape** and content were transferred

Large vacuoles



Selection criteria:

- Female partner younger than 40 years
- Three or more retrieved M-II ova in the present **IVF-IMSI** cycle
- **IMSI** outcome, including delivery, confirmed 10 months after the microinjection.

Results 1:

The experimental and control groups were statistically **similar** in number of retrieved and injected ova, fertilization rate, percentage of top embryos and number of transferred embryos

Results 2:

Vacuolated group exhibited significantly **higher** values of **abortion rate** and significantly **lower** values of the **delivery rate**, as compared to the control one (100% VS 11%, Pearson Chi square= 9.2, and 0% VS 88%, Pearson Chi square=7.0, respectively; $p \leq 0.01$)

Conclusions:

Sperm morphology in IVF-ICSI may affect the axis of embryonic development from ovum fertilization to delivery.

In cases of spermatozoa with normal nuclear shape but large vacuoles, this axis is probably normal at the beginning, but the embryo survival in the later stages is impaired

Final conclusion:

These studies implies that sperm nuclear factor, observed by MSOME may have an impact on overall embryo quality beyond fertilization in ICSI