


# *Sperm DNA matters:* Finding the best sperm

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# Aims & Questions:

- To define sperm quality
  - To explain the rationale in needing to find the best sperm among several millions
  - To identify molecular abnormalities related to sperm function, mainly based on sperm DNA integrity
  - To describe the techniques of sperm selection that are under investigation showing an improvement to clinical outcomes from **evidence-based medicine**
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What do we  
quality?

Why do we

- Firstly, because of IVF treatments.
- Regardless of the success of several attempts.
- Goal: from a single sperm and egg to have an embryo that develops.



but sperm

h?

riding ART

ent succeeds and

m to have an embryo

**Are there evidence-based sperm selection methods improving sperm DNA quality and subsequently reproductive success?**





PUBMED and EMBASE  
related terms

850

Very contradictory results

Guidelines from  
ESHRE & ASRM

Existing data do not support a consistent strong relationship between abnormal DNA integrity and reproductive outcomes.

At present, the results of sperm DNA integrity testing alone do not predict pregnancy achieved through natural conception or with IUI, IVF, or ICSI.

RECOMMENDATION

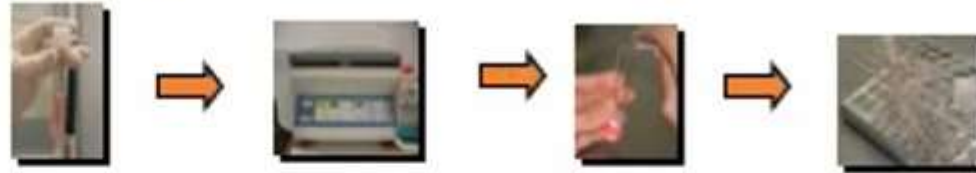
There is insufficient evidence to recommend the routine use of sperm DNA integrity tests in the evaluation and treatment of the infertile couple (Level C: There is insufficient evidence to support a recommendation, either for or against.).

reproductive outcomes

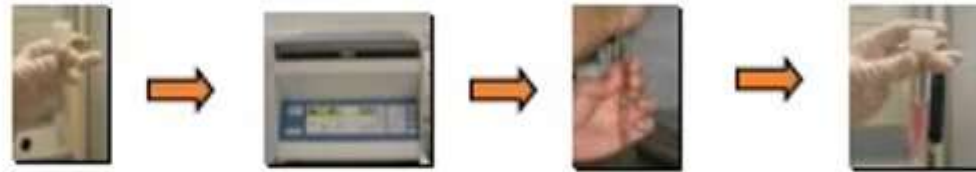
*Classical sperm selection methods, based on motility and morphology*

✓ “self-selection” (IUI and IVF): operator independent

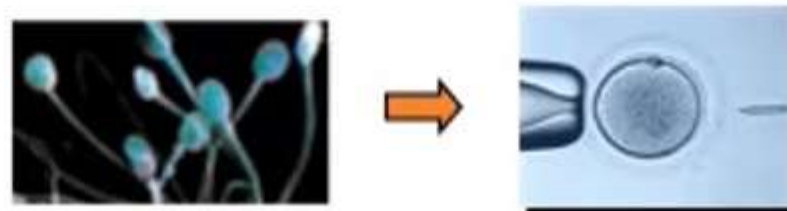
SWIM UP





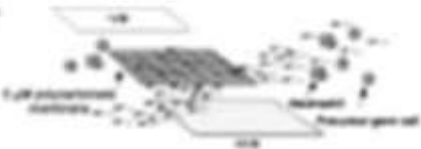
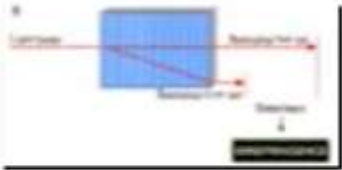

DENSITY GRADIENT CENTRIFUGATION



✓ subjective sperm selection (ICSI): operator dependent



## Advanced sperm selection:

	<i>Method</i>	<i>Basis</i>	
<i>IMSI</i>		<i>Morphology</i>	Good scientific evidences Costs of the equipment Embryologists training Time-consuming (new microinjecting schedules?)
<i>PICSI</i>		<i>HA receptors</i>	Limited to micromanipulation Newborns Insufficient data
<i>Electrophoresis*</i>		<i>Negatively charged cells</i>	Improves DNA quality Newborns Insufficient data
<i>Birrefringence</i>		<i>Light deviation</i>	Limited to micromanipulation Insufficient data
<i>Microfluidics</i>		<i>Motility</i>	Only a motility-based sperm selection method Not available information regarding the sperm employment after selection

# IMSI

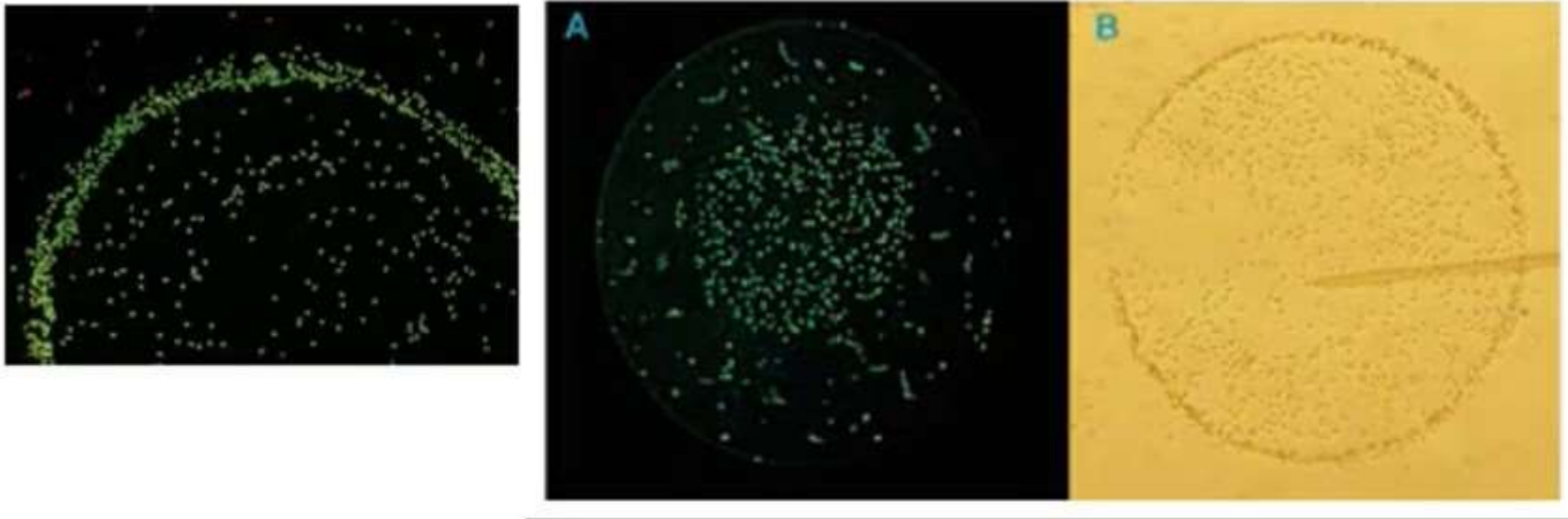
- A method of unstained, real-time, high magnification motile sperm organellar morphology examination (MSOME)





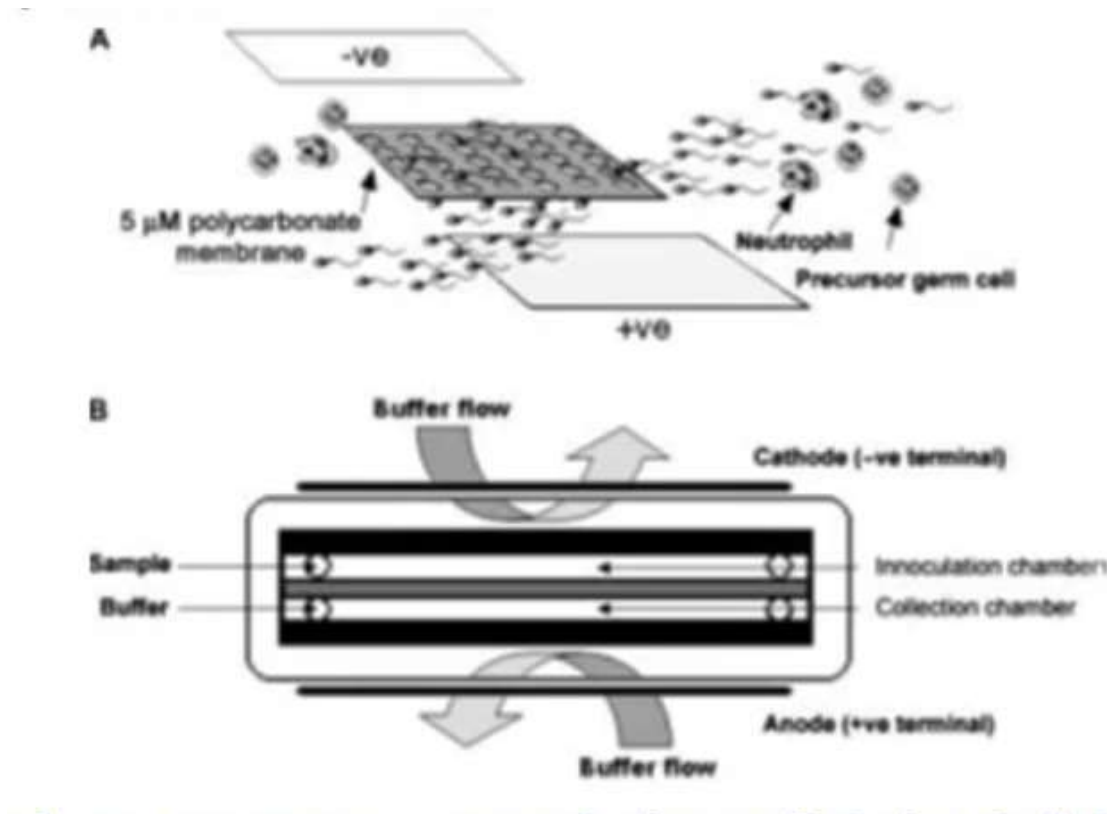
# PICSI

- Hyaluron binding



# Electrophoresis: the basis

- The highest quality spermatozoa within the ejaculate also carry the greatest net negative charge and can be separated from other electronegative cell, such as leucocytes and immature germ cells by virtue of their smaller cross-sectional size



# Birefringence

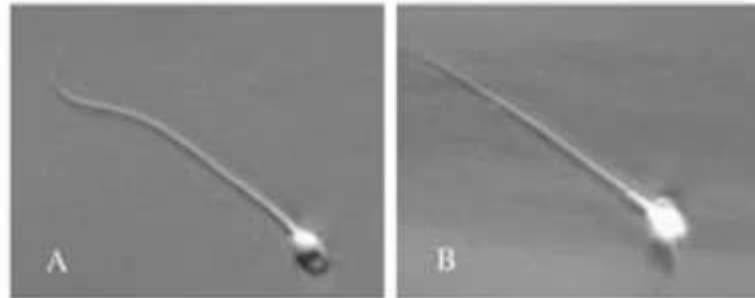
- Based on the ability of light to pass through the sperm head

## Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for intracytoplasmic sperm injection

*Luca Gianaroli, M.D.,<sup>a</sup> M. Cristina Magli, M.Sc.,<sup>a</sup> Anna P. Ferraretti, M.D.,<sup>a</sup> Andor Crippa, Ph.D.,<sup>a</sup> Michela Lippi, B.Sc.,<sup>a</sup> Serena Capitani, Ph.D.,<sup>b</sup> and Baccio Baccetti, M.D.<sup>b</sup>*

**FIGURE 2**

**(A)** The localization of birefringence in the postacrosomal region indicates that the acrosome reaction has already occurred. **(B)** The presence of birefringence in both compartments of the head, acrosome, and nucleus, identifies an intact acrosome in a nonreacted spermatozoon. The birefringence in the midpiece is clearly evident in **B**.



Gianaroli. Selection of reacted spermatozoa for ICSI. Fertil Steril 2005.

# Magnetic activated cell sorting

- Select non-apoptotic sperm based on the fact that apoptosis is most prevalent in infertile patients



**Apoptosis: related with male infertility**

**Symptoms: phosphatidylserine externalization in the outer membrane**

**Selection: annexin V bound beads**

**Results: non-apoptotic sperm enriched fraction  
Improvement in livebirth-rates (Romany et al., 2011)**

## Testicular sperm

- Reported benefit of using testicular sperm in certain cases





# Conclusions & Recommendations

- Advanced sperm selection methods lack good scientific evidence supporting generalized use.
  - Many of the sperm selection techniques have not yet been related to live birth rate
  - Success rates in reproductive results lead us to think that there is still much room for improvement
  - Sperm selection is a relevant step in the process, so selecting sperm the way we currently do is yielding relatively poor results
  - There is no 'one fits all' sperm selection method able to significantly improve success rates.
  - Finding good sperm is possible but finding the best sperm, not yet.
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