

IMSI and PICSI: do they maximize your ICSI?

Laura Rienzi

ESHRE campus – Reproductive Andrology:
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Sperm quality and ICSI

1995 Success rates of intracytoplasmic sperm injection is independent of basic sperm parameters.

Human Reproduction vol.10 no.5 pp.1123-1125, 1995

The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters.

Human Reproduction vol.11 no.5 pp.1019-1022, 1996

The outcome of intracytoplasmic sperm injection is unrelated to 'strict criteria' sperm morphology

Peter Svalander¹, Ann-Helene Jakobsson, Ann-Sofie Forsberg, Anna-Carin Bengtsson and Matts Wikland

Sperm quality and ICSI


2007 Sperm parameters associated with intracytoplasmic sperm injection outcome:

- ultramorphology of subcellular organelles (transmission and scanning electron microscopy) Berkovitz *et al.*, 1999; Mitchell *et al.*, 2006

- chromosomal stability (FISH analysis) Calogero *et al.*, 2001; Burrello *et al.*, 2003; Nagvenkar *et al.*, 2005


- nuclear integrity (SCSA, TUNEL assay, SCD test) Larson *et al.*, 2000 Virro *et al.*, Fernandez *et al.*, 2003; 2004; Greco *et al.*, 2005a,b

THESE METHODS CAN NOT BE CONDUCTED IN REAL TIME

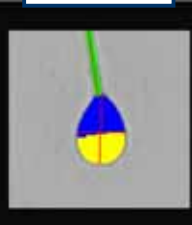
 **Sperm quality and ICSI**

A) real time FINE SPERM MORPHOLOGY ASSESSMENT

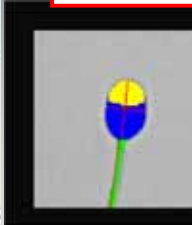
B) real time SPERM BINDING ABILITY ASSESSMENT


 **Sperm morphology and ICSI**

NORMAL



ABNORMAL



 **Sperm morphology and ICSI**

FERTILITY AND STERILITY
VOL. 79, N°1, JANUARY 2003

Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection.

De Vos A, Van De Velde H, Joris H, Verheyen G, Devroey P, Van Steirteghem A.
Centre for Reproductive Medicine, University Hospital, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Belgium.

g.en.e.r.a.

Sperm morphology and ICSI

	Normal sperm morphology (ejaculated)	Abnormal sperm morphology (ejaculated)
No. of oocytes injected	4,406	418
Fertilization rate (%)	72.5 ± 25.1	64.4 ± 38.0 *
Embryo quality	73.6 ± 29.8	72.5 ± 35.2
N° transfers	1226	41
Female age	34.1 ± 5.4	32.3 ± 6.7
Pregnancy rate (%)	37.0	22.0 *
Clinical pregnancy rate(%)	33.0	22.0 *
Implantation rate (%)	19.0 ± 31.7	11.2 ± 23.2 *
Live birth rate (%)	14.9 ± 28.4	7.9 ± 18.1 *

* Significantly different De Vos et al., 2003

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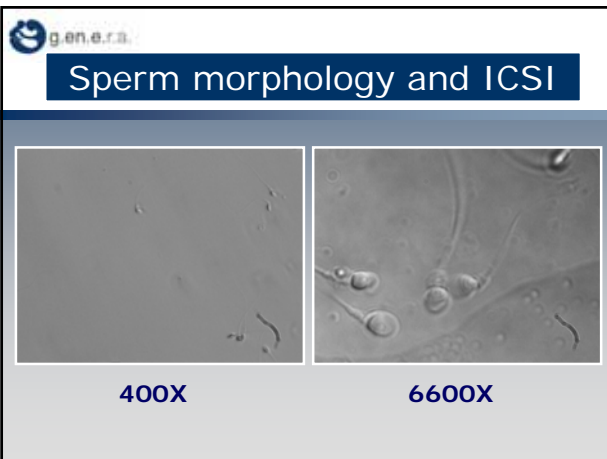
Sperm morphology and ICSI

Letter to New England Journal of Medicine:

“Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection”

Benjamin Bartoov et al. (2001)

IMSI (intracytoplasmic morphologically selected sperm injection) is a new method based on motile sperm organellar morphology examination (MSOME) performed with an inverted light microscope equipped with high-power nomarski optics enhanced by digital imaging to achieve a magnification of 6600x.



IMSI: sperm preparation

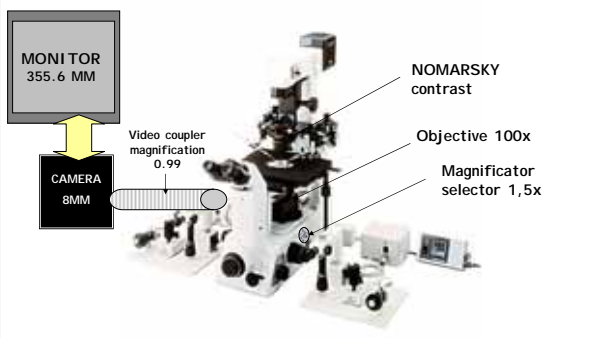
- use of a density gradient in the preparation prior to selection
- use of PVP (different concentration)
- low temperature (according to sperm motility)

POOR SPERM MOTILITY → temperature 37°C, no PVP and supplemented with 6% of human serum albumin
HIGH SPERM MOTILITY → temperature 20°C and concentration of PVP about 8%.

- glass-bottomed dish over the top of an 100x objective lens covered by a droplet of immersion oil
- examination of individual sperm at high magnification by the inverted microscope equipped with high-power nomarski optics enhanced by digital imaging
- sperm selection according to MSOME criteria

Bartoov et al., 2002

IMSI: equipment



IMSI: equipment

CALCULATION OF THE TOTAL MAGNIFICATION ON THE TELEVISION MONITOR IS BASED ON 4 PARAMETRES:

1. OBJECTIVE MAGNIFICATION 100X
2. MAGNIFICATION SELECTOR 1.0X, 1.5X, 2.0X
3. VIDEO COUPLER MAGNIFICATION 0.99
4. CCD CHIP DIAGONAL DIMENSION 8MM AND TELEVISION MONITOR DIAGONAL DIMENSION 355.6MM.

TOTAL MAGNIFICATION:

$$100 \times 1.5 \times 0.99 \times (355.6\text{MM}/8\text{MM}) = 6600$$

IMSI: sperm assessment

Motile Sperm Organellar Morphology Examination CRITERIA for SPERMATOOZA SUITABLE for IMSI

The MSOME criteria for the morphological normalcy of the sperm nucleus were defined as below:

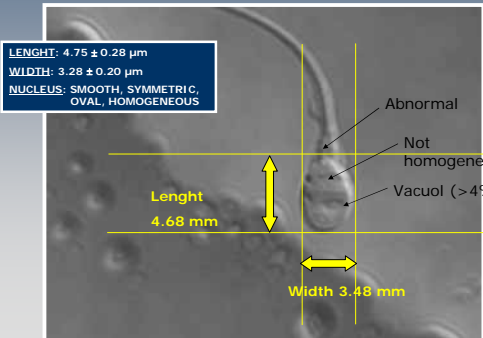
- SMOOTH
- SYMMETRIC
- OVAL CONFIGURATION
- HOMOGENEITY OF THE NUCLEAR CHROMATIN MASS (no more than one vacuole / less than 4% of the nuclear area)

The average length and width limits in 100 spermatozoa with a normally looking nucleus, are estimated as follow:

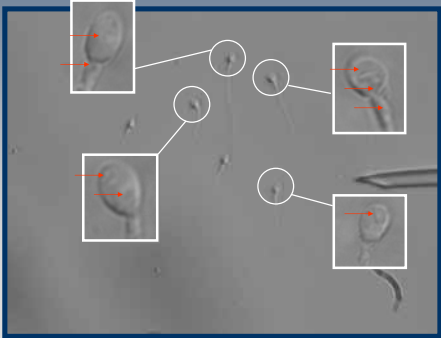
- LENGTH: $4.75 \pm 0.28 \mu\text{m}$
- WIDTH: $3.28 \pm 0.20 \mu\text{m}$

Bartoov et al., 2003

IMSI: sperm assessment



IMSI: sperm assessment



IMSI: results

Some studies have recently analysed the impact of IVF-IMSI procedure on ICSI outcome in terms of: fertilization rate, embryo development, pregnancy rate, implantation rate and abortion rate.

REVIEW OF THE LITERATURE (2003-2007):

IMSI: results

STUDY DESIGN

Bartoov et al., 2003

- Only male factor infertility
- Female <37 years old
- At least two previous failed ICSI cycles.
- More than 3 MII oocytes available.

80 couples recruited

- 18 couples excluded because no "normal" spermatozoa found...

- 50 matched with couples who underwent a routine ICSI procedure at the same IVF center and exhibited the same number of previous ICSI failure

- 12 unmatched.

IMSI: results

Bartoov et al., 2003

	CONTROL (n=50)		EXPERIMENTAL (n=50)	
	Previous procedure (ICSI)	Current procedure (ICSI)	Previous procedure (ICSI)	Current procedure (IMSI)
Retrieved oocytes	13.3 ± 5.2	13.2 ± 5.2	13.4 ± 5.5	13.6 ± 5.8
Injected oocytes	10.3 ± 4.7	10.2 ± 5.5	10.1 ± 4.6	10.6 ± 4.4
Fertilization rate (%)	63.7 ± 18.9	65.5 ± 21.5	63.1 ± 25.3	65.5 ± 17.5
Percentage of top embryos (%)	31.1 ± 16.4	31.0 ± 19.5	31.7 ± 21.6	45.2 ± 28.2 *
Transferred embryos	3.6 ± 1.1	3.5 ± 1.2	3.6 ± 1.2	3.8 ± 1.1
Implantation rate	—	9.5	—	27.9 *
Pregnancy rate	—	30	—	66 *
Abortion rate	—	33	—	9 *

IMSI: results

STUDY DESIGN

Berkovitz et al. 2004

- Female <40 years old
- More than 3 MII oocytes available in the present IVF-ICSI cycles

309 IVF / IMSI cycles recruited

- 145 cases (47%) oocytes were injected with spermatozoa exhibiting the preferred nuclear morphology **POSITIVE GROUP**

- 38 (12%) of the cycles, injections were performed with the 'second' best morphologically evaluated sperm cells (compromised criteria) **NEGATIVE GROUP**

- 126 (41%) of the cycles, the number of spermatozoa with morphologically normal nuclei was insufficient for all oocytes retrieved within one cycle **MIXED GROUP**

IMSI: results

Berkovitz et al. 2004

	STUDY GROUPS	
	Positive (n= 38) per cycle* (total)	Negative (n= 38) per cycle* (total)
No. of retrieved oocytes	13.3 ± 5.7 (506)	13.1 ± 6.8 (488)
No. of injected oocytes	10.1 ± 4.5 (382)	9.2 ± 4.0 (334)
Fertilization rate (%)	71.3 ± 20.8 (264) *	50.3 ± 24.1 (174)
Percentage of top embryos (%)	34.9 ± 31.3 (81) *	19.4 ± 27.1 (32)
No. Embryos transferred	3.3 ± 1.2 (125)	3.5 ± 1.4 (113)
Implantation rate (%)	25.0 ± 25.9 (31) *	5.9 ± 12.9 (8)
Pregnancy rate per transfer (%)	52.6 (20) *	18.4 (7)
Abortion rate (%)	10.0 (2) *	57.1 (4)

* Significantly different (P ≤ 0.05)

IMSI: results

Berkovitz et al. 2004

choice	Specific Nuclear Malformations	No. of Patients	Frequency (%) of malformation within each patient		Pregnancy outcome		
			Mean ± SD	Range	D	O	A
1	Large oval	5	77.9 ± 8.6	68-89	2	0	0
1	Small oval	2	80.0 ± 28.3	60-100	0	0	0
2	Wide forms (>3.7µm width)	2	100		0	0	0
2	Narrow forms (<2.9µm width)	7	83.5 ± 15.5	60-100	1	0	0
3	Regional disorder	2	80.0 ± 28.3	60-100	0	0	0
4	Large vacuoles + normal shape/size	18	86.1 ± 15.4	63-100	0	0	3
5	Narrow forms + large vacuoles	2	70.9 ± 15.5	60-82	0	0	1

D = Delivered; O = Ongoing pregnancy; A = Abortion

IMSI: results

STUDY DESIGN

Hazout et al. 2005

- Female <40 years old
- Two or more previous ICSI attempts failed

125 couples recruited:

- 88 exhibiting male factor (WHO criteria)
- 37 exhibiting normal semen (WHO criteria)

72 cases were also examined for sperm DNA integrity (TUNEL test)

IMSI: results


Hazout et al. 2005

Outcome variable	ICSI attempt	
	Conventional	High magnification
Oocytes injected	9.5 + 2.7	9.4 + 2.5
2PN zygotes	6.2 + 1.9	6.4 + 1.8
Cleaved embryos	5.9 + 1.1	6.3 + 1.0
Good-morphology embryos	3.1 + 1.1	4.0 + 1.2

IMSI: results

Hazout et al. 2005


	ICSI attempt		P value
	Conventional	High magnification	
Transfer procedure (n)	125	125	-
Embryos transferred (n)	265	261	>0.05
Total pregnancies (n)	8	51	>0.001
Clinical pregnancies (n)	3	47	>0.001
Deliveries (n)	0	42	>0.001
Gestational sacs with heartbeat (n)	2	53	>0.001
Babies born (n)	0	46	>0.001
Pregnancy rate (%)	6.4	40.8	>0.001
Clinical pregnancy rate (%)	2.4	37.6	>0.001
Clinical implantation rate (%)	0.8	20.3	>0.001
Delivery rate (%)	0	33.6	>0.001
Birth rate (%)	0	17.6	>0.001

 **IMSI: results**

Hazout et al., 2005


ICSI attempt	% DNA-fragmented spermatozoa					
	< 30 % (n = 51)		30-40 % (n = 11)		> 40 % (n = 10)	
	Implantation rate ^a	Birth rate ^b	Implantation rate ^a	Birth rate ^b	Implantation rate ^a	Birth rate ^b
Conventional (%)	1/107 (0.9)	0/107 (0)	0/25 (0)	0/25 (0)	1/24 (0.7)	0/24 (0)
High magnification (%)	25/106 (23.6)	20/106 (18.9)	4/23 (17.4)	4/23 (17.4)	7/21 (33.3)	6/21 (28.6)
P value	<0.001	<0.001	<0.05	<0.05	<0.01	<0.01

^aNumber of gestational sacs per number of embryos transferred
^bNumber of babies born per number of embryos transferred

 **IMSI: results**

STUDY DESIGN **Bartoov et al., 2006**


1. Comparison between 80 couples, who underwent an IVF-IMSI trial with matched couples, who underwent IVF-ICSI procedure
2. Comparison performed between matched IMSI groups:
 - Embryos obtained from injection by spermatozoa with normal nuclei. 'BEST' GROUP
 - Embryos obtained from injection by spermatozoa with minimal morphological impairment. 'SECOND' BEST GROUP

 **IMSI: results**

Bartoov et al., 2006

	MATCHED STUDY GROUPS	
	IMSI (n = 80)	ICSI (n = 80)
Nuber of retrieved ova	12.1 ± 6.0	11.9 ± 5.9
Number of injected ova	9.2 ± 5.0	9.3 ± 5.0
Fertilization rate (%)	67.4 ± 20.8	69.1 ± 22.6
Top embryos (%)	38.7 ± 31.6 *	25.7 ± 28.3
Number of embryos transferred	3.1 ± 1.3	3.1 ± 1.1
Implantation rate	31.3 ± 36.3 *	9.4 ± 17.4
Pregnancy rate per transfer	48 (60) *	20 (25)
Abortion rate per pregnancy	7 (14) *	8 (40)


* significantly different (P ≤ 0.05)

 **IMSI: results**

Bartoov et al., 2006

	STUDY GROUPS	
	'BEST' (n = 70)	'SECOND BEST' (n = 70)
Number of retrieved oocytes	12.3 ± 4.8	13.0 ± 5.9
Number of injected oocytes	9.5 ± 4.1	8.9 ± 3.9
Fertilization rate (%)	74.1 ± 20.5 *	62.3 ± 24.3
Top embryos (%)	26.7 ± 20.5	16.2 ± 26.0
Number of embryos transferred	3.3 ± 1.3	3.2 ± 1.4
Implantation rate	26.1 ± 26.8 *	8.3 ± 15.1
Delivery rate per cycle	52.8 *	17.1
Pregnancy rate per cycle	58.6 *	25.7
Abortion rate per pregnancy	9.8 *	33.3


* significantly different (P ≤ 0.05)

 **IMSI: results**

Bartoov et al., 2006

Hierarchy of choice	Specific nuclear malformations	No. of patients	IMSI outcome (delivery)		
			P	A	D
1	Large oval	14	6	3	3
1	Small oval	18	6	1	5
2	Wide forms (>3.7 µm width)	1	0		
2	Narrow forms (< 2.9 µm width)	8	1	0	1
3	Regional disorder	1	0		
4	Large vacuoles + normal shape / size	25	4	2	2
5	Large vacuoles + abnormal forms	3	1	0	1

P = Pregnancy; A = Abortion; D = Delivery

 **IMSI: results**

STUDY DESIGN **Berkovitz et al., 2006**

EXPERIMENTAL ICSI GROUP:

- Spermatozoa with a normal nuclear shape but large **VACUOLES**.

CONTROL GROUP:

- Only spermatozoa with strictly defined morphologically normal nuclei

28 IVF-ICSI cycles per group, based on MSOME, selected from 878 trials

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IMSI: results

Berkovitz et al. 2006

	Experimental (n=28)	Control (n=28)
Retrieved oocytes	13.0 ± 5.0	12.1 ± 4.4
Injected oocytes	8.1 ± 3.6	8.4 ± 3.2
Fertilization rate (%)	68.7 ± 20.3	72.8 ± 18.5
Top quality embryos (%)	23.0 ± 31.1	27.1 ± 29.4
Embryos transferred	3.0 ± 1.3	3.2 ± 0.7
Pregnancy rates	18%	50% *
Abortion rate per pregnancies obtained	80%	7% *

* significantly different (P ≤ 0.05)

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IMSI: results

Bach et al. Oral Presentation ESHRE 2007

STUDY DESIGN

1. Comparison of embryo development between ICSI and IMSI
2. Influence on sperm vacuoles on blastocyst development

282 MII oocytes injected (151 standard ICSI, 131 IMSI)

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IMSI: results

Bach et al. Oral Presentation ESHRE 2007

	ICSI	IMSI
Fertilization rate	72.2	70.2
Cleavage rate on day 3	79.5	81.7
Blastocyst formation rate	27.5	52.0

Moreover, blastocyst formation rate was directly correlated with the type of injected spermatozoa:

Type	Percentage	Notes
G1	73.3%	(no abnormalities)
G2	45.0%	(small vacuoles: <4%, normal shape)
G3	9.3%	(large vacuoles: >4%, normal shape)
G4	0.4%	(large vacuoles: >4%, abnormal shape)


 **IMSI: do it maximize your ICSI?**

- it makes possible to exclude from selection for ICSI spermatozoa with nuclear and/or flagellar defects (which may decrease the probability of accidental injection of a DNA-damaged spermatozoon to the oocyte).
- it seems to improve laboratory and clinical outcomes (BF: 52% vs 27.5%; IR: 30% vs 10%).

BUT:


- it is not always possible to select "normal" sperm, in such cases the IR is dramatically affected
- time consuming technique, need for additional highly trained man power, additional cost of the procedure.

Prospective randomized studies are not yet available.


 **Sperm quality and ICSI**

A) real time FINE SPERM MORPHOLOGY ASSESSMENT

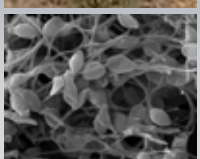
B) real time SPERM BINDING ABILITY ASSESSMENT


 **Sperm binding assay**

Hyaluronan and oocyte
 – Major component of cumulus oophorus (Drahorad *et al.* 1991)



Hyaluronan and sperm
 – Cellular maturity and viability (Huszar *et al.* 2003)




 **Sperm binding assay**

Jakab et al., 2005


HYPOTHESIS


- Mature spermatozoa may selectively bind to HA.
- Diminished sperm maturity (failure of spermatogenic membrane remodeling) may be related to increased levels of chromosomal aberrations.
- Solid-state HA binding would facilitate the selection of individual mature sperm with low levels of chromosomal aneuploidies.

 **Sperm binding assay**

HBA assay basics

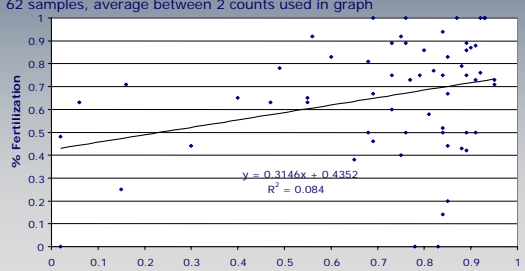
- Contains thin layer of Hyaluronan
- Selected spermatozoa bind to solid state HA



 **Sperm binding and IVF**

Sperm binding and conventional IVF

62 samples, average between 2 counts used in graph



$y = 0.3146x + 0.4352$
 $R^2 = 0.084$

P = 0.022

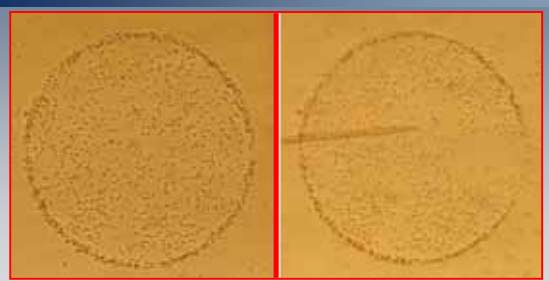
Sperm binding and ICSI

SPERM PREPARATION

- a drop of washed spermatozoa is placed close to the edge of the HA spot (in the special Petri dish)
- spermatozoa are allowed to migrate spontaneously
- HA-bounded spermatozoa are collected with the ICSI micropipette

PETRI DISH ICSI = PICSI

Sperm binding and ICSI



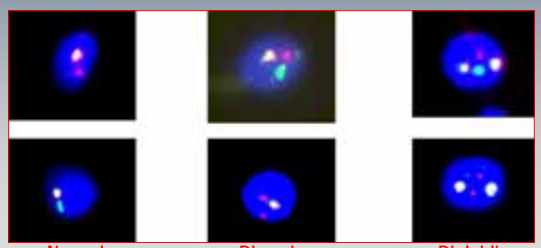
Washed sperm placed near solid HA spot bonded to Petri dish (Biocat) in HTF

Incubation, room temp., 10 min

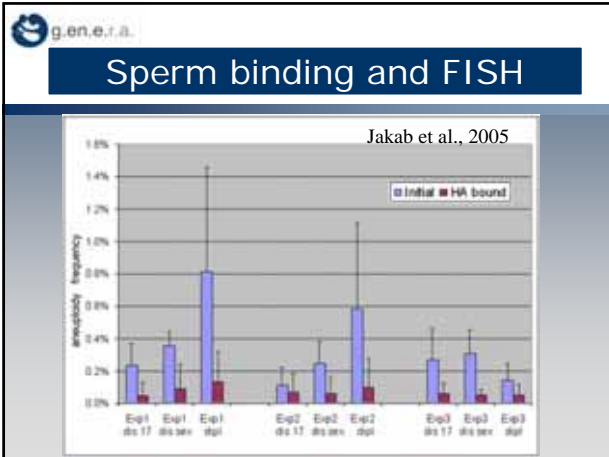
Collect bound sperm with ICSI micropipette

Sperm binding and FISH

Multicolor FISH on decondensed sperm nuclei
(centromeric probes X, Y and 17)



Normal Disomies Diploidies



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Sperm binding and ICSI

Sanchez et al. Oral Presentation ESHRE 2005

	PICSI (n=18)
Fertilization rate	67.0%
Top quality embryos	51.7%
Pregnancy rates	33.0%
Abortion rate per pregnancies obtained	0%
Take home baby	33.0%


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Sperm binding and ICSI

WorriIow et al., 2006

Study on sibling oocytes (n=273)


	ICSI (n=26)	PICSI (n=26)
Fertilization rate	66.6%	61.1%
Blistocyst formation	53.9%	51.9%
Pregnancy rates	25%	57.1%

 **Sperm binding and ICSI**

Janssens et al., 2006


Study on sibling oocytes (n=291)

	ICSI (n=20)	PICSI (n=20)
Fertilization rate	66.9%	72.9%
Oocyte degeneration rate	13.8%	9.0%
Top quality embryos	NS	NS

 **Sperm binding and ICSI**

Nagy et al., personal communication

	ICSI (n=6)	PICSI (n=6)
Fertilization rate	75.8%	79.2%
Top quality embryos	52.1%	42.4%
Pregnancy rates	66.6%	83.3%
Abortion rates	0	0
Implantation rates	50%	71.4%

 **IMSI and PICSI**

Objective of the techniques is to maximize ICSI improving sperm selection

IMSI: morphological parameter

- sperm maturity
- DNA integrity

PICSI: biochemical parameter

- sperm maturity
- euploidy

FULLY MATURE SPERMATOZOA: extrusion of cytoplasm, plasma membrane remodelling:

- normal head morphology
- fully formed binding sites for the zona pellucida



IMSI and PICSI

IMSI and PICSI: do they maximize your ICSI?

UNTILL NOW, NO SUFFIENT DATA ARE AVAILABLE!!!



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