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Is there an impact of IMSI on reproductive outcome?

Laura Rienzi
Senior Clinical Embryologist

Salzburg, Austria – 1,2 April 2011

No commercial activities are related to this presentation

Learning objectives

1) Sperm selection procedure: how to do it?
2) Clinical outcomes related to sperm selection procedure: is there a possible improvement?
3) Sperm phenotype: what should we look for?
4) Conclusions: do we have enough evidences to conclude on this aspect?

Sperm morphology and ICSI

1995

Success rates of intracytoplasmatic sperm injection is indipendent 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.

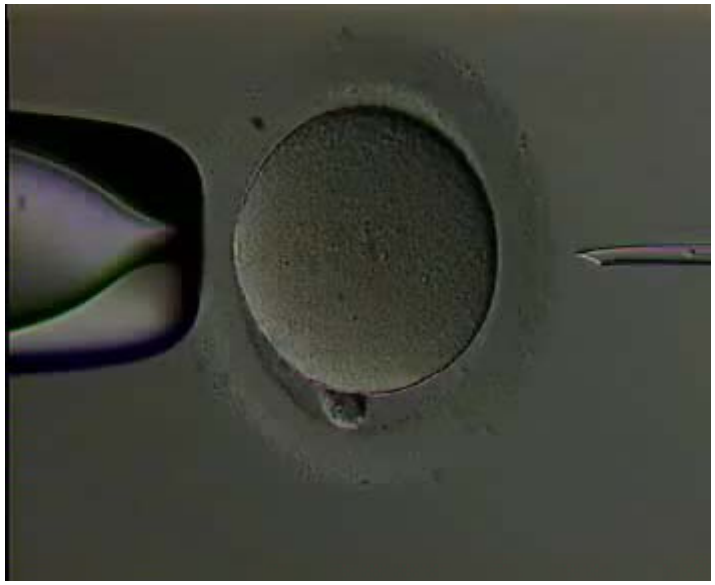
Nagy ZP, Liu J, Joris H, Verheyen G, Tournaye H, Camus M, Derde MC, Devroey P, Van Steirteghem AC.

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

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

Peter Svalander[1], Ann-Helene Jakobsson, Ann-Sofie Forsberg, Anna-Carin Bengtsson and Matts Wikland

Sperm morphology and ICSI



The establishment of a pregnancy even with compromised ejaculated (dysfunctional and/or with high rates of DNA fragmentation) may be attributed to the corrective role of selecting a single spermatozoon for ICSI.

Virro, Larson-Cook et al. 2004

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 VeldeH, JorisH, VerheyenG, DevroeyP, Van Steirteghem A.

Centre for Reproductive Medicine, University Hospital, Dutch-speakingBrussels Free University (VrijeUniversiteitBrussel), Belgium.

Sperm morphology and ICSI

Retrospective study

662 consecutive ICSI cycles

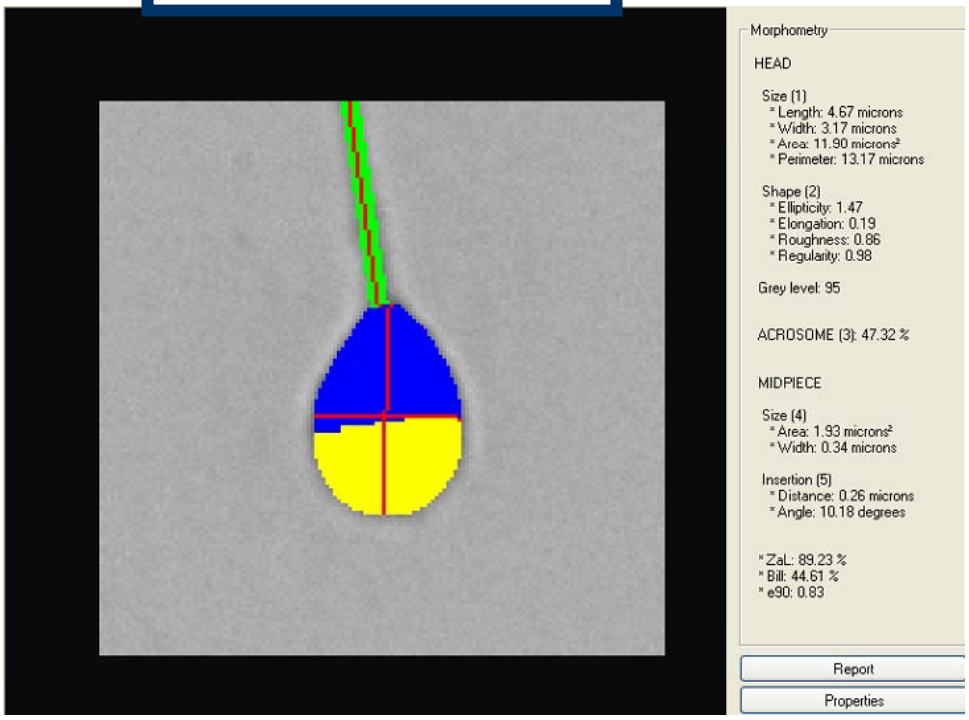
	Normal sperm morphology (ejaculated)	Abnormal sperm morphology (ejaculated)
No. Of oocytes injected	4,406	418
Fertilization rate (%)	72.5 ± 25.1	64.4 ± 38.0 *
Embryo quality	73.6 ± 29.8	72.5 ± 35.2
N°transfers	1226	41
Female age	34.1 ± 5.4	32.3 ± 6.7
Pregnancy rate (%)	37.0	22.0 *
Clinical pregnancy 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

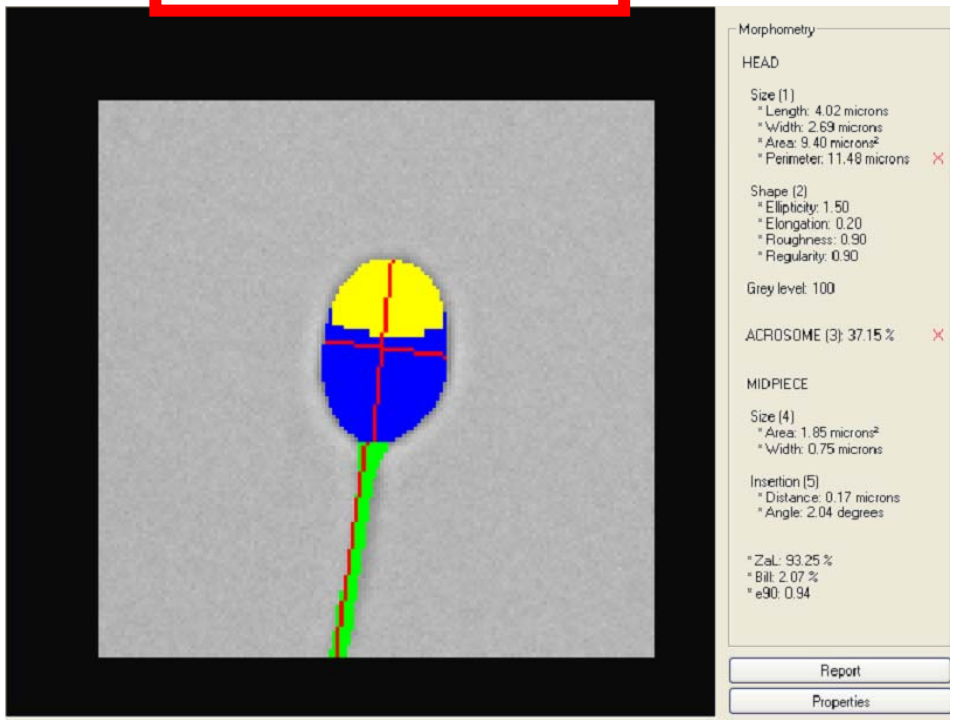
De Vos *et al.*, 2003

Sperm quality and ICSI

NORMAL



ABNORMAL



REAL TIME FINE SPERM MORPHOLOGY ASSESSMENT

Intracytoplasmic Morphologically Selected Sperm Injection

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)

Introduction of a new concept to observe spermatozoa called 'motile-sperm organelle-morphology examination' (MSOME) and to evaluate the fine nuclear morphology of motile spermatozoa in real time.

Intracytoplasmic Morphologically Selected Sperm Injection

(IMSI)

1. **Optical resolution**
2. **Image contrast**
3. **Maximal optical magnification**
4. **Magnification of the video system**

NOMARSKY

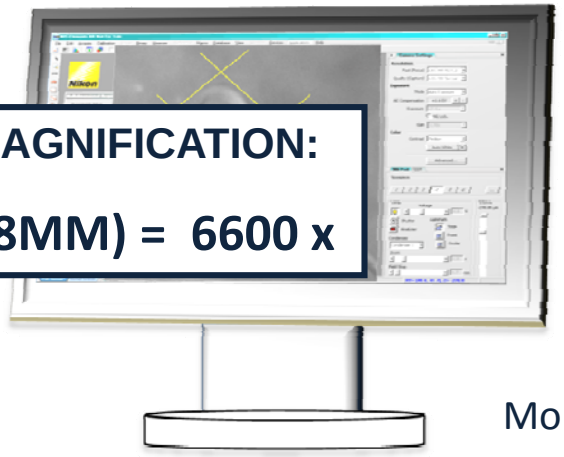
contrast

DIC

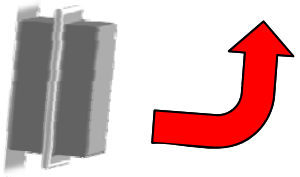
FINAL DIGITALLY ENHANCED MAGNIFICATION:

100 X 1.5 X 0.99 X (355.6MM/8MM) = 6600 x

100X Objective



Monitor



Video coupler magnification 0.99

Camera 8mm

IMSI: Sperm preparation

Bartoov et al., 2002

- Use of a density gradient in the preparation prior to selection

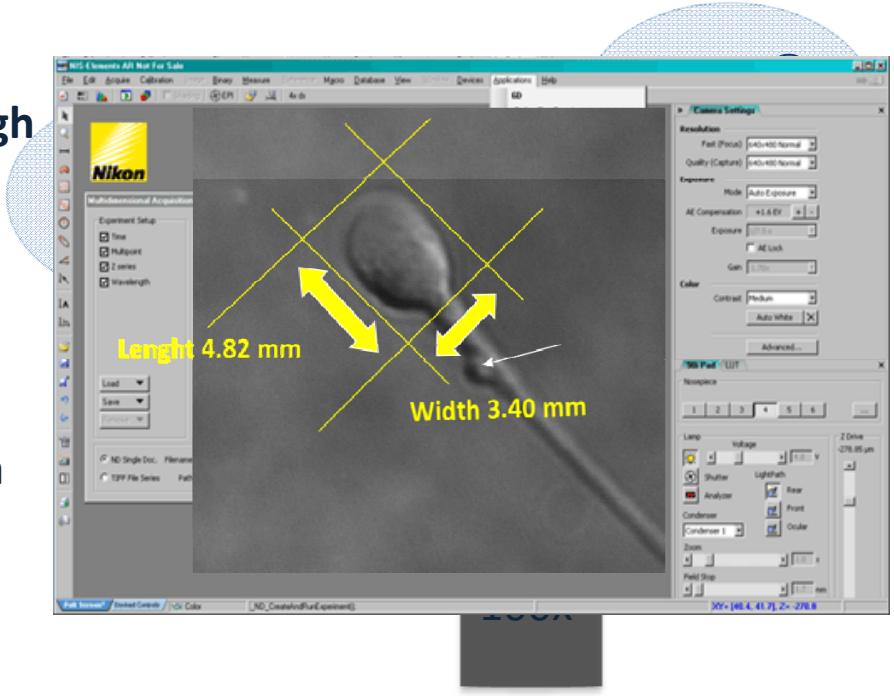
- Use of PVP (different concentration)

-low temperature (according to sperm motility)

-glass-bottom dish over the top of an 100x objective lens covered by a droplet of immersion oil

- Examination of individual spermatozoa at high magnification by the inverted microscope equipped with high-power nomarski optics enhanced by digital imaging

- sperm selection according to MSOME criteria



IMSI: Sperm assessment

Motile Sperm Organellar Morphology Examination

CRITERIA to select SPERMATOZOA SUITABLE for IMSI

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

- **SMOOTH**
- **SYMMETRIC**
- **OVAL CONFIGURATION**
- **HOMOGENEITY OF THE NUCLEAR CHROMATIN MASS**

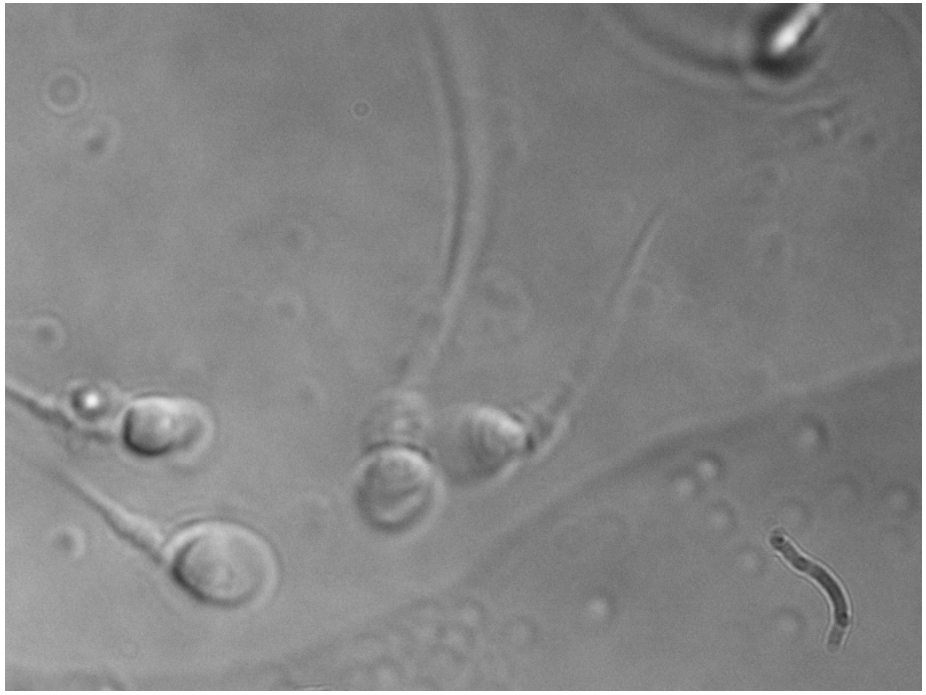
(no more than one vacuole / less than 4% of the nuclear area)

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

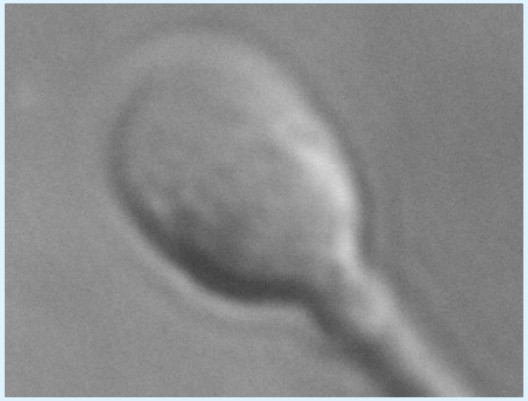
- **LENGHT: 4.75 ± 0.28 µm**
- **WIDTH: 3.28 ± 0.20 µm**

Bartoov et al., 2003

IMSI: Sperm assessment



IMSI: Sperm assessment



Time expensive technique

Highly trained embryologists required

Additional cost to upgrade the equipment

IMSI: Clinical results

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

META-ANALYSIS (Souza Setti et al., 2010):

Studies included in the review

Trial	Design	Participants	Numbers		Outcomes
			Experimental (IMSI)	Control (ICSI)	
Bartoov et al. (2003)	Comparative	50 couples undergoing IMSI (male factor infertility, female age <37 years, more than three retrieved metaphase II oocyte in the last ICSI cycle, at least two previous consecutive failed ICSI cycles), matched with 50 couples undergoing ICSI	50	50	Fertilization rate, top-quality embryo rate, implantation rate, pregnancy rate, miscarriage rate
Berkovitz et al. (2006)	Comparative	80 couples (male factor infertility, female age <37 years, at least two previous consecutive failed ICSI cycles), matched with 80 couples undergoing ICSI	80	80	Fertilization rate, top-quality embryo rate, implantation rate, pregnancy rate, miscarriage rate
Antinori et al. (2008)	Randomized	446 couples (at least two previous diagnosis of severe oligoasthenozoospermia, at least 3 years of primary infertility, female age <35 years and undetected female factor) randomly allocated to receive ICSI and IMSI treatments	227	219	Fertilization rate, implantation rate, pregnancy rate, miscarriage rate

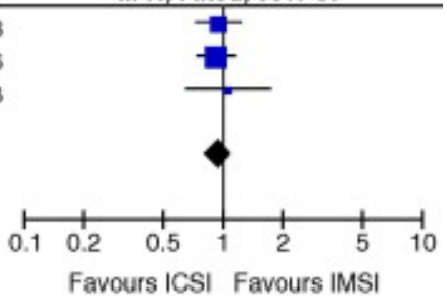
Laboratory outcome

A

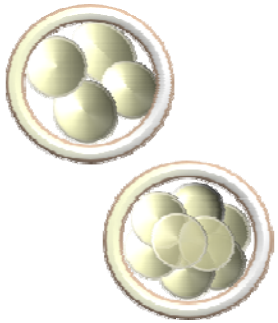
Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
Bartoov et al., 2003	341	530	334	510	37.3%	0.95 [0.74, 1.23]	2003
Berkovitz et al., 2006	528	784	514	744	52.9%	0.92 [0.74, 1.14]	2006
Antinori et al., 2008	624	658	605	640	9.7%	1.06 [0.65, 1.72]	2008
Total (95% CI)		**1972**		**1894**	**100.0%**	**0.95 [0.81, 1.11]**	
Total events	1493		1453				

Heterogeneity: Chi² = 0.27, df = 2 (P = 0.87); I² = 0%

Test for overall effect: Z = 0.69 (P = 0.49)



Events = number of fertilized oocytes; Total = number of injected oocytes.

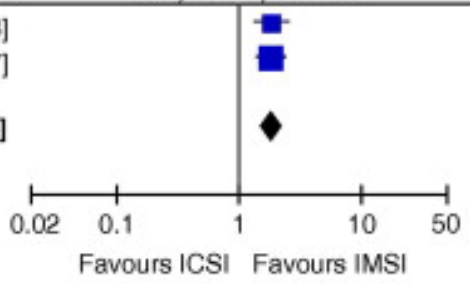


B

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Bartoov et al., 2003	154	341	103	334	41.0%	1.85 [1.35, 2.53]
Berkovitz et al., 2006	204	528	132	514	59.0%	1.82 [1.40, 2.37]
Total (95% CI)		**869**		**848**	**100.0%**	**1.83 [1.50, 2.24]**
Total events	358		235			

Heterogeneity: Chi² = 0.00, df = 1 (P = 0.95); I² = 0%

Test for overall effect: Z = 5.86 (P < 0.00001)



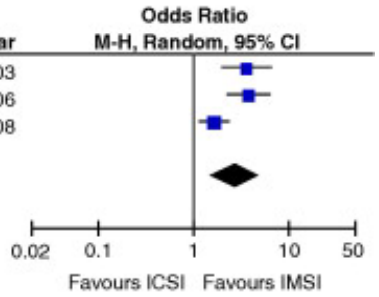
Events = number of top quality embryos; Total: number of obtained embryos.

Clinical outcome

C

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Bartoov et al., 2003	53	190	17	175	30.1%	3.60 [1.99, 6.50]	2003
Berkovitz et al., 2006	69	248	23	248	32.6%	3.77 [2.26, 6.29]	2006
Antinori et al., 2008	97	560	59	521	37.3%	1.64 [1.16, 2.32]	2008
Total (95% CI)		**998**		**944**	**100.0%**	**2.72 [1.50, 4.95]**	
Total events	219		99				

Heterogeneity: $Tau^2 = 0.22$; $Chi^2 = 9.37$, df = 2 (P = 0.009); $I^2 = 79\%$
Test for overall effect: Z = 3.29 (P = 0.001)

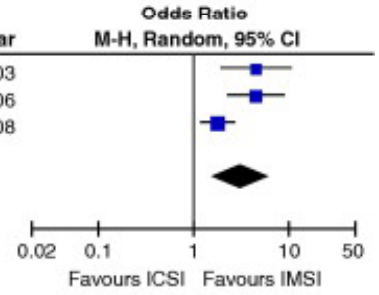


Events = number of gestational sacs; Total: number of transferred embryos.

D

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Bartoov et al., 2003	33	50	15	50	27.7%	4.53 [1.95, 10.51]	2003
Berkovitz et al., 2006	48	80	20	80	32.3%	4.50 [2.29, 8.84]	2006
Antinori et al., 2008	89	227	58	219	40.1%	1.79 [1.20, 2.67]	2008
Total (95% CI)		**357**		**349**	**100.0%**	**3.12 [1.55, 6.26]**	
Total events	170		93				

Heterogeneity: $Tau^2 = 0.27$; $Chi^2 = 7.48$, df = 2 (P = 0.02); $I^2 = 73\%$
Test for overall effect: Z = 3.19 (P = 0.001)

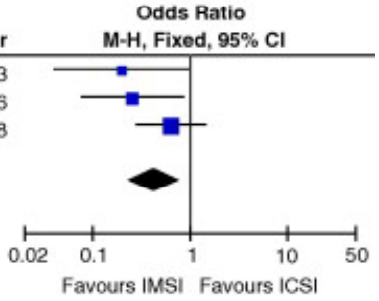


Events = number of pregnancies; Total= number of cycles.

E

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
Bartoov et al., 2003	3	33	5	15	20.8%	0.20 [0.04, 0.99]	2003
Berkovitz et al., 2006	7	48	8	20	32.2%	0.26 [0.08, 0.85]	2006
Antinori et al., 2008	15	89	14	58	47.0%	0.64 [0.28, 1.44]	2008
Total (95% CI)		**170**		**93**	**100.0%**	**0.42 [0.23, 0.78]**	
Total events	25		27				

Heterogeneity: $Chi^2 = 2.47$, df = 2 (P = 0.29); $I^2 = 19\%$
Test for overall effect: Z = 2.74 (P = 0.006)



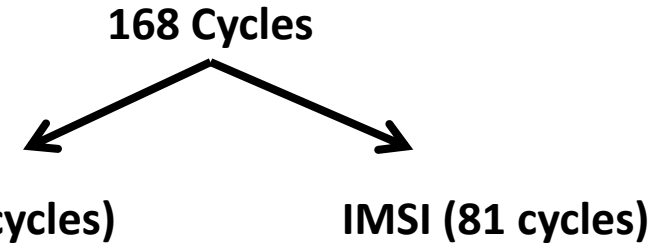
Events = number of miscarriages; Total = number of pregnancies.

IMSI: Prospective randomized study

Clinical outcome of intracytoplasmıc injection of spermatozoa morphologically selected under high magnification: a prospective randomized study

Balaban et al. RBM online 2011

IMSI: Prospective randomized study



Characteristics	ICSI	IMSI
Female age	28.80±4.08	29.67±4.03
Male age	32.53±4.87	33.97±5.52
Aetiology of infertility		
Male factor	39(48.1)	38(43.7)
Ovulatory	1 (1.2)	2 (2.3)
Tubal	10 (12.3)	7 (8.0)
Unexplained	24 (29.6)	30 (34.5)
Multiple factors	7 (8.6)	10 (11.5)

Balaban et al. RBM online 2011

Sperm parameters and oocyte characteristics

Characteristics	ICSI	IMSI
Sperm Parameters		
Sperm Count (million/ml)	41.96 ± 39.42	38.30 ± 34.38
Ejaculate volume (ml)	2.83 ± 1.18	2.64 ± 1.34
Motility (% total count)	41.35 ± 16.68	40.74 ± 17.22
Morphologically normal spermatozoa (% total count)	2.89 ± 1.68	2.89 ± 1.59
Spermatozoa with a vacuolar nucleus (%)	32.72 ± 16.81	34.88 ± 18.45
Oocyte characteristics		
No. Of oocytes collected	12.30 ± 4.75	11.47 ± 3.96
No. Of metaphaseII oocytes	9.28 ± 3.43	8.71 ± 2.95

Balaban et al. RBM online 2011

Laboratory and clinical outcome

Outcome	ICSI	IMSI	P-value
Duration of ICSI procedure (min)	13.55 ± 5.43	20.54 ± 9.43	< 0.001
Fertilization rate (%)	80.97 ± 15.06	81.60 ± 10.65	NS
Grade 1 and 2 embryos on transfer day (%)	4.84 (63.95)	5.01 (66.44)	NS
Mean no.of embryos transferred	2.76 ± 0.46	2.72 ± 0.48	NS
Clinical pregnancy per initiated cycle (%)	36/81 (44.4)	47/87 (54.0)	NS
Live birth rate per initiated cycle (%)	31/81 (38.3)	38/87 (43.7)	NS
Implantation rate (%)	42/215 (19.5)	66/228 (28.9)	NS
Multiple pregnancy rate (%)	6/36 (16.7)	16/47(34.0)	<0.001

Balaban et al. RBM online 2011

Prospective randomized study: standard ICSI *vs* IMSI in OAT patients

Inclusion criteria:

- female age < = 42 years
- basal serum FSH (< = 10 mIU/ml)
- severe OAT (<= 4% according to Kruger)

Exclusion criteria:

Less than 3 MII obtained

IMSI vs ICSI in OAT: Material and methods

Patient

hCG a

day of

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ICSI)

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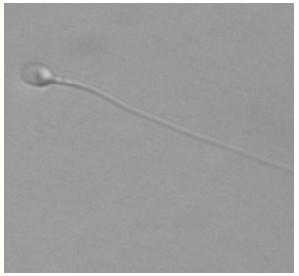
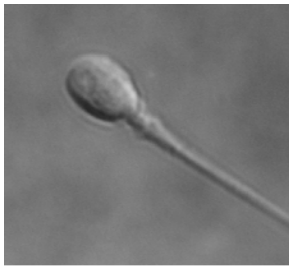
Bartoov





Preliminary Results

	GROUPS	
	IMSI (n = 17)	ICSI (n = 16)
Female age	35.2 ±2.8	34.9 ±3.4
Number of retrieved oocytes	11.3 ± 4.6	10.4 ± 3.8
Number of retrieved MII	9.3 ± 4.4	8.6 ± 3.0
Number of injected oocytes	49 (2.9 ± 0.3)	48 (3.0 ±0)
Fertilization rate (%)	42/49 (85.7)	40/78(83.3)
Top embryos (%)	29/42 (68.2)	27/40 (67.6)
Number of embryos transferred	2.5 ± 0.5	2.5 ± 0.8
Embryo transfers performed	17/17	15/16
Implantation rate	7/42 (16.7)	8/40 (20.0)
Clinical pregnancyrate per cycle	5/17 (29.4)	6/16 (37.5)
Abortion rate per clinical pregnancy	1/5 (20.0)	1/6 (16.7)

Which sperm phenotype does really reflect competence?

Does the presence of sperm nuclear vacuoles affect ICSI outcome?

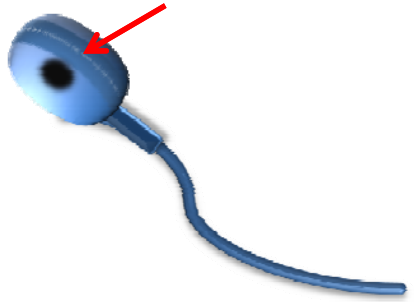
Hierarchy of choice	Specific nuclear malformations	No. of patients	IMSI outcome (delivery)		
			P	A	D
1	Large oval	14	6	3	3
1	Small oval	18	6	1	5
2	Wide forms (>3.7 µm width)	1	0		
2	Narrow forms (< 2.9 µm width)	8	1	0	1
3	Regional disorder	1	0		
4	Large vacuoles + normal shape / size	25	4	2	2
5	Large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

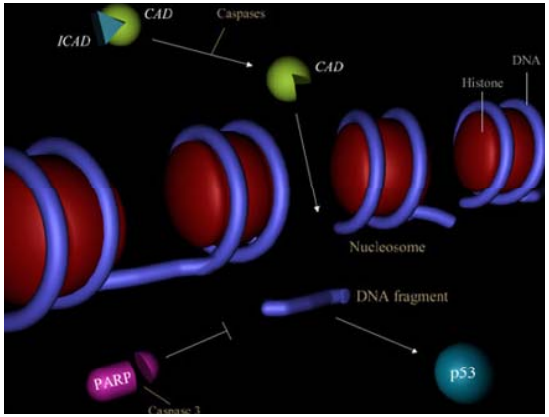
P = Pregnancy; A = Abortion; D

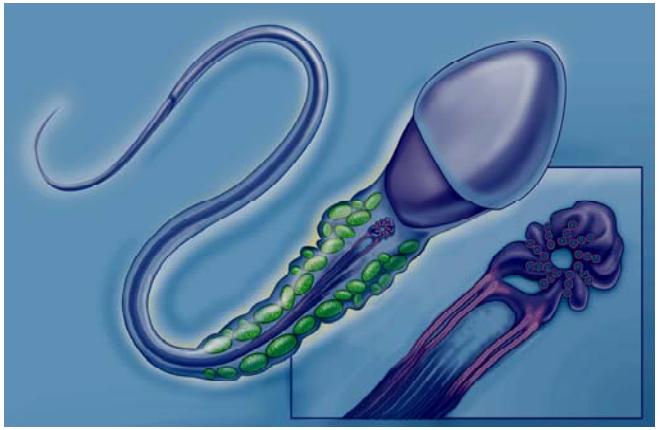
Berkovitz et al. 2006

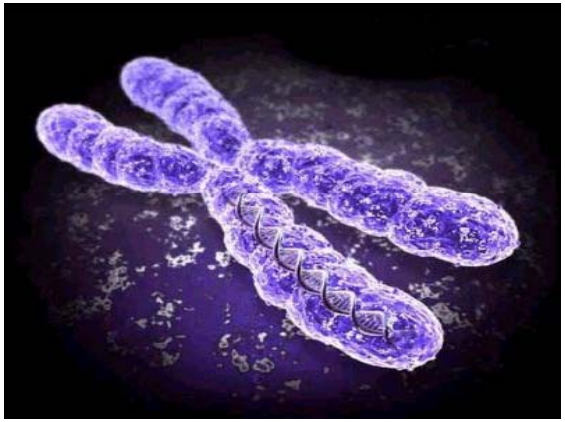
Nuclear vacuoles and sperm competence

- DNA Integrity
- Mitochondrial function
- Chromosomal aberrations









Effect of paternal DNA damage

An increased percentage of spermatozoa with fragmented DNA has been related to:

- Compromised embryo ability to develop

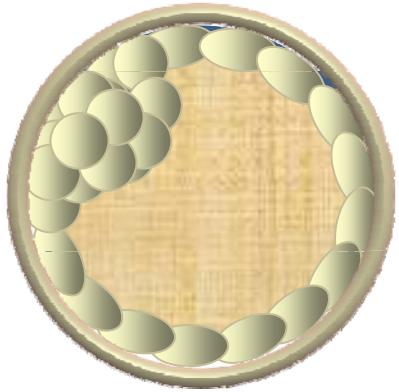
  Tesarik et al., 2004

- Aberrant growth, premature aging, abnormal behavior, and mesenchymal tumors.

  Fernandez-Gonzalez et al., 2008

Does the presence of nuclear vacuoles influence the embryo's competence to develop to the blastocyst stage?

Characteristics	*Value*
No. of patients	25
Women's age (years, mean ± SD)	36.2 ± 2.5
No. of oocytes (mean ± SD)	247 (9.9 ± 1.6)
No. of MII oocytes (mean ± SD)	198 (7.9 ± 1.8)
No. of MII oocytes for injection (mean ± SD)	164 (6.6 ± 1.4)

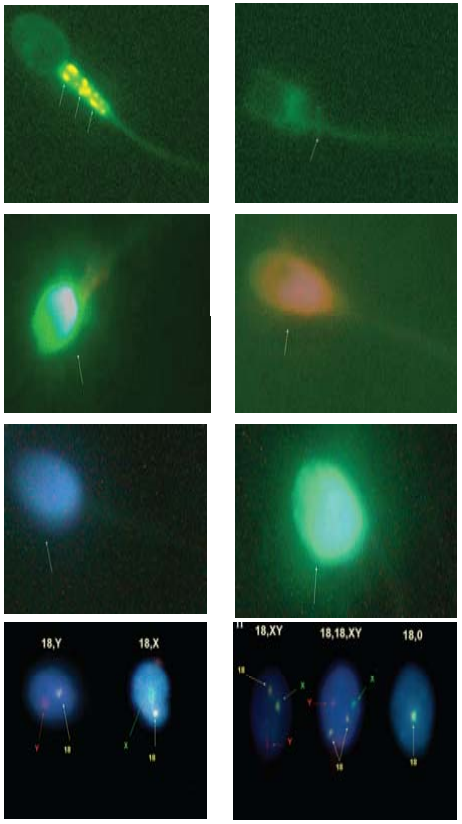


Results *Type of injected spermatozoa*	*Grade I/II*	*Grade III/IV*	*P-value*
No. of injected oocytes (mean ± SD)	86 (3.4 ± 0.9)	78 (3.12 ± 1.0)	NS
Percentages (no.) of embryos per injected oocyte			
Zygotes	89.5 (77)	84.6 (66)	NS
Day-3 embryos	88.4 (76)	82.1 (64)	NS
Good quality day-3 embryos	43.0 (37)	30.8 (24)	NS
Blastocysts	60.5 (52)	3.8 (3)	<0.001
Good quality blastocysts	37.2 (32)	1.3 (1)	<0.001

Late paternal effect that impacts embryo development after the onset of paternal DNA content contribution to embryonic development

Vanderzwalmen et al., 2008

Sperm morphology and physiological status



Test	*Whole sperm samples*			*TD patients, single sperm*	
	Controls (n=10)	*PO (n=10)*	*TD (n=10)*	*Group A (100 cells)*	*Group B (100 cells)*
Mitosensor (%)	15.5 ± 6.1	31.6± 14.1[a]	48.7±15.3[bc]	13.3 ± 4.9	52.2 ± 14.7[e]
Acridine orange (%)	15.7 ± 6.1	29.8 ± 8.8[c]	77.9± 3.3[c,d]	5.3 ± 3 .0	71.9 ± 11.1[e]
TUNEL (%)	14.0 ± 6.4	28.9± 12.7[a]	58.0± 1.1[b,c]	9.3 ± 4.8	40.1 ± 11.6[e]
Aneuploidies (%)	1.2 ± 0.4	1.3 ± 0.5	14.5± 8.4[c,d]	0.0	5.1 ± 3.1

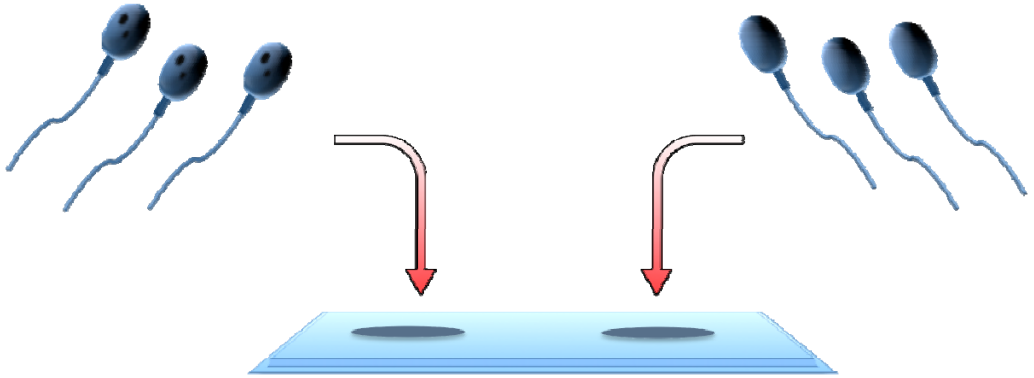
TD=testicular damage: P0= partial obstruction

a=*P*< 0.01 versus controls; b=*P*< 0.01 versus PO; c=*P*< 0.001 versus controls;

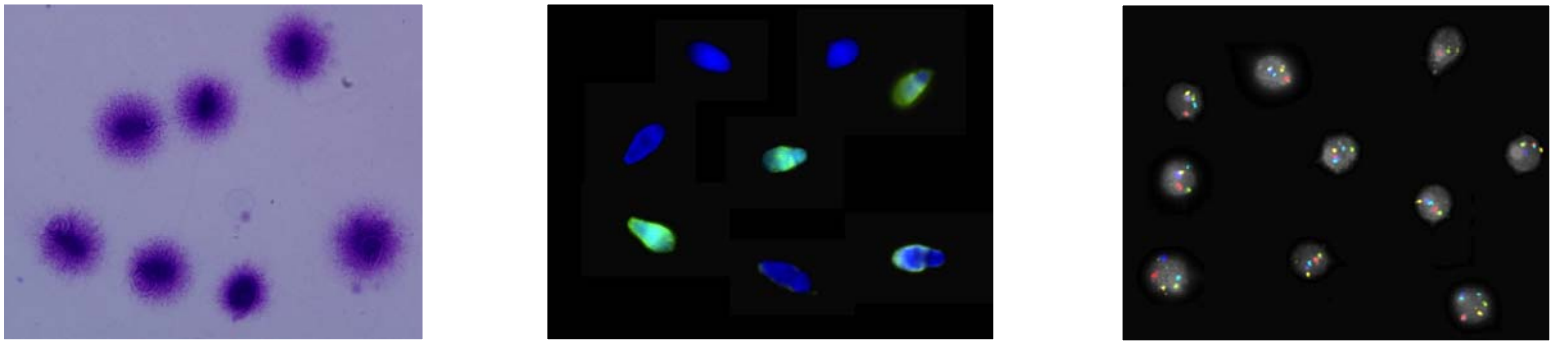
D=*P*< 0.001 versus PO; e=*P*< 0.001 versus group A.

Garolla et al., 2008

Are sperm vacuoles responsible for DNA damage?



6600x



Sperm DNA fragmentation

Basic sperm parameters

Concentration (x10^6/ml)	63.5 ± 26.3
Motility (M% ± SD)	56.9± 1.7
Morphology (M% ± SD)	3.4 ± 3.2
Vacuolization	67%

	Vacuolated	Control
Total sperm	576	486
Fragmented (%)	23 (3.9)	22 (4.5)

Sperm DNA fragmentation

Basic sperm parameters

Concentration (x10^6/ml)	79.5 ± 56.7
Motility (M% ± SD)	52.9± 5
Morphology (M% ± SD)	4.0 ± 2
Vacuolization	65%

	Vacuolated	Control
Total sperm	697	592
Fragmented (%)	68 (9.8)	61 (10.3)

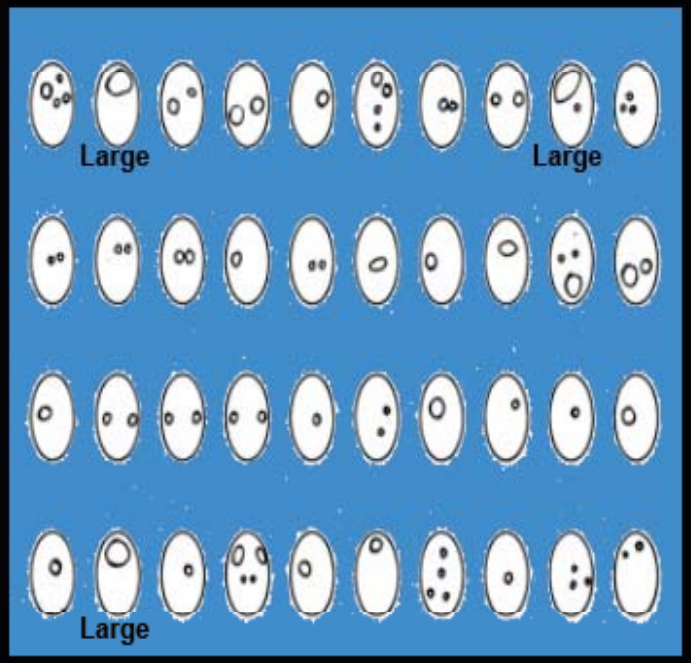
Chromosomal content

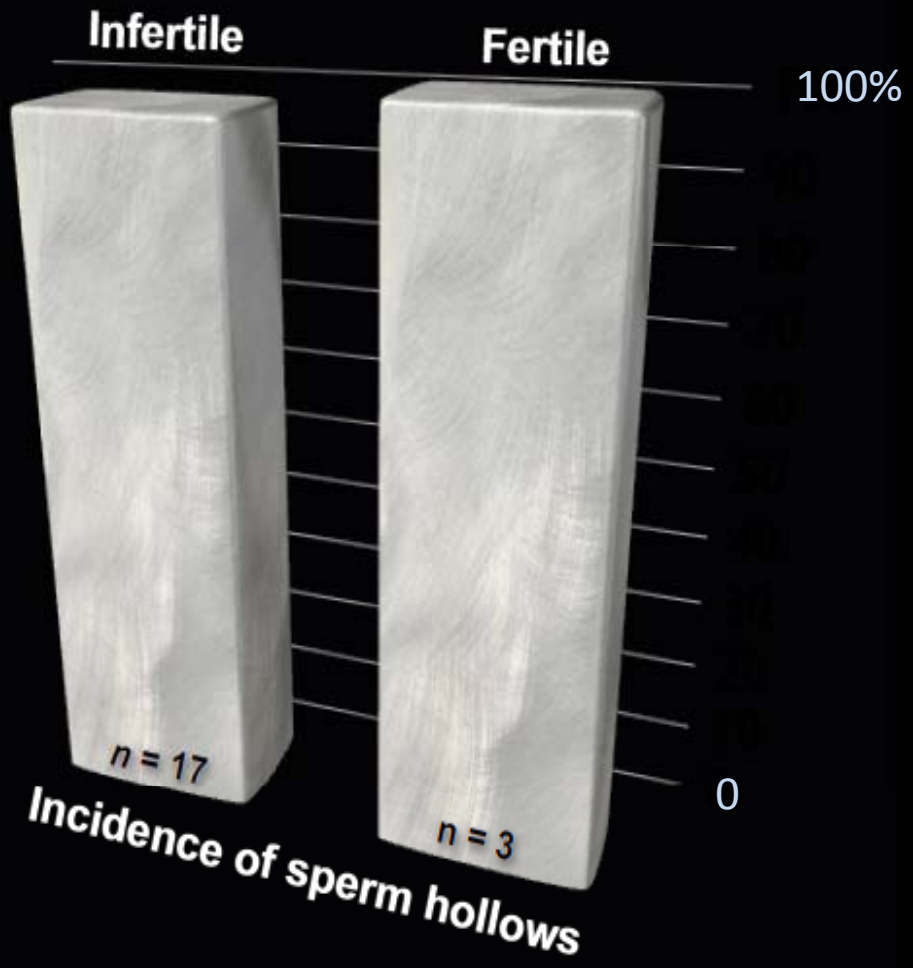
(X, Y, 13, 15, 16, 17, 18, 21, 22)

Basic semen parameters	
Concentration (x10^6/ml)	45.9 ± 17
Motility (M% ± SD)	56.5± 9.1
Morphology (M% ± SD)	4.2± 1.5
Vacuolization	57%

	Vacuolated	Control
Total sperm	623	575
Aneuploid (%)	10 (1.5)	7 (1.1)

No relationship between chromosome aberrations and vacuole-like structures on human sperm head

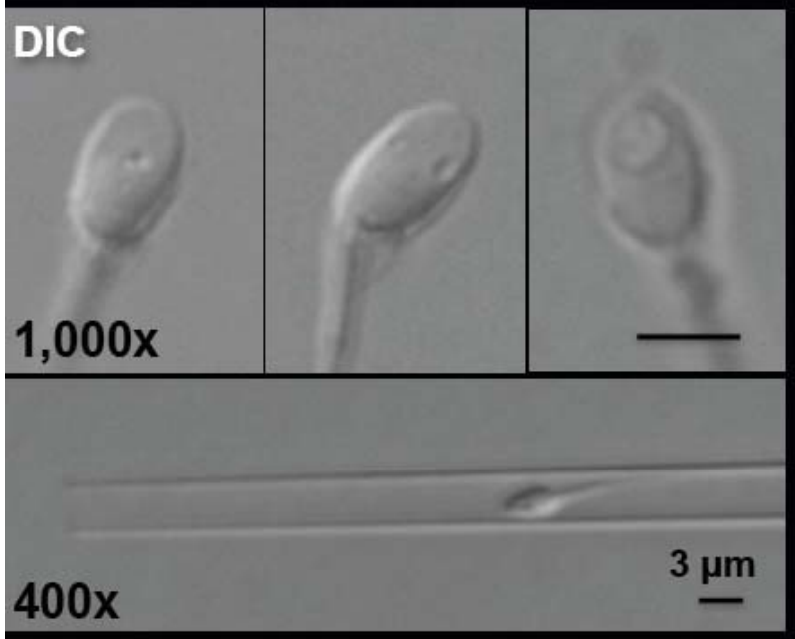




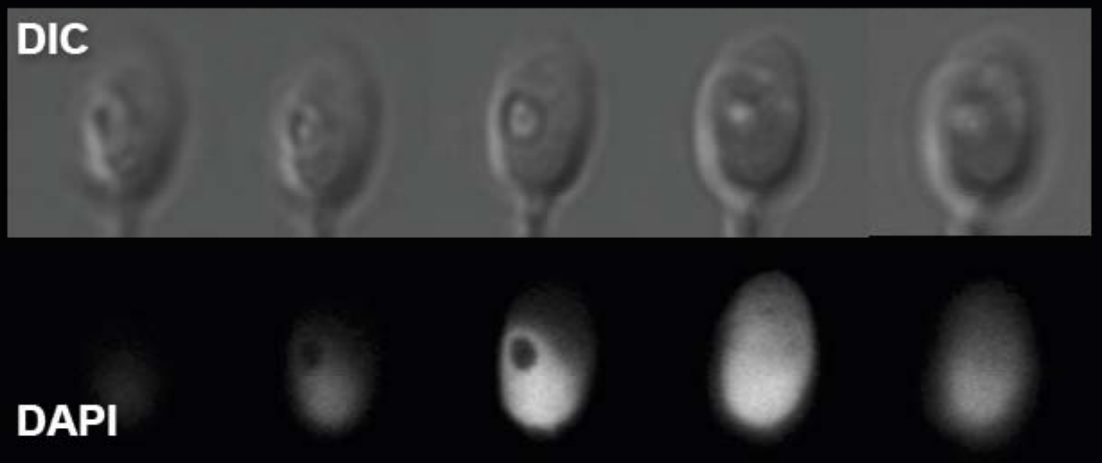
Watanabe et al., 2009

No relationship between chromosome aberrations and vacuole-like structures on human sperm head

IMSI



IMSI Confocal

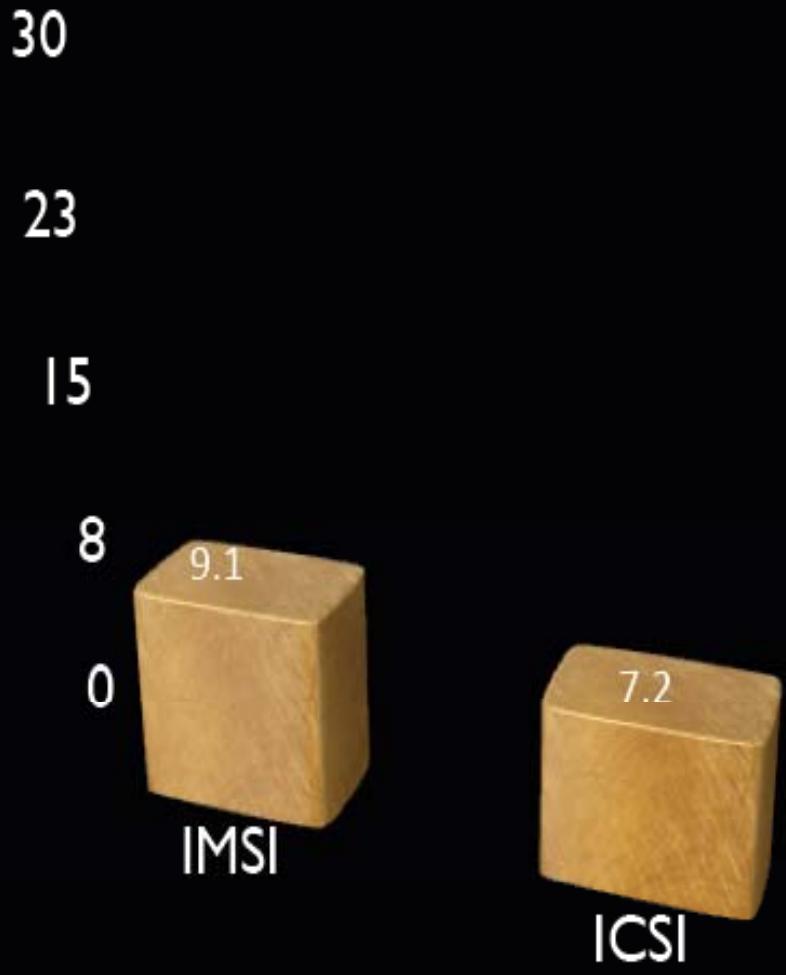


Z-section images captured every 0.2 μm

Watanabe et al., 2009 ESHRE Amsterdam
Hirosaki University Graduate School of Medicine, Japan

No relationship between chromosome aberrations and vacuole-like structures on human sperm head

Chromosome spread in a hybrid oocyte



Watanabe et al., 2009

Lesson from IMSI approach

Sperm quality may affect ICSI results in terms of embryo development (blastocyst formation) and clinical outcome.

No clear evidences have been published yet (evidence-based medicine, prospective randomized studies, enough power, identification of a specific category of patients) about the real efficacy of IMSI approach.

Lesson from IMSI approach

Moreover contradictory results have been recently found from different groups about the role of i.e. vacuoles (?) on sperm competence

The presence of sperm head defects assessed by high magnification microscopy did not directly translate to chromosomal abnormalities or presence of DNA breakage.

We need to investigate better this aspect and try to find different aspects other than sperm morphology that can have an impact on ICSI outcome

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CLINICA VALLE GIULIA, Roma
SALUS – ASI MEDICAL, Marostica
GENERA UMBERTIDE, Perugia

Gynecology:

Filippo Ubaldi

Elena Baroni

Silvia Colamaria

Maddalena Giuliani

Fabio Sapienza

ginecologia
endocrinologia
embriologia
riproduzione
assistita

Embryology:

Laura Rienzi

Stefania Romano

Laura Albricci

Antonio Capalbo

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