XXXVII Congresso
Brasileiro Urologia
Curitiba | PR
24 a 27 AGOSTO | 2019
Expo Unimed Curitiba

Seleção de espermatozoides para ICSI

Edson Borges Jr.

FERTILITY

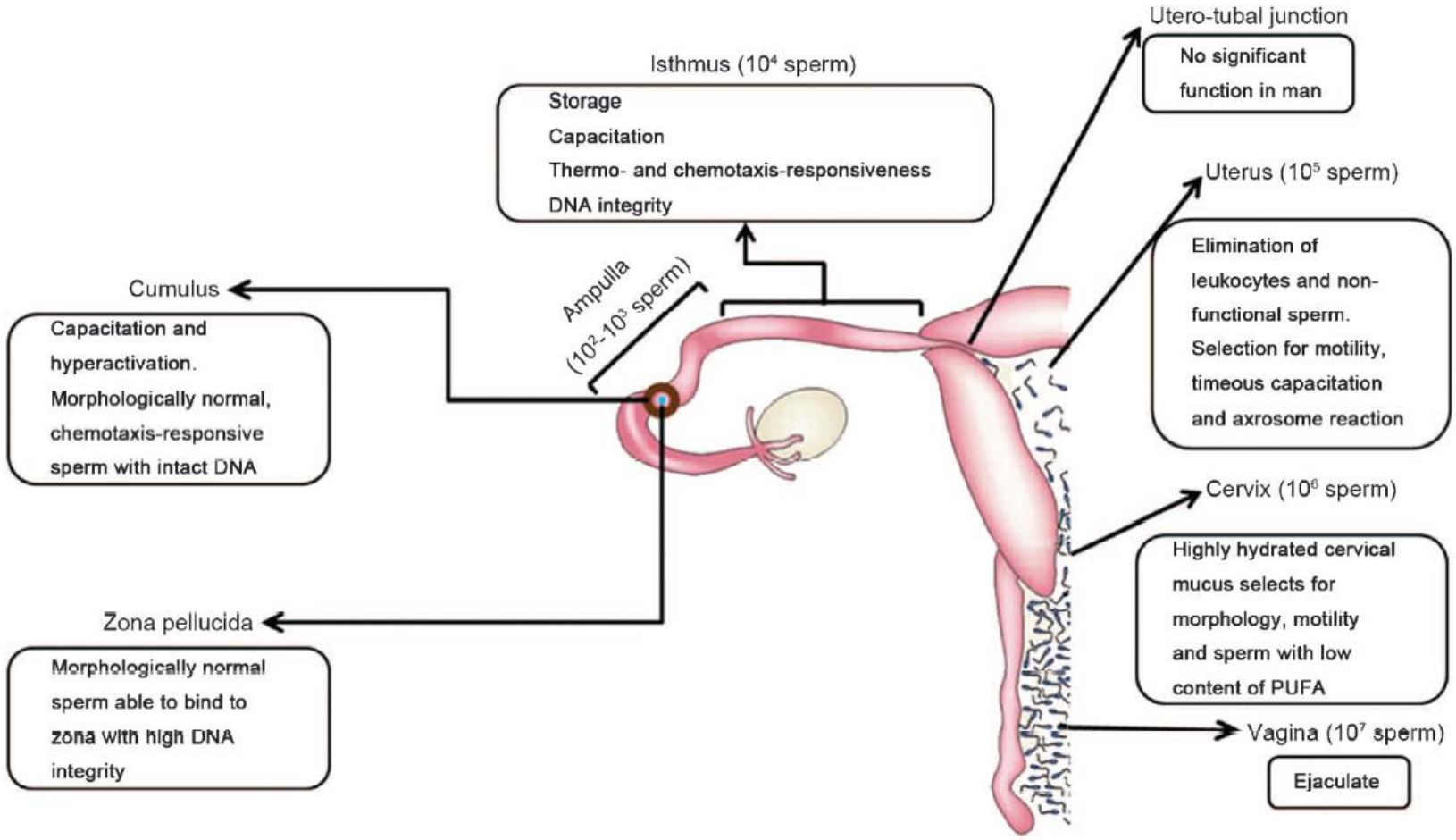
Declaração:

Declaro o recebimento de honorários para palestras e/ou ensaios clínicos da Merck, Ferring e Abbott (não relacionados ao assunto desta palestra).

Nenhum outro conflito de interesse para divulgar.

Resolução do Conselho Federal de Medicina nº 1.595/2.000

FERTILITY



Asian Journal of Andrology (2012) 14, 260–269

FERTILITY

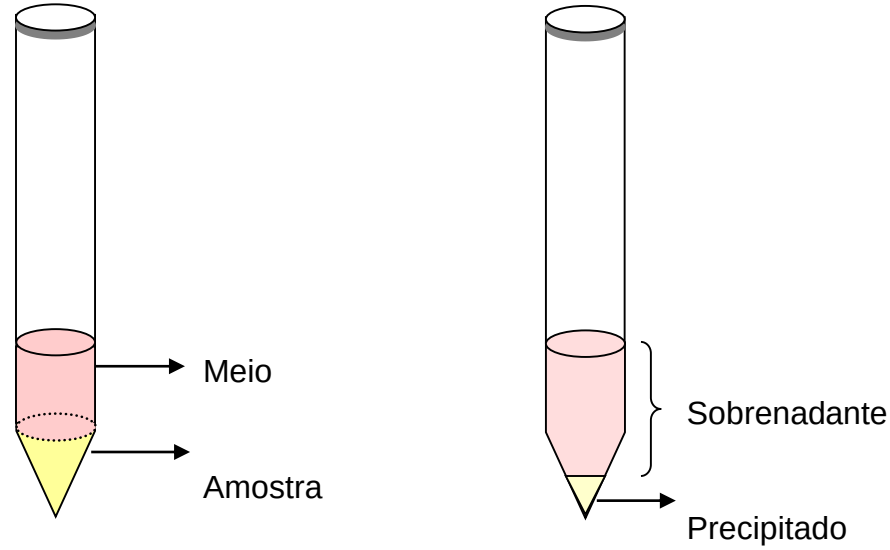
Técnicas de Processamento Seminal

- *Sperm-wash*
- Gradiente descontínuo de densidade
- *Swim-up*
- Filtração em lã de vidro
- *TEST yolk buffer*

FERTILITY

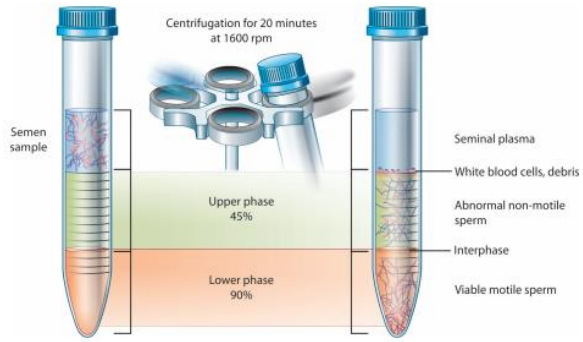
Processamento Seminal

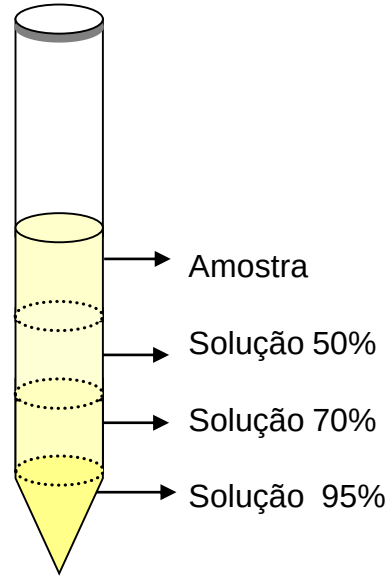
- ***Sperm wash***
- **Gradiente descontínuo de densidade**
- ***Swim-up***

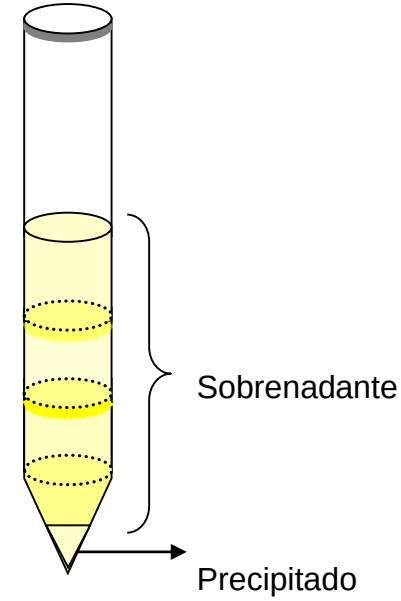


Processamento Seminal

- *Sperm wash*
- **Gradiente descontínuo de densidade**
- *Swim-up*

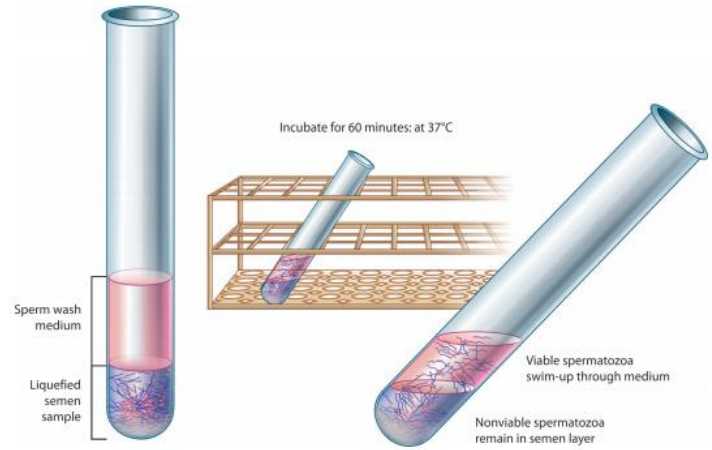


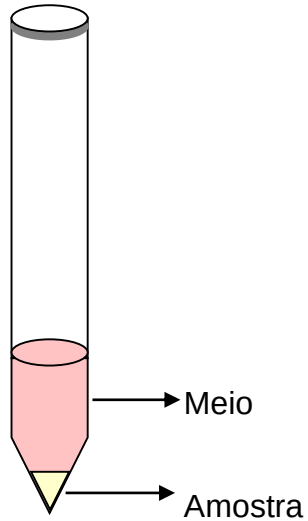


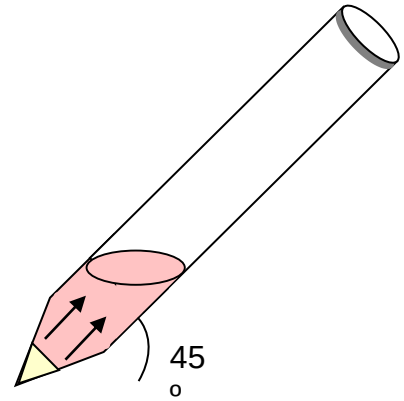


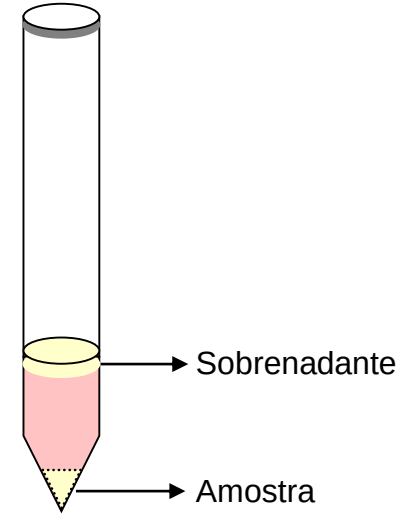
Processamento Seminal

- *Sperm wash*
- Gradiente descontínuo
- ***Swim-up***



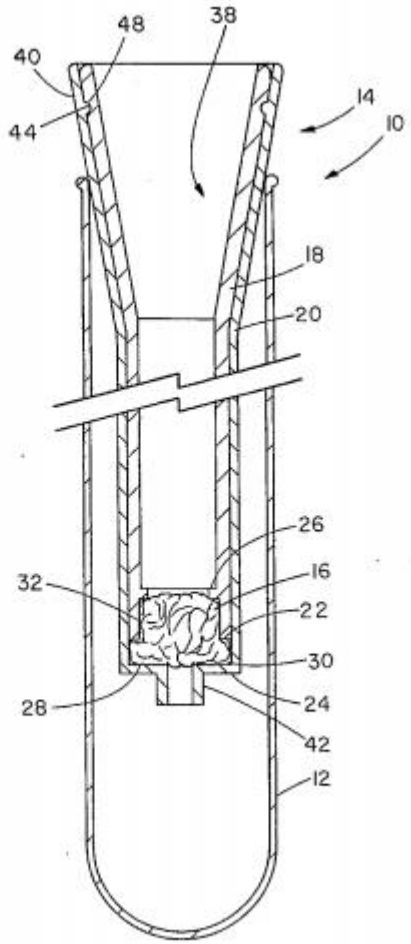






Processamento Seminal

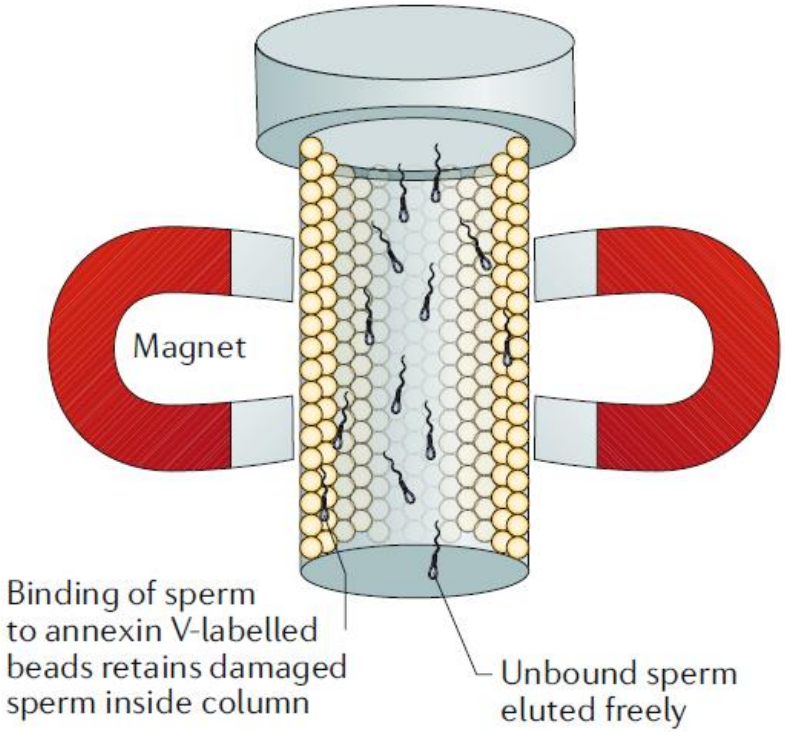
- **Filtração em lã de vidro**
- ***TEST yolk buffer***

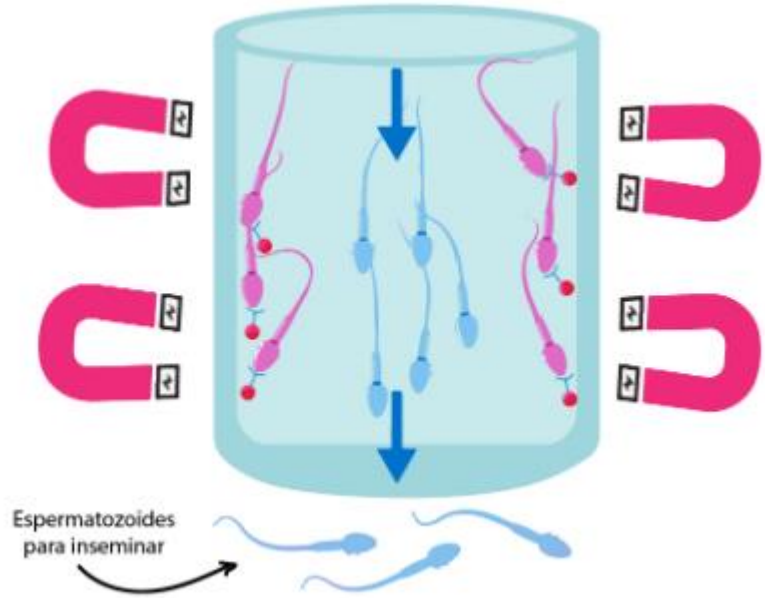


Procedure	Pros/benefits	Cons/risks
Swim-up (SU) procedure	• Concentrates motile sperm, which migrate to the higher viscosity medium • Leaves nondynamic factors behind • Separates sperm exhibiting double-stranded DNA damage from normal motile sperm	• Collecting motile sperm does not guarantee fertilizing ability • Does not remove ROS, which decreases fertilization and pregnancy rates, from the sample • Does not separate competent sperm from WBCs, cellular debris, and immature sperm
Continuous or discontinuous density-gradient (DG) procedure	• Effectively separates motile from nonmotile sperm • Provides a higher concentration of motile sperm • Industry standard to select sperm for ART • Lower ROS and reduced oxidative stress • Separates out WBCs • Mature motile sperm are collected in the resulting pellet • Separates out both double- and single-stranded DNA damage	• Cannot be used for low sperm concentrations, highly viscous samples, and those with a lot of cellular debris
Glass wool filtration prcedure	• Yields higher concentrations of sperm than SU or DG procedures • Nonviable sperm "stick" or adhere to the glass wool column, allowing functionally intact motile sperm to pass through • Good for highly viscous semen samples • Good for asthenozoospermic and oligozoospermic samples • Higher concentration of intact acrosomes • Higher chromatin integrity • Higher zona-free hamster oocyte sperm penetration assay results • Higher percentage fertilization of oocytes in IVF compared with SU	• Some are concerned about glass wool debris getting into the sample, but this has not been shown to have a detrimental effect • Less familiar technique for ART
TEST yolk buffer treatment	• Good for couples with failed fertilization (<10% fertilization rate) or idiopathic infertility • Initiates sperm capacitation • Increases fertilization, acrosome reaction, and IVF outcomes • No apparent deleterious effects	• Requires thermal treatment with unclear mechanism; subsequent cooling seems to improve the membrane cholesterol-phospholipid ratio

Técnicas de adesão molecular

- **MACS:** ***magnetic-activated cell sorting***

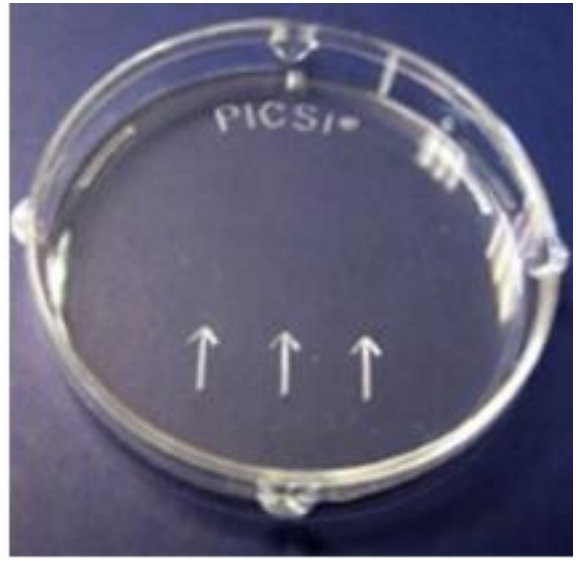




FERTILITY

Técnicas de adesão molecular

- **PICSI (Petri Dish ICSI, Petri dish for ICSI)**



adesão em HA (ácido hialurônico)

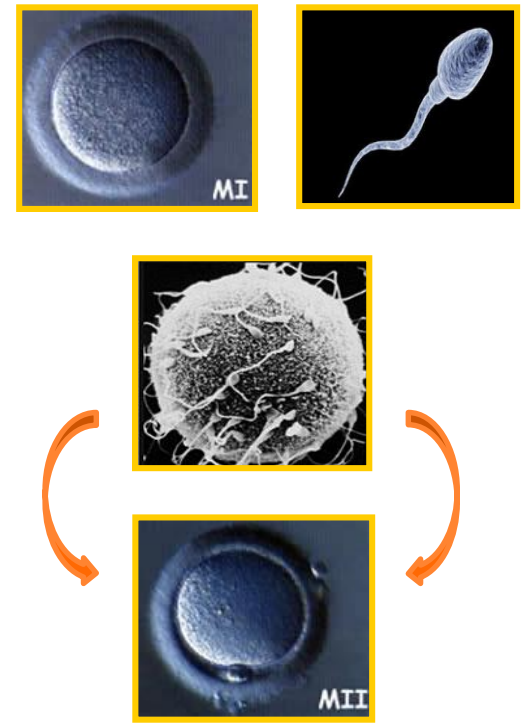
FERTILITY

Procedure	Pros/benefits	Cons/risks
Magnetic-activated cell sorting (MACS)	• Separates out sperm with apoptosis • Assumes a lower rate of DNA damage • Improves sperm morphology	• Reduces motility • No improvement in ICSI outcomes compared with SU or DG • No difference in implantation or miscarriage rate • No difference in outcomes when using donor eggs • Limited studies have been completed
Membrane maturity procedures (PICSI: plastic culture dish with microdots of hyaluronic acid (HA) on its inner surface; Sperm Slow method, a viscous medium containing HA)	• Sperm with HA receptors are mature, have normal shapes, low DNA fragmentation rates, and low frequency of chromosomal aneuploidies • Superior embryo quality with HA-ICSI sperm	• No improvements in fertilization rates or pregnancy rates • No difference in embryo development in time-lapsed studies

RBM Online - Vol 19. No 6. 2009 802–807 Reproductive BioMedicine Online; www.rbmonline.com/Article/4116 on web 30 September 2009

Article

Outcome of ICSI using zona pellucida-bound spermatozoa and conventionally selected spermatozoa



Dr Edson Borges Jr

Daniela Paes de Almeida Ferreira Braga[1,2], Assumpto Iaconelli Jr[1,2], Rita de Cássia Sávio Figueira[1], Camila Madaschi[1], Luciana Semião-Francisco[1], Edson Borges Jr[1,2,3]

[1]Fertility – Assisted Fertilization Center, Av Brigadeiro Luis Antonio, 4545 São Paulo, SP, Brazil; [2]Sapientiae Institute–Educational and Research Center in Assisted Reproduction, Rua Vieira Maciel, 62 São Paulo, SP, Brazil

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FERTILITY

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Article

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Table 1. Comparison of fertilization, embryo quality and embryo transfer rates in oocytes undergoing routine intracytoplasmic sperm injection (control) or injected with zona pellucida-bound spermatozoa.

Parameter	*Control (n = 194)*	*ZP-bound spermatozoa (n = 194)*	P-*value*
Fertilization rate	76.8 (149/194)	77.3 (150/194)	NS
High-quality embryo rate	70.0 (104/149)	83.3 (125/150)	0.003
Embryo transfer rate	43.6 (65/149)	54.7 (82/150)	0.004

Values are percentage (number/total); NS = not statistically significant at the 5% level; ZP = zona pellucida.

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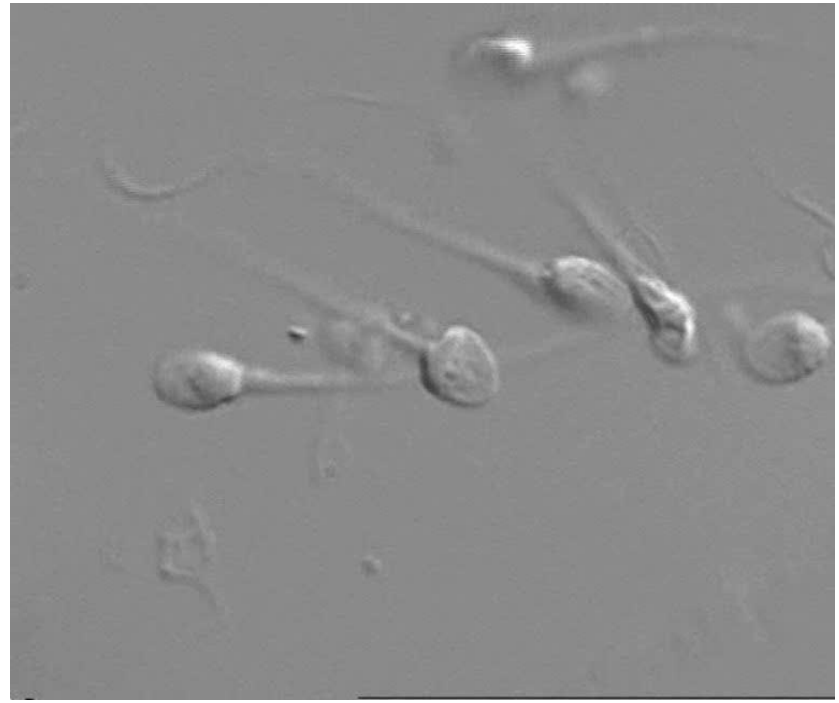
- This finding was confirmed using a binary logistic regression, showing that the use of **zona pellucida-bound spermatozoa for ICSI was determinant of the likelihood of the embryo quality (OR = 2.23; CI 95% = 1.30–3.81; P = 0.004);**

- This result was also confirmed by the logistic regression model, which demonstrated a nearly **twofold increase in embryo transfer rates in embryos derived from the ZP-binding group (OR = 1.94; IC 95% = 1.21–3.13; P = 0.006).**

- **MSOME** *Motile Sperm Organellar Morphology Examination*
- **IMSI** *Intracytoplasmic Morphologically Select Sperm Injection*



FERTILITY

Reproductive BioMedicine Online (2010) 21, 450–455

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REVIEW

Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis

Amanda Souza Setti [a], Renata Cristina Ferreira [b], Daniela Paes de Almeida Ferreira Braga [a,b], Rita de Cássia Sávio Figueira [a,b], Assumpto Iaconelli Jr [b], Edson Borges Jr [a,b,*]

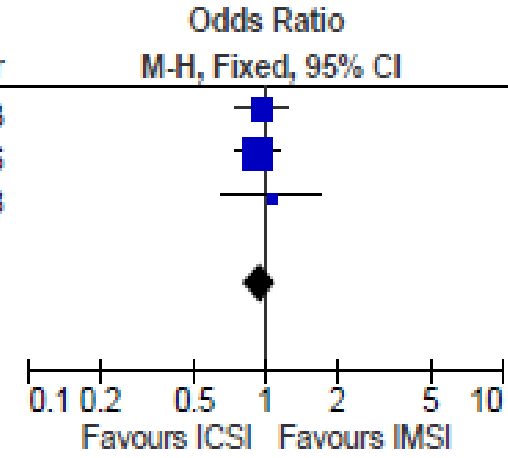
FERTILITY

Fertilização

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)



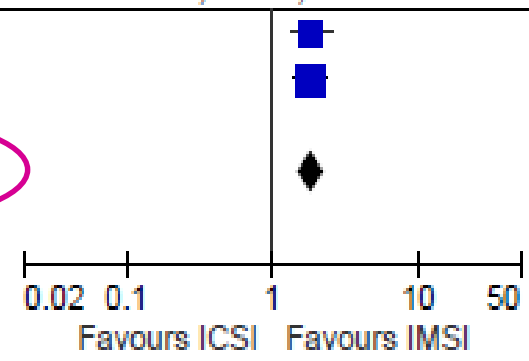
ausência de significância

Bons Embriões

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)



OR: 1.83 (1.50 – 2.24), p<0.00001

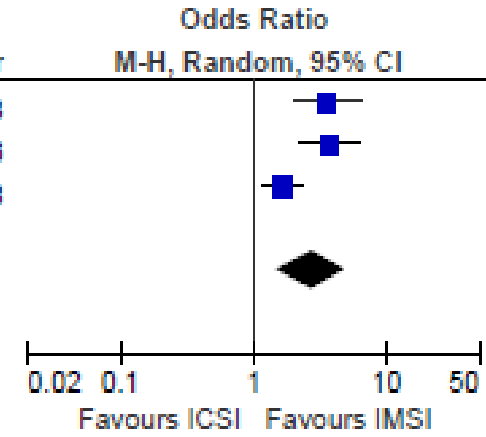
FERTILITY

Implantação

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: Tau² = 0.22; Chi² = 9.37, df = 2 (P = 0.009); I² = 79%

Test for overall effect: Z = 3.29 (P = 0.001)



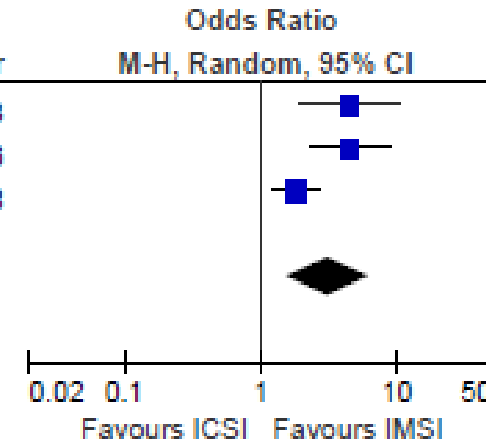
OR: 2.72 (1.50 – 4.95), p=0.001

Gestação

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: Tau² = 0.27; Chi² = 7.48, df = 2 (P = 0.02); I² = 73%

Test for overall effect: Z = 3.19 (P = 0.001)



OR: 3.12 (1.55 – 6.26), p=0.001

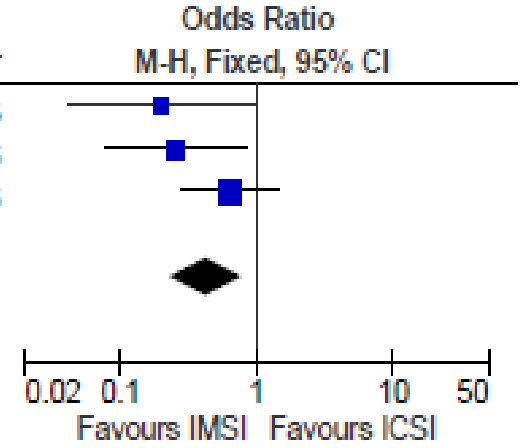
FERTILITY

Aborto

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: Chi² = 2.47, df = 2 (P = 0.29); I² = 19%

Test for overall effect: Z = 2.74 (P = 0.006)



OR: 0.42 (0.23 – 0.78), p=0.006

FERTILITY

European Journal of Obstetrics & Gynecology and Reproductive Biology 171 (2013) 286–290

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Intracytoplasmic morphologically selected sperm injection is beneficial in cases of advanced maternal age: a prospective randomized study

A.S. Setti [a,b], R.C.S. Figueira [b], D.P.A.F. Braga [a,b], T. Aoki [c], A. Iaconelli Jr. [a,b], E. Borges Jr. [a,b,*]

ICSI x IMSI

- Implantação: 12,1% x 38,3% (p=0.026)
- Gestação: 13,8% x 60,0% (p<0,001)
- IMSI influência positiva na:
  - ✓ formação de blastocisto (RC:15,00 – p<0,001)
  - ✓ Implantação (RC:24,04 – p=0,027)
  - ✓ chances de gestação (OR=9,0 – p=0,001)

FERTILITY

European Journal of Obstetrics & Gynecology and Reproductive Biology 183 (2014) 96–103

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Review

Intracytoplasmic morphologically selected sperm injection results in improved clinical outcomes in couples with previous ICSI failures or male factor infertility: a meta-analysis

Amanda S. Setti [a,b,c], Daniela P.A.F. Braga [a,b], Rita C.S. Figueira [b,c], Assumpto Iaconelli Jr.[a,b], Dr.Edson Borges [a,b,*]

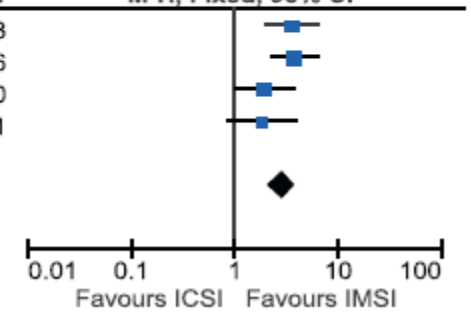
2.1 Previous ICSI failures

a) Implantation

Study or Subgroup	IMSI Events	IMSI Total	ICSI Events	ICSI Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
Bartoov et al.	53	190	17	175	24.0%	3.60 [1.99, 6.50]	2003
Berkovitz et al.	69	248	23	248	31.2%	3.77 [2.26, 6.29]	2006
Gonzalez-Ortega et al.	35	78	25	84	25.0%	1.92 [1.01, 3.67]	2010
Oliveira et al.	24	164	11	128	19.8%	1.82 [0.86, 3.88]	2011
Total (95% CI)		**680**		**635**	**100.0%**	**2.88 [2.13, 3.89]**	
Total events	181		76				

Heterogeneity: $Chi^2 = 4.52$, df = 3 (P = 0.21); $I^2 = 34\%$

Test for overall effect: Z = 6.88 (P < 0.00001)

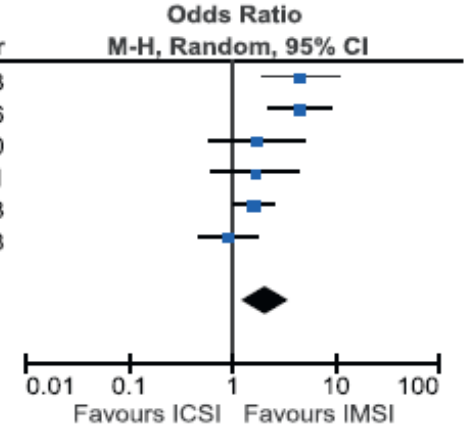


b) Pregnancy

Study or Subgroup	IMSI Events	IMSI Total	ICSI Events	ICSI Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Bartoov et al.	33	50	15	50	15.3%	4.53 [1.95, 10.51]	2003
Berkovitz et al.	48	80	20	80	17.8%	4.50 [2.29, 8.84]	2006
Gonzalez-Ortega et al.	19	30	15	30	12.8%	1.73 [0.62, 4.84]	2010
Oliveira et al.	14	63	8	55	13.8%	1.68 [0.65, 4.37]	2011
Klement et al.	52	127	97	322	21.6%	1.61 [1.05, 2.46]	2013
El Khattabi et al.	22	90	34	130	18.7%	0.91 [0.49, 1.70]	2013
Total (95% CI)		**440**		**667**	**100.0%**	**2.07 [1.22, 3.50]**	
Total events	188		189				

Heterogeneity: Tau² = 0.29; Chi² = 16.40, df = 5 (P = 0.006); I² = 70%

Test for overall effect: Z = 2.71 (P = 0.007)

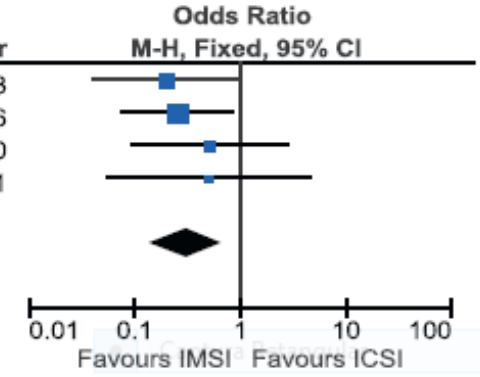


c) Miscarriage

Study or Subgroup	IMSI Events	IMSI Total	ICSI Events	ICSI Total	Weight	Odds Ratio M-H, Fixed, 95% CI	Year
Bartoov et al.	3	33	5	15	28.6%	0.20 [0.04, 0.99]	2003
Berkovitz et al.	7	48	8	20	44.2%	0.26 [0.08, 0.85]	2006
Gonzalez-Ortega et al.	3	19	4	15	17.2%	0.52 [0.10, 2.77]	2010
Oliveira et al.	2	14	2	8	10.0%	0.50 [0.06, 4.47]	2011
Total (95% CI)		**114**		**58**	**100.0%**	**0.31 [0.14, 0.67]**	
Total events	15		19				

Heterogeneity: Chi² = 0.92, df = 3 (P = 0.82); I² = 0%

Test for overall effect: Z = 2.96 (P = 0.003)



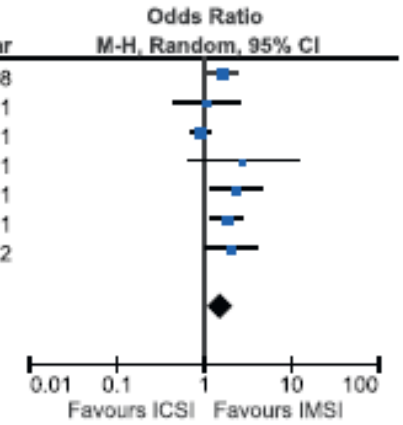
2.2 Male factor

a) Implantation rate

Study or Subgroup	IMSI Events	IMSI Total	ICSI Events	ICSI Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Antinori et al.	97	560	59	521	19.9%	1.64 [1.16, 2.32]	2008
Oliveira et al.	11	93	13	116	9.6%	1.06 [0.45, 2.50]	2011
Setti et al.	158	664	156	613	22.0%	0.91 [0.71, 1.18]	2011
Knez et al.	6	35	3	44	4.4%	2.83 [0.65, 12.24]	2011
Balaban et al.	32	108	16	105	12.6%	2.34 [1.19, 4.59]	2011
Wilding et al.	86	355	48	324	18.8%	1.84 [1.24, 2.72]	2011
Knez et al.	27	93	19	115	12.7%	2.07 [1.06, 4.02]	2012
Total (95% CI)		**1908**		**1838**	**100.0%**	**1.56 [1.11, 2.18]**	
Total events	417		314				

Heterogeneity: $Tau^2 = 0.12$; $Chi^2 = 17.98$, df = 6 (P = 0.006); $I^2 = 67\%$

Test for overall effect: Z = 2.58 (P = 0.010)

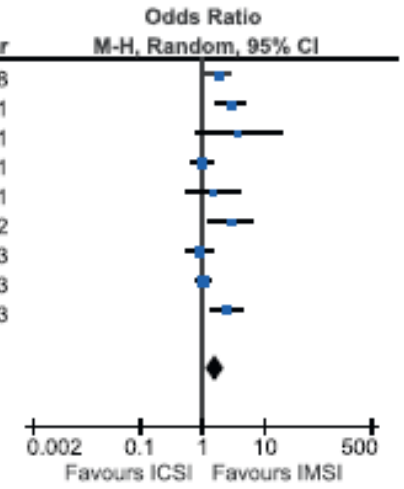


b) Pregnancy rate

Study or Subgroup	IMSI Events	IMSI Total	ICSI Events	ICSI Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Antinori et al.	89	227	58	219	14.0%	1.79 [1.20, 2.67]	2008
Wilding et al.	80	122	44	110	12.0%	2.86 [1.68, 4.87]	2011
Knez et al.	5	20	3	37	3.5%	3.78 [0.80, 17.89]	2011
Setti et al.	93	250	92	250	14.5%	1.02 [0.71, 1.46]	2011
Oliveira et al.	12	37	11	45	6.9%	1.48 [0.56, 3.90]	2011
Knez et al.	25	52	17	70	8.8%	2.89 [1.34, 6.24]	2012
De Vos et al.	43	125	51	139	12.4%	0.90 [0.55, 1.50]	2013
Klement et al.	126	269	473	1033	15.8%	1.04 [0.80, 1.37]	2013
El Khattabi et al.	61	132	33	126	12.1%	2.42 [1.43, 4.09]	2013
Total (95% CI)		**1234**		**2029**	**100.0%**	**1.61 [1.17, 2.23]**	
Total events	534		782				

Heterogeneity: $Tau^2 = 0.15$; $Chi^2 = 27.87$, df = 8 (P = 0.0005); $I^2 = 71\%$

Test for overall effect: Z = 2.90 (P = 0.004)



MALE FACTOR

990 Fertility and Sterility® Vol. 95, No. 3, March 1, 2011

Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes

Rita de Cássia S. Figueira, M.Sc.,[a] *Daniela P. A. F. Braga, M.Sc.,*[a,b] *Amanda S. Setti, B.Sc.,*[b] *Assumpto Iaconelli, Jr., M.D.,*[a] *and Edson Borges, Jr., M.D., Ph.D.*[a,b]

[a] Fertility-Assisted Fertilization Centre; and [b] Sapientiae Institute-Educational and Research Centre in Assisted Reproduction, São Paulo, Brazil

Patient(s): Couples who underwent IVF-PGS cycle, as a result of advanced maternal age, were randomly allocated into two groups: intracytoplasmic sperm injection (ICSI; n = 60) or intracytoplasmic morphologically selected sperm injection (IMSI; n = 60).

FERTILITY

990 Fertility and Sterility® Vol. 95, No. 3, March 1, 2011

Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes

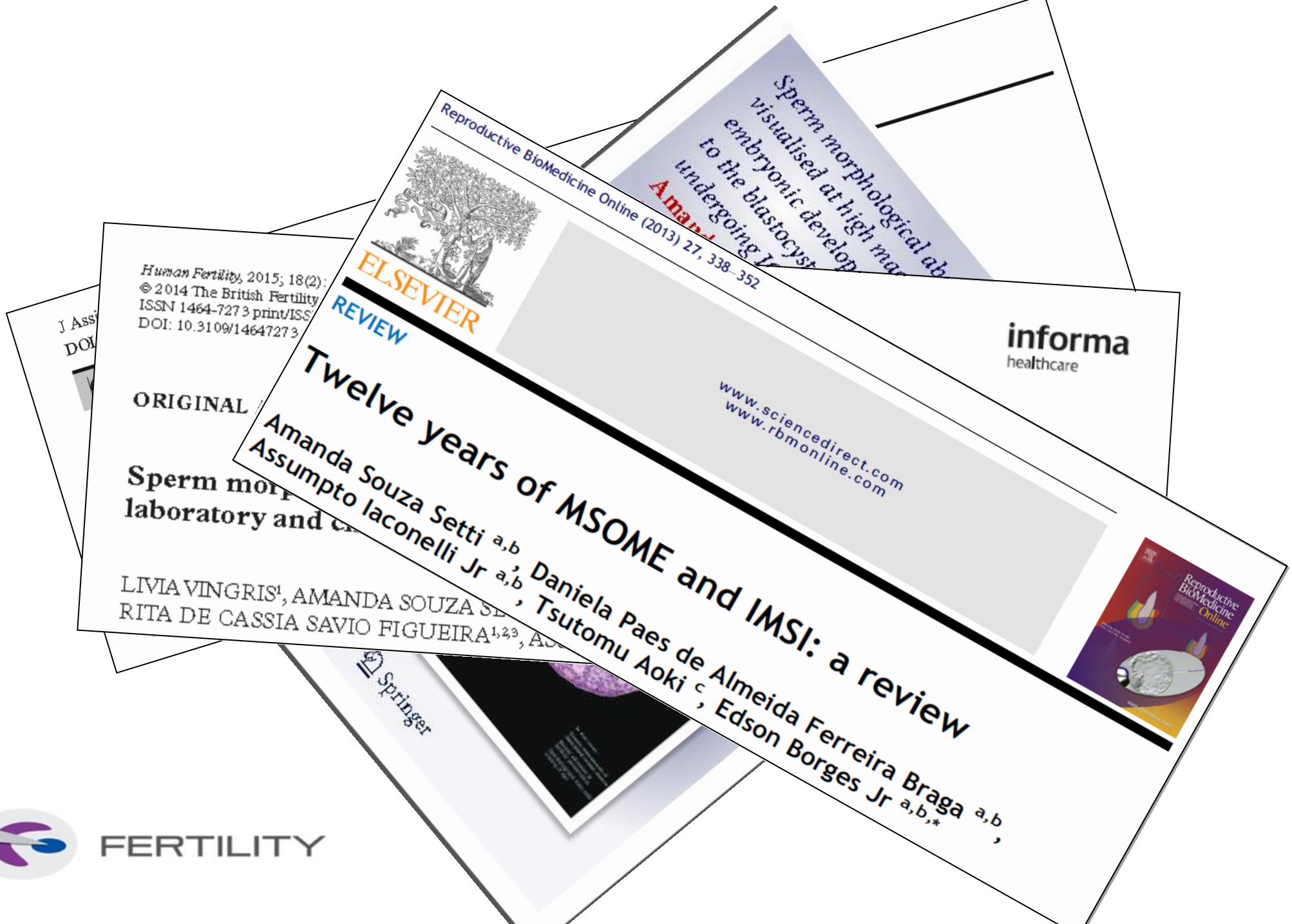
Edson Borges Jr., *et al*

IMSI:

- Lower incidence of sexual chromosomes aneuploidy (23,5% x 15,0%) OR= 0,57 (0,37-0,90; p= 0,015)
- Lower incidence of chaotic embryos (27,5% x 18,8%) OR=0,64 (0,43-0,96; p=0,032)
- Lower chance of cycle cancellation (11,8 % x 2,5%) OR=0,26 (0,11-0,62; p=0,001)

Conclusion(s): Spermatozoa free of nuclear morphological malformations were found to be significantly associated with the lower incidence of aneuploidy in derived embryos, resulting in lower rates of cycle cancellation.

FERTILITY


Sperm morphological ab
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embryonic develop
to the blastocyst
undergoing
Reproductive BioMedicine Online (2013) 27, 338–352
ELSEVIER
REVIEW
www.sciencedirect.com
www.rbmonline.com
Twelve years of MSOME and IMSI: a review
Amanda Souza Setti a,b, Daniela Paes de Almeida Ferreira Braga a,b,
Assumpto Iaconelli Jr a,b, Tsutomu Aoki c, Edson Borges Jr a,b,*
Reproductive BioMedicine Online
Human Fertility, 2015; 18(2):
© 2014 The British Fertility
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DOI: 10.3109/1464727 3
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LIVIA VINGRIS[1], AMANDA SOUZA S
RITA DE CASSIA SAVIO FIGUEIRA[1,2,3], AS
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FERTILITY

ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Keywords:
blastocyst, intracytoplasmic sperm injection, male infertility, motile sperm organelle morphology examination, semen analysis

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Sperm morphological normality under high magnification is correlated to male infertility and predicts embryo development

[1,2]B. F. Zanetti, [1,2]D. P. A. F. Braga, [1]R. R. Provenza, [1]R. C. S. Figueira, [1,2]A. Iaconelli Jr. and [1,2]E. Borges Jr.

[1]Fertility Medical Group, São Paulo, SP, Brazil, and [2]Instituto Sapientiae – Centro de Estudos e Pesquisa em Reprodução Humana Assistida, São Paulo, SP, Brazil

MSOME I+II

Grade I:
- Normal form
- No vacuoles

Grade II:
- Normal form
- ≤ 2 small vacuoles

Grade III:
- Normal form
- > 2 small vacuoles or at least one large vacuole

Grade IV:
- Abnormal head shapes or other abnormalities
- Large vacuole

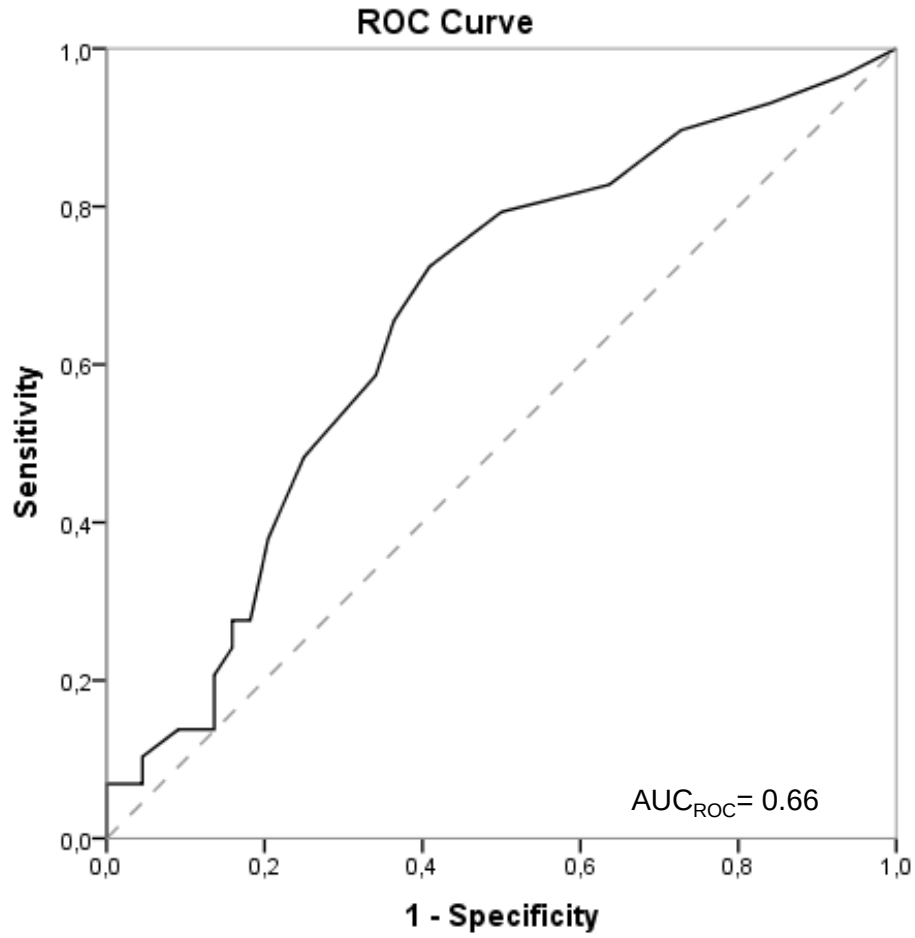
FERTILITY

Logistic regression analyses of MSOME grades correlation with ICSI outcomes

	MSOME I+II		MSOME III		MSOME IV	
	β	*p*	β	*p*	β	*p*
Fertilization rate	0.197	0.044	0.150	0.134	-0.192	0.052
High-quality embryos rate	0.306	0.013	0.379	0.002	-0.378	0.002
Blastocyst rate	0.248	0.047	0.008	0.954	-0.195	0.130
Implantation rate	-0.098	0.405	-0.137	0.252	0.138	0.244
	95% CI	***p***	**95% CI**	***p***	**95% CI**	***p***
Cancelation rate	0.95; 1.07	0.817	0.94; 1.12	0.557	0.95; 1.03	0.716
Pregnancy rate	0.90; 1.05	0.493	0.84;1.09	0.528	0.96; 1.09	0.396

FERTILITY MEDICAL GROUP

ROC Curve of MSOME grades I+II and blastocyst formation rate (below or equal and above 50%)



AUC: 0,66

MSOME I+II

cut-off: 5,5%

sensitivity of 0.72

specificity of 0.41

FERTILITY MEDICAL GROUP

Descriptive statistic of ICSI outcomes per MSOME I+II normality classification

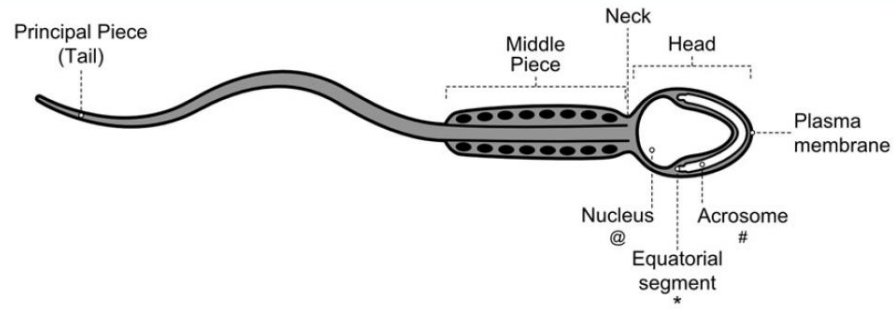
	Normal (MSOME I+II ≥5.5%)	Abnormal (MSOME I+II <5.5%)	p
Female age (years)	36.18 ± 4.29	36.66 ± 3.58	0.527
Total dose of FSH administered (IU)	2346.38 ± 680.43	2422.61 ± 704.55	0.560
Number of follicles	14.28 ± 12.49	14.06 ± 10.97	0.925
Number of retrieved oocytes	9.92 ± 9.95	10.81 ± 7.74	0.608
Fertilization rate	86.94 ± 19.04	84.59 ± 14.79	0.708
High-quality embryos rate	41.78 ± 16.04	38.40 ± 21.73	0.463
Blastocyst rate	50.14 ± 5.05	28.53 ± 5.69	0.005
Implantation rate (%)	20.10 ± 35.59	24.24 ± 37.05	0.618
Pregnancy rate (%)	28.26	36.36	0.472

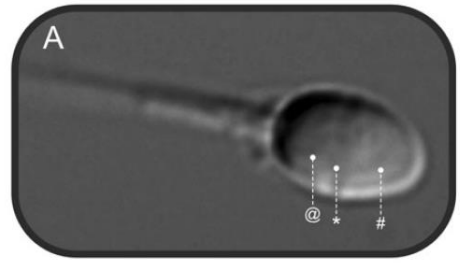
Human Reproduction, Vol.33, No.4 pp. 563–571, 2018
Advanced Access publication on February 6, 2018 doi:10.1093/humrep/dey019

human reproduction ORIGINAL ARTICLE *Andrology*

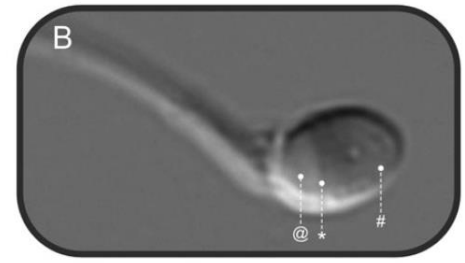
The significance of human spermatozoa vacuoles can be elucidated by a novel procedure of array comparative genomic hybridization

Arie Berkovitz[1,*], Yaron Dekel[2,3,4,5], Revital Goldstein[3,4], Shhadeh Bsoul[3], Yossy Machluf[6], and Dani Bercovich[3,4]

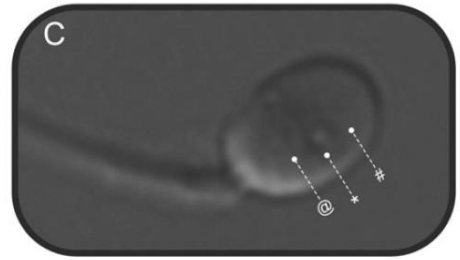




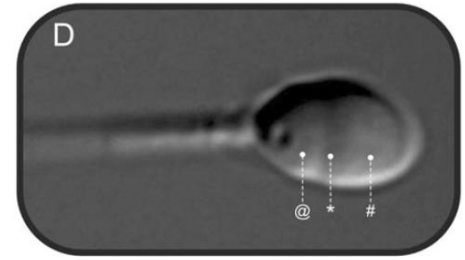
No vacuoles
No Suspicion of nuclear damage
Total CNVs: 234



A small acrosomal vacuole
No Suspicion of nuclear damage
Total CNVs: 245



Two small equatorial vacuoles
Suspected of nuclear damage: Vacuole location
Total CNVs: 285



Small and deep nuclear vacuole
Suspected of nuclear damage: Vacuole location & depth
Total CNVs: 744