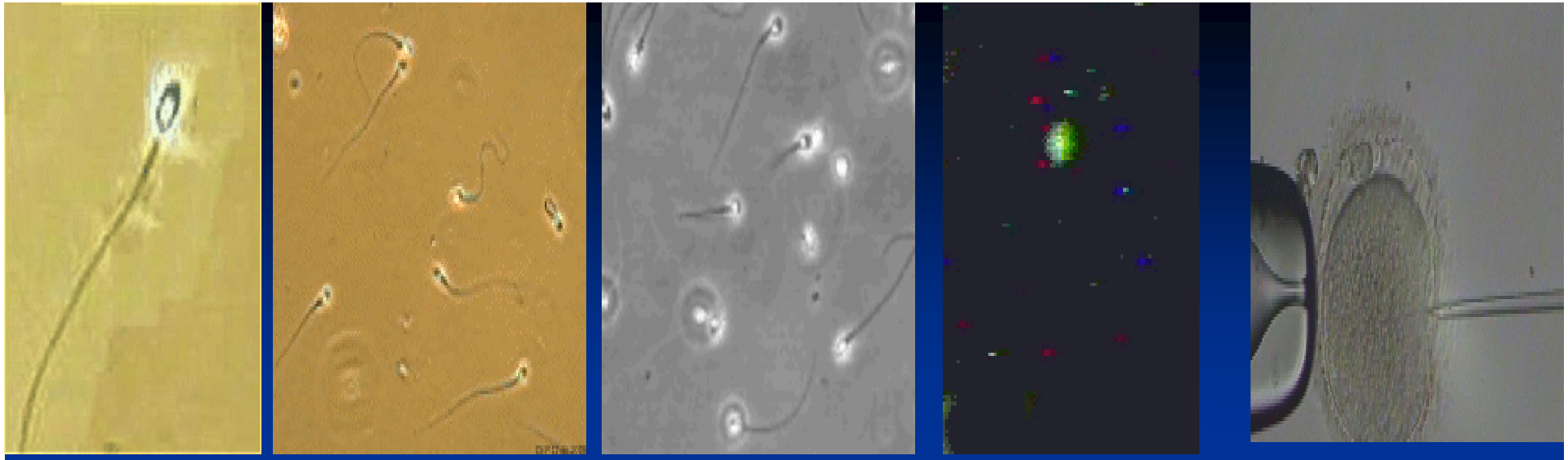
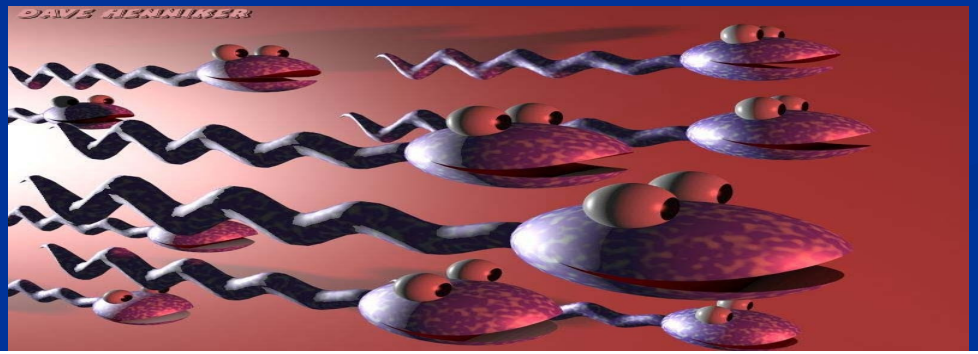
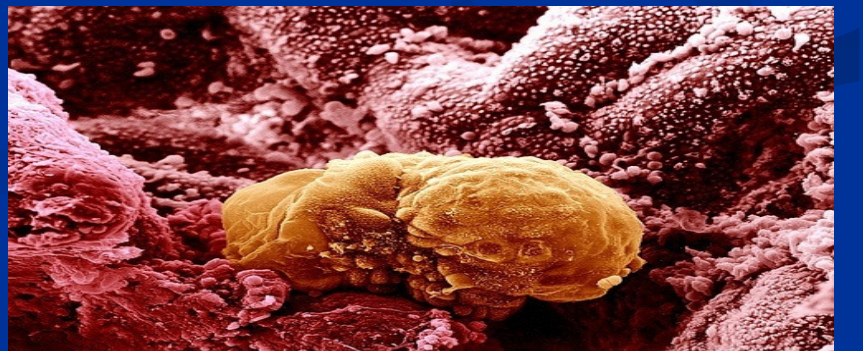


Does the Origin of the Spermatozoa Affect ICSI Outcome ?

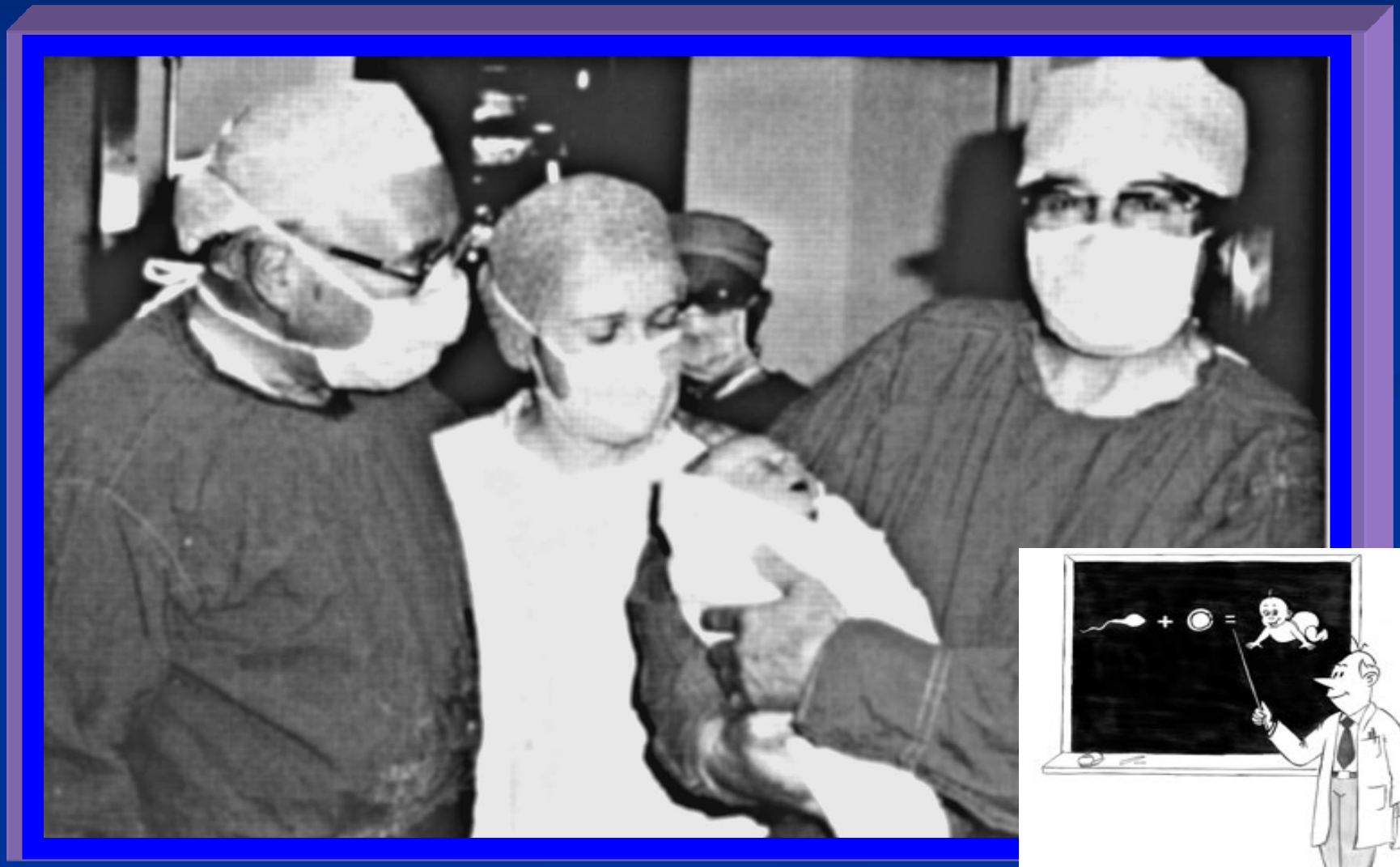
Timur Gürgan M.D.

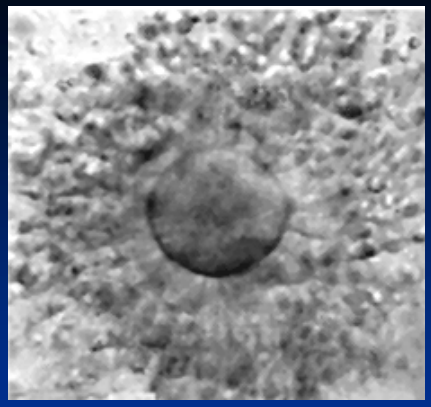
Dept. Of Ob&Gyn, Faculty of Medicine, Hacettepe University, Ankara, Turkey





Robert EDWARDS
25 JULY 1978





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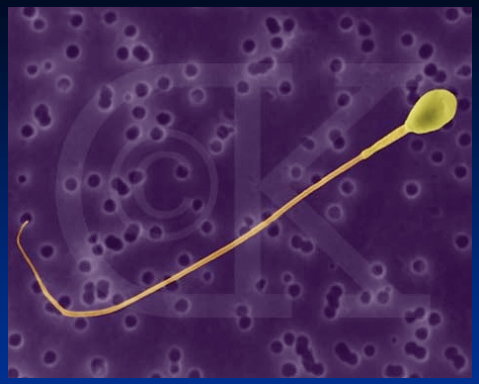
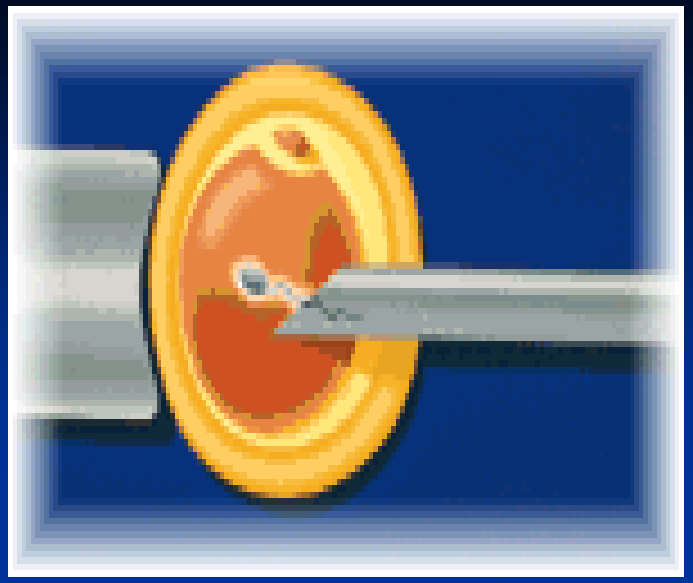
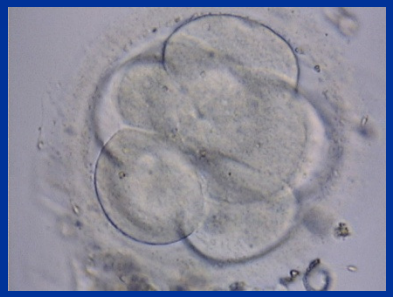






Success of ICSI

- The outcome of ICSI depend upon
  1. Etiology of the male problem
  2. Sperm origin
  3. Sperm status being fresh or after cryopresentation and thawing
  4. Factors related to the female partner such as age and ovarian reserve

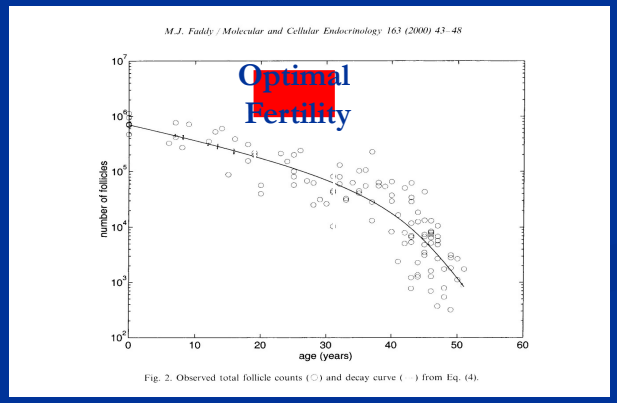


Fig. 2. Observed total follicle counts (○) and decay curve ( ) from Eq. (4).

Testicular Environment

Internal

Hormonal Status
Varicocele etc.
Infections

External

Temperature, Chemicals,
Drugs, Radiation, Physical
damage, **Stress**

Fertile

Age

Descreptive
****

SPERM QUALITY

Infertile

Subfertile

GENETIC

Ploidy, Nondisjunction
Translocations

DNA Integrity

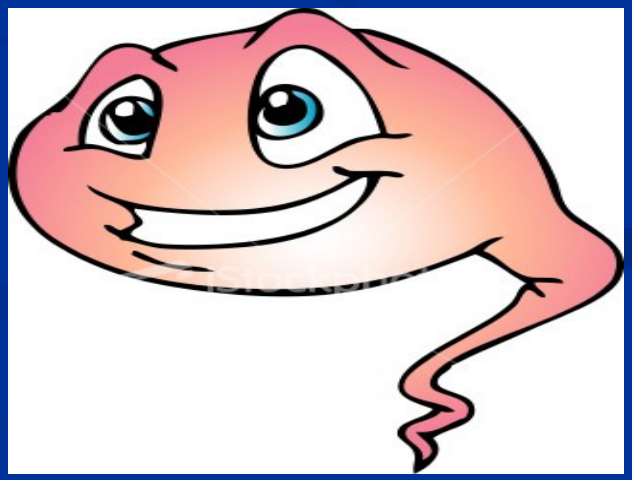
Deletions etc

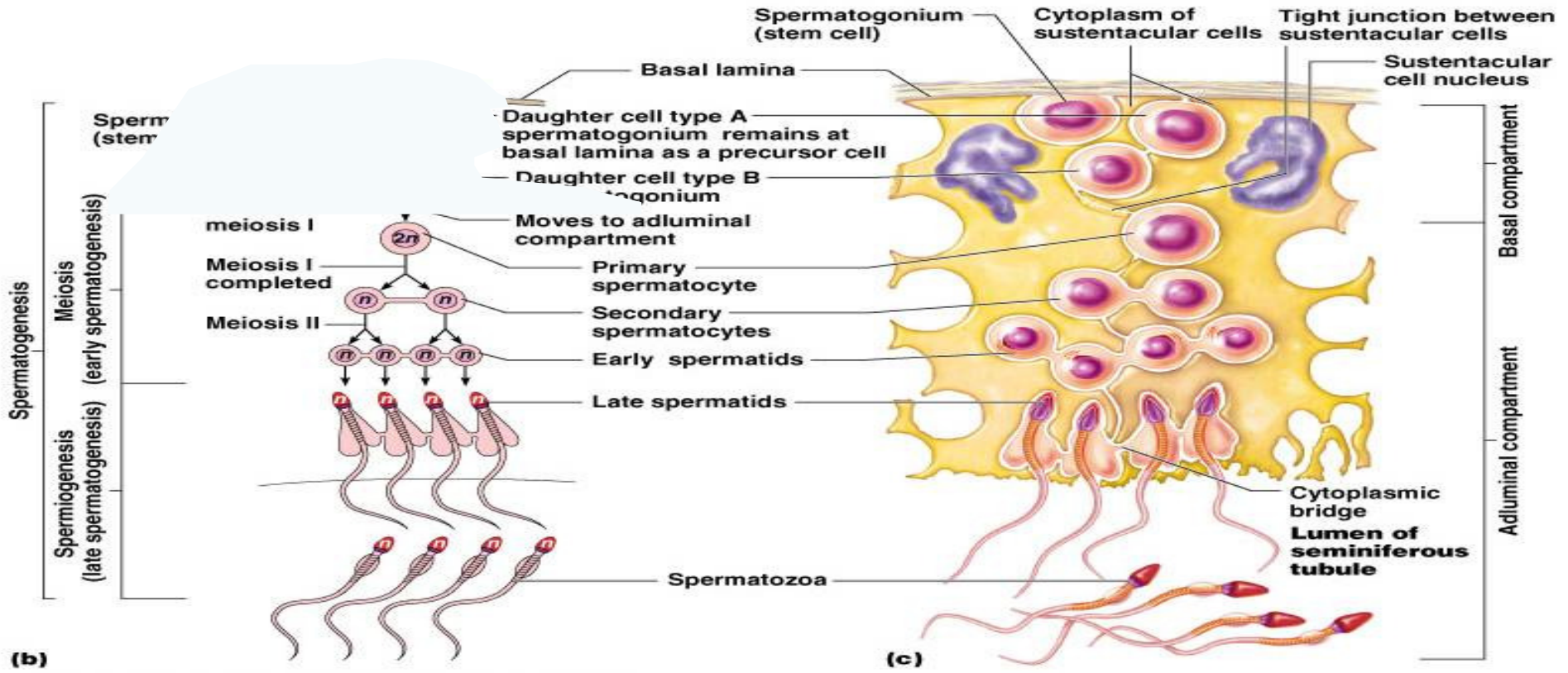
EPIGENETIC

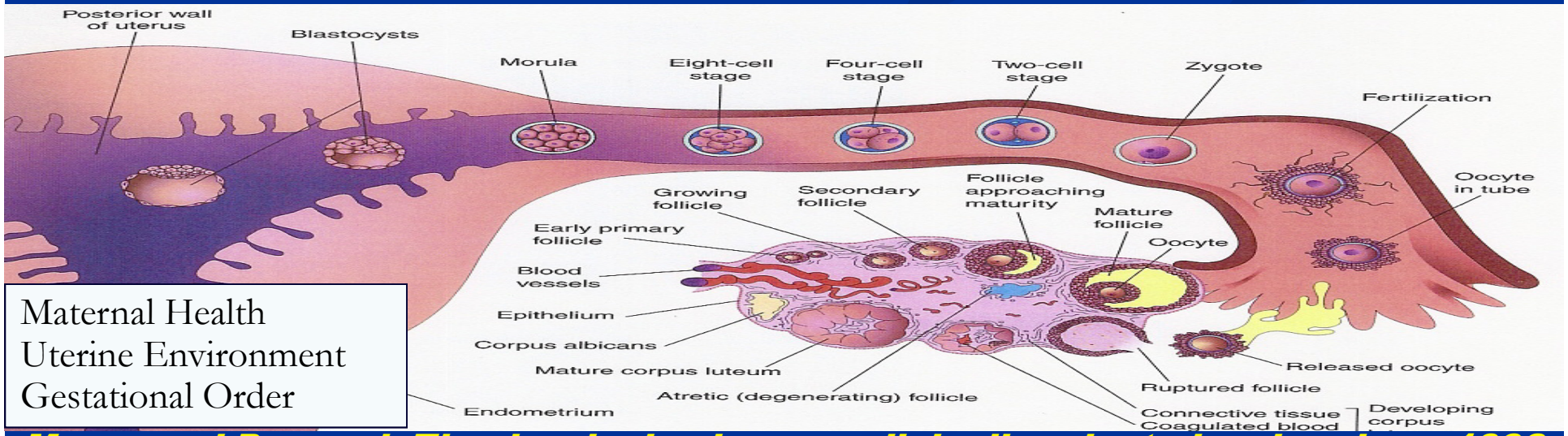
Centrosome
Mitochondrial
Chromatin Packaging
Cytosolic egg activation

Paternal affects

- Fertilization
- Early embryo development
- Genomic activation
- Clinical pregnancy







Maternal Health
Uterine Environment
Gestational Order

Moore and Persaud. The developing human, clinically oriented embryology. 1998

- ***SPERMATOGONIES***

46 chromosomes: 2n ADN

- ***SPERMATOCYTES I***

46 chromosomes: 4n ADN

MITOSE

- ***SPERMATOCYTES II***

23 chromosomes : 2n ADN

- ***SPERMATIDES***

23 chromosomes : n ADN

MEIOSE

- ***SPERMATOZOÏDES***

23 chromosomes : n ADN

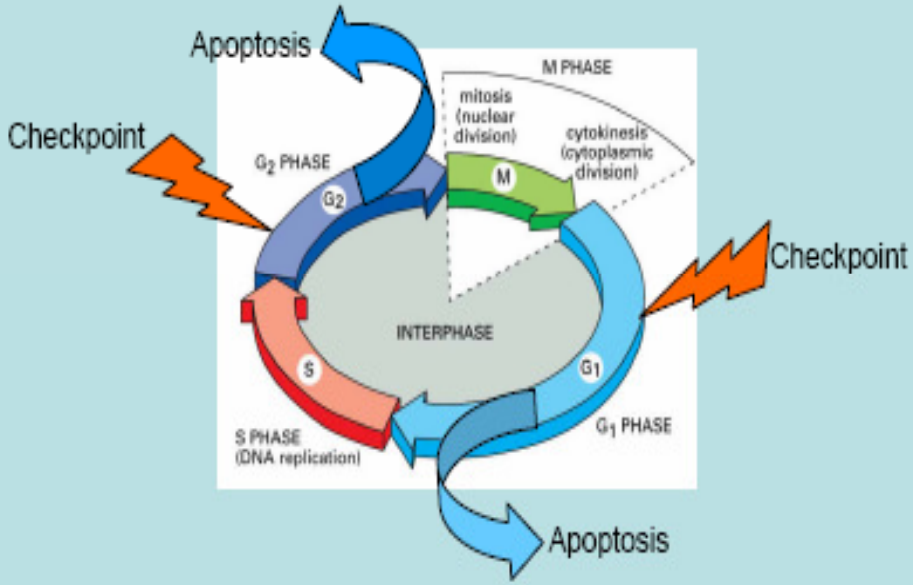
SPERMIOGENESE

Decision points

&

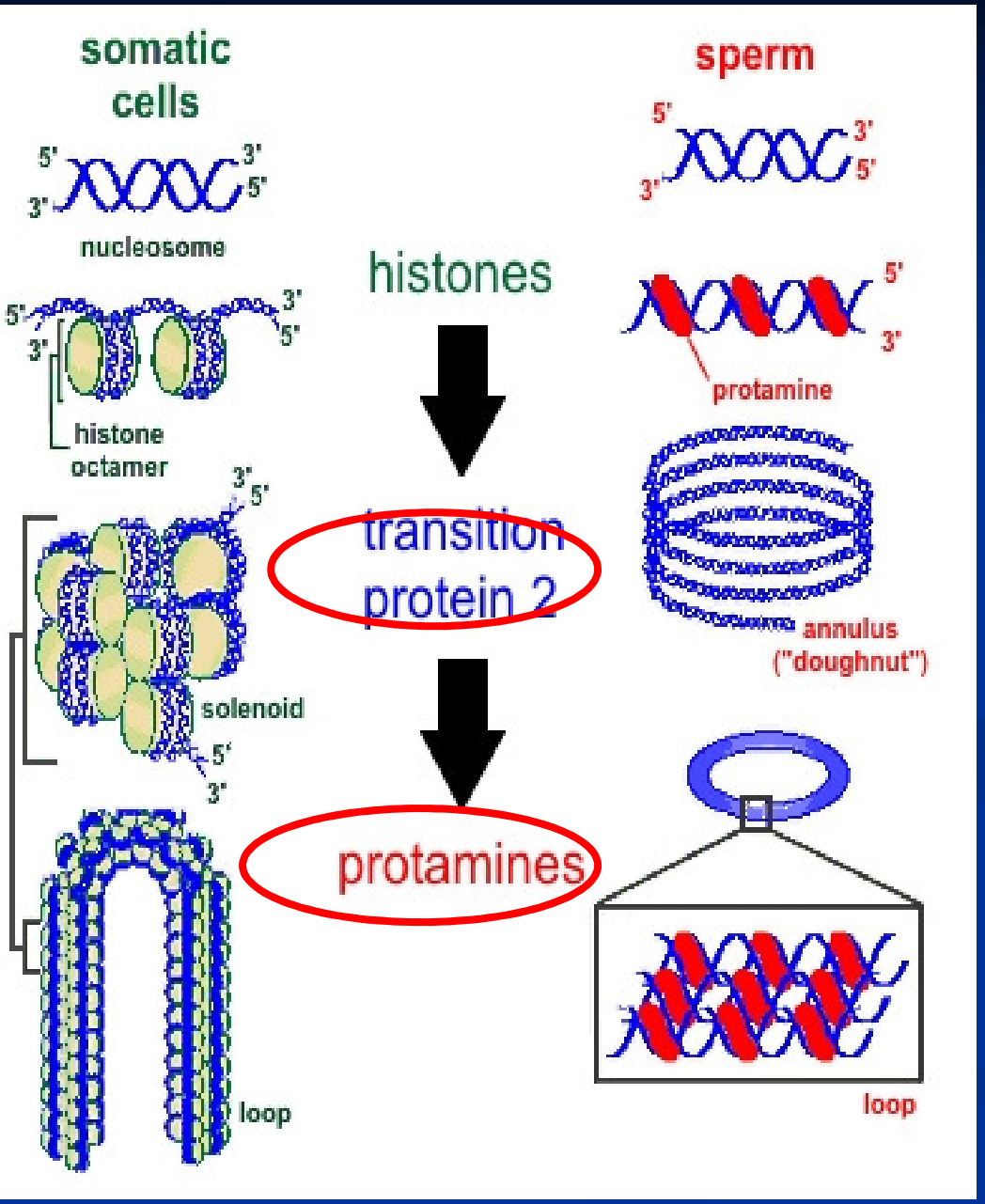
Checkpoints

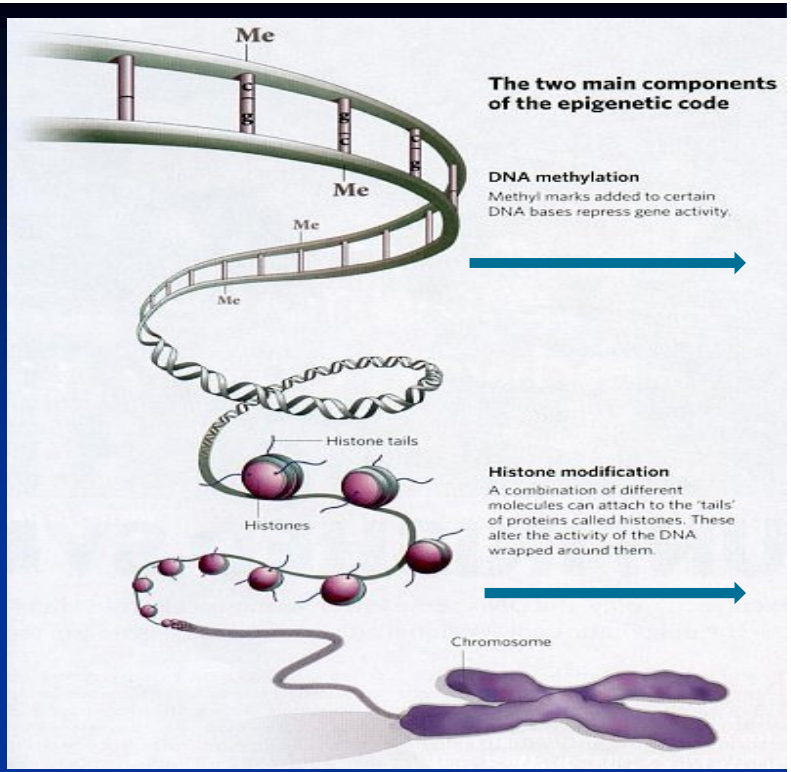
The cell has several systems for interrupting the cell cycle if something goes wrong.

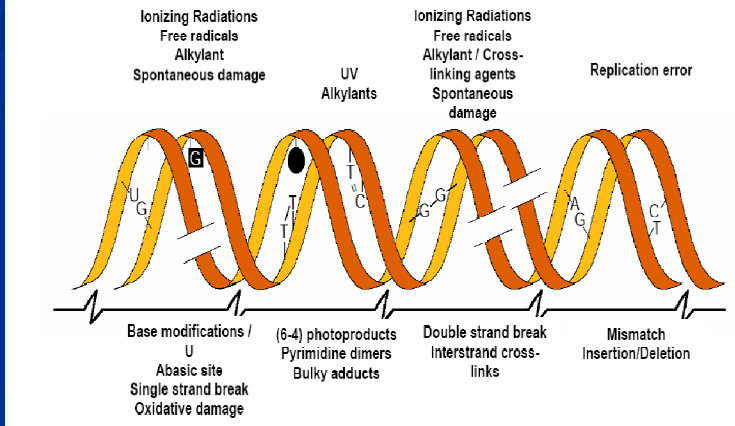


Abortive Spermatogenesis : Quantitative /Qualitative

Abnormalities:Chromosomal , Genetic, Epigenetics , Protamins,Maturation and Competency







Nucleotide Excision Repair (NER)

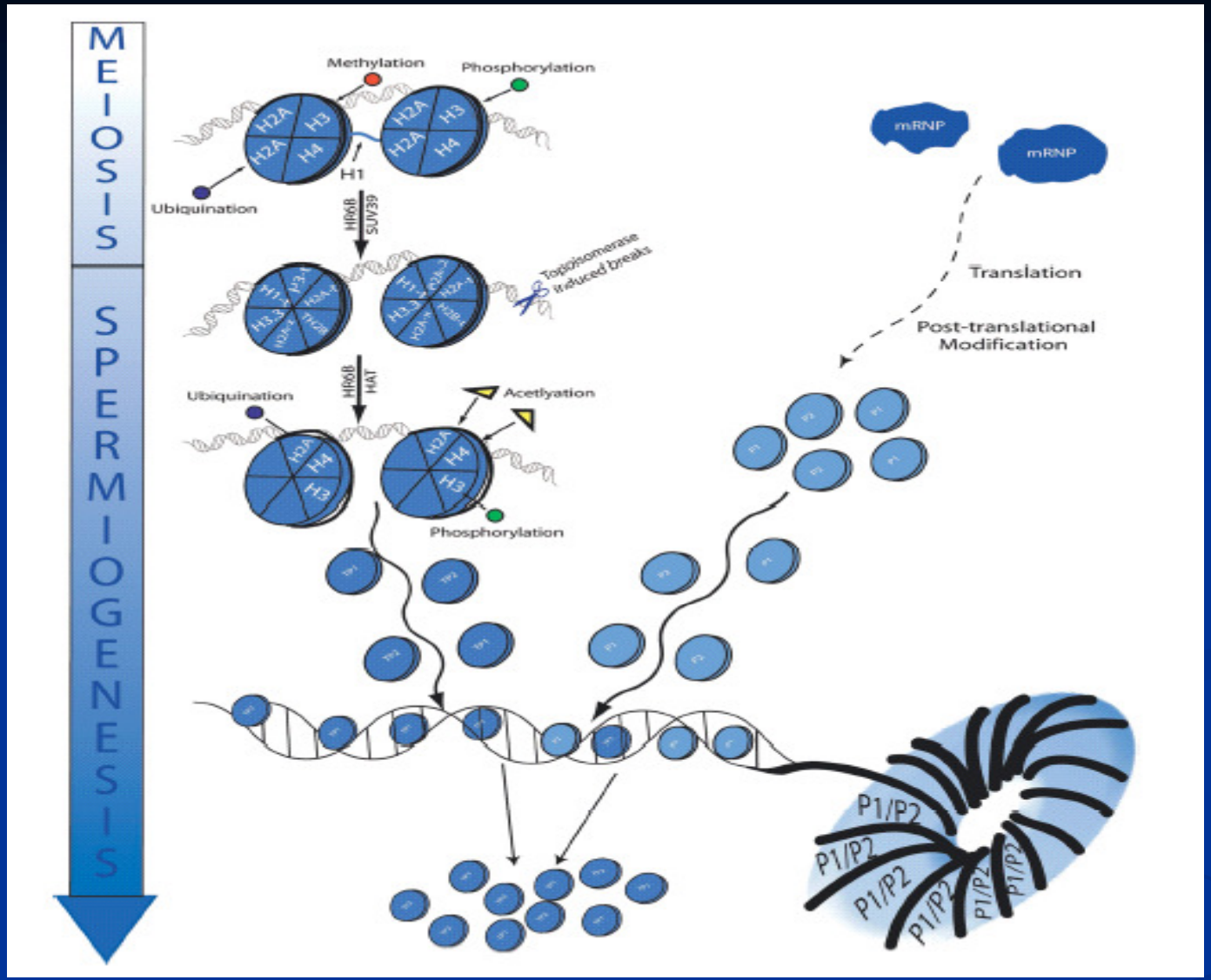
Mismatch Repair

Base Excision Repair (BER)

Recombination (HR & NHEJ)

Genetics of Sperm

- **Aneupoloid** spermatoza are able to fertilize oocyte but both embryo quality and implantation rates are reduced (Shi&Martin,2000)
- **High grade DNA fragmentation**. The existance of a cytoplasmic droplet in sperm head could mean indicator of genetic disorder (Henkel et al.,2003)
- **Sperm with insufficient exchange of nuclear proteins** (histones) with more condenced protamines during spermatogenesis . Protamine-1 to protamine-2 ratio in sperm is crucial for oocyte fertilization (Hammadeh,1999;Nasr-Esfahani et aal.,2004;Stager,2003) Disturbance of histone-chromatin exchance occurs during spermatogenesis . Aberrant changes in this ratio seems to have influance on fertiliztion capacity

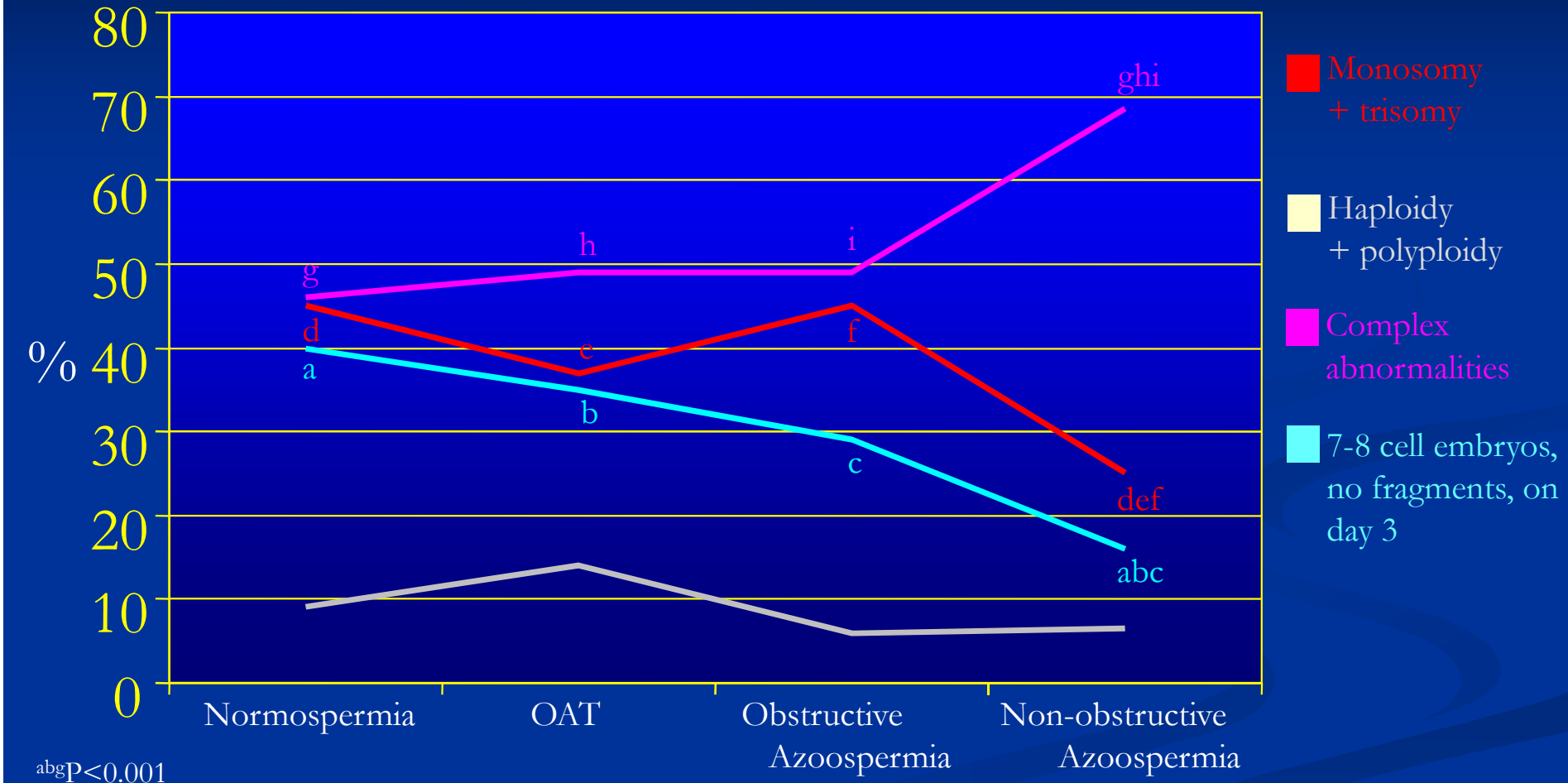


Altered protamine expression and diminished spermatogenesis: what is the link?

Douglas T.Carrell[1,2,3,4], Benjamin R.Emery[1,2] and Sue Hammoud[1,2]

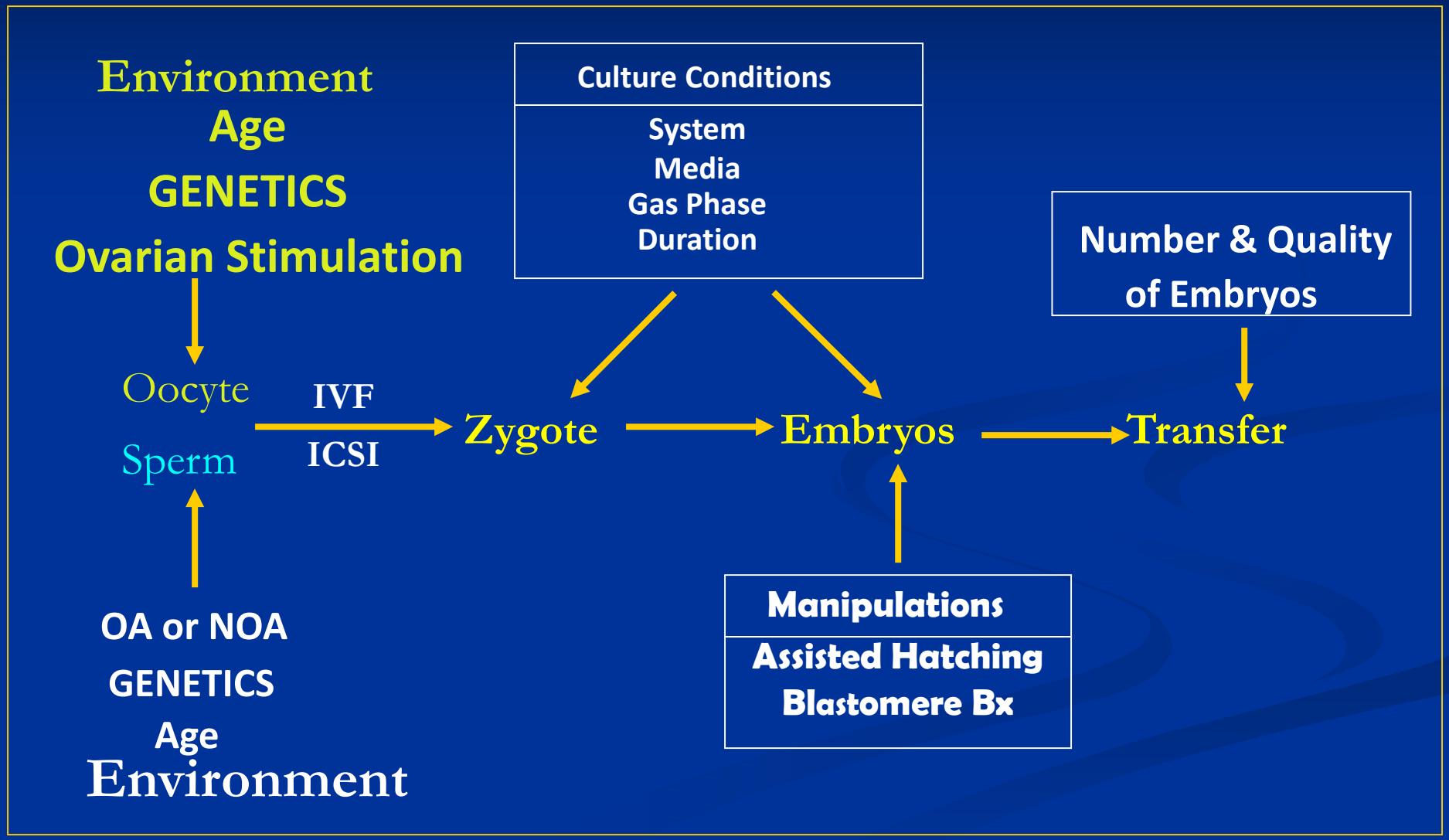
CHROMOSOMALLY ABNORMAL EMBRYOS ACCORDING TO SPERM INDICES

n=1549

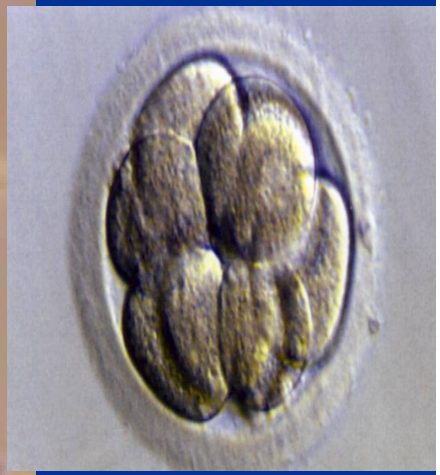


[abg]P<0.001
[ci]P<0.025
[de]P<0.05
[f]P<0.01
[h]P<0.005

Possible Causes of Adverse Outcomes



Oocyte :An recombinant factory



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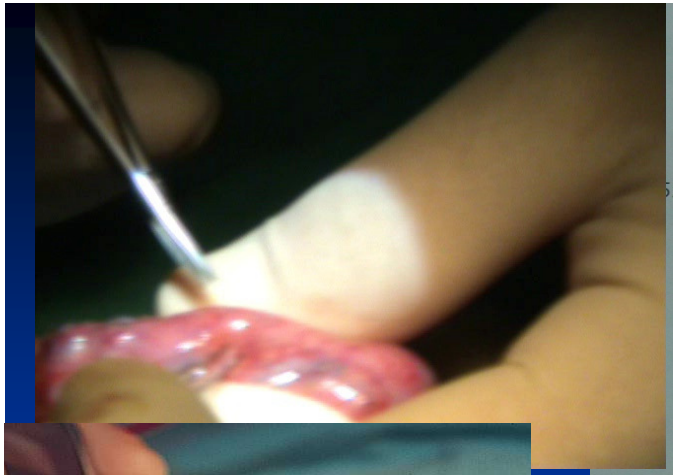
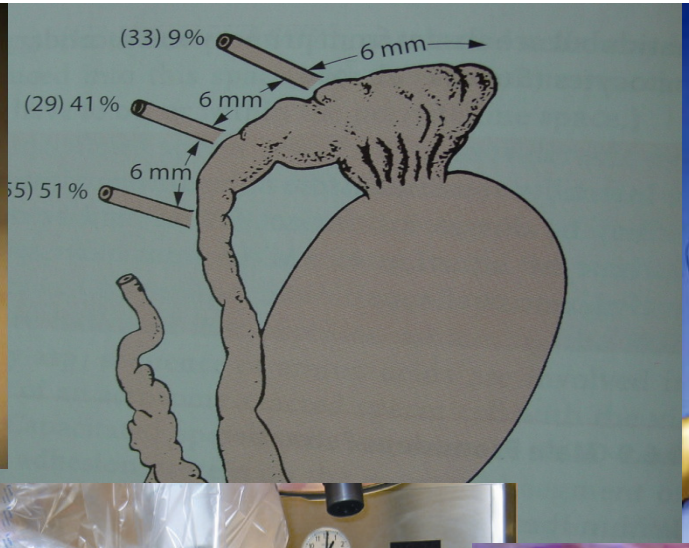
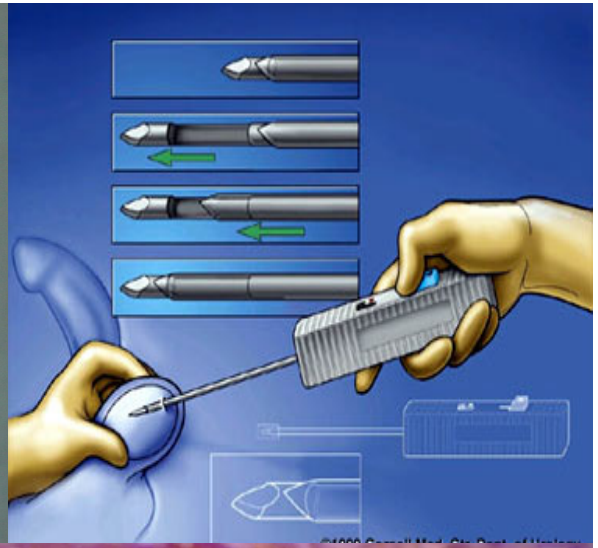
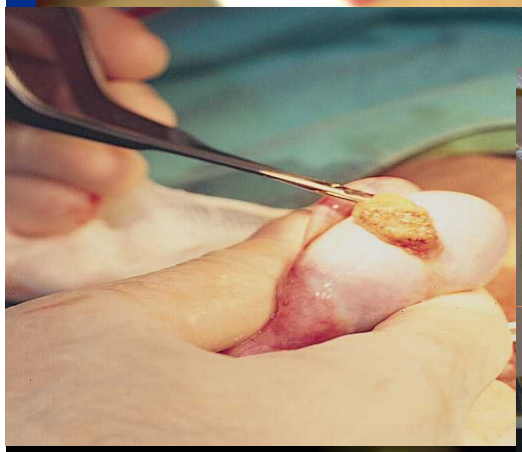
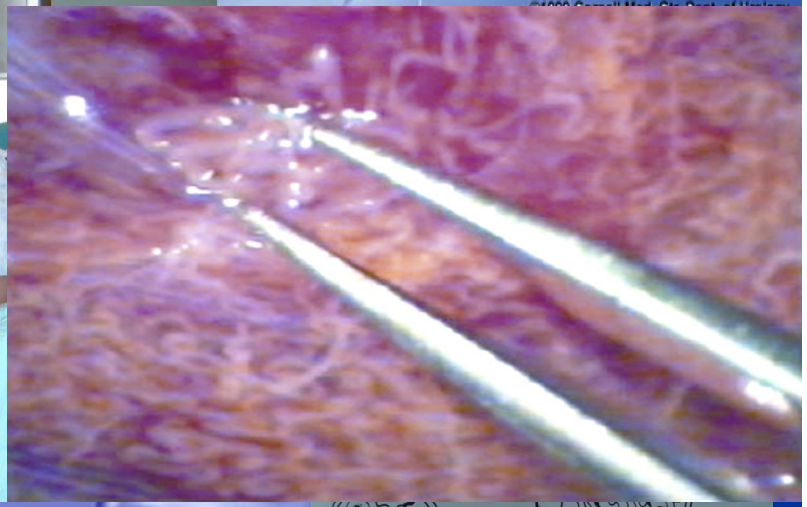
Good quality oocyte can repair sperm DNA fregmantations during the early embryonic devalopment But if the DNA fragmentation is more than 30% this will not be possible

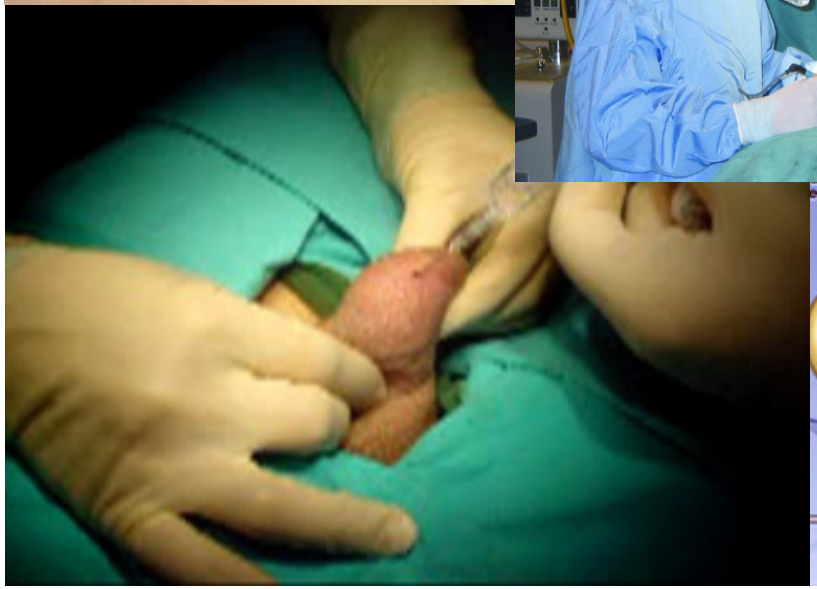


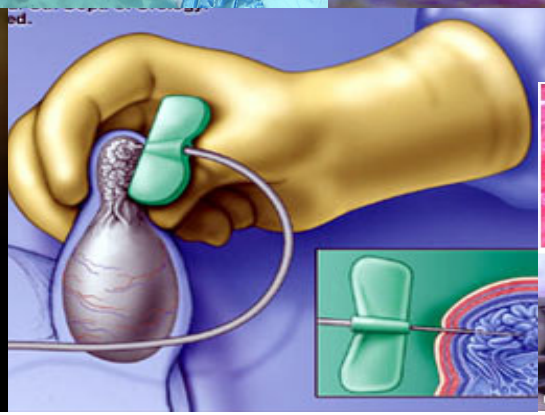

(33) 9%
6 mm
(29) 41%
6 mm
6 mm
5) 51%

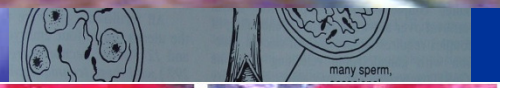


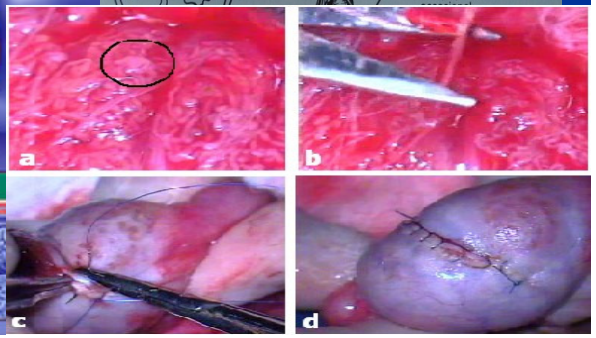









many sperm,


a
b
c
d

Sperm Origin and ICSI

- **Ejeculated sperm**

Morphology

Motility

Oligo/astheno/terato /crypto spermia

Chromozomal abnormalities

Genetic competancy

- **Obstructive Azoospermia**
- **Nonobstructive azoopermia**
- **Causes meybe different :** Hormonal ,Infectious, Genetic,Enviromental,CABVD,Cryptorchism,Cancer Therapy,Operations etc.

Morphology and ICSI outcome

French DB, et al. Fertil Steril.2009

Kruger strict morphology and ICSI cycle outcome.

	% normal sperm morphology						
	0%	1%	2%	3%	4%	5%–7%	>7%
Transfers	138	167	137	129	113	236	154
Maternal age (mean ± SD)	32.3 ± 3.2	32.7 ± 2.7	32.4 ± 2.9	32.5 ± 2.5	32.0 ± 3.9	32.3 ± 3.5	32.8 ± 3.8
No. of oocytes (mean ± SD)	13.3 ± 6.1	14.4 ± 6.8	14.2 ± 6.8	13.5 ± 6.5	13.1 ± 6.7	13.8 ± 6.8	12.9 ± 6.1
No. of oocytes injected (mean ± SD)	10.2 ± 5.0	11.0 ± 5.7	11.0 ± 5.7	10.6 ± 5.3	10.1 ± 4.9	10.3 ± 4.8	9.3 ± 4.8
Fertilization rate	75%	75%	77%	77%	74%	76%	77%
No. of embryos transfered (mean ± SD)	2.3 ± 0.48	2.4 ± 0.65	2.4 ± 0.57	2.5 ± 0.57	2.3 ± 0.58	2.3 ± 0.51	2.4 ± 0.60
Blastulation rate with spare embryos	50%	43%	44%	47%	41%	42%	45%
Blastocysts of high quality suitable for freezing	37%[a]	29%	26%	31%	27%	28%	29%
Clinical pregnancy rate	60%	59%	56%	49%	57%	54%	56%
Implantation rate	36%	39%	32%	31%	41%	34%	33%
Live birth rate per transfer	56%	54%	47%	44%	52%	49%	52%
Miscarriage rate	4%	5%	9%	5%	5%	5%	4%
Total deliveries	77	90	64	57	59	115	80
Singleton	55	49	37	35	30	72	53
Twins	19	38	24	20	23	39	27
Triplets	3	3	3	2	6	4	0

[a] Significantly different from other morphology subgroups ($P < .05$).

French. Severe teratospermia and ICSI clinical outcomes. Fertil Steril 2009.

No difference in terms of sperm morphology

FERTILITY AND STERILITY®
VOL. 79, NO. 1, JANUARY 2003

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

Anick De Vos, Ph.D., Hilde Van De Velde, Ph.D., Hubert Joris, M.T., Greta Verheyen, Ph.D., Paul Devroey, M.D., Ph.D., and André Van Steirteghem, M.D., Ph.D.

Centre for Reproductive Medicine, University Hospital, Dutch-speaking Brussels Free University, Brussels, Belgium

Objective: To evaluate the influence of morphology of individual spermatozoa on fertilization and pregnancy outcome.

Design: Retrospective analysis.

Setting: An IVF center in an institutional research environment.

Patient(s): Fertilization and embryo quality according to individual sperm morphology were analyzed in 662 consecutive ICSI cycles. Pregnancy outcome was evaluated for these cycles and an additional 1005 consecutive ICSI cycles.

Intervention(s): ICSI was performed using sperm cells of ejaculated, epididymal, or testicular origin. Observation through an inverted microscope was used to prospectively classify injected sperm cells as normal or morphologically abnormal.

Main Outcome Measure(s): Oocyte fertilization, embryo morphology, and pregnancy outcome of unmixed embryo transfers.

Result(s): Injection of morphologically abnormal spermatozoa (irrespective of origin) resulted in a lower fertilization rate (60.7%) than did injection of morphologically normal spermatozoa (71.7%). Embryo cleavage quality did not differ between groups. Higher pregnancy and implantation rates were obtained in patients with normal sperm morphology (36.7% and 18.7%, respectively) than in those with abnormal sperm morphology (20.2% and 9.6%).

Conclusion(s): Individual sperm morphology assessed at the moment of ICSI correlated well with fertilization outcome but did not affect embryo development. The implantation rate was lower when only embryos resulting from injection of an abnormal spermatozoon were available. (Fertil Steril® 2003;79:42–8. ©2003 by American Society for Reproductive Medicine.)

Sperm morphology and ICSI

Retrospectivestudy

	Normal sperm morphology	Abnormal sperm morphology
No. Of oocyte sinjected	4,406	418
Fertilization rate (%)	72.5 ± 25.1	64.4 ± 38.0 *
Embryoquality	73.6 ± 29.8	72.5 ± 35.2
N°transfers	1226	41
Femaleage	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 *

De Vos *et al.*, 2003

*

ELSEVIER

European Journal of Obstetrics & Gynecology and Reproductive Biology 94 (2001) 250–255

EUROPEAN JOURNAL OF OBSTETRICS & GYNECOLOGY AND REPRODUCTIVE BIOLOGY

www.elsevier.com/locate/ejogrb

The origin of spermatozoa does not affect intracytoplasmic sperm injection outcome

Orhan Bukulmez*, Aykan Yucel, Hakan Yarali, Ibrahim Bildirici, Timur Gurgan

Hacettepe University School of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Ankara, Turkey

Abstract

Objective: To assess whether the origin of spermatozoa, ejaculate or testicular, affects intracytoplasmic sperm injection (ICSI) outcome. *Study design:* Retrospective study of 890 consecutive first ICSI and embryo transfer cycles done for male infertility. The ICSI outcome of ejaculated spermatozoa (n=780) and testicular spermatozoa retrieved from patients with obstructive azoospermia (n=43), non-obstructive azoospermia (n=53) and severe oligoasthenoteratozoospermia (n=14) were compared by using chi-square test, independent t-test and ANOVA with Bonferroni test. *Results:* All azoospermic males had a diagnostic testicular biopsy at least 6 months before the ICSI procedure. Spermatozoa were successfully retrieved in all 43 patients with obstructive azoospermia and in 72.6% of 73 non-obstructive cases. The cycle characteristics of the four groups were similar apart from a younger mean female age in the non-obstructive azoospermia group when compared with the ejaculated spermatozoa group. The fertilization, implantation and clinical pregnancy rates were comparable among the four groups. *Conclusion:* Testicular spermatozoa recovered from patients with obstructive and all types of non-obstructive azoospermia were as much as effective as ejaculated spermatozoa in ICSI. © 2001 Elsevier Science

TABLE 3

Normal fertilization, implantation, and pregnancy rates in cycles with different sperm characteristics.

Variables evaluated	Normal-fertilization rate	Implantation rate	Pregnancy rate
Normozoospermia			
Fresh (%)	74.9	16.3	39.3
Cryopreserved (%)	79.6	16	25
P value	.284	.977	.484
Oligozoospermia			
Fresh (%)	73.5	26.6	60
Cryopreserved (%)	73.6	25.9	50
P value	.991	.964	.714
Asthenozoospermia			
Fresh (%)	75.3	12.5	60
Cryopreserved (%)	50.5	15.5	50
P value	.001	.702	.741
Oligoasthenozoospermia			
Fresh (%)	72.7	15.1	45
Cryopreserved (%)	57.9	13.5	45.5
P value	.004	.702	.741

58 patients with ejeculated spermatozoa
79 cycles with fresh and 61 cycles with frozen sperm
NO DIFFERENCE in IR and PR
In astheno and oligoastheno groups FR is lower in cryo groups

Borges E Jr. Fertil Steril.2007

Clinical Urology
International Braz J Urol Vol. 34(1): 49-56, January - February, 2008

Sperm Defect Severity Rather Than Sperm Source Is Associated With Lower Fertilization Rates after Intracytoplasmic Sperm Injection

Sidney Verza Jr, Sandro C. Esteves

313 ICSI cycles 1) Ejaculated (group 1; n = 220) and 2) Testicular/Epididymal (group 2; n = 93). Ejeculated group was subdivided into four subgroups 1) single defect (oligo-[O] or astheno- [A] or teratozoospermia-[T], n = 41), 2) double defect (a combination of two single defects, n = 45), 3) triple defect (OAT, n = 48), and 4) control (no sperm defects; n = 86)

Table 2 – ICSI outcomes with ejaculated sperm with and without sperm defects, and with epididymal and testicular spermatozoa from men with obstructive and non-obstructive azoospermia.

	Group 1 – Ejaculated Sperm (n = 220)				Group 2 – Testicular and Epididymal Sperm from Azoospermic Men (n = 93)		
	Normal (n = 86)	Single Defect (n = 41)	Double Defect (n = 45)	Triple Defect (n = 48)	OA (n = 39)	NOA (n = 54)	p Value
Fertilization (%2PN)	71.3 ± 24.1[a]	73.2 ± 22.1[b]	72.1 ± 19.6[c]	63.4 ± 26.9[d]	73.6 ± 20.7[e]	52.2 ± 29.3[f]	< 0.05[d,f versus a,b,c,e]
Cleavage (%)	92.8 ± 18.1[a]	89.6 ± 21.4[b]	93.5 ± 17.4[c]	88.1 ± 28.6[d]	94.8 ± 10.2[e]	77.7 ± 34.0[f]	< 0.05[f versus a,b,c,d,e]
Good embryo quality* (%)	48.4 ± 34.8[a]	50.5 ± 30.9[b]	46.9 ± 31.1[c]	48.3 ± 35.8[d]	46.3 ± 30.0[e]	35.7 ± 27.4[f]	< 0.05[f versus a,b,c,d,e]
Clinical pregnancy (%)	40.9[a]	36.6[b]	44.4[c]	51.0[d]	51.3[e]	25.9[f]	0.01[f versus a,b,c,d,e]
Miscarriage (%)	14.9	9.1	12.5	12.0	20.0	14.3	NS

*One-way ANOVA was used to compare clinical and laboratorial parameters among groups, and the chi-square test was used to compare pregnancy and miscarriage rates, $p < 0.05$ considered significant; NS = not significant. * = 7-9 blastomeres of similar size, and grades I or II cytoplasmic fragmentation on the day of embryo transfer (day 3) (17). OA = obstructive azoospermia; NOA = non-obstructive azoospermia, values are mean ± standard deviation.*

Use of surgical sperm retrieval in azoospermic men: a meta-analysis

James D. M. Nicopoullos, M.B.B.S.,[a] Carole Gilling-Smith, Ph.D.,[a] Paula A. Almeida, Ph.D.,[a] Julian Norman-Taylor, M.R.C.O.G.,[a] Ian Grace, B.Sc.,[b] and Jonathan W. A. Ramsay, M.S.[a,c]

Assisted Conception Unit, Chelsea and Westminster Hospital, London, United Kingdom

Objective: To compare the outcome of intracytoplasmic sperm injection (ICSI) cycles [1] using epididymal and testicular sperm in patients with obstructive azoospermia (OA); [2] using surgically retrieved sperm in patients with OA and nonobstructive azoospermia (NOA); and [3] using fresh and frozen-thawed sperm.

Design: Meta-analysis of published data.

Setting: Assisted conception unit.

Patient(s): Ten reports (734 cycles: 677 transfers) were identified as suitable to assess source of sperm; 9 reports (1,103 cycles: 998 transfers) to assess etiology; and 17 reports (1,476 cycles: 1,377 transfers) to assess the effect of cryopreservation.

Intervention(s): Surgical sperm retrieval/ICSI.

Main Outcome Measure(s): Fertilization rate (FR), implantation rate (IR), clinical pregnancy rate (CPR), and ongoing pregnancy rate (OPR) per embryo transfer.

Result(s): Meta-analysis demonstrated no significant difference in any outcome measure between the use of epididymal or testicular sperm in men with OA. Meta-analysis showed a significantly improved FR (relative risk [RR] 1.18; 95% confidence interval [CI]: 1.13–1.23) and CPR (RR 1.36; 95% CI: 1.10–1.69) in men with OA as compared to NOA with a nonsignificant increase in OPR. There was no difference in either IR or miscarriage rate between the two groups. Comparing fresh with frozen-thawed epididymal sperm there was no difference in FR or IR, a significantly higher CPR (RR 1.20; 95% CI: 1.0–1.42), and no difference in OPR. No difference in fertilization or pregnancy outcome was noted when the testicular cycles were analyzed separately, but IR was significantly impaired using frozen-thawed sperm (RR 1.75; 95% CI: 1.10–2.80).

Conclusion(s): Meta-analysis of published data confirms that etiology of azoospermia and cryopreservation of surgically retrieved sperm impacts on ICSI outcome, and allows us to make several recommendations for clinical practice. Origin of sperm, in men with similar etiology, does not affect outcome. (Fertil Steril® 2004; 82:691–701. ©2004 by American Society for Reproductive Medicine.)

CONCLUSSION - I

- Testicular pathology resulted in a decreased implantation rate without affecting fertilization and erly pre-implantation development
- In patient with NOA ,pregnancy rates are lowest for maturation arrest 20% but not significantly different from Sertoli cell-only or hypoplasia (15%)
- There is usually no differances on FR , PR, IR comparing ICSI using fresh or frozen and thawed epididymal or testicular sperm in OA and NOA patients . But issue should be taken cautiously since one should not be so confidant with spermatozoa derived from NOA men !

CONCLUSION - II

- Significantly lower FR,IR and CPR in cycles from men with NOA
- Embryos generated spermatozoa in men with NOA result in a lower blastulation and IR than the embryos generated from ejeculated sperm or from men with OA
- Even with normal karyotyping,spermatozoa from men with NOA have a higher incidance of chromozomal abnormalities

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ICSI outcomes in obstructive azoospermia: influence of the origin of surgically retrieved spermatozoa and the cause of obstruction

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BACKGROUND: Spermatozoa can be retrieved from the testis and epididymis of men with obstructive azoospermia (OA) and used for ICSI. However, it is unknown whether the outcome of ICSI depends on the cause of obstruction or the origin of surgically retrieved spermatozoa. METHODS: A cohort of 171 men with OA and normal spermatogenesis were included in this retrospective study. They were divided into three groups according to the site and origin of obstruction: 83 men had congenital bilateral absence of vas deferens; 55 and 33 had acquired epididymal and deferent duct obstructions, respectively. The outcome of 368 ICSI cycles was determined and compared according to the origin of spermatozoa: epididymal (n = 253) or testicular (n = 115). RESULTS: Fertilization and clinical pregnancy rates did not differ between spermatozoa of different origin (58.9% versus 51.9% and 22.1% versus 24.3% with epididymal and testicular spermatozoa, respectively). However, the miscarriage rate was significantly higher for testicular spermatozoa (35.7% versus. 12.5% $P < 0.05$, χ^2 test). Findings were similar whatever the aetiology of the OA. CONCLUSION: This study suggests that the use of testicular spermatozoa, even those generated during normal spermatogenesis, alters embryonic development and that epididymal spermatozoa should be preferentially used, irrespective of the aetiology of OA.

Table I. ICSI outcomes according to the origin of spermatozoa from 171 obstructive azoospermic patients

A	Testicular spermatozoa	Epididymal spermatozoa
No. of oocyte retrievals	115	253
Age of women (years) (mean ± SD)	33.0 ± 4.5	33.0 ± 4.2
Age of men (years) (mean ± SD)	38.2 ± 7.5	36.0 ± 5.7
No. of oocytes retrieved (mean ± SD)	1164 (10.1 ± 6.2)	2623 (10.4 ± 6)
No. of metaphase II (MII) oocytes injected (mean ± SD)	874 (7.6 ± 4.8)	1889 (7.5 ± 4.4)
No. of 2 pronuclei (PN) oocytes (%)[a]	454 (51.9%)	1112 (58.9%)
No. of cleaved embryos (%)[a]	483 (55.2%)	1192 (63.1%)
No. of embryos with < 20% fragmentation (%)[b]	208 (43%)	501 (42%)

[a]Percentage of the total number of MII oocytes injected; [b]Percentage of the total number of embryos.

B	Testicular spermatozoa	Epididymal spermatozoa	p[d]
No. of embryo transfers	110	235	
No. of transferred embryos (mean ± SD/transfer)	232 (2.1 ± 0.7)	526 (2.2 ± 0.8)	
No. of embryos implanted (%)[a]	33 (14.2%)	68 (12.9%)	
No. of clinical pregnancies (%)[b]	28 (24.3%)	56 (22.1%)	
No. of miscarriages (%)[c]	10 (35.7%)	7 (12.5%)	< 0.05
No. of extra-uterine pregnancies	1	1	
No. of medical abortion	0	2	
No. of deliveries (% per cycle)	17 (14.8%)	46 (18.2%)	
No. of newborns	20	53	
No. of newborns with malformation	1	3	

[a]Ratio between the number of gestational sacs and the number of transferred embryos; [b]Ratio between the number of clinical pregnancies and the number of oocyte retrievals; [c]Ratio between the number of spontaneous pregnancy losses before 20 weeks of gestation and number of the clinical pregnancies; [d]χ^2 test.

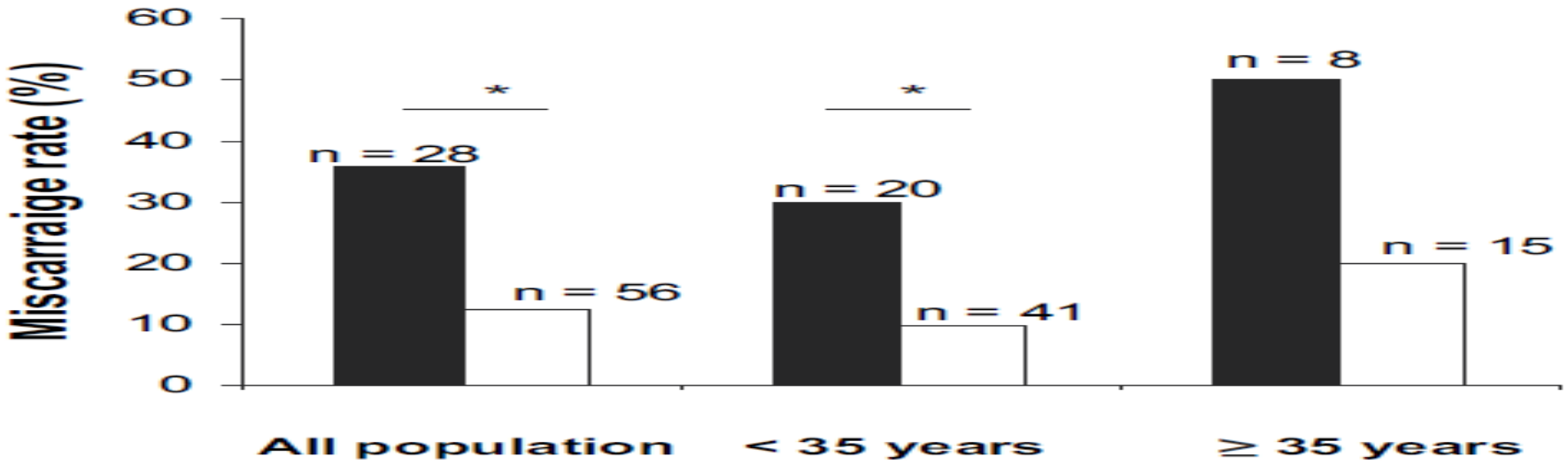

Figure 1. Miscarriage rate (MR) after ICSI according to the sperm origin (■ testicular spermatozoa □ epididymal spermatozoa) and age of women in obstructive azoospermia. *n* = number of clinical pregnancies; **P* < 0.05.

Table III. Outcome of transfers of thawed embryos according to the source of spermatozoa

	Testicular spermatozoa	Epididymal spermatozoa	*P*
Age of women (years) (mean ± SD)	32.2 ± 4.7	32.4 ± 4.2	
Age of men (years) (mean ± SD)	37.4 ± 7.8	36.0 ± 5.6	
No. of embryo transfers	55	136	
No. of transferred embryos (mean ± SD/transfer)	101 (1.8 ± 0.7)	256 (1.8 ± 0.7)	
No. of thawed embryos implanted (%)[a]	6 (5.9%)	27 (10.5%)	<0.05[e]
No. of clinical pregnancies (%)[b]	5 (8.4%)	23 (16.3%)	<0.05[e]
No. of miscarriages (%)[c]	3 (60%)	4 (17.4%)	<0.05[f]
No. of deliveries (%)[d]	2 (3.6%)	19 (13.9%)	
No. of newborns	3	21	

[a]Ratio between the number of gestational sacs and the number of transferred embryos; [b]Ratio between the number of clinical pregnancies and the number of oocyte

Does the outcome of ICSI in cases of obstructive azoospermia depend on the origin of the retrieved spermatozoa or the cause of obstruction? A comparative analysis

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Objective: To compare the outcomes of intracytoplasmic sperm injection (ICSI) for men with obstructive azoospermia and normal spermatogenesis, according to the use of epididymal or testicular spermatozoa and the cause of obstruction.

Design: Retrospective study.

Setting: Private infertility center.

Patient(s): A detailed chart review of a cohort of 1,121 men with obstructive azoospermia who underwent intracytoplamic sperm injection (ICSI) was performed.

Intervention(s): Patients were grouped according to the origin of spermatozoa: epididymal (n =331) or testicular (n =790). They were further classified into two subgroups according to the cause of obstruction: congenital bilateral absence of vas deferens (CBAVD; n = 434), and other causes of obstruction (n= 687).

Main Outcome Measure(s): Fertilization, clinical pregnancy, and miscarriage rates.

Result(s): Fertilization (64.2% vs. 68.0%), clinical pregnancy (42.3% vs. 43.2%), and miscarriage (17.6% vs. 18.4%) rates did not differ between epididymal spermatozoa and testicular spermatozoa, respectively. Fertilization, clinical pregnancy, and miscarriage rates were also similar in the patients with CBAVD or due to other causes of obstruction.

Obstructive Azoospermia

- The use of testicular spermatozoa for ICSI is associated with a rate of pregnancy loss that is higher than that of epididymal spermatozoa/
- Immaturity and or chromozomal abberations of testicular spermatozoa affect embryo development

  DNA damage is higher in epididymal spermatozoa in man with obstructive azoospermia (Ramos et al.,2002;EiGreco et al.,2005) Other causes may be responsible for higher abortion rates using testicular spermatozoa

- There is no differance in ICSI outcomes related to use of fresh or frozen-thawed spermatozoa ,whether epididymal or testicular ??

  (Friedler et al.,2002;Nicopoullos et al.,2004) ; (Buffat et al.,2006)

- The cause of obstruction has an effect on ICSI out come/Pregnancy rates lower in the cases of aquired epididymal obstruction caused by infection and inflamation compared with CABVD

  (Nicopoullos et al.,2004;Pasqulotto et al.,2005)

Motility of Spermatozoa

- Vitality of the sperm seems to be most significant factor to achieve successful results in ICSI
- Motility is the most significant sign of vitality
- Immotile sperms can be death
- In extreme crytozoospermia or testicular tissue extraction it is difficult to identify motile sperm

*Hypoosmotic swelling test

*Diode laser

*Mechanical touch system

	Group 1	Group 2	Group 3	Group 4
Cycles with embryo transfer	2.317	79	62	135
Mean age of the women	33.03	32.6	33.17	33.2
Mean oocytes punctured	8.39	9.14	8.21	7.86
Fertilized oocytes (%)	67.1	49.8	68.3	47.8
Mean number of PN score 4 per ET	2.0	0.6	1.8	0.4
Embryo score	14.5	11.8	14.5	10.8
% Pregnancy rate (three embryos)	35.7	18.9*	37.5	19.7**
% Abortion rate per pregnancy	26.8	23.1	28.6	57.9

*$P < 0.001$; **$P < 0.01$.

Grup 1:motile ejeculated(2317)
Grup 2: immotile ejeculated(79)
Grup 3: Motile testicular (62)
Grup 4: immotile testicular(135)

Stalf T, et al.Andrologia.2005

Immotile Sperm

- Death spermatozoa are the cells without intact membranes
- Harmful subtances like reactive oxygene species (ROS) can penatrate into the cells and damage its DNA decreasing pregnancy rates (Agarwal&Saleh, 2002;Henkel et al.,2003)
- Factors in the cytoplasmic layer of the spermatozoon which are not yet identified but supposed to play role in activation of oocyte and ferilization reaction migth be lost
- Immotile sperm from the testis may represent a stage of biochemical immaturity thus having less reproductive capacity than motile sperm/ immaturity may play a role in the genetic integrity of the spermatozoa

  (Silber et al.,2003)

Therefore motility of testicular spermatozoa is more important than vitality since produce better embryos to implant

DNA Damage

- The integrity of sperm DNA is important for the success of fertilization
- DNA strand breaks can cause abnormalities in chromatin packaging
- Higher levels of DNA damage may cause developmental arrest, apoptosis prior to implantation or early pregnancy failure

  (Evenson et al., 1999; Morris et al., 2002)
- Correlation between DNA strand breaks and
  - age
  - sperm count
  - motility

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doi:10.1093/humrep/den321

Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis

Armand Zini[1,4], Jason M. Boman[1], Eric Belzile[2] and Antonio Ciampi[2,3]

11 studies involved 1549 cycles of treatment (808 IVF and 741 ICSI cycles) with 640 pregnancies (345 IVF and 295 ICSI) and 122 pregnancy losses. OR: 2.48 (95% CI 1.52, 4.04,P < 0.0001) indicates that sperm DNA damage is predictive of pregnancy loss after IVF and ICSI

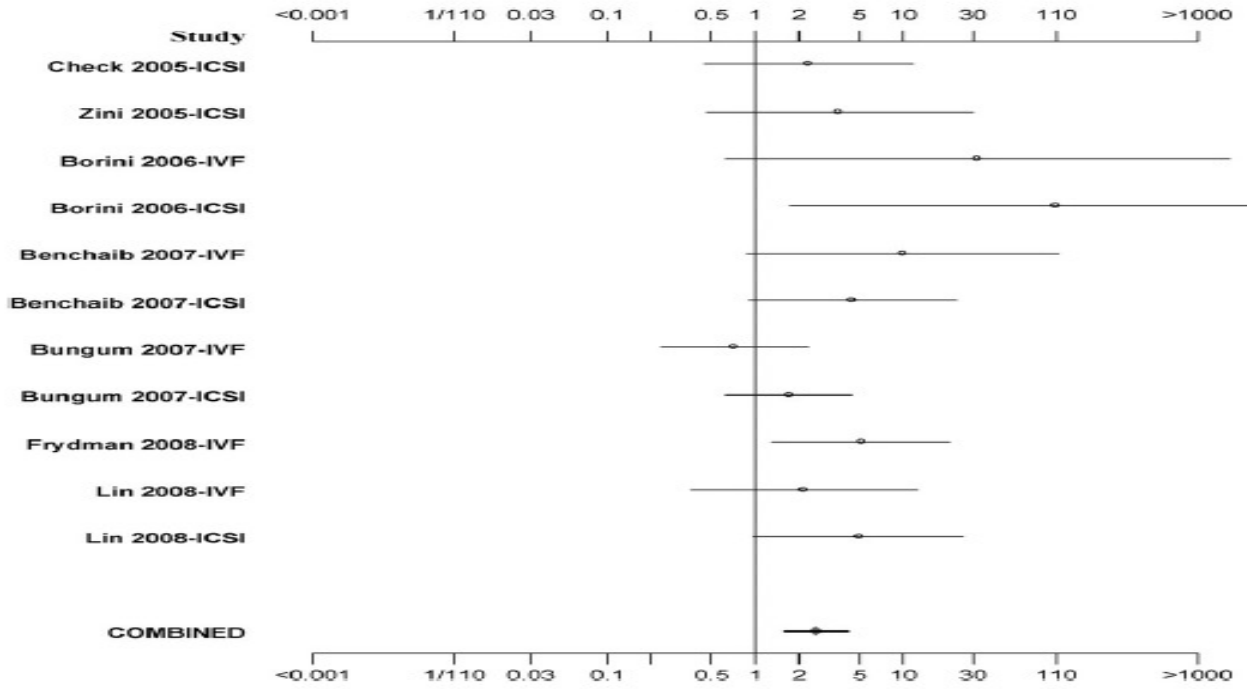
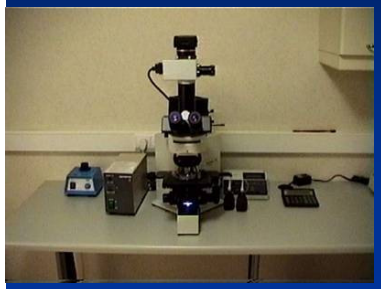
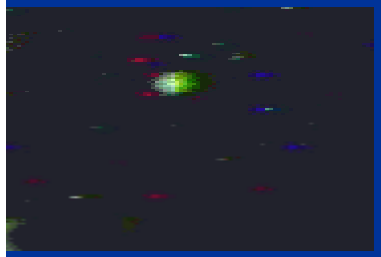


Figure 1: Forest plot depicting odds ratio (OR) and 95% confidence interval (CI) of the 11 studies and the combined OR from the meta-analysis (note: scale is logarithmic).





Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF : a meta-analysis

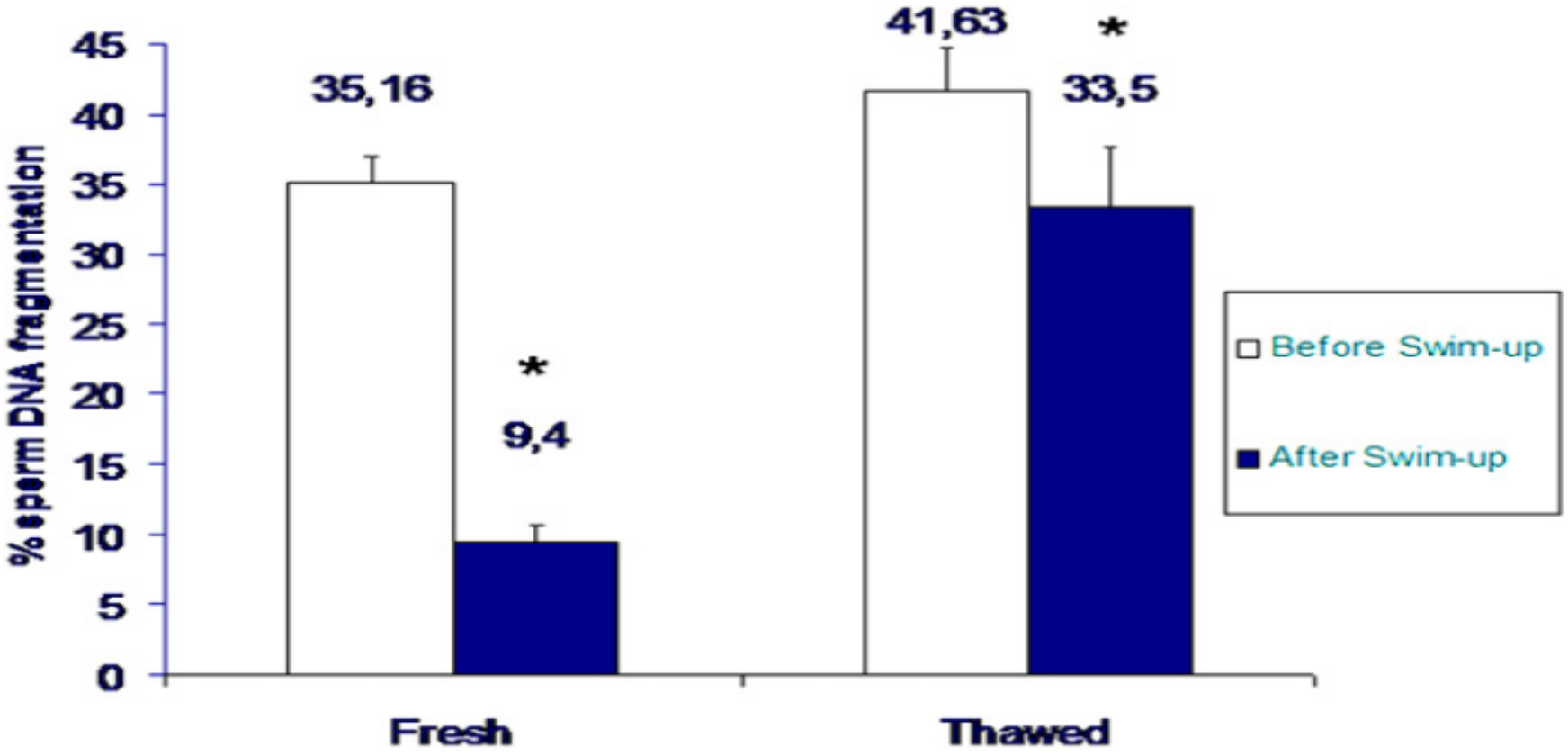
- Sperm DNA damage is associated with a significantly increased risk of pregnancy loss after IVF and ICSI.

- Clinical indication for the evaluation of sperm DNA damage prior to IVF or ICSI and a rationale for further investing the association between sperm DNA damage and pregnancy loss.

Armand Zini et al. 2008

FIGURE 1

Percentage of cells with sperm DNA fragmentation (SDF) with respect to sperm origin (fresh or frozen thawed). Levels of SDF before and after swim-up preparation before ART are also represented. *Statistically significant difference, $P < .05$.



Meseguer. Sperm DNA damage conditioned by oocyte. Fertil Steril 2010.

Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality

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Objective: To quantify the effect of sperm DNA fragmentation (SDF) on reproductive outcome by evaluating the most statistically significant bias factors using logistic regression.

Design: Prospective blind observational cohort study.

Setting: University affiliated private IVF unit.

Patient(s): Two hundred ten male partners of couples undergoing in vitro fertilization (IVF) or first intracytoplasmic sperm injection (ICSI) cycles with fresh or thawed sperm with the women's own or donated oocytes.

Intervention(s): None.

Main Outcome Measure(s): SDF determined before and after swim-up (n = 420), odds ratio calculated of the effect of an increase of one unit of SDF on pregnancy, and stratified regression analysis performed to evaluate the confusion effect of oocyte quality, sperm origin, and the fertilization procedure.

Result(s): The effect of SDF on pregnancy was not affected by sperm origin (fresh or thawed) or fertilization procedure when measured both before and after swim-up. When oocytes from infertile patients were employed, SDF had a statistically significant negative impact on chance of pregnancy. For every 10% increase in SDF, the probability of not achieving pregnancy increased by 1.31. When donated oocytes were employed, SDF did not have a statistically significant effect.

Conclusion(s): The effect of SDF on the probability of pregnancy can be calculated independent of the fertilization procedure or sperm origin. Oocyte quality conditions the extent of the negative impact of SDF on pregnancy; this can be overcome when good quality oocytes are employed. (Fertil Steril® 2010;■:■–■. ©2010 by American Society for Reproductive Medicine.)

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Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa

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BACKGROUND: Sperm DNA damage (fragmentation) is a recently discovered cause of male infertility for which no efficient treatment has yet been found. Previous findings have suggested that clinically relevant sperm DNA damage may occur at the post-testicular level. This study was undertaken to assess the clinical usefulness of ICSI with testicular spermatozoa in this indication. METHODS: The percentage of spermatozoa with fragmented DNA, assessed by terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling assay, and ICSI outcomes were compared in two sequential attempts performed, respectively, with ejaculated and testicular spermatozoa in 18 men with increased sperm DNA fragmentation. RESULTS: The incidence of DNA fragmentation was markedly lower in testicular spermatozoa as compared with ejaculated spermatozoa. No differences in fertilization and cleavage rates and in embryo morphological grade were found between the ICSI attempts performed with ejaculated and with testicular spermatozoa. However, eight ongoing clinical pregnancies (four singleton and four twin) were achieved by ICSI with testicular spermatozoa (44.4% pregnancy rate; 20.7% implantation rate), whereas ICSI with ejaculated spermatozoa led to only one pregnancy which was spontaneously aborted. CONCLUSIONS: These data show that ICSI with testicular spermatozoa provides the first efficient assisted reproduction treatment option for men with high levels of sperm DNA damage.

Table II. Fertilization and embryo development after ICSI with ejaculated and testicular spermatozoa

Sperm source	Attempts	Oocytes injected	Normal zygotes[a]	Fertilization rate[b]	Cleaved embryos[c]	Good-morphology embryos[d]
Ejaculate	18	185	131	70.8%[e]	124 (94.7%)[e]	59 (47.6%)[e]
Testis	18	187	140	74.9%[e]	133 (95.0%)[e]	68 (51.1%)[e]

[a]With two equal-sized pronuclei.
[b]Percentage of injected oocytes that developed to normal zygotes.
[c]Percentages are calculated from the number of normal zygotes.
[d]Embryos with normal pronuclear morphology on day 1, ≥6 cells on day 3, equal sized blastomeres and <10% of the intrazonal space occupied by fragments. The percentages are calculated from the number of cleaved embryos.
[e]The differences between data for the two sperm sources are not significant ($P > 0.05$).

Table III. Implantation and pregnancy after ICSI with ejaculated and testicular spermatozoa

Sperm source	Attempts	Embryos transferred	Clinical pregnancies[a]	Pregnancy rate[b]	Gestational sacs[c]	Implantation rate[d]
Ejaculate	18	56	1	5.6%[e]	1	1.8%[f]
Testis	18	58	8	44.4%[e]	12	20.7%[f]

[a]With at least one gestational sac with cardiac activity.
[b]Percentage of attempts resulting in a clinical pregnancy.
[c]With cardiac activity.
[d]Percentage of embryos transferred that gave rise to a gestational sac with cardiac activity.
[e]$P < 0.05$.
[f]$P < 0.01$.

Obstetric Outcome of Pregnancies According to Sperm Origin and Quality

- In ejeculated sperm group , intrauterine death is higher in severely defective sperm group than better quality sperm groups

  Aytoz A et al.,1998

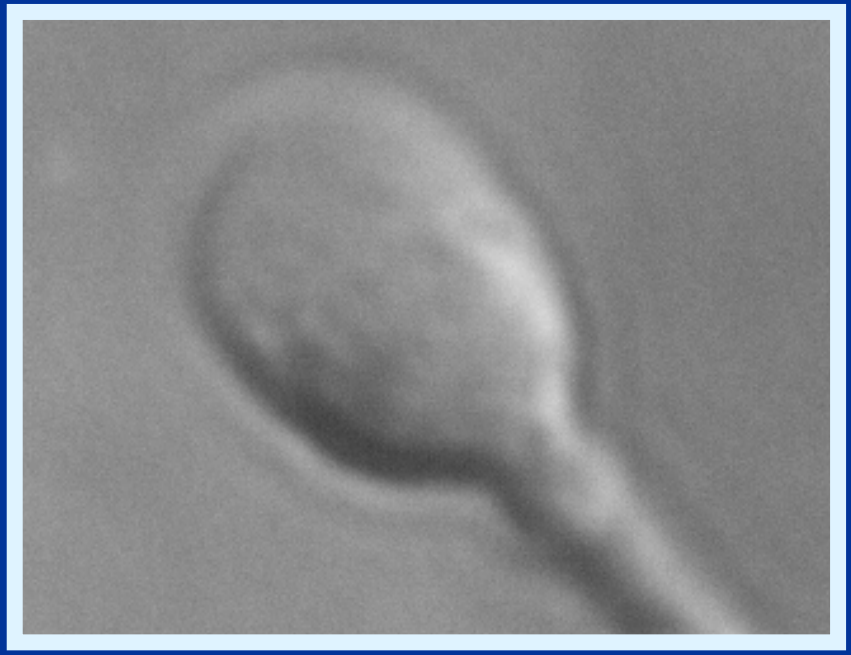
- Obstetric outcomes is not influenced by sperm origin or quality in ICSI pregnancies

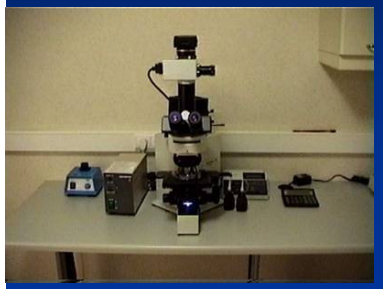
  Wennerholm UB et al.,2000

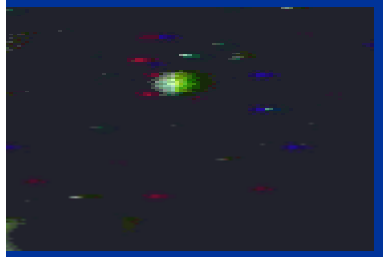
- The course of pregnancy as well as the outcome after ICSI neither affected by the origin of sperm nor by the number of sperm in ejeculate

  Ludwig M et al.,2003

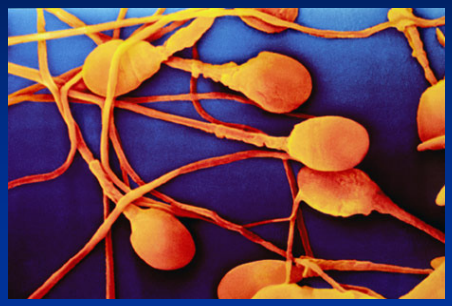
Can sperm selection improve pregnancy rate?



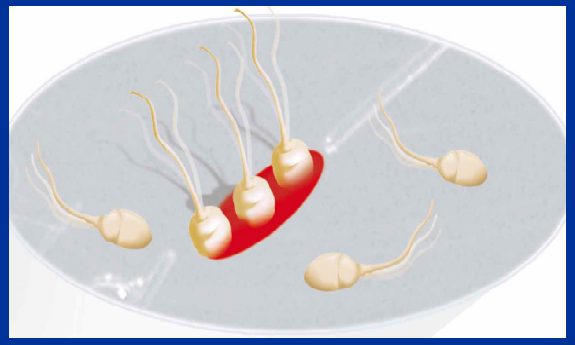


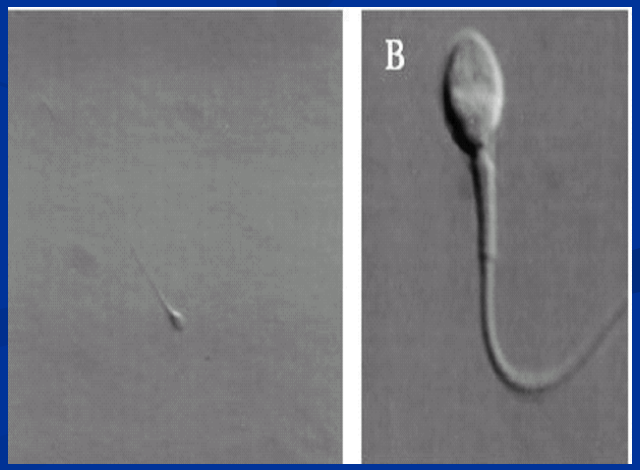


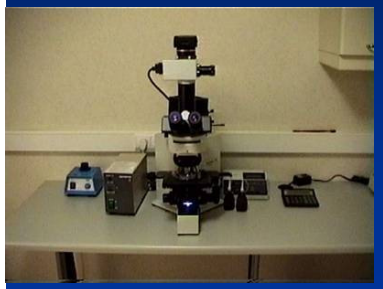
Andrology Labratory Applications

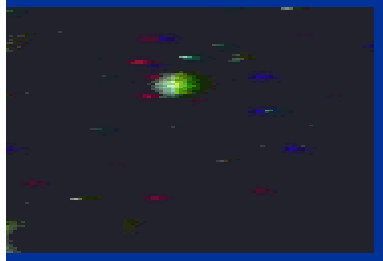


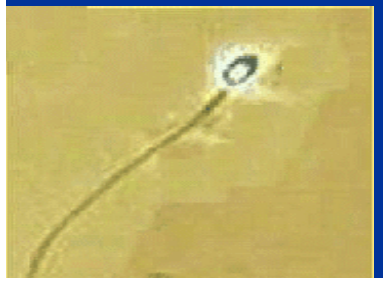
- Sperm analysis and counts
- Sperm DNA screenings
- Sperm function tests
- Sperm Aneuploidy and damage analysis
- Sperm selection with IMSI
- Sperm selection with BİREFRİGRENCE
- PTX applications











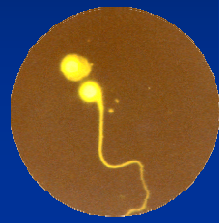
Diagnosis of sperm abnormalities in Andrology Lab

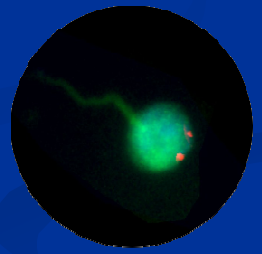
Acridine orange test
Aniline blue test
SCSA

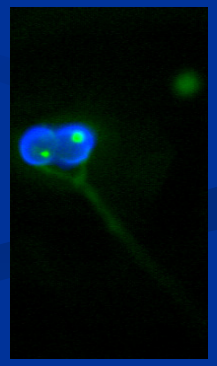
Tunnel test
Halo sperm
Comet assay

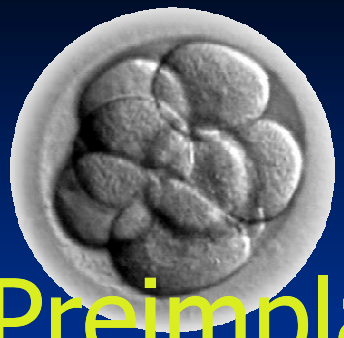
Sperm FISH test

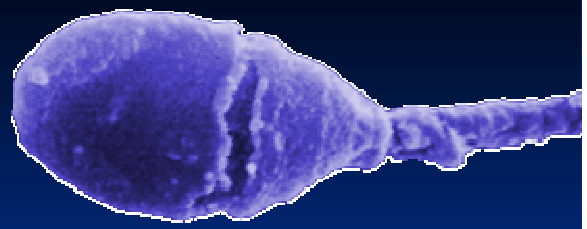
Hemizona Binding Assay
Hyaluronan Binding Assay





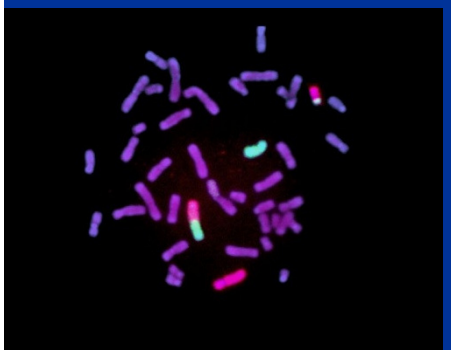


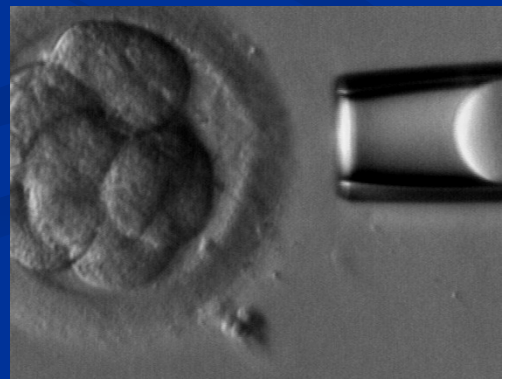




Preimplantation Genetic Diagnosis for Male Infertility

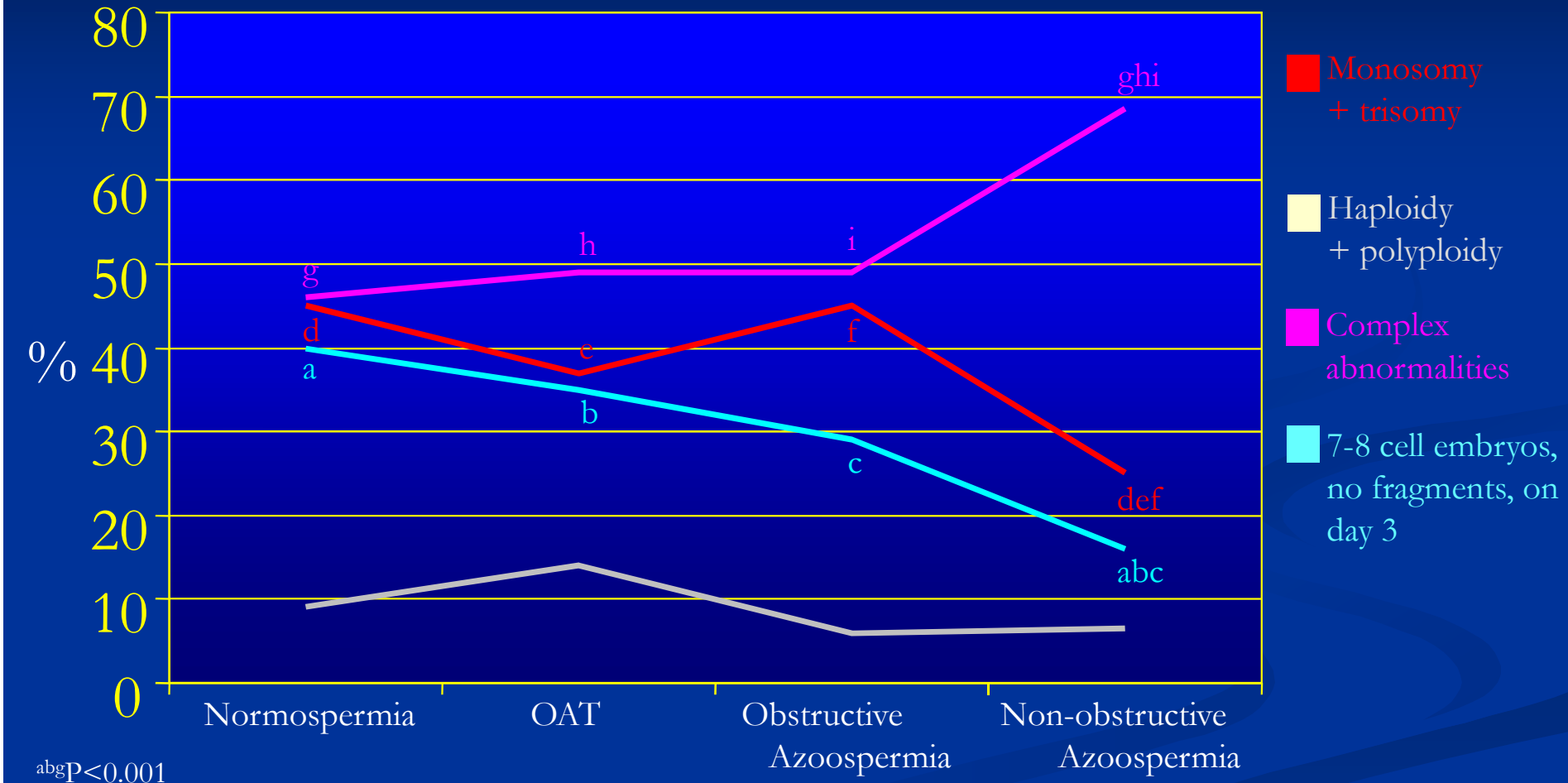
(Aneuploidy)





CHROMOSOMALLY ABNORMAL EMBRYOS ACCORDING TO SPERM INDICES

n=1549



[abg]P<0.001
[ci]P<0.025
[de]P<0.05
[f]P<0.01
[h]P<0.005

Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies

Attila Jakab, M.D.,[a] *Denny Sakkas, Ph.D.,*[b] *Elena Delpiano, M.D.,*[a] *Sevil Cayli, M.S.,*[a] *Ertug Kovanci, M.D.,*[a] *David Ward, Ph.D.,*[c] *Alberto Ravelli, M.D.,*[a] *and Gabor Huszar, M.D.*[a]

The [a] Sperm Physiology Laboratory and [b] In Vitro Fertilization Laboratory, Department of Obstetrics and Gynecology, and the [c] Department of Genetics, Yale University School of Medicine, New Haven, Connecticut

Objective: To test a newly invented intracytoplasmic sperm injection (ICSI) sperm selection method based on sperm hyaluronic acid (HA) binding.

Design: Comparison of chromosomal disomy and diploidy frequencies in sperm arising from semen and in HA-bound sperm.

Setting: Academic andrology laboratory.

Patient(s): Men presenting for semen analysis.

Intervention(s): Washed sperm fractions of 32 semen samples were applied to Petri dishes or glass slides coated with immobilized HA. The unbound sperm were rinsed gently, and the HA-bound sperm were removed with an ICSI pipette. The control sperm population was the unselected sperm. Both HA-selected and unselected sperm were treated with fluorescence in situ hybridization with centromeric probes for the X, Y, and 17 chromosomes.

Main Outcome Measure(s): Chromosomal disomy and diploidy frequencies.

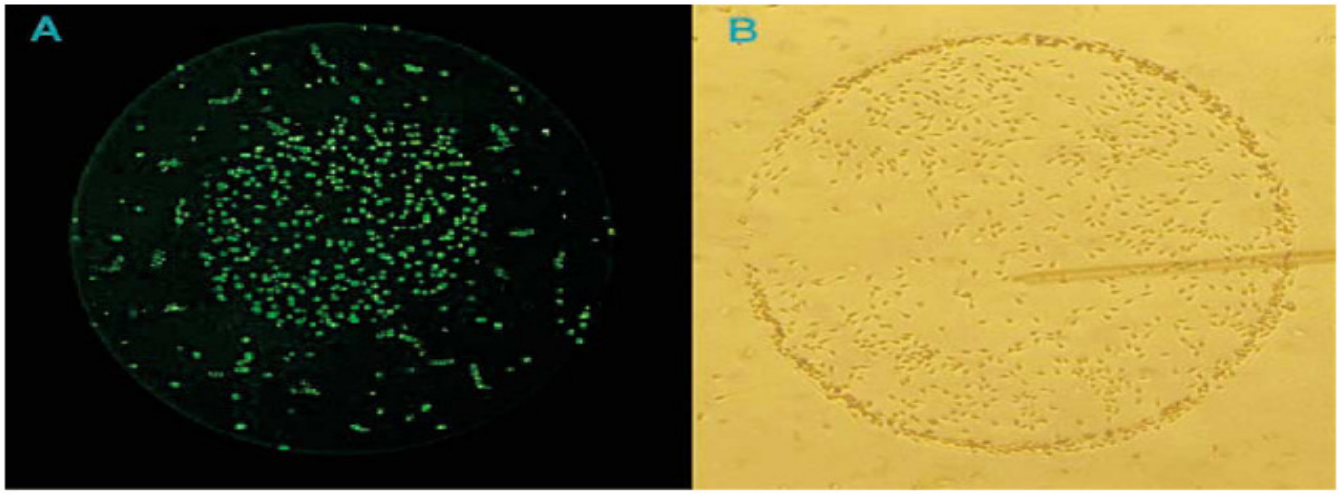
Result(s): In the HA-bound sperm (495–2,079 per man, 41,670 in all) compared with unselected sperm (4,770 per man, 162,210 in all), the chromosomal disomy frequencies were reduced to 0.16% from 0.52%, diploidy to 0.09% from 0.51%, and sex chromosome disomy to 0.05% from 0.27% (a 5.4-fold reduction vs. 4-fold respective increase in ICSI offspring).

Conclusion(s): The HA sperm selection method for ICSI, which is based on a relationship between sperm receptors for zona pellucida and HA, will likely reduce the potential genetic complications and adverse public health effects of ICSI. (Fertil Steril® 2005;84:1665–73. ©2005 by American Society for Reproductive Medicine.)

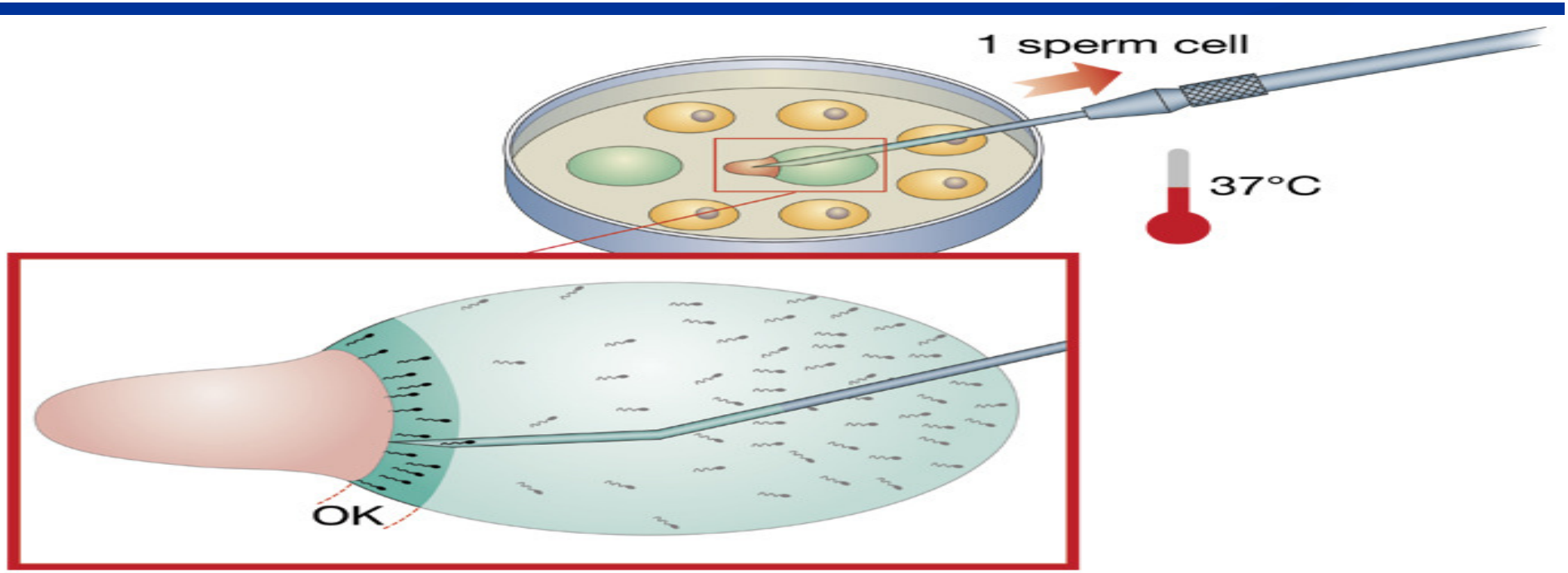
Key Words: Sperm maturity, chromosomal aneuploidies, ICSI sperm selection, genetic integrity, paternal contribution

FIGURE 1

(**A**) Sperm approach from the periphery and then bind to the HA-spot. (**B**) Sperm being picked up with the ICSI pipette.



Jakab. A novel method for ICSI sperm selection. Fertil Steril 2005.



IMSI: Sperm assessment

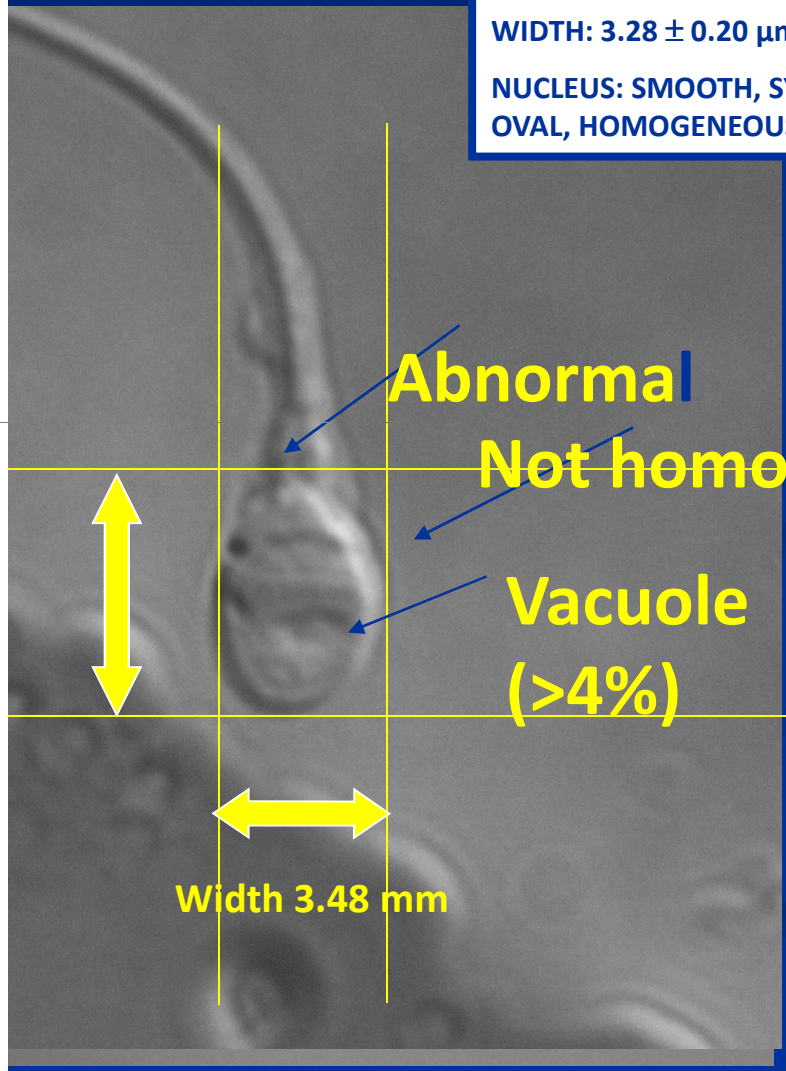
Table 3. Comparison of clinical outcome variables between the last conventional intracytoplasmic sperm injection (ICSI) attempt and the subsequent ICSI attempts with high-magnification sperm selection.

Outcome variable	*ICSI attempt*		*P-value*
	Conventional	*High-magnification*	
Transfer procedures (*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

LENGHT: 4.75 ± 0.28 µm

WIDTH: 3.28 ± 0.20 µm

NUCLEUS: SMOOTH, SYMMETRIC, OVAL, HOMOGENEOUS



Reproductive BioMedicine Online (2010) 21, 450–455

www.sciencedirect.com
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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,*]

Abstract The development of a modified intracytoplasmic sperm injection (ICSI), called intracytoplasmic morphologically selected sperm injection (IMSI), demonstrated that a profound morphological investigation of the spermatozoon, under the magnification of 6600×, enables outcome improvement. The aim of this study was to compare ICSI outcome with IMSI outcome. The meta-analysis results demonstrated no significant difference in fertilization rate between ICSI and IMSI groups. However, a significantly improved implantation (odds ratio (OR) 2.72; 95% confidence interval (CI) 1.50–4.95) and pregnancy rate (OR 3.12; 95% CI 1.55–6.26) was observed in IMSI cycles. Moreover, the results showed a significantly decreased miscarriage rate (OR 0.42; 95% CI 0.23–0.78) in IMSI cycles as compared with ICSI cycles. This is the first meta-analysis of published data to evaluate the potential benefits of IMSI. The pooled data of IMSI cycles demonstrate a statistically significant improvement in implantation and pregnancy rates and a statistically significant reduction in miscarriage rates. However, more randomized controlled trials are needed to confirm these results.

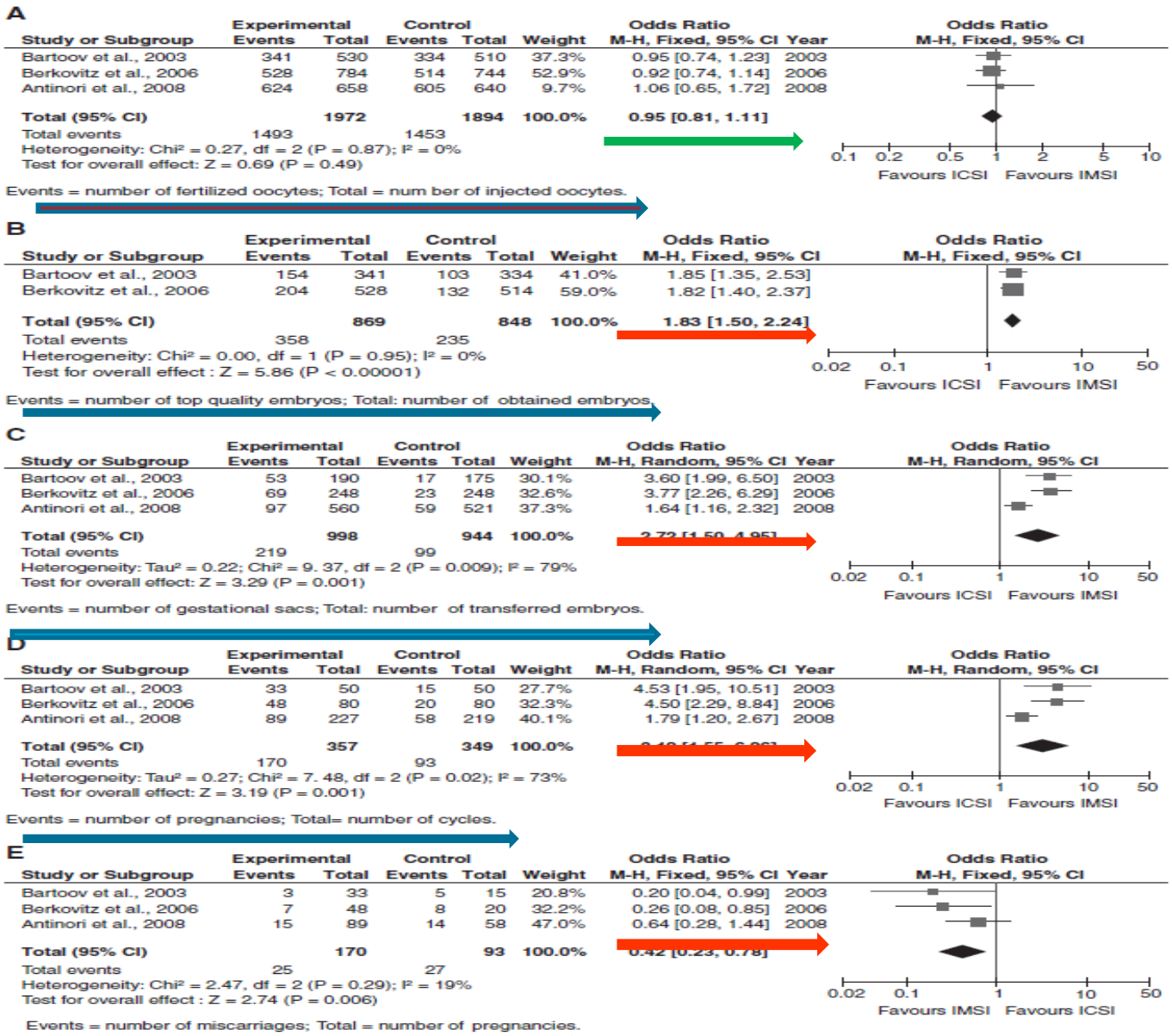
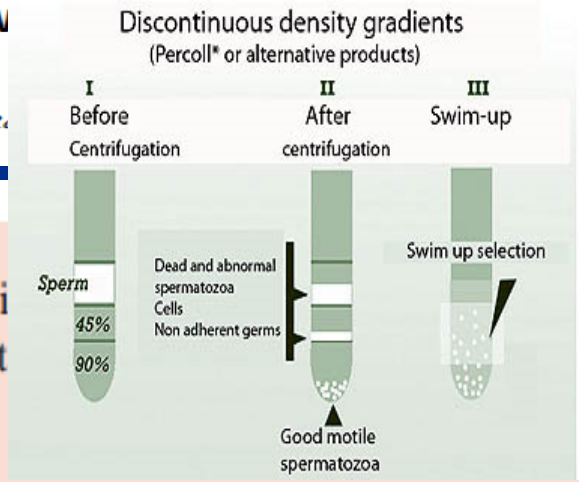


Figure 2 Meta-analysis comparing intracytoplasmic sperm injection (ICSI) versus intracytoplasmic morphologically selected sperm injection (IMSI) outcomes, expressed as odds ratios (OR) with 95% of confidence intervals (CI). **(A)** Fertilization rate (0.95, 0.81—1.11), **(B)** top-quality embryo rate (1.83, 1.50—2.24), **(C)** implantation rate (2.72, 1.50—4.95), **(D)** pregnancy rate (3.12, 1.55—6.26), and **(E)** miscarriage rate (0.42, 0.23—0.78). Individual studies are displayed with a square. The horizontal line through the squares indicates the 95% CI. When the CI crosses the vertical line with OR = 1, there is no significant difference between the groups. The diamond in the last row of the graph illustrates the overall result of the meta-analysis. When the diamond does not cross the vertical line, the difference between the groups is considered as statistically significant. Fixed and random models assume homogenous and heterogeneous studies, respectively.

TECHNIQUES AND INSTRUMENTATION

Use of high-magnification microscopy for the assessment of sperm recovered after tw sperm processing methods

Ana Laura Monqaut, Ph.D.,[a] Christabell Zavaleta, B.Sc.,[b] Gemma López, B.Sc.,[a] R and Mario Brassesco, M.D.[a]



Objective: To compare the quality of sperm samples obtained after density-gradi performing a nuclear structural analysis with high magnification microscopy at

Design: Prospective and randomized split-sample study.

Setting: Reproductive Medicine Center.

Patient(s): Sperm samples from 53 male-partners of couples undergoing infertility treatment.

Intervention(s): Samples were analyzed by high magnification microscopy before and after preparation and classified according the level of nuclear vacuolization.

Main Outcome Measure(s): Recovery rate, motility rate and percentage of sperm scoring each of 4 grades of vacuolization in fresh and processed sample.

Result(s): Both sperm processing methods, swim-up and density gradient centrifugation, allowthe selection of sperm with lower nuclear vacuolization and presumably lower DNA fragmentation than the whole ejaculate.

Conclusion(s): Swim-up produces samples with less vacuolization, but the recovery rate is also lower. The choice of processing technique would then depend on whether intrauterine insemination, IVF or ICSI/IMSI is to be performed. A prospective randomized study scoring fertility rates would be necessary to directly access the influence of these methods on the fertility outcome. (Fertil Steril® 2010;■:■–■. ©2010 by American Society for Reproductive Medicine.)

IMSI: Clinical results

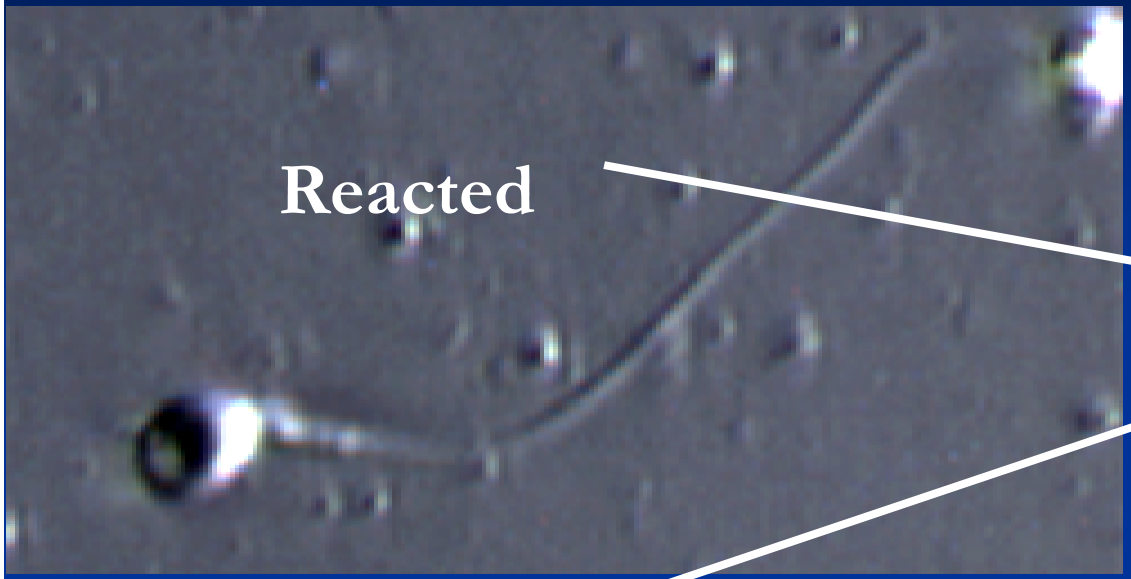
Several publications report that the selection of spermatozoa with normal nuclear shapes at high magnification is positively associated with pregnancy rates:

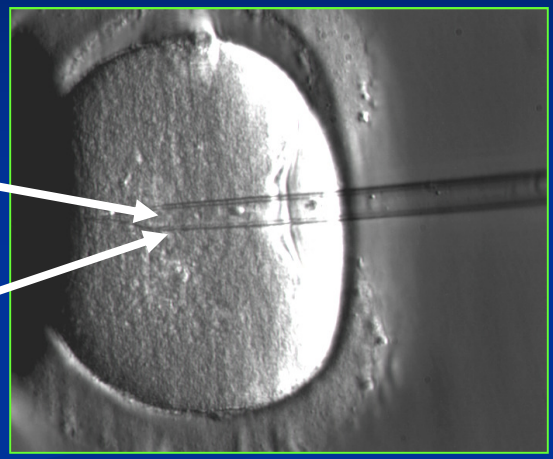
- **Couples with previous and repeated implantation failure**
  Bartoov et al., 2002, 2003; Berkovitz et al. 2006
- **Patients with high degree of sperm DNA fragmentation rate**
  Hazout et al., 2006
- **Repeated poor quality embryonic development in ICSI cycles**
- **Aging males ?**

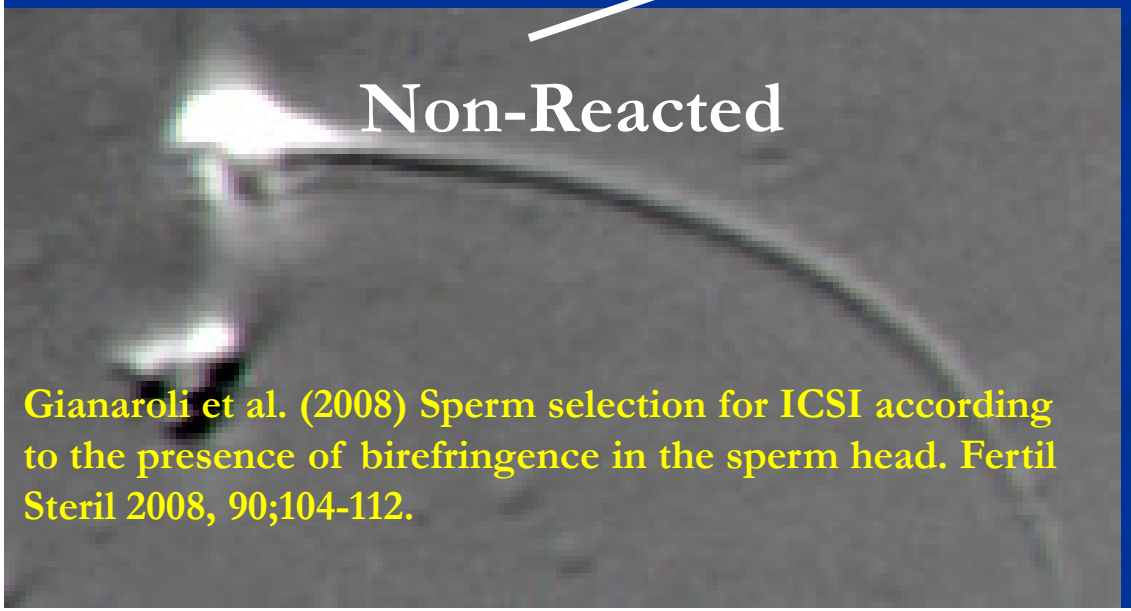
BIREFRINGENCE IN SPERM HEAD

CLINICAL APPLICATION IN sev. OAT AND TESE

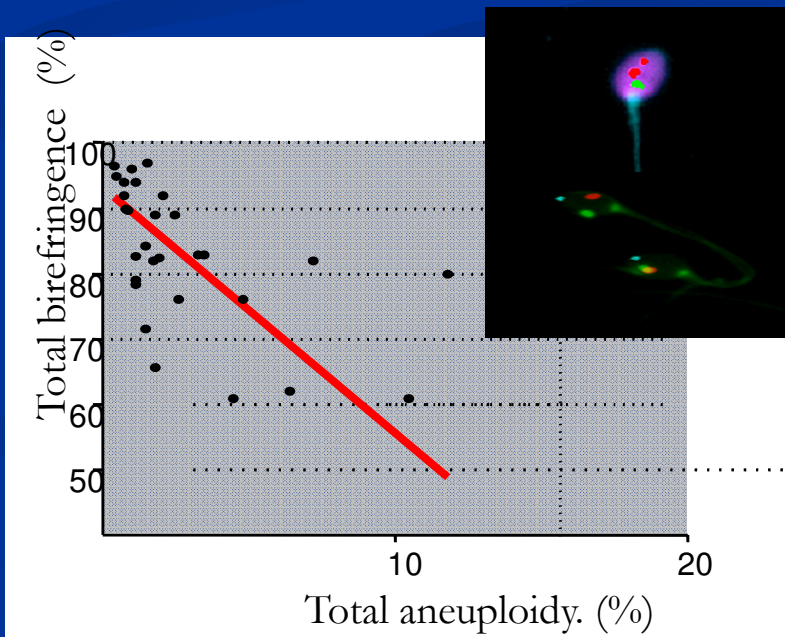
Human spermatozoa possess characteristics of birefringence due to the anisotropy of their protoplasmic texture.







Gianaroli et al. (2008) Sperm selection for ICSI according to the presence of birefringence in the sperm head. Fertil Steril 2008, 90;104-112.



Gianaroli et al.,2010	Reacted	Non-reacted	Mixed
No. cycles	23	26	22
No. transferred cycles (%)	22 (96)	21 (81)	20 (91)
No. clinical pregnancies (% /cycle)	12 (55)[a]	3 (14)[ab]	8 (40)[a]
Implantation rate (%)	(39.0)[c]	(8.6)[cd]	(24.4)[d]
Ongoing pregnancy rate / cycle (%)	11 (48)[e]	2 (8)[ef]	7 (32)[f]

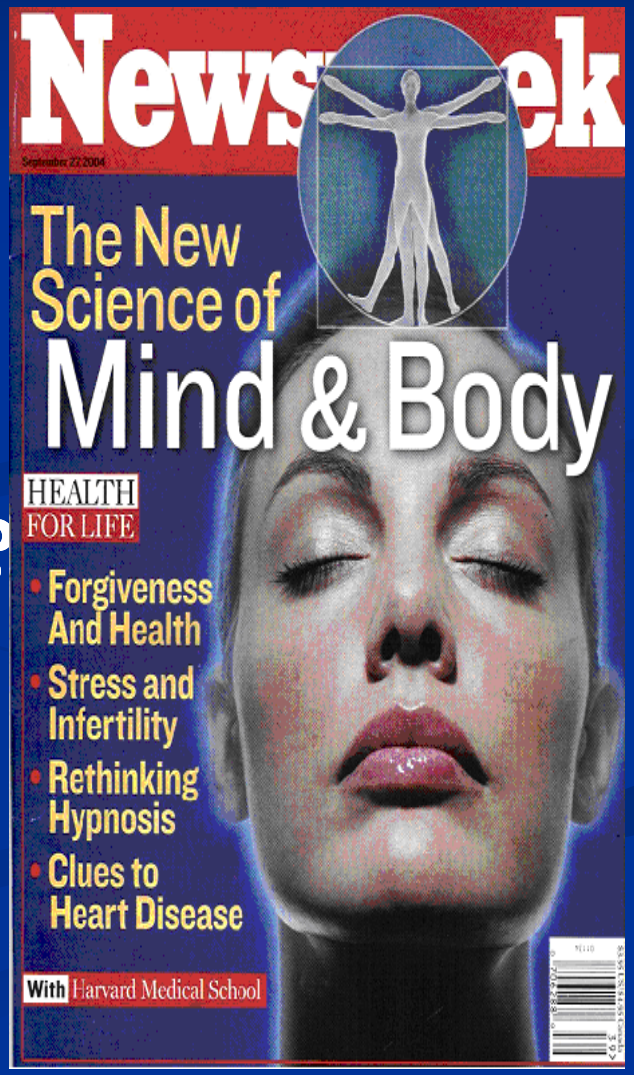
[a]P=0.006 [b]P=0.05 [ce]P=0.002 [d]P=0.048 [f]P=0.033

SPERM HEAD BIREFRINGENCE IN RELATION TO MORPHOLOGY

Gianaroli et al.,2007 No. Spermatozoa =1908	Normal morphology	Abnormal morphology	
	679	1229	
Total head birefringence (%)	62	37	
Partial head birefringence (%)	31	38	
No head birefringence (%)	7	25	

Life Style Changes & Drugs

1.Cigarette Smoking
2.Alchol Consumption
4.BMI : Obesitiy
5.Enviromental factors
6.Stress Reduction
7. FSH,Testosteron,tamoxifen..?



Acupuncture and herbal medicine in IVF: evidence for clinical practice

Ying Cheong[1], Luciano G. Nardo[2, 3], Tony Rutherford[4], William Ledger[5]

Current evidence suggests that consideration should be given to using acupuncture around the time of ET in women undergoing IVF.

Human Reproduction Vol.20, No.9 pp. 2590–2594, 2005
Advance Access publication June 2, 2005
doi:10.1093/humrep/dei091

ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment

Ermanno Greco[1], Stefania Romano[1], Marcello Iacobelli[1], Susanna Ferrero[1], Elena Baroni[1], Maria Giulia Minasi[1], Filippo Ubaldi[1], Laura Rienzi[1] and Jan Tesarik[2,3]

[1]Centre for Reproductive Medicine, European Hospital, Via Portuense 700, 00149 Rome, Italy and [2]MAR&Gen, Molecular Assisted Reproduction and Genetics, Gracia 36, 18002 Granada, Spain

BACKGROUND: Most studies examining the use of ICSI for cases of elevated sperm DNA fragmentation report poor pregnancy and implantation rates. ICSI with testicular sperm samples has recently been suggested for these cases. Here we test a less invasive approach based on oral antioxidant treatment prior to ICSI with ejaculated spermatozoa. METHODS: Thirty-eight men with an elevated (≥15%) percentage of DNA-fragmented spermatozoa in the ejaculate were treated with antioxidants (1 g vitamin C and 1 g vitamin E daily) for 2 months after one failed ICSI attempt. In 29 (76%) of these cases this treatment led to a decrease in the percentage of DNA-fragmented spermatozoa, and a second ICSI attempt was performed. Outcomes of the two attempts were compared. RESULTS: No differences in fertilization and cleavage rates or in embryo morphology were found between the ICSI attempts performed before and after the antioxidant treatment. However, a marked improvement of clinical pregnancy (48.2% versus 6.9%) and implantation (19.6% versus 2.2%) rates was observed after the antioxidant treatment as compared with the pretreatment ICSI outcomes. CONCLUSIONS: Oral antioxidant treatment appears to improve ICSI outcomes in those patiens with sperm DNA damage, in whom this treatment reduces the percentage of damaged spermatozoa.

Table II. Comparison of basic sperm parameters and the incidence of DNA fragmentation in the antioxidant-responsive group before and after the treatment period[a]

Time of analysis	Sperm concentration ($\times 10^6$/ml)	Sperm motility (%)	Normal sperm forms (%)	TUNEL-positive spermatozoa (%)
Before treatment	17.9 ± 16.3	40.6 ± 24.8	10.5 ± 8.3	24.0 ± 7.9
After treatment	18.3 ± 17.9[b]	39.9 ± 19.0[b]	9.6 ± .4[b]	8.2 ± 4.3[c]

[a]Data are mean ± SD.
[b]$P > 0.05$.
[c]$P < 0.001$.

Table III. Comparison of basic sperm parameters and the incidence of DNA fragmentation in the antioxidant-non-responsive group before and after the treatment period[a]

Time of analysis	Sperm concentration ($\times 10^6$/ml)	Sperm motility (%)	Normal sperm forms (%)	TUNEL-positive spermatozoa (%)
Before treatment	19.1 ± 17.4	39.9 ± 24.8	11.4 ± 7.9	25.1 ± 8.5
After treatment	27.5 ± 24.6[b]	41.6 ± 22.0[b]	8.0 ± 7.1[b]	23.8 ± 9.2[b]

[a]Data are mean ± SD.
[b]$P > 0.05$.

Table IV. Fertilization and embryo development in two sequential ICSI attempts performed in the antioxidant-responsive group before and after antioxidant treatment

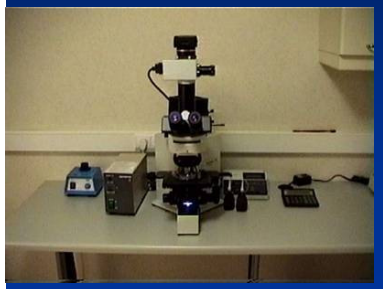
Time of attempt	Attempts	Oocytes injected	Normal zygotes[a]	Fertilization rate (%)[b]	Cleaved embryos [n (%)][c]	Good-morphology embryos [n (%)][d]
Before treatment	29	288	199	69.1[e]	188 (94.5)[e]	86 (45.7)[e]
After treatment	29	276	195	70.7[e]	181 (92.8)[e]	92 (50.8)[e]

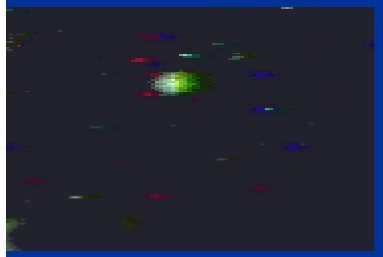
[a]With two equal-sized pronuclei.
[b]Percentage of injected oocytes that developed to normal zygotes.
[c]Percentages are calculated from the number of normal zygotes.
[d]Embryos with normal pronuclear morphology on day 1, six or more cells on day 3, equal-sized blastomeres, and < 10% of intrazonal space occupied by fragments. The percentages are calculated from the number of cleaved embryos.
[e]The differences between data for the two sperm sources are not significant ($P > 0.05$)

Table V. Implantation and pregnancy in two sequential ICSI attempts performed in the antioxidant-responsive group before and after antioxidant treatment

Time of attempt	Attempts	Embryos transferred	Clinical pregnancies[a]	Pregnancy rate (%)[b]	Gestational sacs[c]	Implantation rate (%)[d]
Before treatment	29	89	2	6.9[e]	2	2.2[f]
After treatment	29	92	14	48.3[e]	18	19.6[f]

[a]With at least one gestational sac with cardiac activity.
[b]Percentage of attempts resulting in a clinical pregnancy.
[c]With cardiac activity.
[d]Percentage of embryos transferred that gave rise to a gestational sac with cardiac activity.
[e]$P < 0.05$.
[f]$P < 0.01$.





THE MAIN GOAL

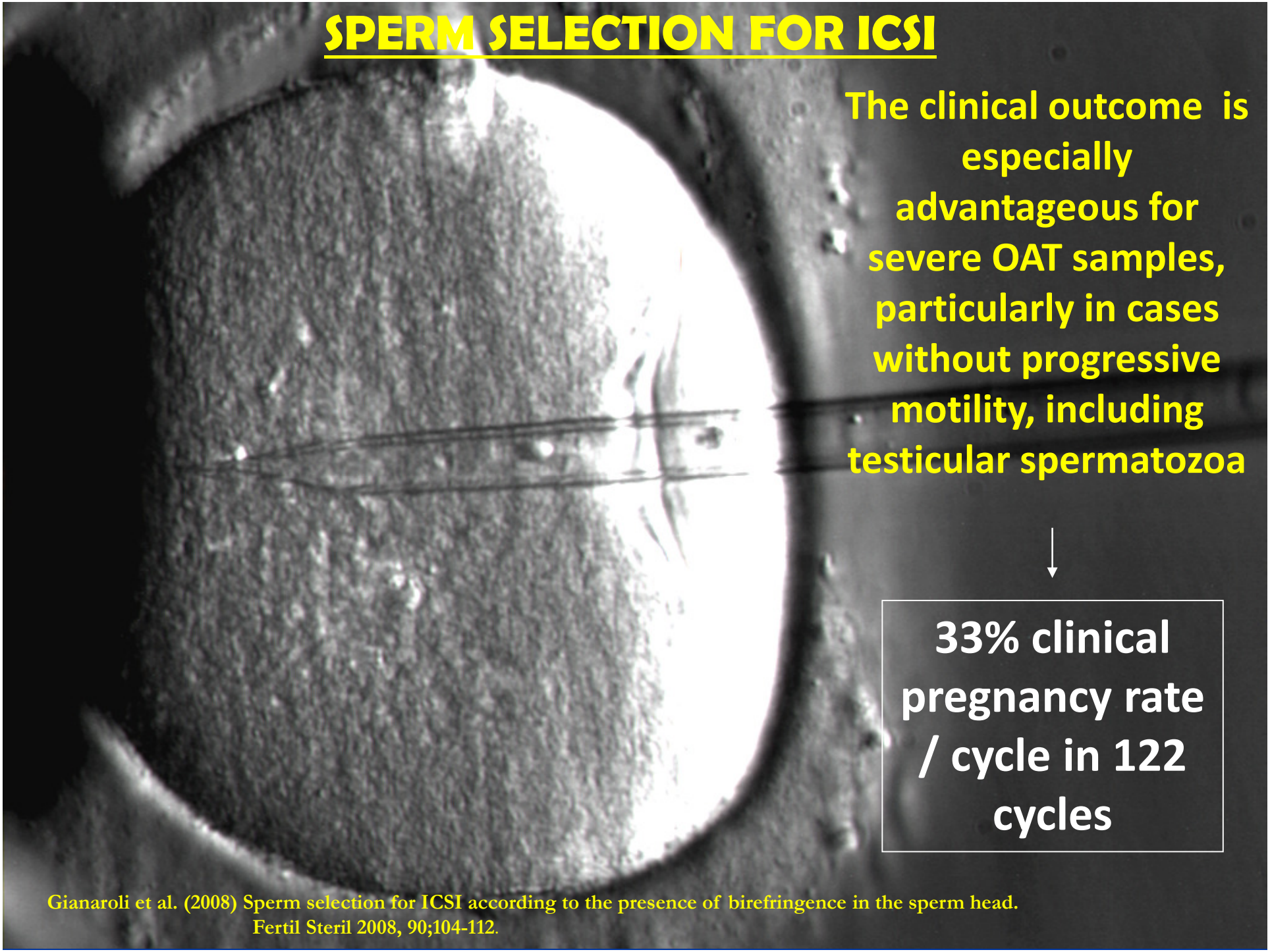
Higher Pregnancy rates
Higher Implantation Rates
Healthy Singleton Pregnancy

Currently, there is no amazing or magic scientific and technological advance to give everybody a healty child

ART's is real art which needs scientific approach which coupled with the experience,talent of the team and the proper use of the technologies avaible in the individualized manner !

THANK YOU

SPERM SELECTION FOR ICSI



The clinical outcome is especially advantageous for severe OAT samples, particularly in cases without progressive motility, including testicular spermatozoa

↓

33% clinical pregnancy rate / cycle in 122 cycles

Gianaroli et al. (2008) Sperm selection for ICSI according to the presence of birefringence in the sperm head. Fertil Steril 2008, 90;104-112.

IMSI: Clinical results

First attempt

	PROSPECTIVE RANDOMIZED STUDY	
	STUDY GROUP IMSI N=227	CONTROL GROUP ICSI N=219
Meanage(years)	31.65 ± 3.23	31.91 ± 3.3
Number of injected oocytes	2.90 ± 0.29	2.92 ± 0.26
Numberof 2PN oocytes	2.75 ± 0.45	2.76 ± 0.42
Number of embryos transferred	2.47 ± 0.68	2.37 ± 0.67
Implantation rate	17.3 *	11.3
Clinical pregnancy rate	39.2 *	26.5
Abortion rate per pregnancy	16.9	24.1

*significantly different (P ≤ 0.05)

Antinori et al., 2008

TABLE 1

Sperm parameters before and after density-gradient centrifugation and swim-up (mean ± standard error of the mean).

Parameter	Whole semen	Density gradient	Swim-up	*P* value
Concentration (million/mL)	76.36 ± 5.68	39.58 ± 2.47	20.38 ± 1.43	< .0001
Motility (%)	58.09 ± 2.16	90.89 ± 0.41	95.96 ± 0.38	< .0001
Magnification grade				
I (%)	2.76 ± 0.32	3.25 ± 0.36	4.58 ± 0.47	< .0001
II (%)	30.23 ± 1.64	34.91 ± 1.24	47.96 ± 1.48	< .0001
III (%)	52.92 ± 1.61	52.24 ± 1.13	39.7 ± 1.59	< .0001
IV (%)	14.09 ± 1.07	9.61 ± 0.8	7.75 ± 0.59	.00069
I + II (%)	32.99 ± 1.83	38.16 ± 1.43	52.55 ± 1.72	< .0001

Notes: The analysis was performed using the Wilcoxon matched paired test. The *P* values represent the statistically significance difference between the two processing methods.

Monqaut. Techniques and instrumentation. Fertil Steril 2010.

European Journal of Obstetrics & Gynecology and Reproductive Biology 150 (2010) 42–46

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

Comparison of day 2 embryo quality after conventional ICSI versus intracytoplasmic morphologically selected sperm injection (IMSI) using sibling oocytes

Ana L. Mauri [a], Claudia G. Petersen [a,b], Joao Batista A. Oliveira [a,b], Fabiana C. Massaro [a], Ricardo L.R. Baruffi [a], Jose G. Franco Jr [a,b,c,*]

[a] Centre for Human Reproduction Prof. Franco Jr., Ribeirão Preto, Sao Paulo, Brazil
[b] Department of Gynaecology and Obstetrics, Botucatu Medical School, Sao Paulo State University, UNESP, Brazil
[c] Paulista Centre for Diagnosis, Research and Training, Ribeirão Preto, Sao Paulo, Brazil

Objective: To evaluate whether intracytoplasmic morphologically selected sperm injection (IMSI) could influence early paternal effects by observing embryo quality at day 2.

Study design: The study included 30 couples with at least one of the following criteria: male factor infertility, at least 2 previous failures of implantation or previous miscarriages after IVF/ICSI. Sibling oocytes of each patient were randomly assigned to either the ICSI group or the IMSI group. For IMSI, spermatozoa were selected at 8400× magnification through an inverted microscope equipped with Nomarski differential interference contrast optics, Uplan Apo 100× oil/1.35 objective lens and variable zoom lens. For conventional ICSI, spermatozoa were selected at 400× magnification. An embryo was defined as top quality if there were four identical blastomeres on day 2 with no fragments or multinucleation of blastomeres. Data were analysed using the Wilcoxon and chi-squared tests. The significance level was set at $P < 0.05$. The variables were analysed in relation to the general population and the subpopulations with or without male factor.

Results: A total of 331 MII oocytes (30 oocyte retrievals) were selected and injected by the ICSI (n: 172) or IMSI (n: 159) procedure. For IMSI, only spermatozoa classified as morphologically normal at high magnification were used. No differences ($P > 0.05$) in fertilisation rate (ICSI: 70.9%; IMSI: 70.4%), early embryo cleavage rate (ICSI: 66.9%; IMSI: 60.4%) or cleavage rate (ICSI: 99.2%; IMSI: 99.1%) were observed. On day 2, as compared to ICSI, IMSI provided a similar proportion of top quality embryos (ICSI: 57.8%; IMSI: 52.2%; $P > 0.05$). These results were not influenced by the presence or absence of male factor.

Conclusion: In terms of embryo quality at day 2, IMSI had the same performance as conventional ICSI. However, we cannot exclude the possibility that IMSI effects occur only as a positive later paternal effect.

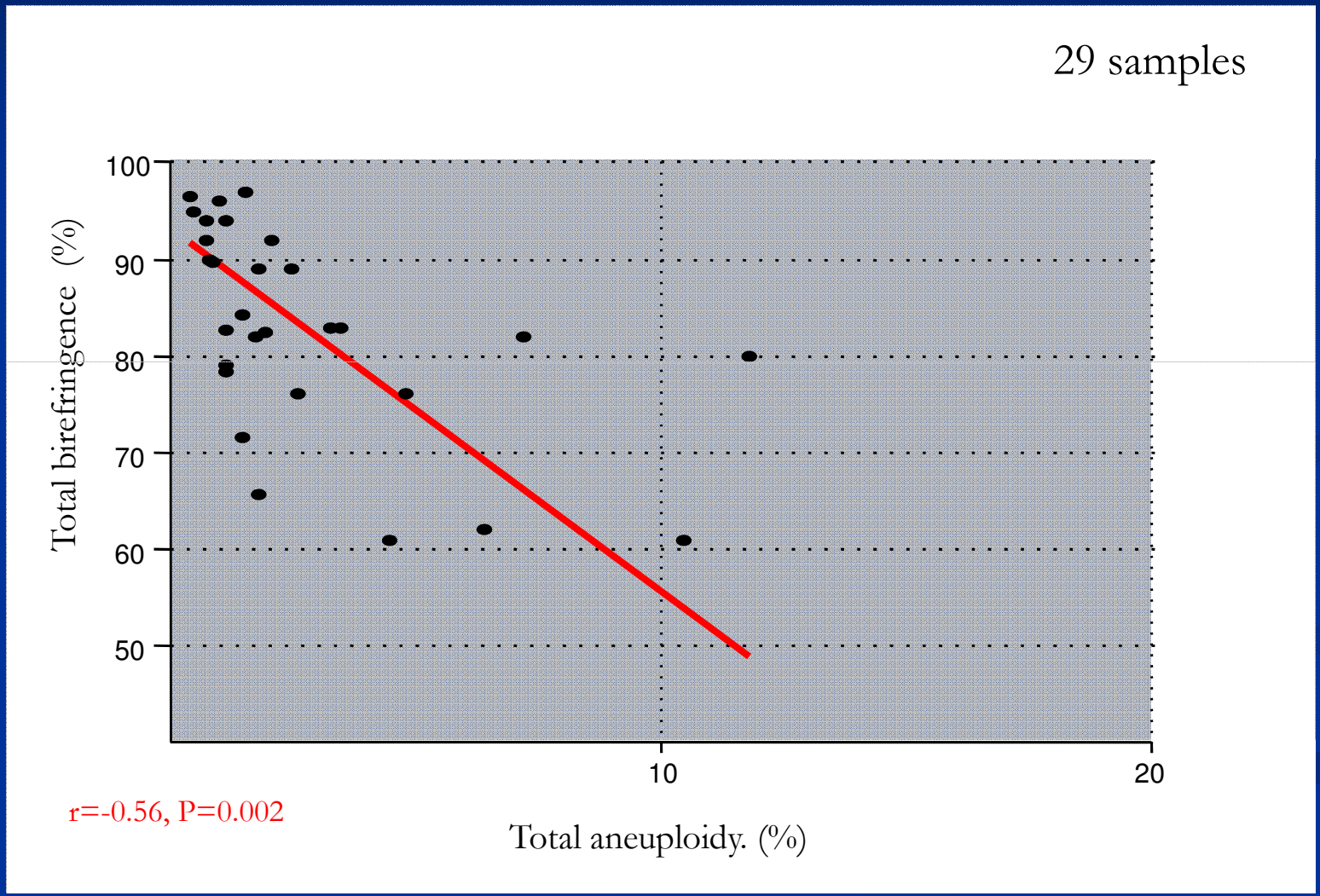
Table 2

Comparison between the morphologically selected sperm injection (IMSI) and conventional intracytoplasmic sperm injection (ICSI) study groups.

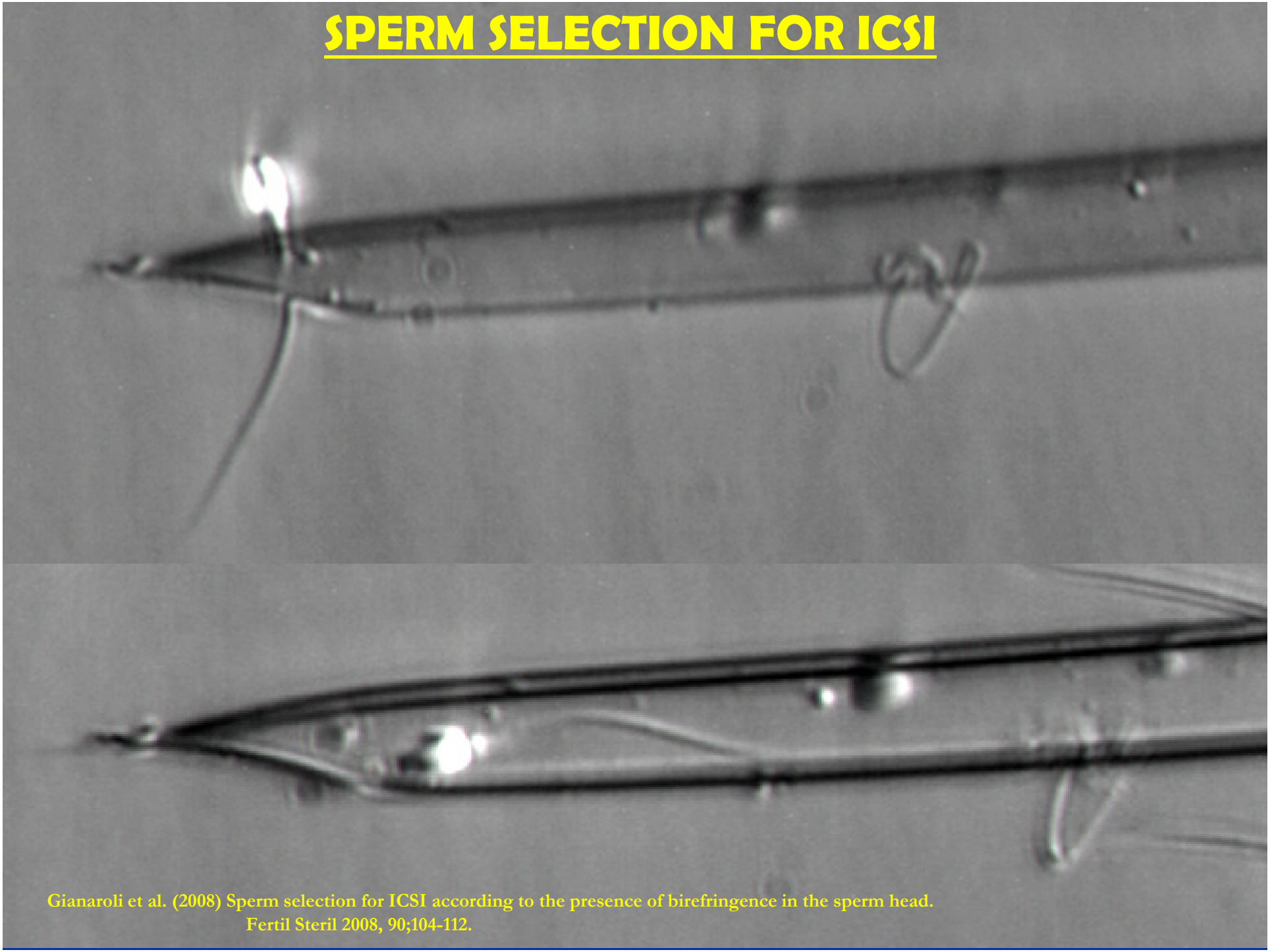
	Total			With male factor infertility			Without male factor infertility		
	IMSI (*n*: 159 oocytes)	ICSI (*n*: 172 oocytes)	*P*	IMSI (*n*: 74 oocytes)	ICSI (*n*: 81 oocytes)	*P*	IMSI (*n*: 85 oocytes)	ICSI (*n*: 91 oocytes)	*P*
Retrieved oocytes (*n*)	6.1 ± 2.3	6.4 ± 2.4	ns	6.0 ± 2.1	6.5 ± 2.5	ns	6.2 ± 2.4	6.4 ± 2.4	ns
Oocytes MII (*n*)	5.3 ± 1.8	5.7 ± 2.3	ns	5.3 ± 2.0	5.8 ± 2.2	ns	5.3 ± 1.6	5.7 ± 2.3	ns
Fertilisation rate	70.4%	70.9%	ns	70.3%	66.7%	ns	70.6%	74.7%	ns
Early cleavage rate	60.4%	66.9%	ns	61.5%	79.6%	ns	56.7%	59.3%	ns
Cleavage rate	99.1%	99.2%	ns	100%	100%	ns	98.3%	98.5%	ns
Top quality day 2 embryos	52.2%	57.8%	ns	50%	57.4%	ns	54.2%	58.2%	ns

ns: not significant ($P > 0.05$).

CORRELATION BETWEEN INCIDENCE OF ANEUPLOIDY AND PRESENCE OF HEAD BIREFRINGENCE



SPERM SELECTION FOR ICSI



Gianaroli et al. (2008) Sperm selection for ICSI according to the presence of birefringence in the sperm head. Fertil Steril 2008, 90;104-112.

Nicopoullous et al, F&S 2004

- Comparing fresh with frozen-thawed epididymal sperm there was no difference in FR or IR, a significantly higher CPR (RR 1.20; 95% CI: 1.0-1.42), and no difference in OPR. No difference in fertilization or pregnancy outcome was noted when the testicular cycles were analyzed separately, but IR was significantly impaired using frozen-thawed sperm (RR 1.75; 95% CI: 1.10-2.80)

Table 3
The cycle characteristics of patients who underwent ICSI with ejaculated and testicular spermatozoa[a]

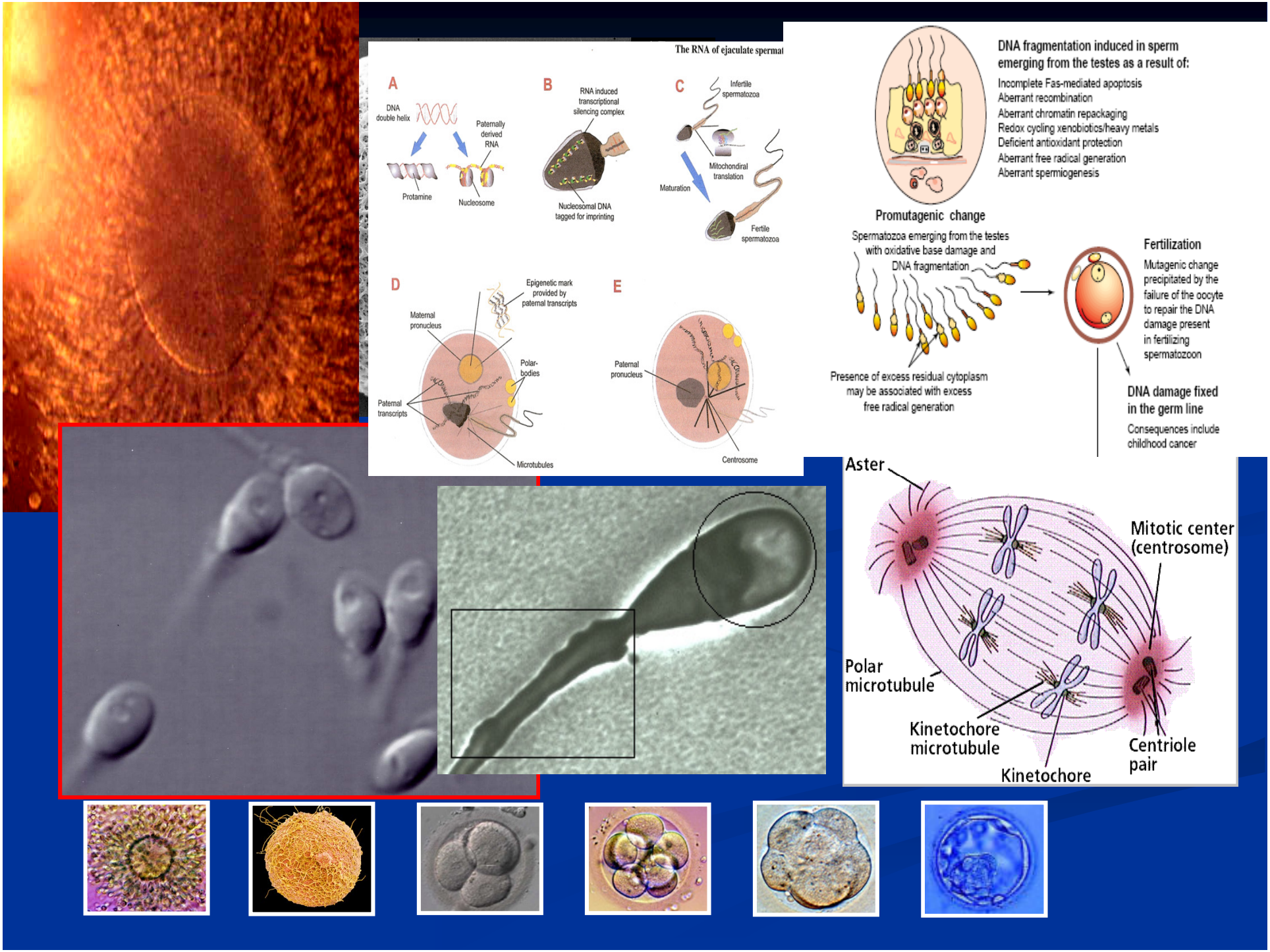
Parameter	Ejaculate sperm ($n=780$)	Obstructive azoospermia ($n=43$)	Non-obstructive azoospermia ($n=53$)	Severe OAT[b] ($n=14$)	P value
Duration of ovarian stimulation (days)	9.9±2.1	10.5±1.9	9.8±1.8	9.6±1.8	NS
Total vials of FSH used	35.7±14.7	36.4±12.7	35.1±10.3	36.6±12.6	NS
E_2 level on day of hCG administration (pmol/l)	5892±4232.5	6648.0±3784.3	5963.2±3148.7	7697.4±2824.4	NS
No. of oocytes retrieved	9.3±5.3	10.5±5.3	9.0±5.5	9.9±3.9	NS
No. of metaphase II oocytes	7.7±4.5	8.9±4.9	7.6±4.0	8.6±3.9	NS
2 pronuclei fertilization rate per injected oocyte (%)	66.5±23.7	59.9±23.1	66.4±21.7	50.8±21.7	NS
Cleavage rate per fertilized oocyte (%)	66.3±24.6	60.1±26.7	68.2±21.0	59.1±22.0	NS
No. of 2-pronuclei oocytes	5.0±3.4	5.5±3.7	4.9±2.9	4.3±3.1	NS
No. of transferable embryos obtained	5.4±4.5	5.8±3.8	5.1±2.5	5.2±3.3	NS
No. of embryos with 0% fragmentation	3.2±2.6	2.9±1.9	3.3±2.2	3.8±2.3	NS
No. of embryos transferred	3.6±1.5	3.7±1.4	4.0±1.4	4.1±1.5	NS
No. of grade 1 embryos transferred	2.5±1.7	2.2±1.6	2.2±1.5	3.1±1.6	NS

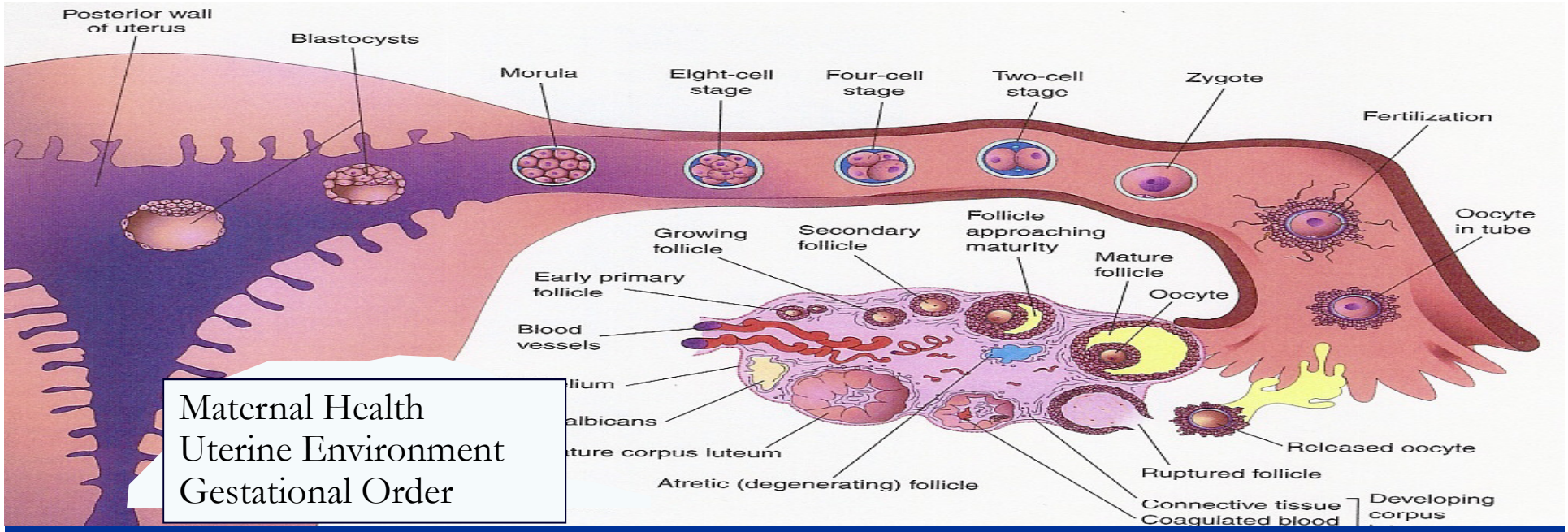
[a] Note: Values are mean±standard deviation.
[b] OAT = Oligoasthenoteratozoospermia.
NS = not significant.

Table 4
The treatment results of ICSI cycles performed with ejaculate and testicular spermatozoa

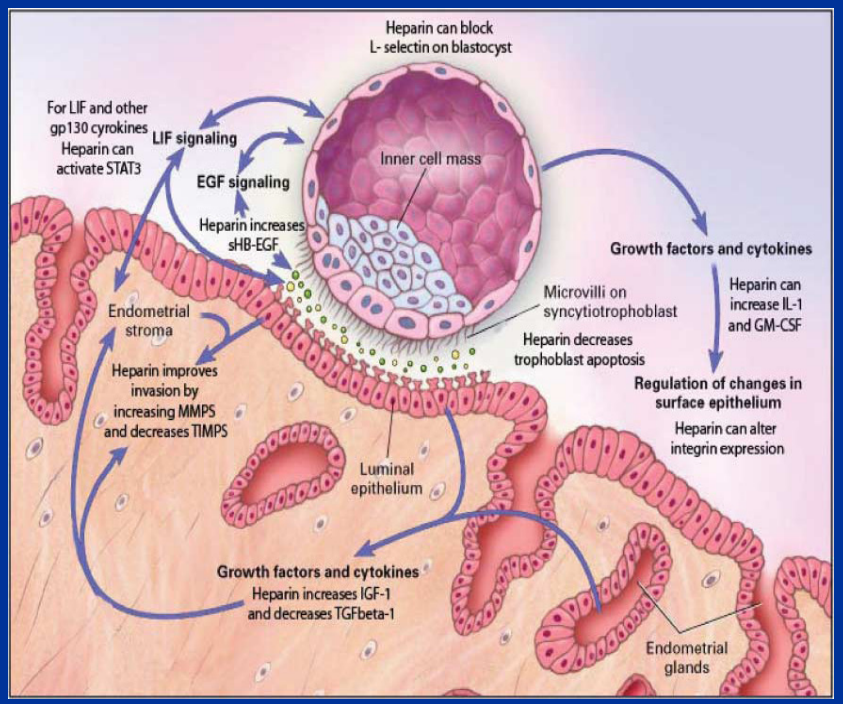
Parameter	Ejaculated spermatozoa ($n=780$)	Obstructive azoospermia ($n=43$)	Non-obstructive azoospermia ($n=53$)	Severe OAT[a] ($n=14$)	P value
Individual implantation rate (%) (mean±S.D.)	25.1±25.5	17.4±25.1	19.7±20.5	36.7±15.3	NS
Implantation rate (%)	367/2802 (13.1)	21/160 (13.1)	27/210 (12.9)	5/58 (8.6)	NS
Clinical pregnancy per embryo transfer (%)	212/780 (27.2)	12/43 (27.9)	18/53 (34.0)	3/14 (21.4)	NS

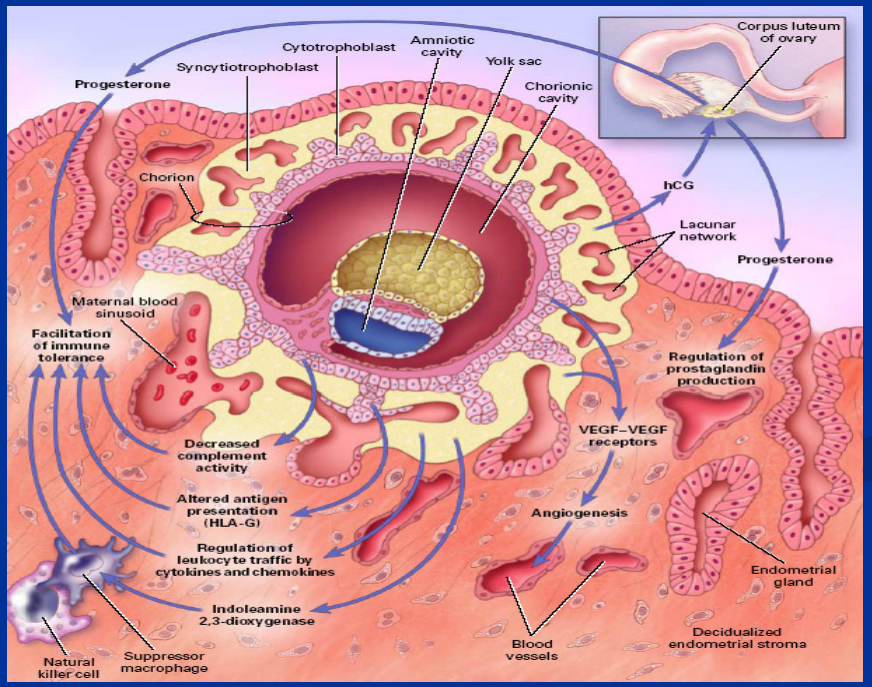
[a] OAT = Oligoasthenoteratozoospermia.


The RNA of ejaculate spermat
A
DNA double helix
Paternally derived RNA
Protamine
Nucleosome
B
RNA induced transcriptional silencing complex
Nucleosomal DNA tagged for imprinting
C
Infertile spermatozoa
Mitochondiral translation
Maturation
Fertile spermatozoa
D
Epigenetic mark provided by paternal transcripts
Maternal pronucleus
Polar-bodies
Paternal transcripts
Microtubules
E
Paternal pronucleus
Centrosome
DNA fragmentation induced in sperm emerging from the testes as a result of:
Incomplete Fas-mediated apoptosis
Aberrant recombination
Aberrant chromatin repackaging
Redox cycling xenobiotics/heavy metals
Deficient antioxidant protection
Aberrant free radical generation
Aberrant spermiogenesis
Promutagenic change
Spermatozoa emerging from the testes with oxidative base damage and DNA fragmentation
Presence of excess residual cytoplasm may be associated with excess free radical generation
Fertilization
Mutagenic change precipitated by the failure of the oocyte to repair the DNA damage present in fertilizing spermatozoon
DNA damage fixed in the germ line
Consequences include childhood cancer
Aster
Mitotic center (centrosome)
Polar microtubule
Kinetochore microtubule
Kinetochore
Centriole pair



Maternal Health
Uterine Environment
Gestational Order





Moore and Persaud. The developing human, clinically oriented embryology. 1998

Journal of Andrology, Vol. 26, No. 5, September/October 2005

The Motility of Epididymal or Testicular Spermatozoa Does Not Directly Affect IVF/ICSI Pregnancy Outcomes

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Table 2. *Cycle stimulation and outcome data for motile and nonmotile sperm groups**

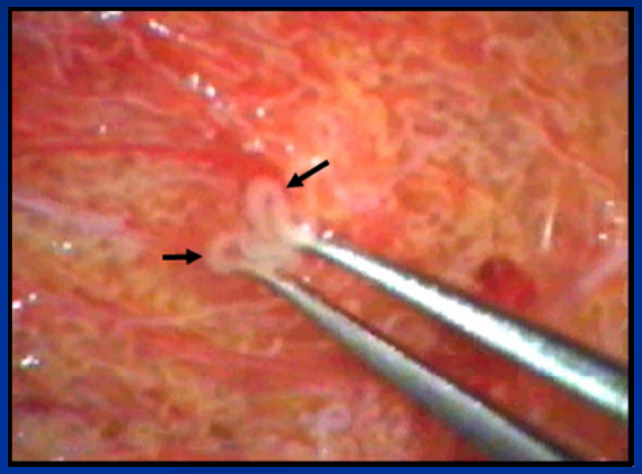
	Motile (n = 39 cycles)	Nonmotile (n = 61 cycles)
Basline serum FSH*	6.3 ± 1.7	5.5 ± 1.7
Total number of ampules used*	37.3 ± 10.3	33.7 ± 9.8
Total number of retrieved oocytes*	12.7 ± 7.1	16.2 ± 7.3†
Total number of mature oocytes*	10.7 ± 5.8	13.4 ± 6.0‡
Fertilization rate	56.7%	59.1%
Cryopreservation rate§	35.9%	39.3%
Number of cells at embryo transfer (72 h)*	6.26 ± 1.46	6.08 ± 1.48
Grade of transferred embryos*	2.01 ± 0.84	2.15 ± 0.73
Number of transferred embryos*	2.97 ± 0.87	3.20 ± 1.06
Implantation rate	51.3%	44.3%
Clinical pregnancy rate	38.5%	31.2%

* Data presented as mean ± SD. FSH indicates follicle-stimulating hormone.
† $P = .02$.
‡ $P = .03$.
§ Percentage of cycles in which at least 1 embryo was frozen.

CONCLUSION - II

- No differance in FR,IR and PR found between epididymal and testicular sperm in patients with similar etiology
- Sperm surgically retrieved from azoospermic men identified significantly impaired outcomes with frozen and thawed sperm

TESE/mic-TESE



- TESE 36% vs Mic-TESE (Schlegel) 68%

RESULTS

- Incidence of DNA fragmentation:
  - In Ejaculate: 23.6 ± 5.1%
  - In Testis: 4.8 ± 3.6%
- No difference in:
  - Fertilization
  - Embryo cleavage
  - Morphology
  - Number of embryos transfered
- Significant increase in pregnancy and implantation rates
  - 5.6% vs. 44.4%
  - 1.8% vs. 20.7%