Birefringence: a tool for sperm selection in ICSI cycles

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Infertile population

- Female factor (40%)
- Male factor (30%)
- Female and male factor (30%)

Male factor (low count, motility, morphology) is present in > 50%

- 70% : mild - moderate
- 30% : severe to extreme (TESE)

Infertile population undergoing ART

Male factor is present in > 50%

- 70% : mild - moderate OAT
- 20% : severe OAT
- 10% : sperms retrieved from the seminal tract duu to obstructive azoospermia (OA) or non-ostructive azoospermia (NOA)

Men with severe OAT and Azoospermia :
population at higher genetic risk compared to normospermic

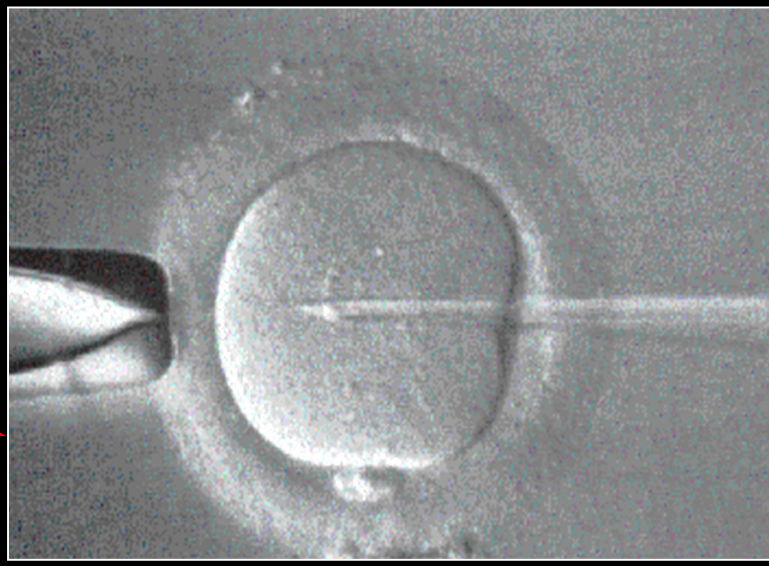
ICSI

ICSI

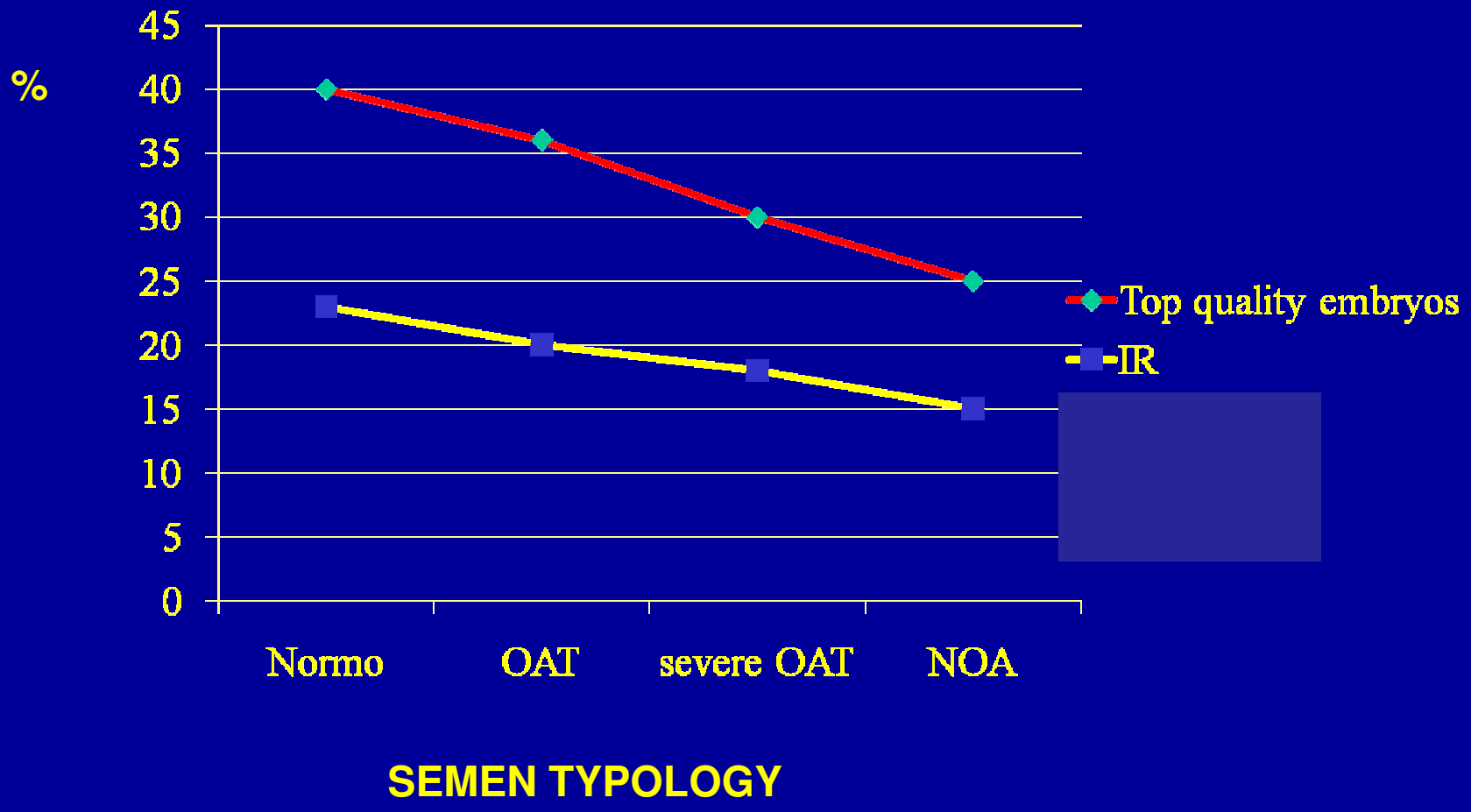
Fertilization rate
80%

No barrier to fertilization

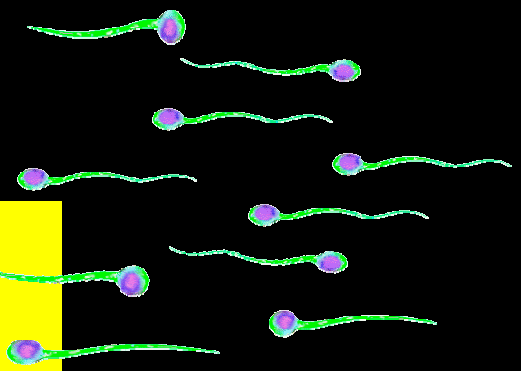
No selection against
DNA damaged sperm

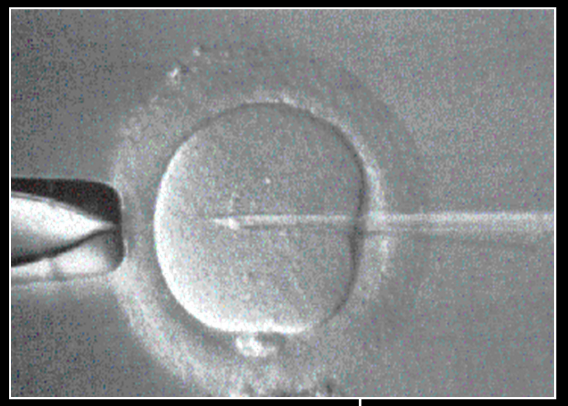


Top quality embryos and Implantation Rate (IR)



Increased risk of chromosomal anomalies





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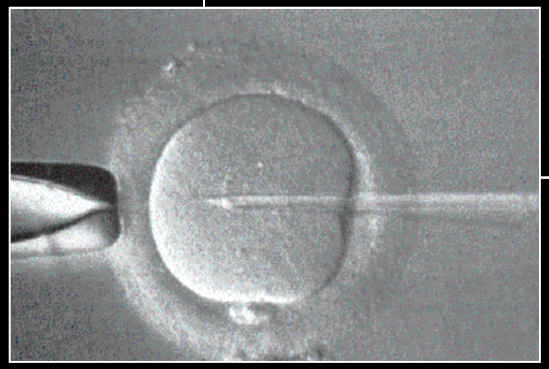
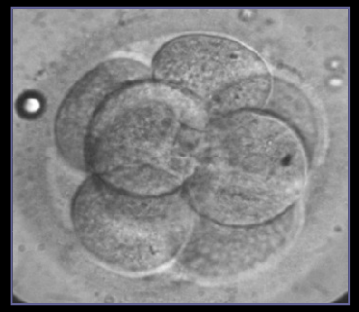
Sperm DNA damage can affect the reproductive process at different levels



Stop

Oocyte repairing



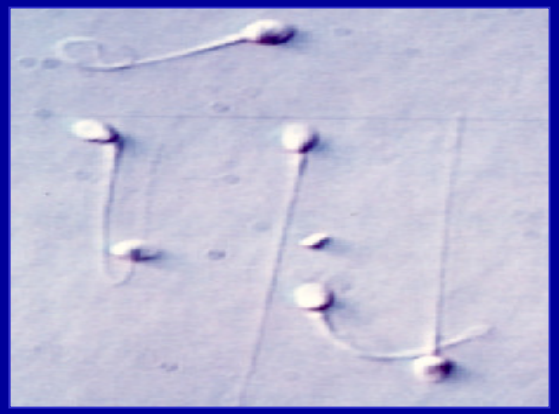
Law regulating ART procedures (Law 40) and integrative Guidelines (DM 21-7-2004) from March 2004 to April 2009

Limitations:

1) Embryo (and 2PN) cryopreservation is not permitted
2) The embryo production techniques – are not allowed to produce a number of embryos higher than the quantity strictly needed for one only simultaneous transfer, anyhow not more than three.
3) PGD-AS embryo's selection is banned
4) Embryos can be discarded only when showing clear signs of degeneration.

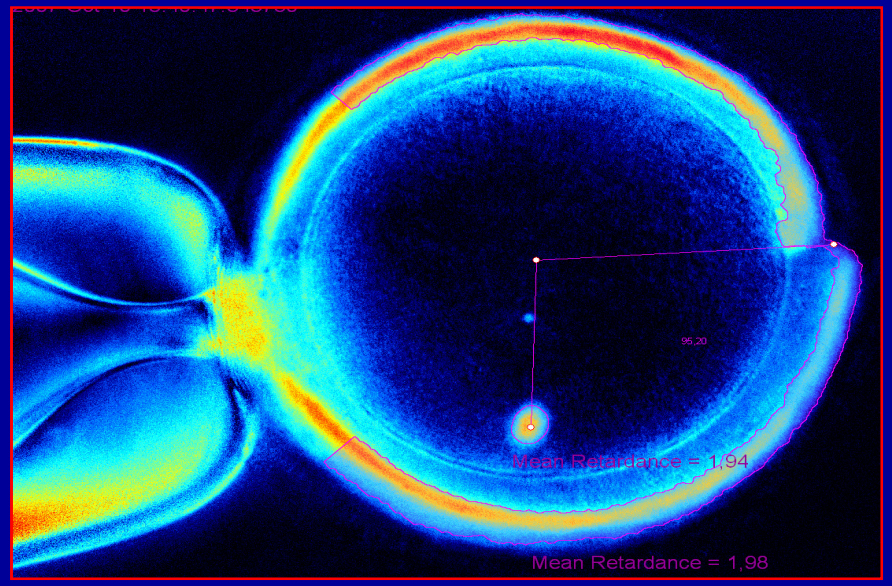
As a direct consequence, a maximum of 3 eggs had be used for insemination

Gametes selection became crucial

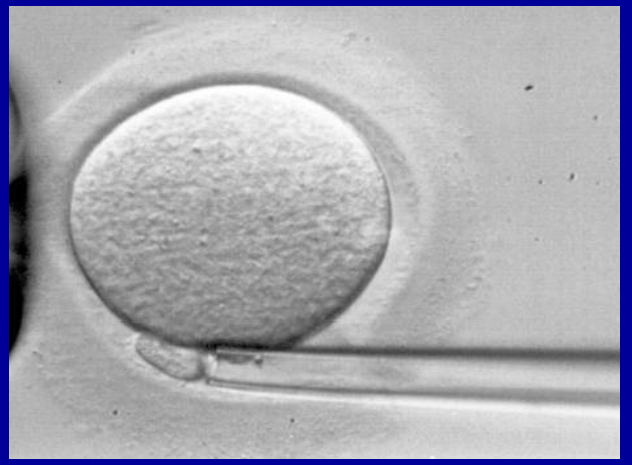


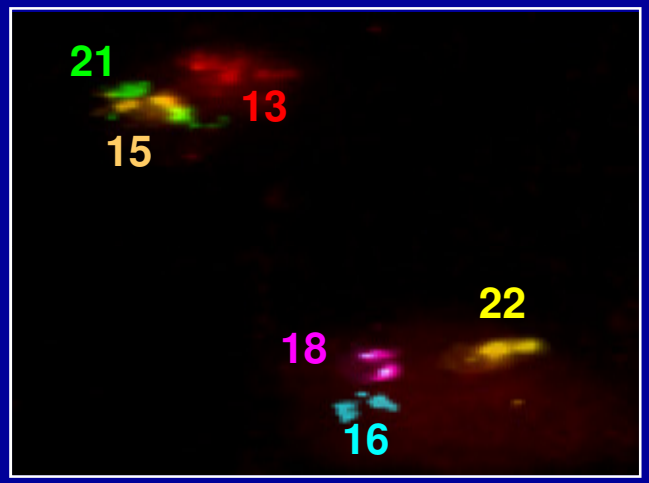
Oocyte selection before insemination

Polscope



First polar body FISH analysis





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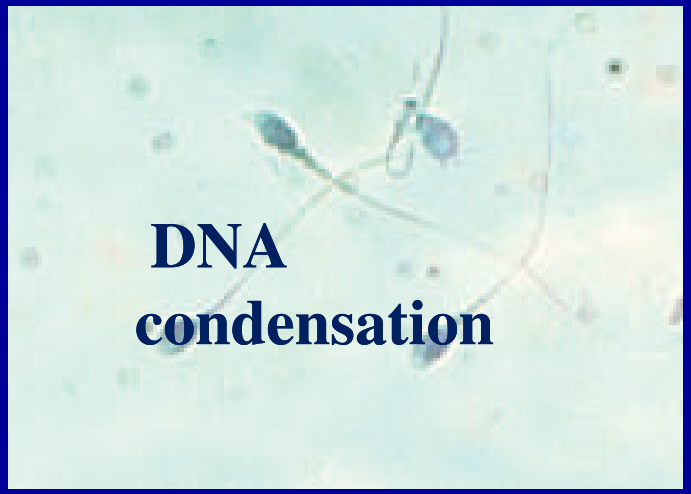
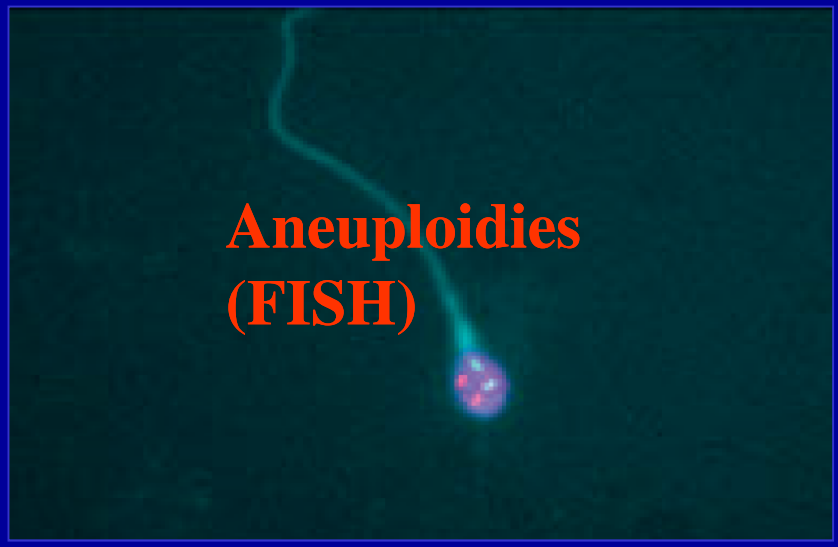
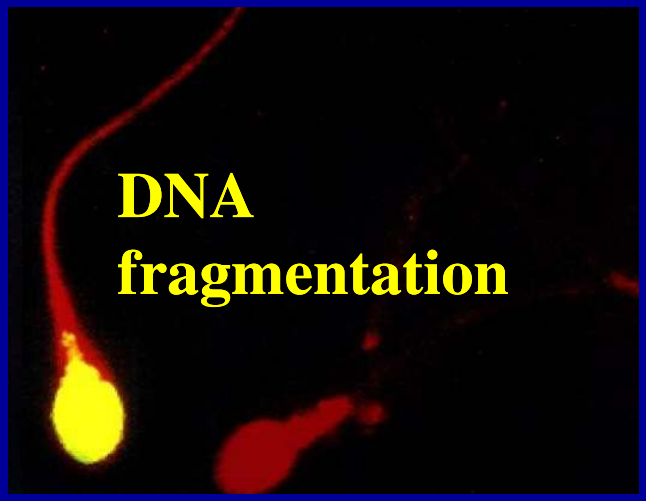
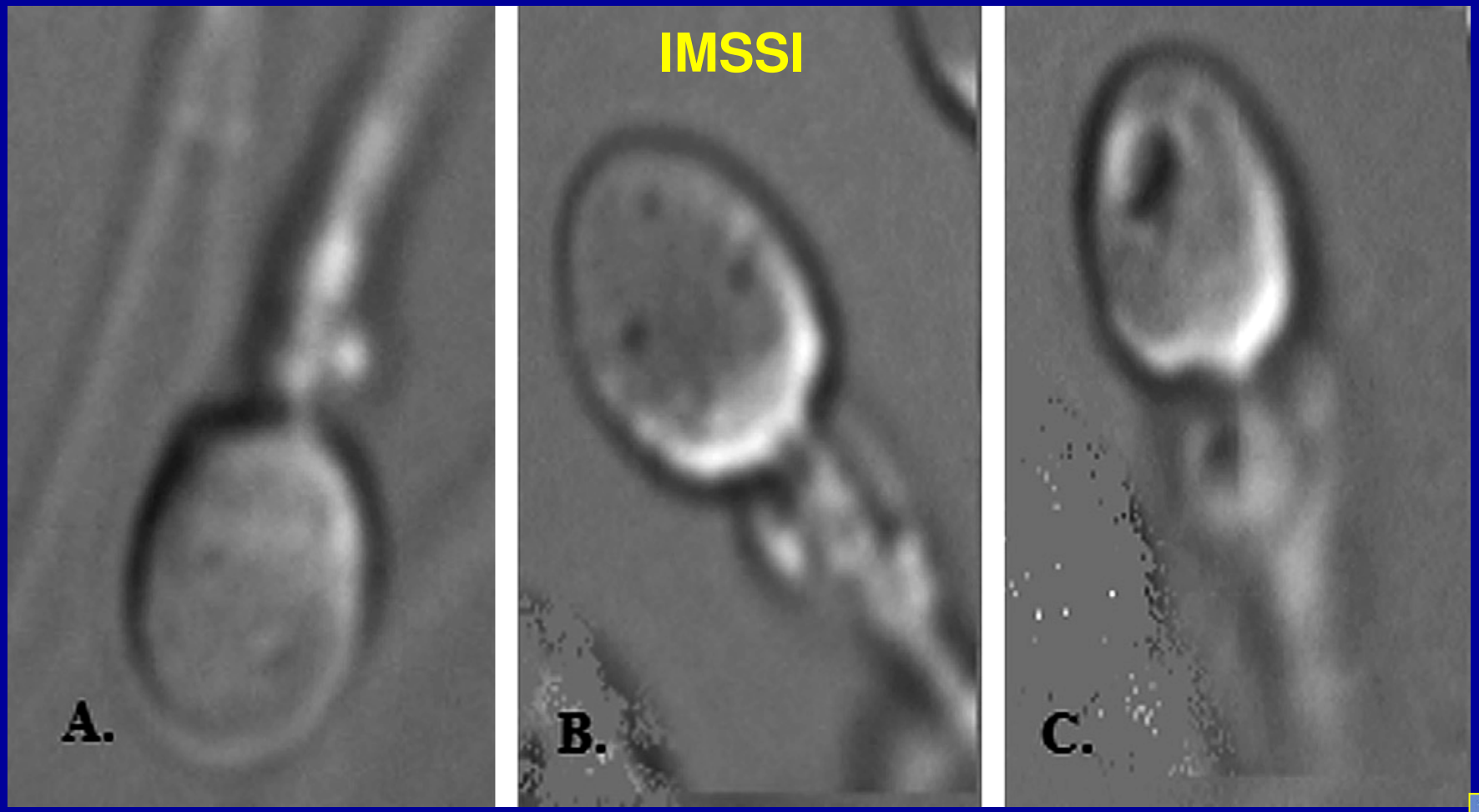
Semen evaluation before treatment







INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION



Peer et al. 2007 Fertil Steril 88, 1589-1594

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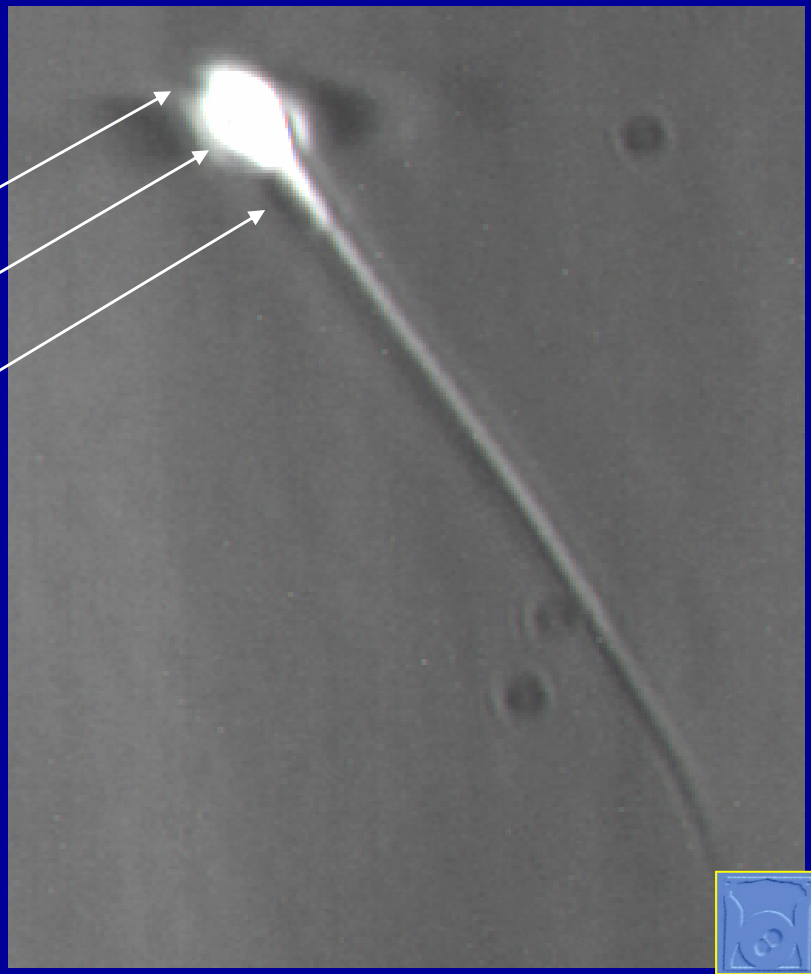
SPERMATOZOA
PROTOPLASMIC STRUCTURE

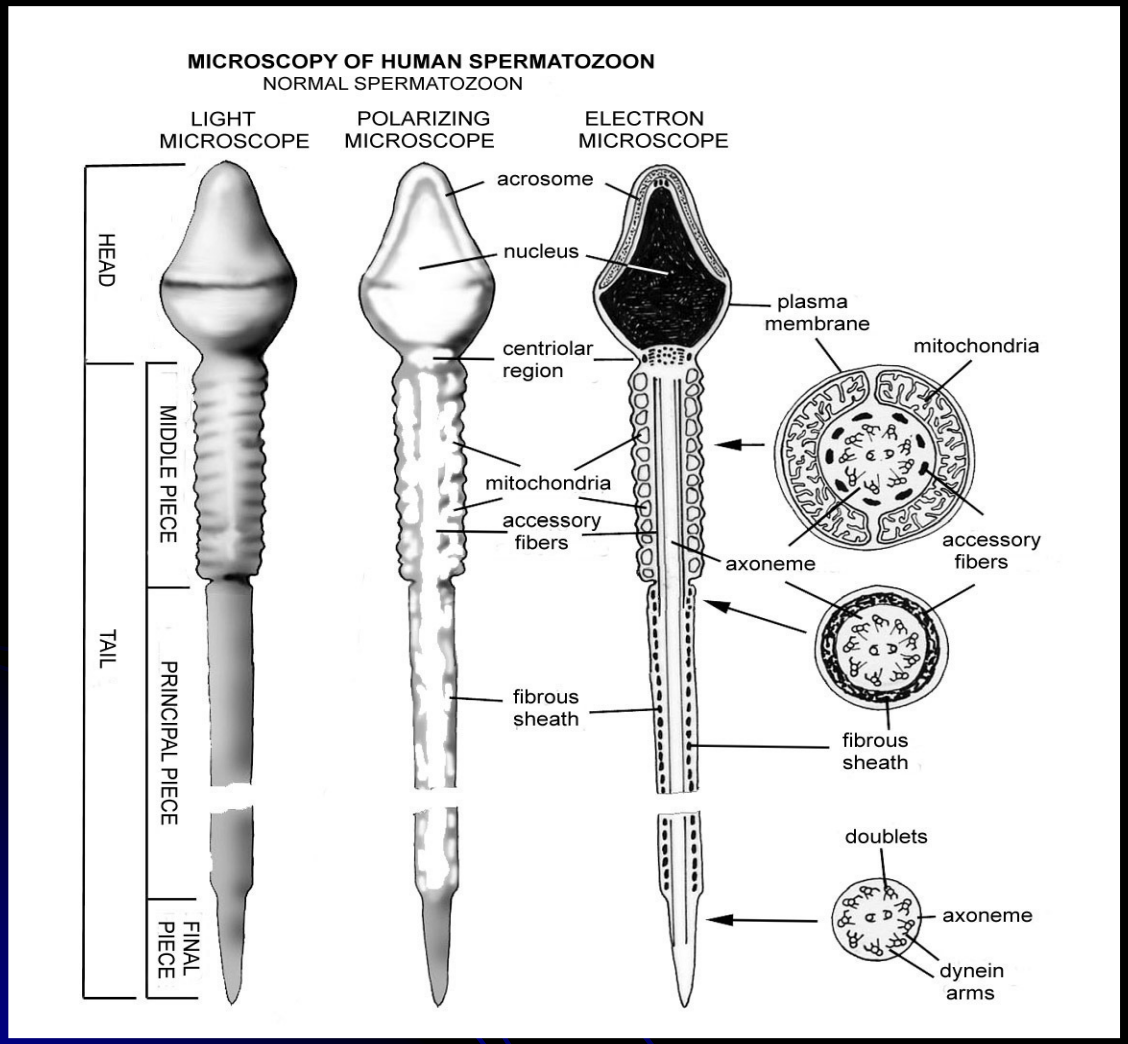
Human spermatozoa possess characteristics of birefringence due to the anisotropy of their protoplasmic texture.



protein subacrosomal filaments - longitudinally oriented

nucleoprotein filaments - arranged in rods and longitudinally oriented

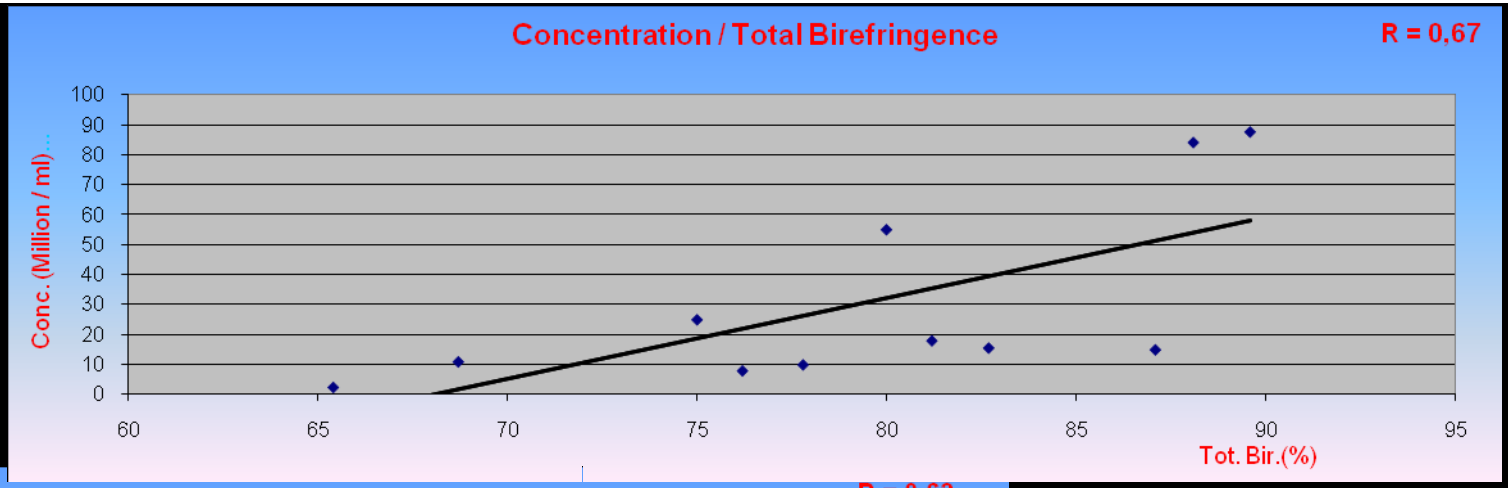
Birefringence

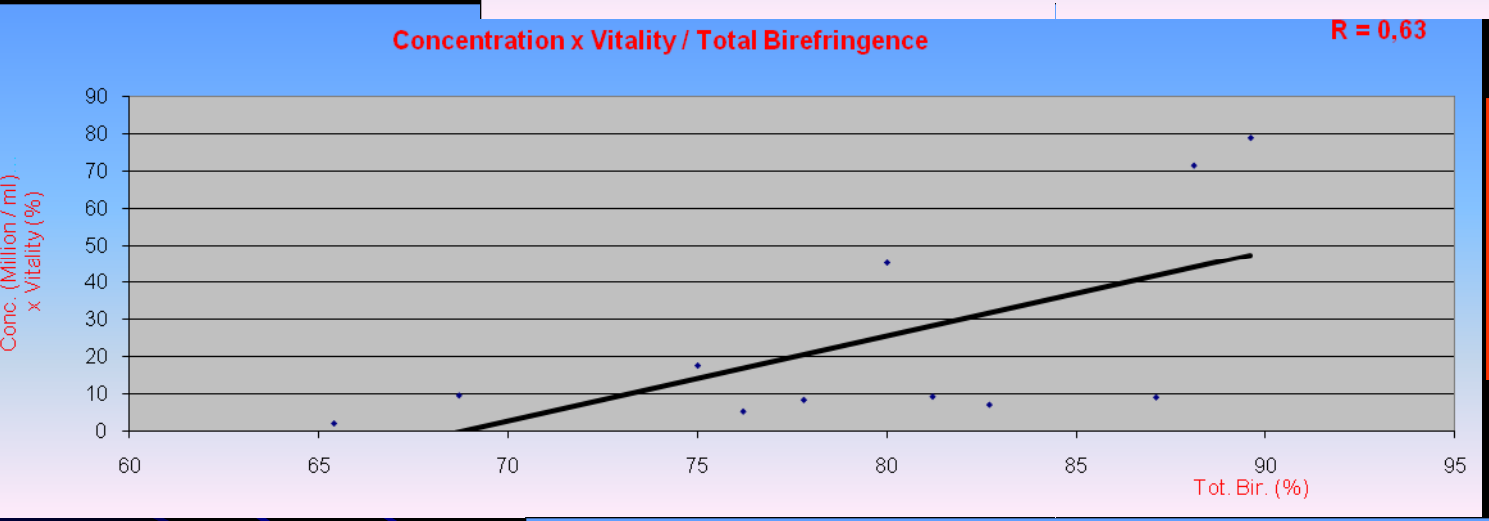


Information on the inner structures that is closer to TEM than that obtained by light microscope

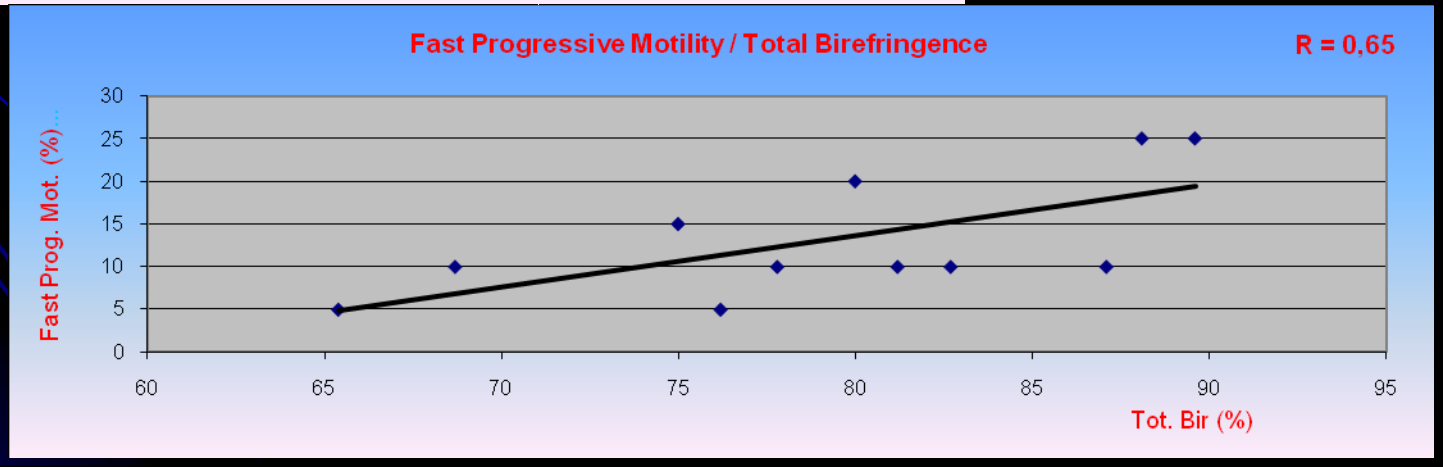
Presence of birefringence is the expression of an organized and very compact texture (nonpyknotic nuclei, normal acrosomes, motile tails)

" good health" of the sperm cell



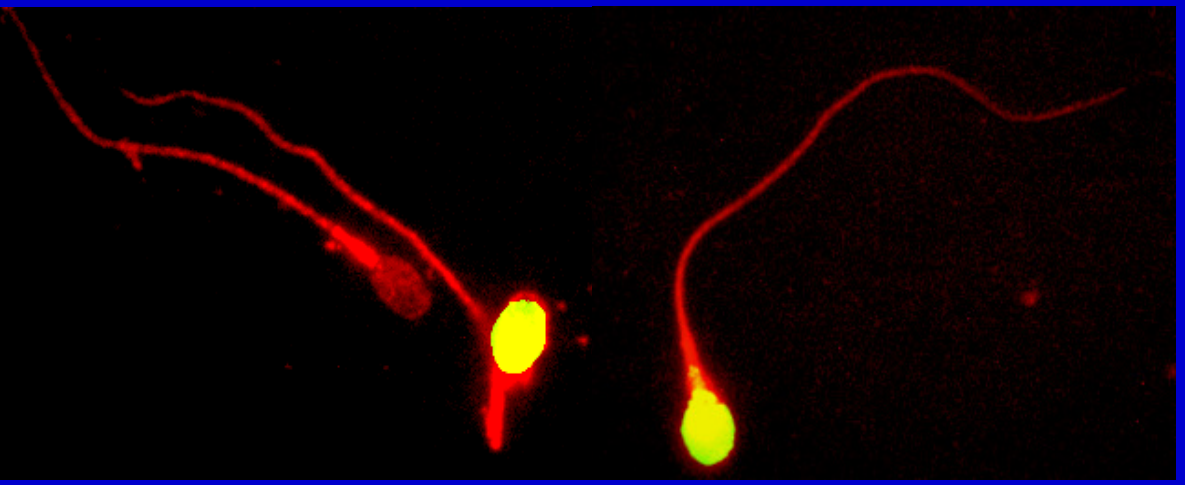


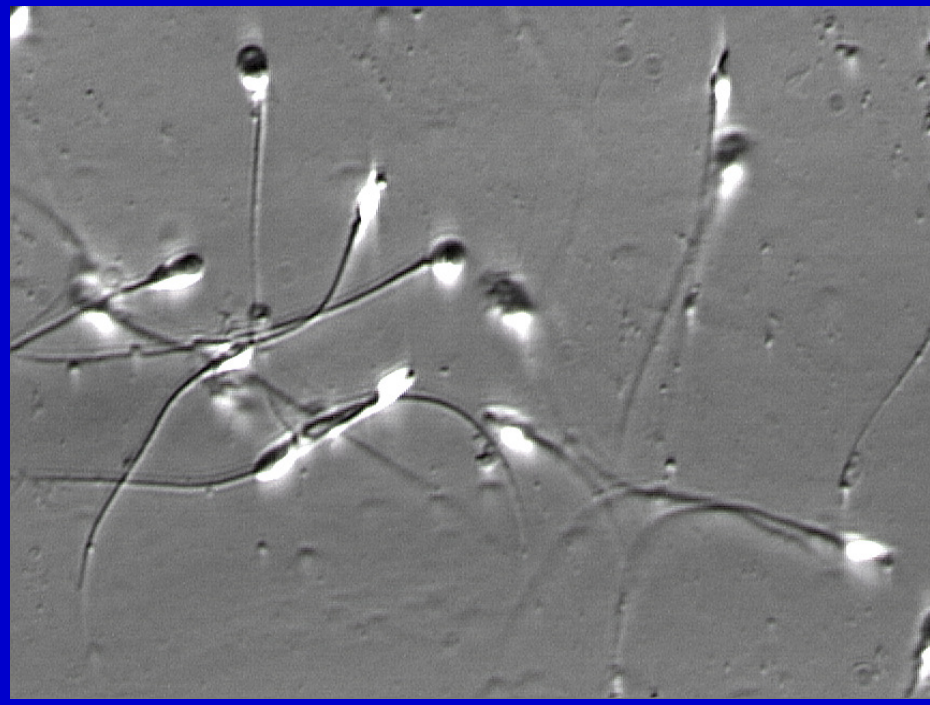
The proportion of birefringent spermatozoa was positively correlated with concentration, vitality and progressive motility



Aim of the study

To establish whether a correlation could be assessed between protoplasmic texture, as identified by **birefringence** properties, and **DNA fragmentation** of sperm cells, determined using the terminal deoxynucleotidyl transferase mediated dUTP-nick end labeling (TUNEL) assay

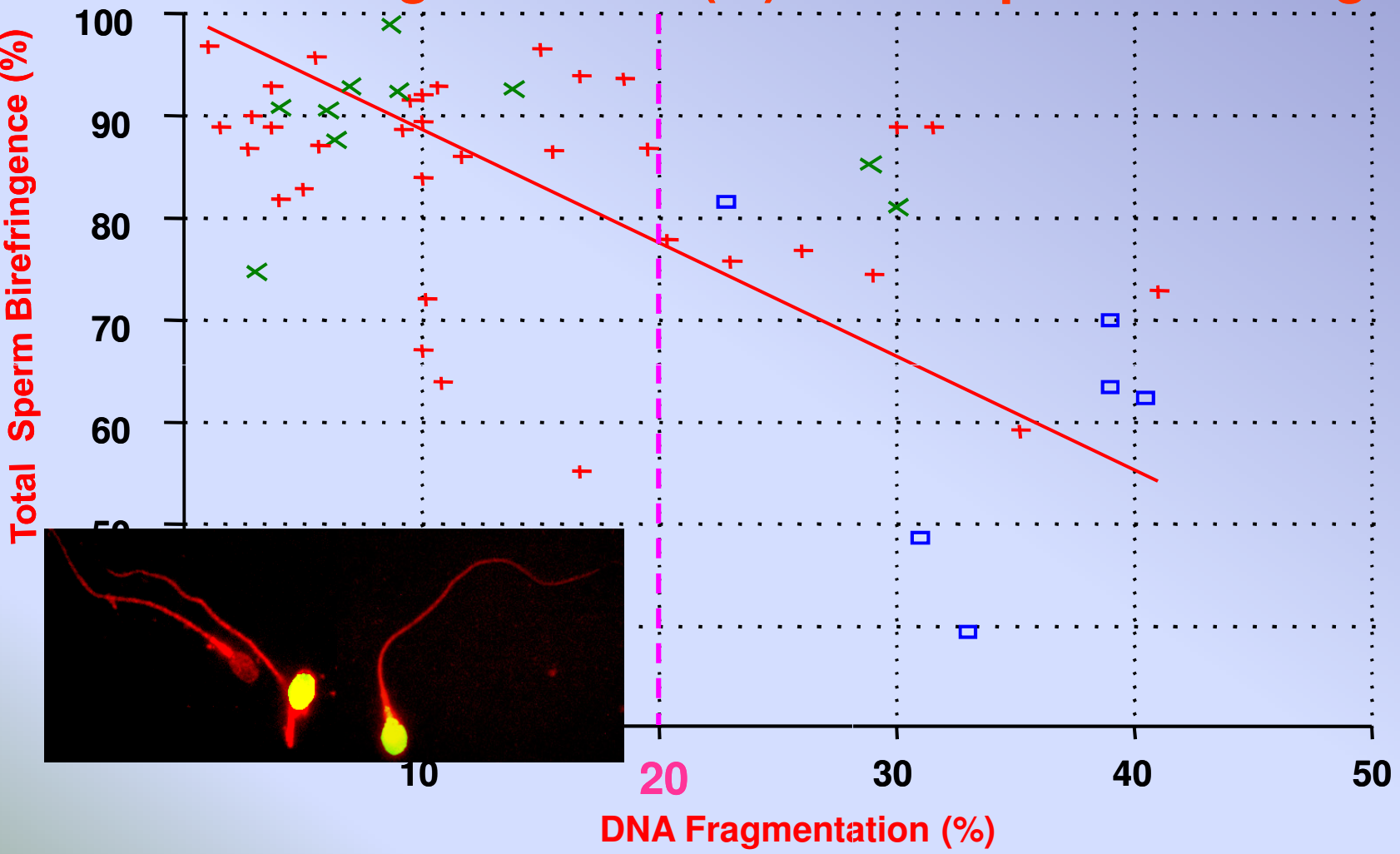




Results

Correlation between sperm head birefringence and DNA fragmentation

DNA Fragmentation (%) / Total Sperm Birefringence (%)



The proportion of total birefringent sperm head was inversely correlated with the incidence of fragmented DNA.

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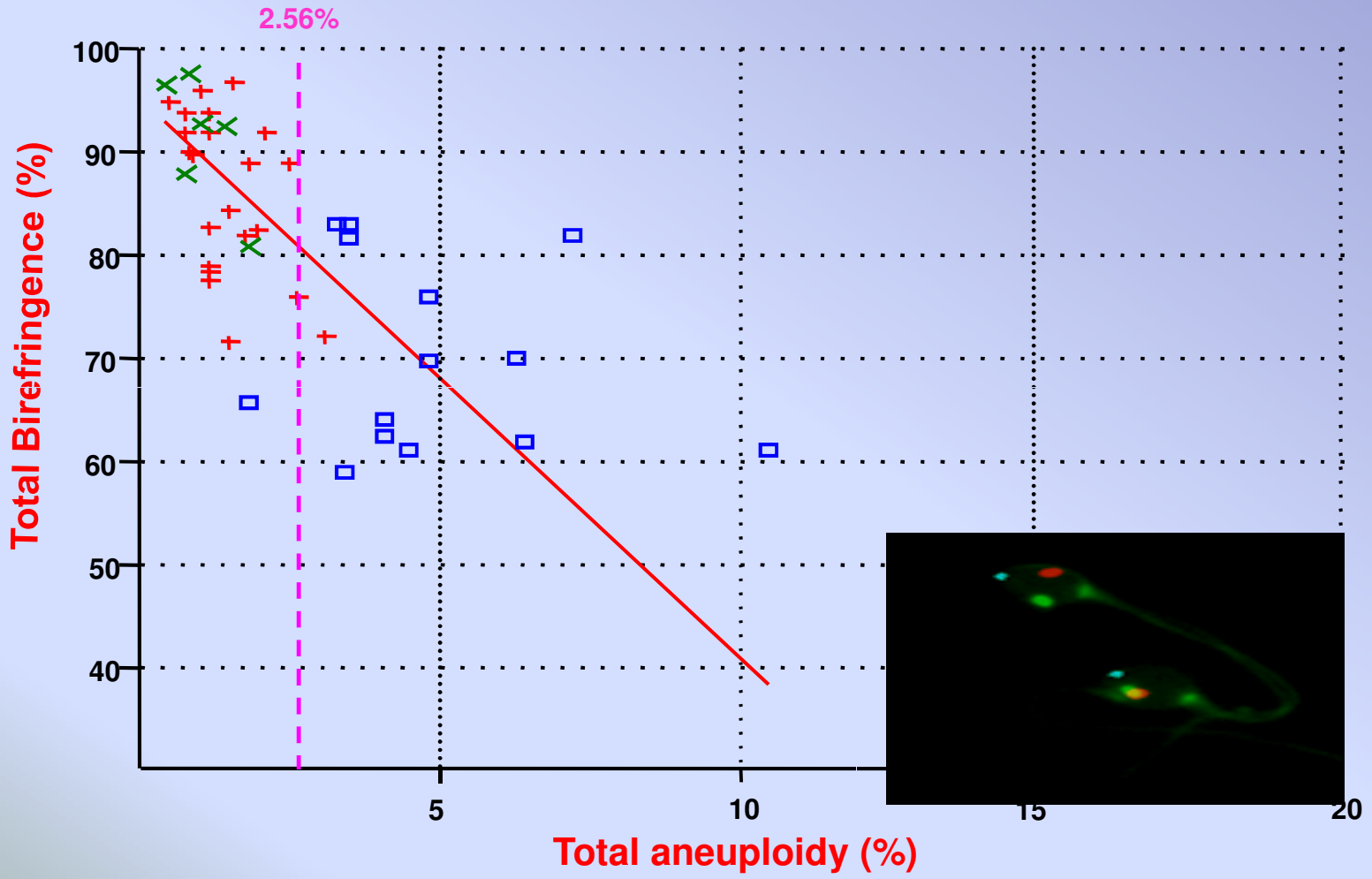
Results

Correlation between sperm head birefringence and aneuploidy

Total aneuploidy (%) / Total Birefringence (%)

R= - 0.68



The proportion of birefringent-head motile spermatozoa was inversely correlated with the frequency of total aneuploidy (P= $9.59E^{-7}$).

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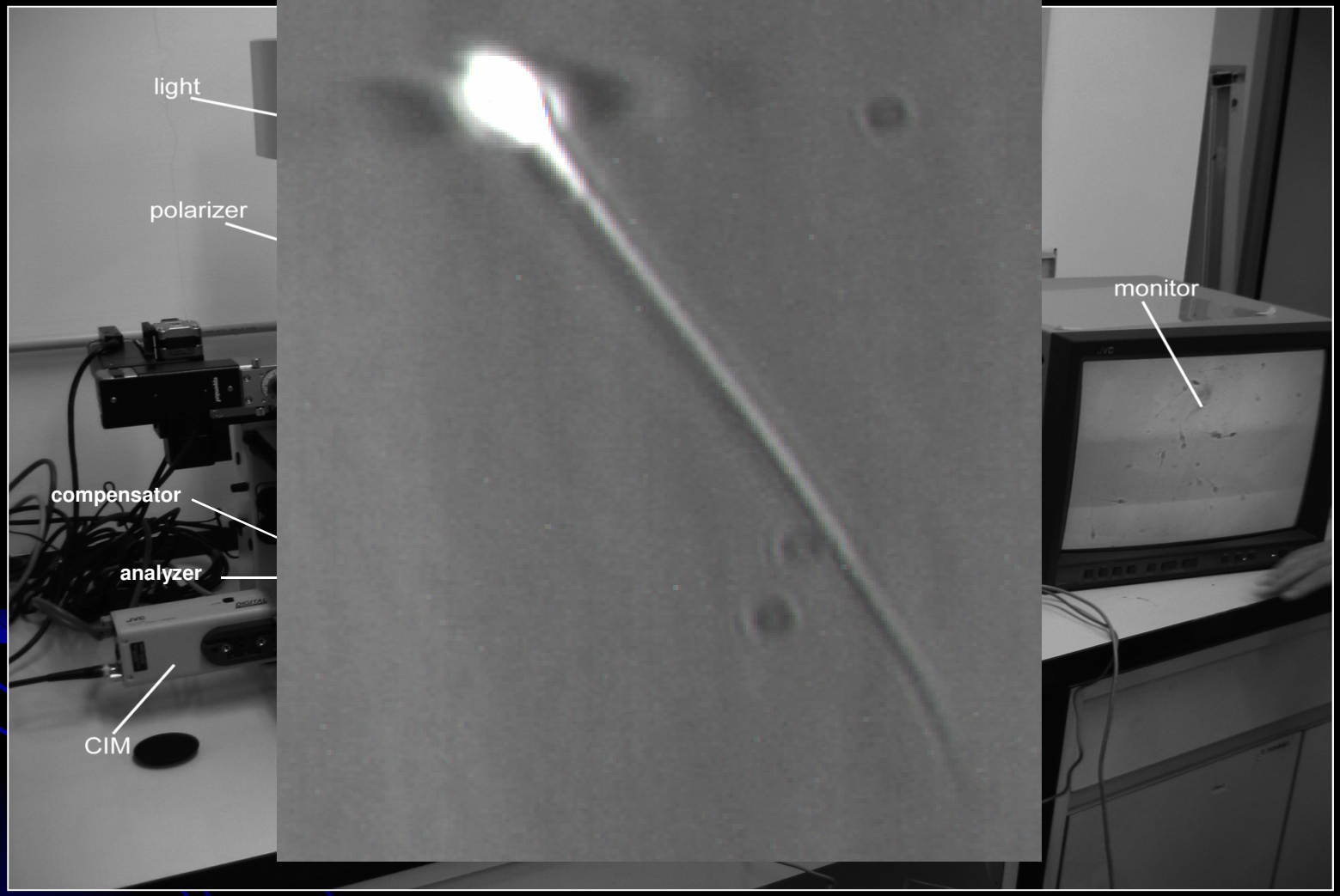
Polarization microscopy



B.Baccetti – Microscopical advances in assisted reproduction - J. Submicrosc. Cytol.Pathol., 36 (3-4), 333-339, 2004.

birefringent (a)

and no birefringent (b).

b

“Diagnosis” of birefringent and not birefringent spermatozoa without affecting their vitality

Prospective controlled trial

Study Group
ICSI by birefrigence

112 cycles
- 9 Normospermic
- 89 OAT
- 14 TESE

85 Fresh Cycles
27 Thawed Oocytes

Control Group
Conventional ICSI

119 cycles
- 9 Normospermic
- 97 OAT
- 13 TESE

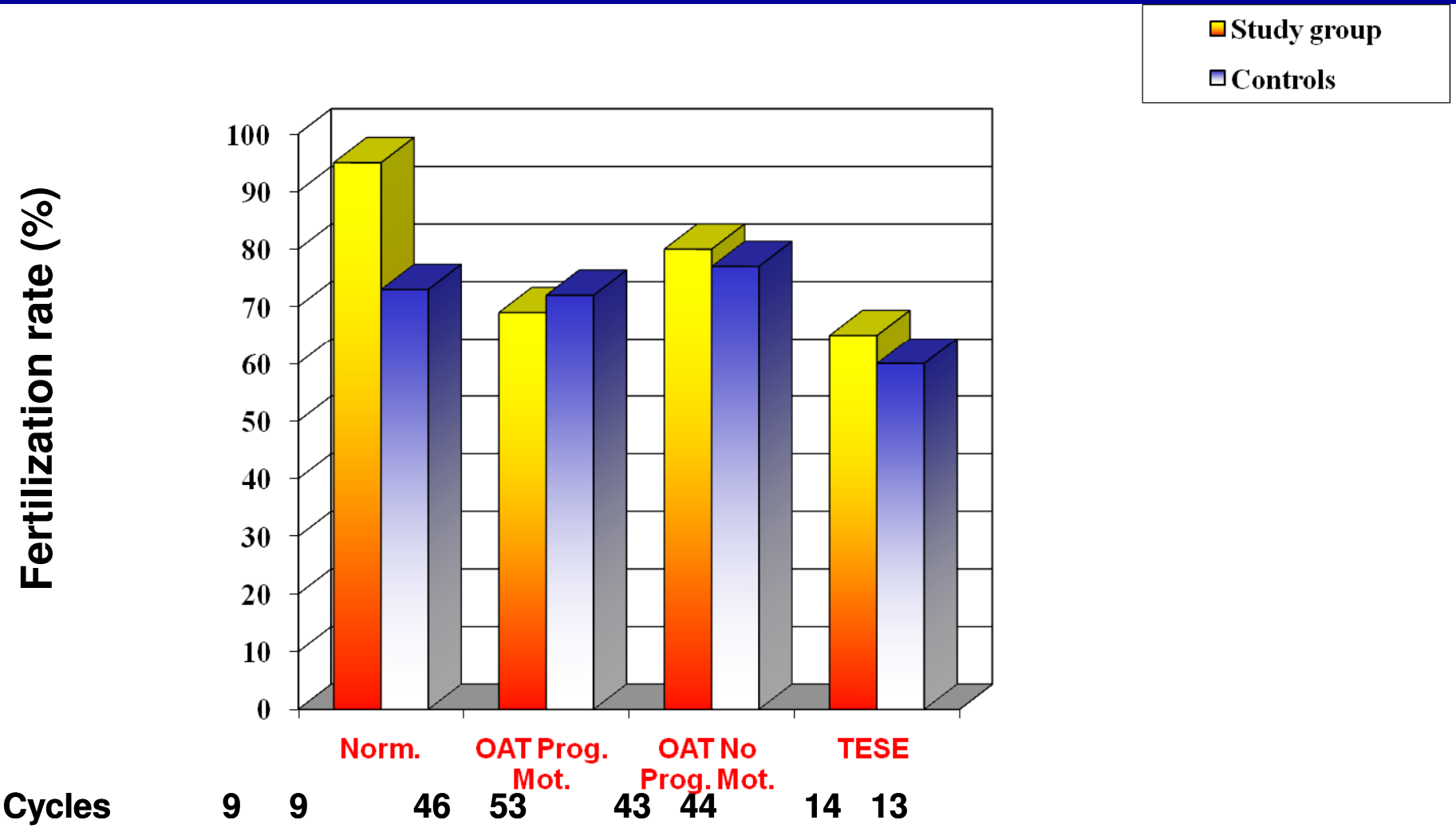
90 Fresh Cycles
29 Thawed Oocytes

According to the Italian law on IVF (2004-2009), only three oocytes could be inseminated per patient and all the generated embryos were transferred.

Gianaroli et al. (2007) Sperm selection for icsi according to the presence of birefringence in the sperm head. Fertil Steril. DOI: 10.1016/jfertnstert.2007.05.078

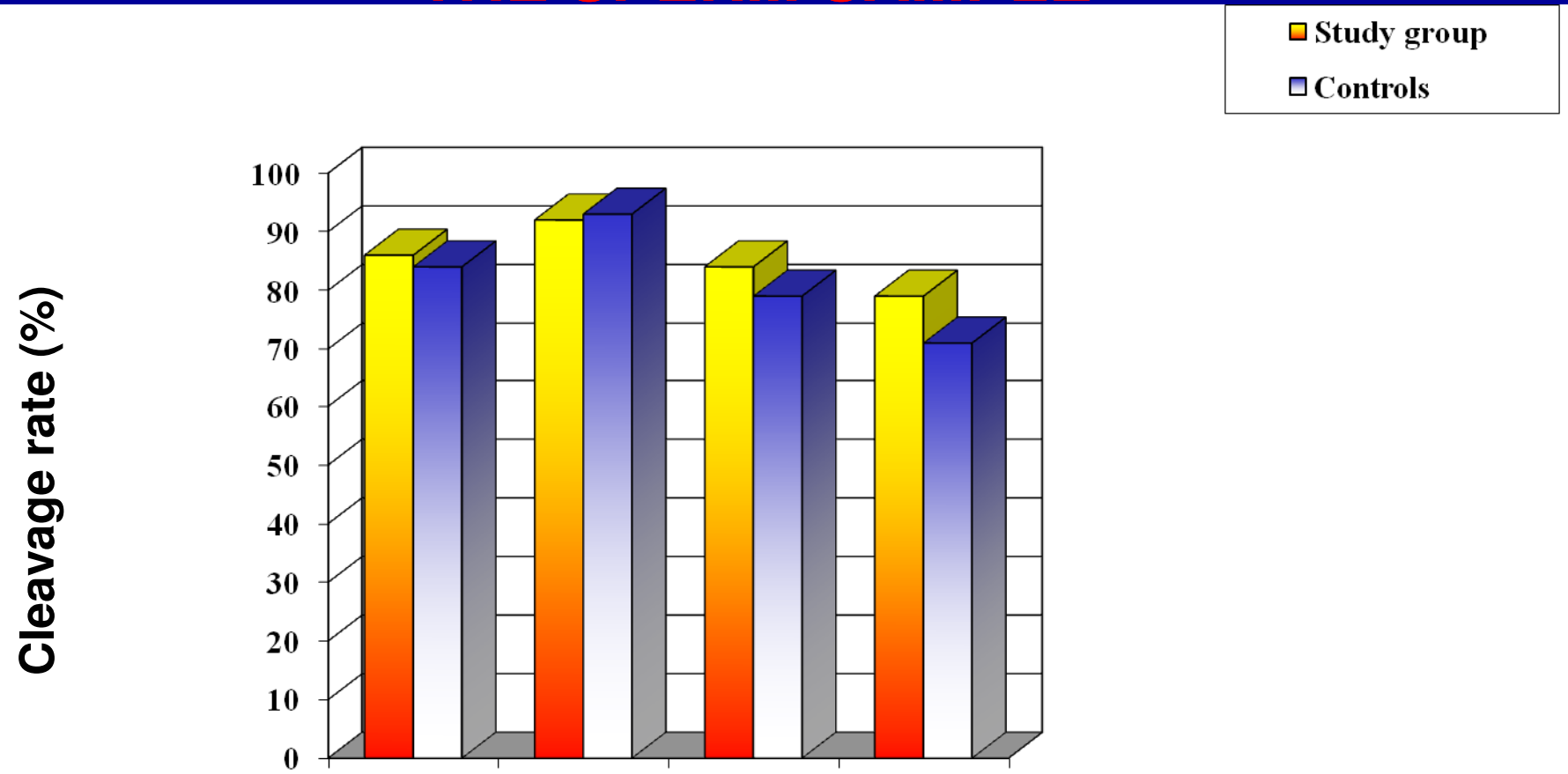
Results

FERTILIZATION RATE ACCORDING TO THE QUALITY OF THE SPERM SAMPLES



Results

CLEAVAGE RATE ACCORDING TO THE QUALITY OF THE SPERM SAMPLE

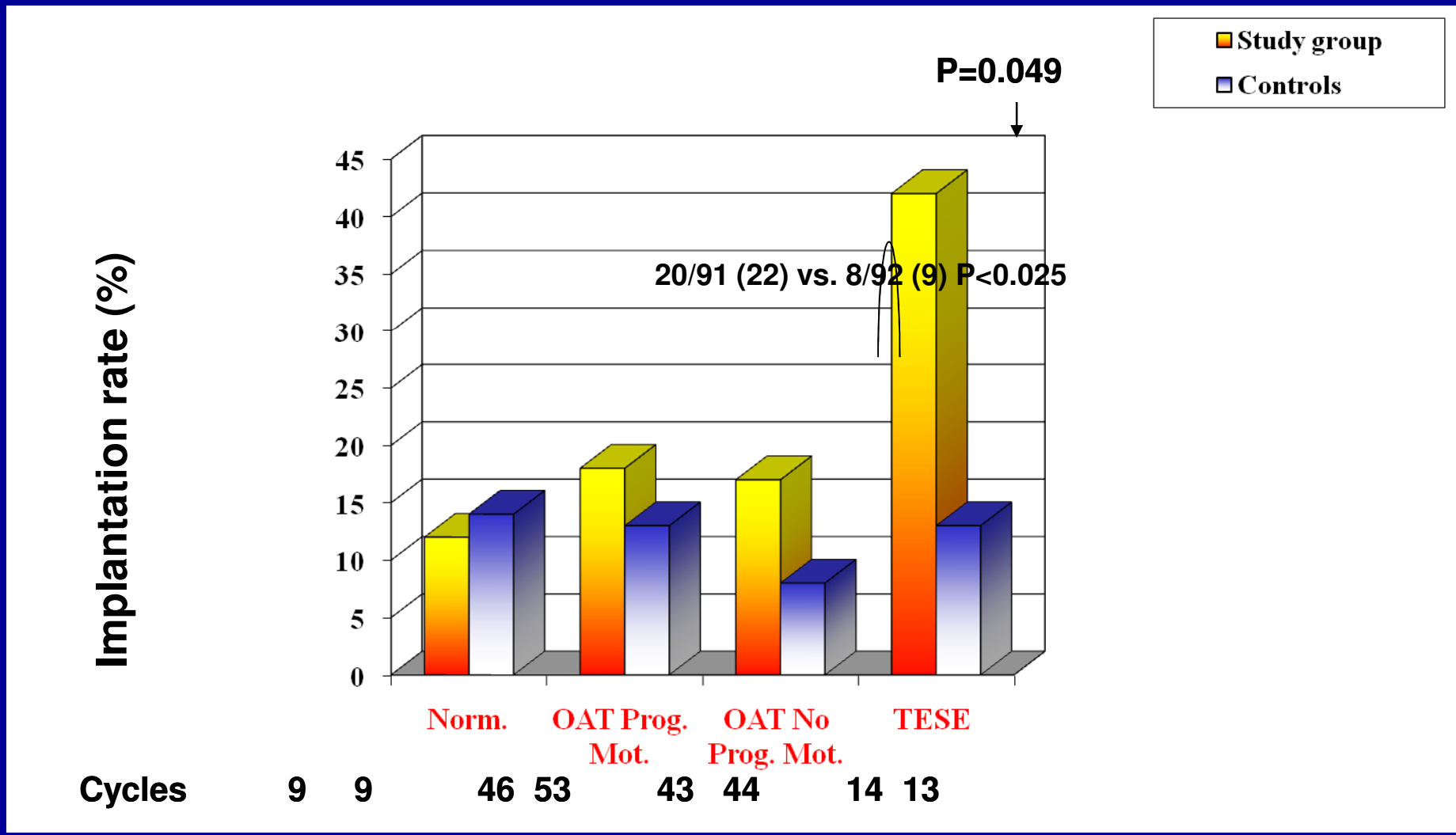


Cumulative Results

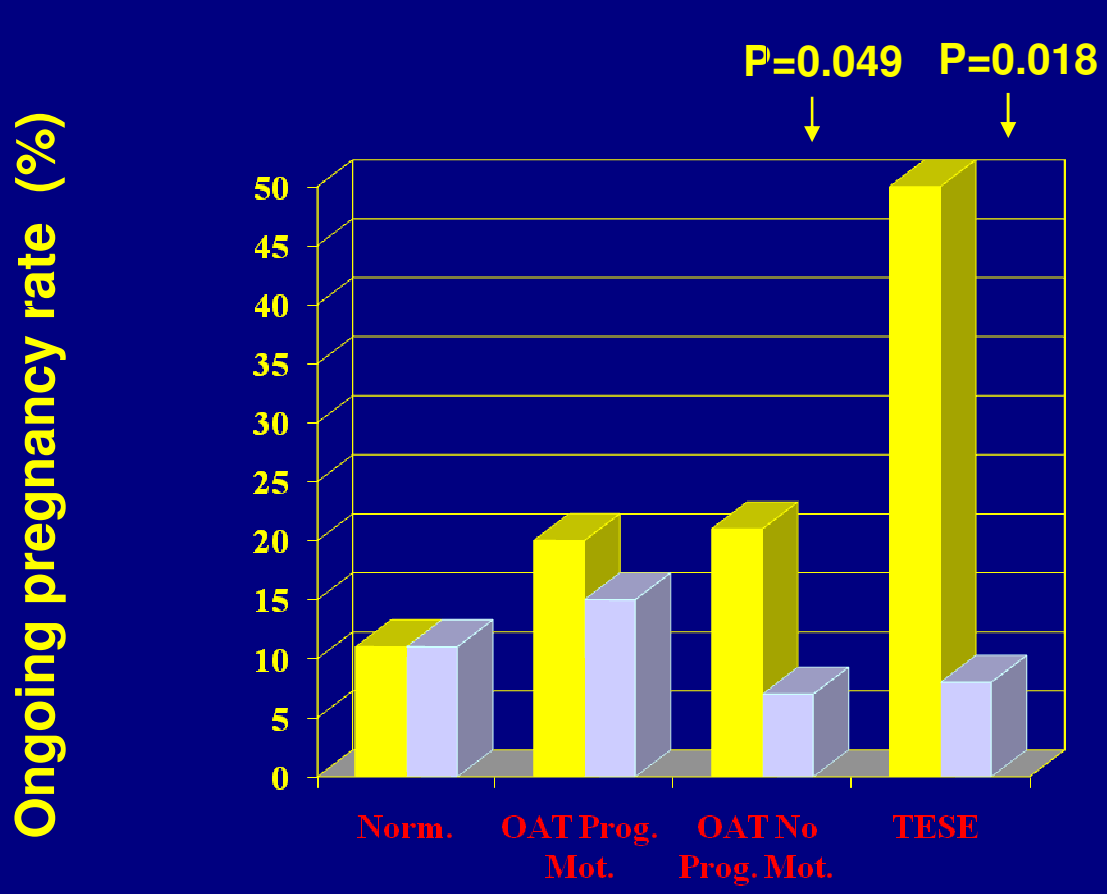
	Study group	Controls	P
No. Cycles	112	119	
Age	34.8±4.4	35.7±4.7	
% birefringent spermatozoa	39.9±30.4	-	
No. Fertilized oocytes (%)	235/317 (74)	248/342 (72)	
No. Embryos	204 (87)	210 (85)	
grade 1 day +2	178 (87)	177 (84)	
4c-1	66 (32)	57 (27)	
grade 1 day +3	144/164 (88)	117/135 (87)	
8c-1	54 (33)	27 (20)	<0.01
No. Transferred cycles	101	104	
No. Transferred embryos	184 (1.8±0.7)	196 (1.9±0.7)	
No. Clinical pregnancies (%)	31 (31)	22 (21)	
Implantation Rate (%)	35/184 (19.0)	22/196 (11.2)	<0.02
Abortions (%)	5 (16)	9 (41)	<0.01
Ongoing pregnancy rate (%)	26/112 (23)	13/119 (11)	<0.01

Results

IMPLANTATION RATE ACCORDING TO THE QUALITY OF THE SPERM SAMPLE

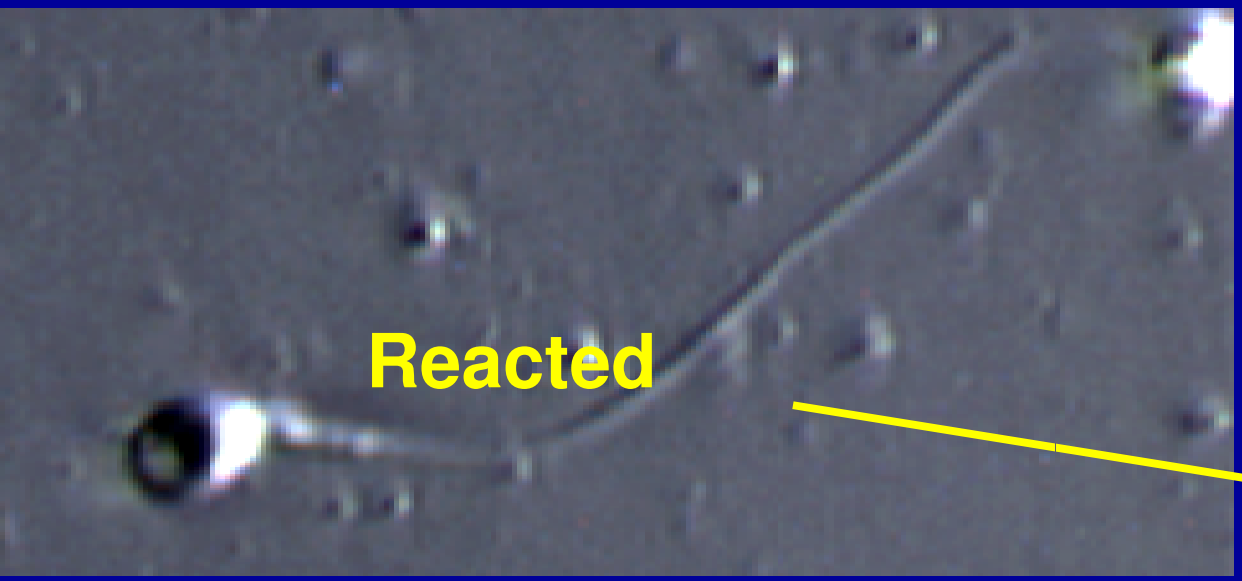


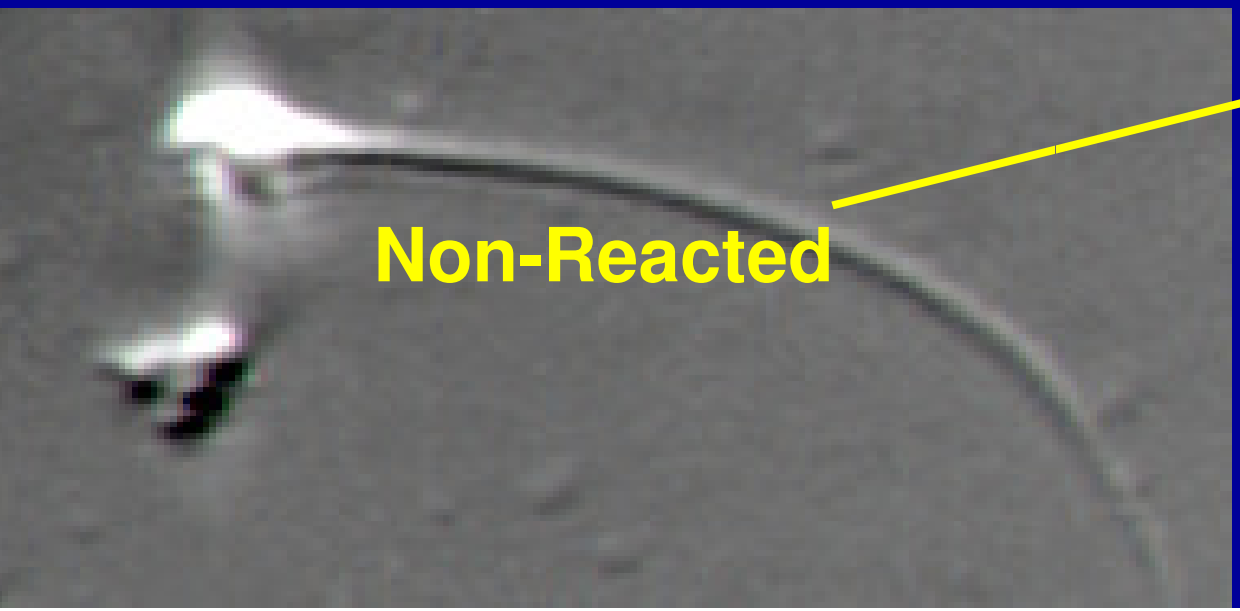
ONGOING PREGNANCY RATE PER CYCLE ACCORDING TO THE QUALITY OF THE SPERM SAMPLE

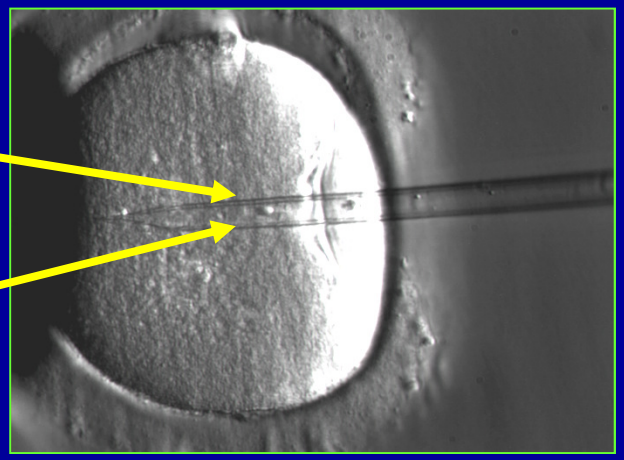


Gianaroli et al. (2007) Sperm selection for icsi according to the presence of birefringence in the sperm head. Fertil Steril. DOI: 10.1016/jfertnstert.2007.05.078

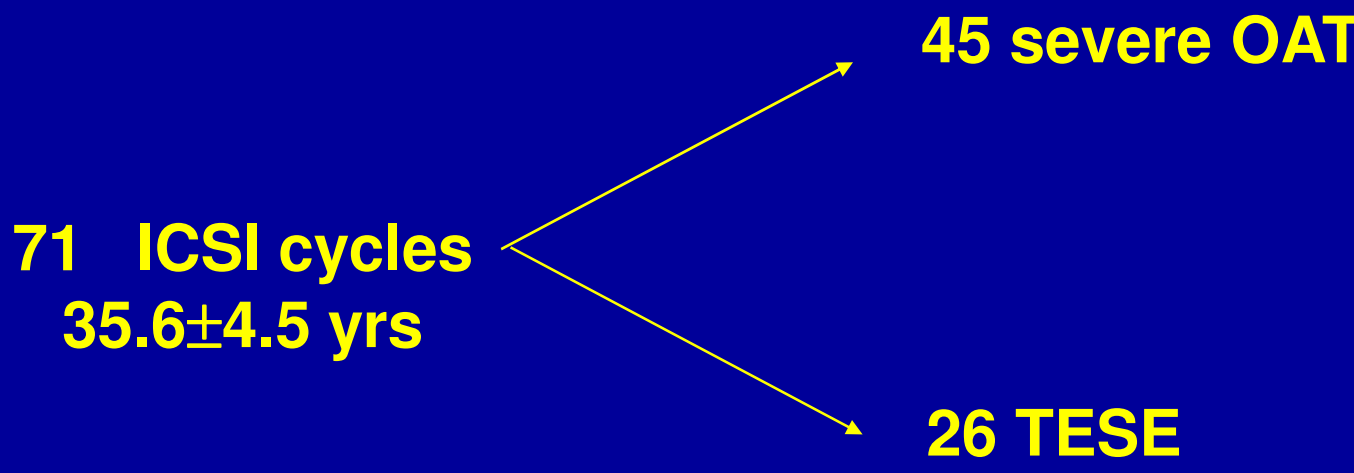
BIREFRINGENCE IN SPERM HEAD







MATERIALS AND METHODS



During ICSI, the type of birefringence of the injected spermatozoa was analyzed to distinguish between reacted and non-reacted spermatozoa.

Up to three oocyte per patient were inseminated.

Gianaroli et al. (2010) Birefringence characteristics in sperm head allow for the selection of reacted spermatozoa for ICSI . Fertil Steril., 93; 807-813)

MATERIALS AND METHODS

Injection with either type of spermatozoa was performed according to an even-odd randomization of patients. Allocation to either group was decided after the oocyte retrieval.

23 cycles – Acrosome reacted spermatozoa

26 cycles – Acrosome non-reacted spermatozoa

22 cycles – Mixed group: Acrosome reacted + Acrosome non-reacted spermatozoa

RESULTS

	Reacted	Non-reacted	Mixed
No. cycles	23	26	22
Age	34.9±4.0	36.3±4.3	35.6±5.3
No. TESE (%)	7 (30)	12 (46)	7 (32)
Fertilization rate (%)	50/72 (69.0)	49/73 (67)	51/69 (74)
Cleavage rate (%)	45/50 (90)	42/49 (86)	44/51 (86)

RESULTS

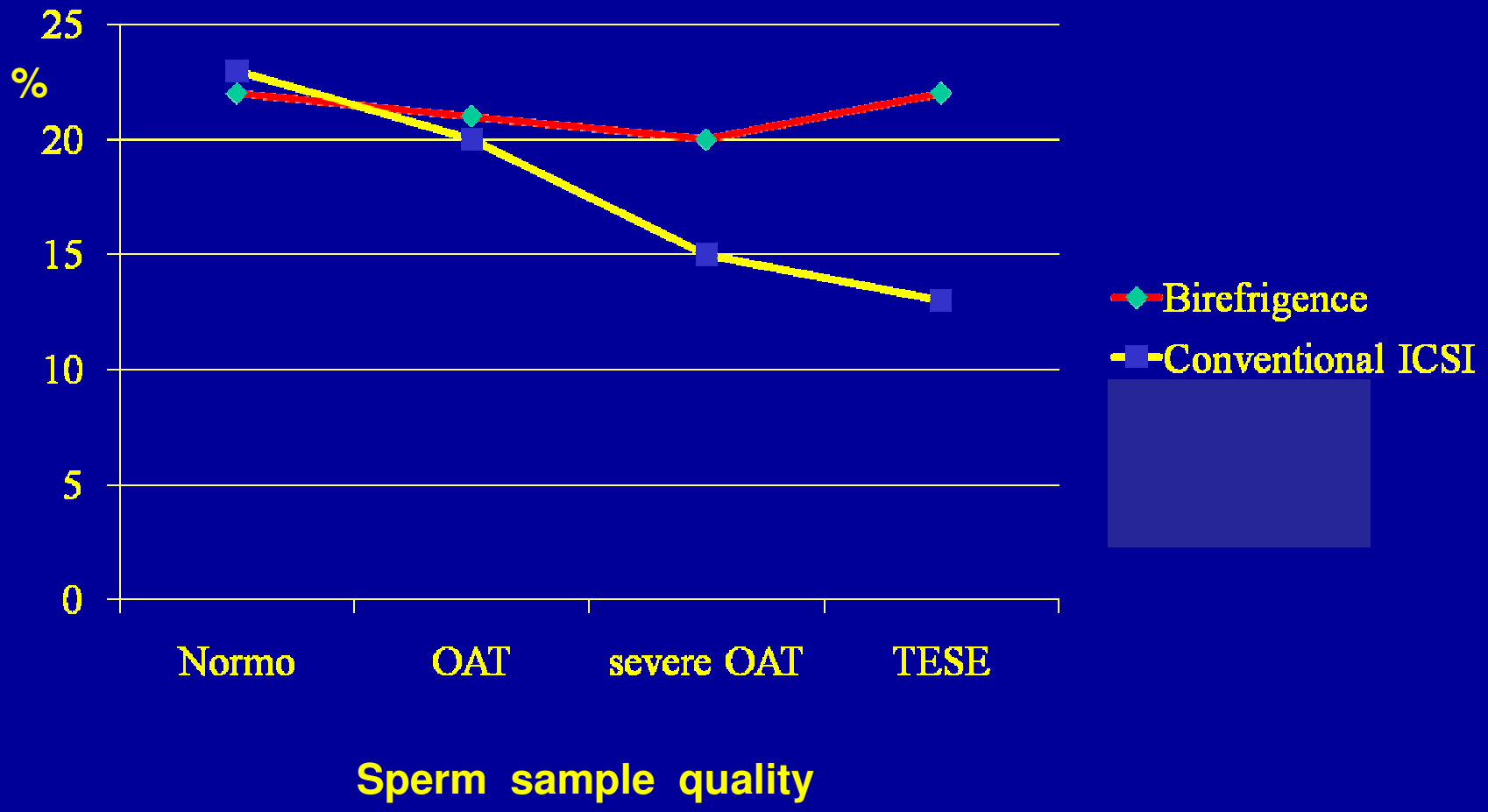
	Reacted	Non-reacted	Mixed
No. cycles	23	26	22
No. transferred cycles (%)	22 (96)	21 (81)	20 (91)
No. clinical pregnancies (% /cycle)	12 (55)[a]	3 (14)[ab]	8 (40)[a]
Implantation rate (%)	(39.0)[c]	(8.6)[cd]	(24.4)[d]
Ongoing pregnancy rate / cycle (%)	11 (48)[e]	2 (8)[ef]	7 (32)[f]

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

SISMER – Birefrigence cumulative data - 2006-2009

	severe OAT	TESE	TOT
Cycles	117	72	189
Transfers	107 (91,4%)	60 (83,3%)	167(88,3%)
Clinical pregnancies (%)	36 (33,6%)	19 (31,6)	55 (33%)
Abortions (%)	5/36 (13,8%)	1 (5,3%)	6 (11%)
I.R. (%)	43/212 (20,3%)	23/97 (23,7%)	66/309 (21,4%)

Implantation Rate (IR)



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