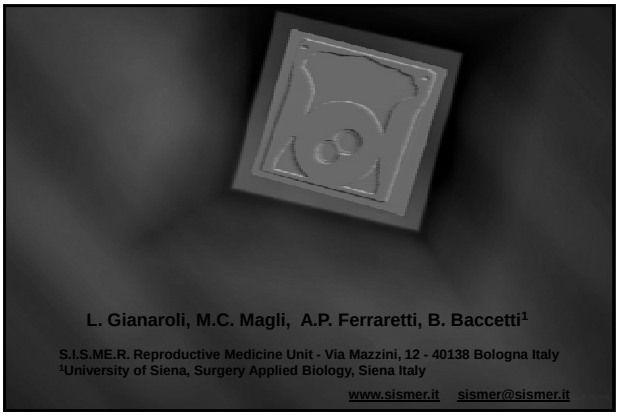

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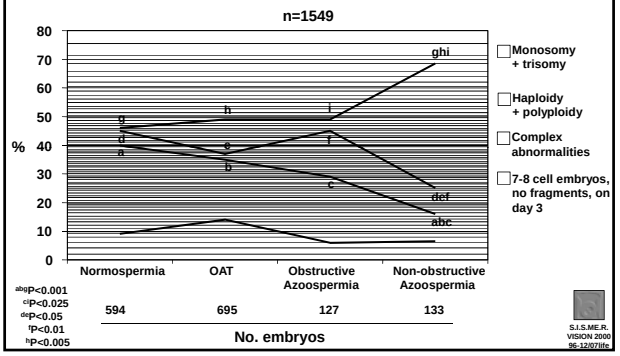
CHROMOSOMALLY ABNORMAL EMBRYOS ACCORDING TO SPERM INDICES

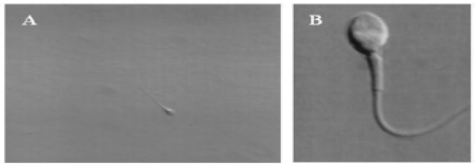


INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION

IMSSI

⇩

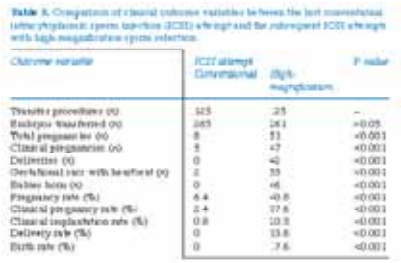
MOTILE SPERM ORGANELLAR EXAMINATION



Bartoov et al 2003 Fertil Steril 2003 80, 1413-1419

INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION

IMSSI



Bartoov 2006, Reprod Biomed Online 12, 634-638

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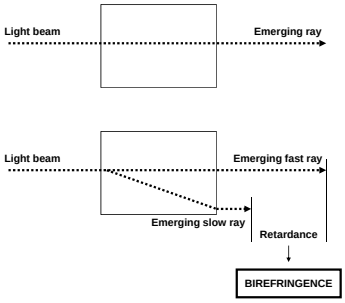
SPERMATOZOA
BIREFRINGENCE EFFECT

When crossing an anisotropic structure, the incident light beam is refracted into two rays traveling at different velocity. The retardance of the slow ray relative to the fast ray generates the birefringence effect.



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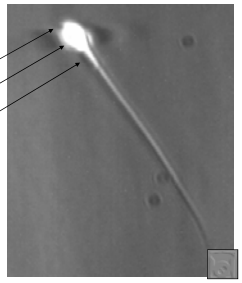
SPERMATOZOA
PROTOPLASMIC STRUCTURE

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

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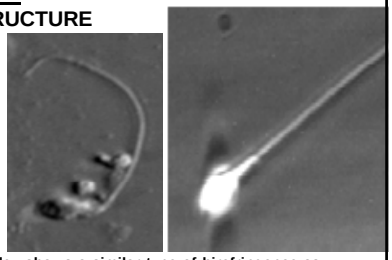
SPERMATOZOA
PROTOPLASMIC STRUCTURE



Living human spermatozoa are naturally birefringent, due to the anisotropy of their protoplasmic texture. In the mature sperm nucleus, there is a strong birefringence associated to nucleoprotein filaments which are arranged in rods and longitudinally oriented.

The mature acrosomal complex shows a similar type of birefringence as the nucleus, indicating the presence of protein subacrosomal filaments which are longitudinally oriented.

The same is for large portions of the tail texture.

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

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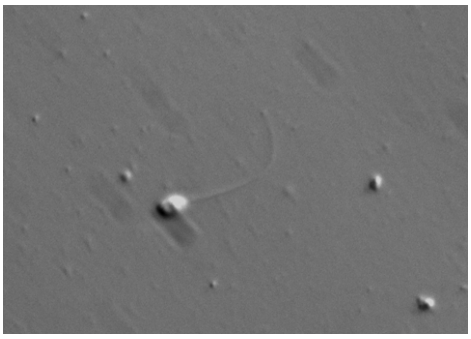
BIREFRINGENCE IN SPERM HEAD



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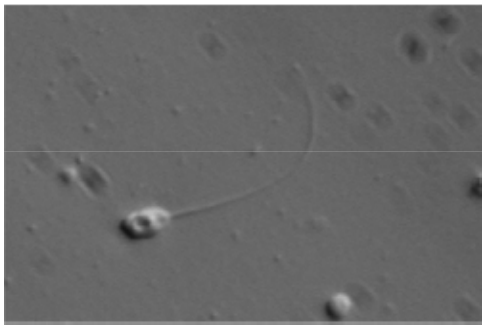
BIREFRINGENCE IN SPERM HEAD



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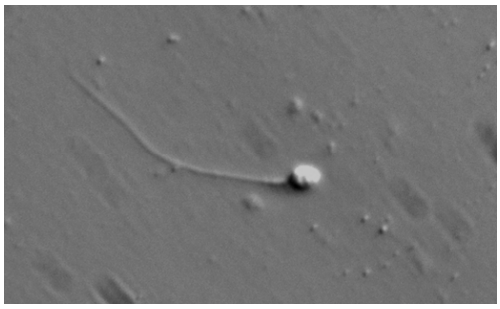
BIREFRINGENCE IN SPERM HEAD



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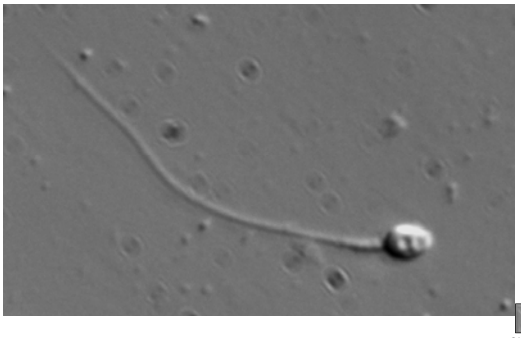
BIREFRINGENCE IN SPERM HEAD



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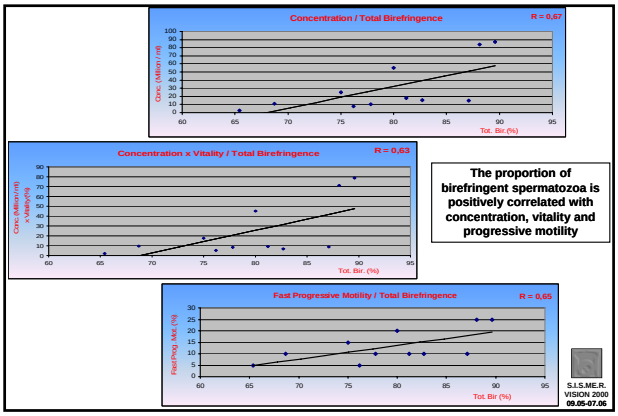
Results

BIREFRINGENCE VALUES AFTER SPERM PREPARATION

9 Normospermic		87.0±11.1
range		65-97
89 OAT		39.3±28.4
range		2-98
14 TESE		13.8±9.1
range		4-32
46 OAT (with progr. motility)		55.3±27.6
range		8-98
43 OAT (0 % progr. motility)		22.1±17.0
range		2-73

p=0.0008
p=2.66595E-06
p=6.92698E-14
p=0.0012479
p=6.84315E-15
p=8.4338E-07
p=0.0866

S.I.S.ME.R.
Vision 2000
09.05-07.06


Concentration / Total Birefringence
R = 0,67
Conc. (Milion / ml)
Tot. Bir.(%)
Concentration x Vitality / Total Birefringence
R = 0,63
Conc. (Milion/ml) x Vitality(%)
Tot. Bir. (%)
Fast Progressive Motility / Total Birefringence
R = 0,65
Rapid Prog. Mot. (%)
Tot. Bir (%)
The proportion of birefringent spermatozoa is positively correlated with concentration, vitality and progressive motility
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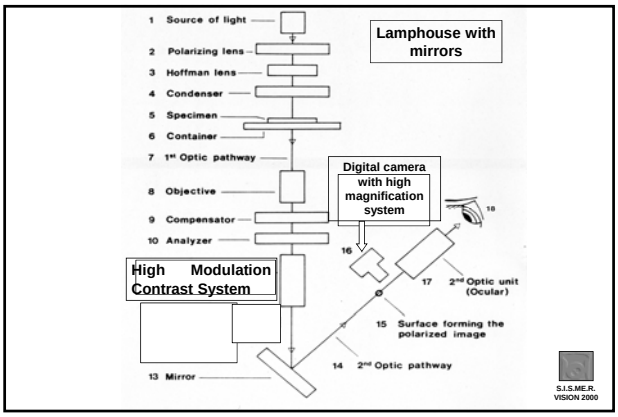
09.05-07.06


1 Source of light
2 Polarizing lens
3 Hoffman lens
4 Condenser
5 Specimen
6 Container
7 1st Optic pathway
8 Objective
9 Compensator
10 Analyzer
13 Mirror
14 2nd Optic pathway
15 Surface forming the polarized image
16
17 2nd Optic unit (Ocular)
18
Lamphouse with mirrors
Digital camera with high magnification system
High Modulation Contrast System
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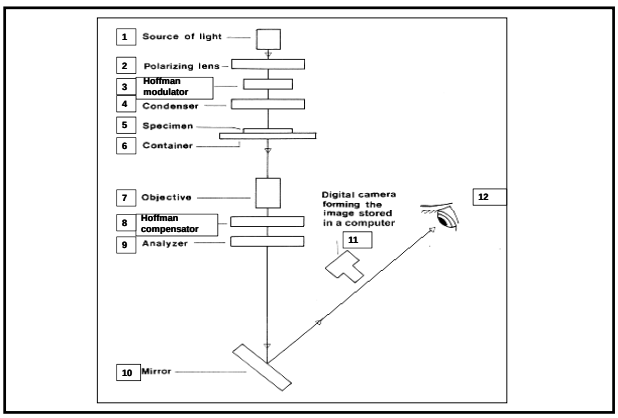
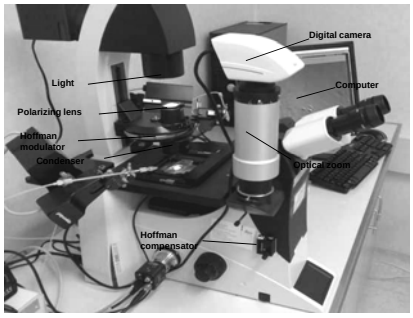
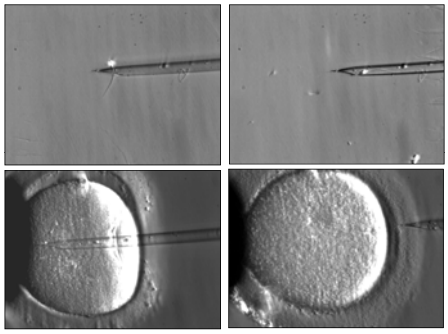

1 Source of light
2 Polarizing lens
3 Hoffman modulator
4 Condenser
5 Specimen
6 Container
7 Objective
8 Hoffman compensator
9 Analyzer
10 Mirror
11
12
Digital camera forming the image stored in a computer



SPERMATOZOA
PROTOPLASMIC STRUCTURE



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

S.I.S.ME.R. VISION 2000

SPERMATOZOA
PROTOPLASMIC STRUCTURE

Study Group		Control Group	
112 cycles	9 Normospermic 89 OAT 14 TESE	119 cycles	9 Normospermic 97 OAT 13 TESE
85 Fresh Cycles	27 Thawed Oocytes	90 Fresh Cycles	29 Thawed Oocytes

According to the current Italian law on IVF, only three oocytes could be inseminated per patient and all the generated embryos were transferred.

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

S.I.S.ME.R. VISION 2000 09.05-07.06

Results

ICSI cycles (Fresh + Thawed)

	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

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

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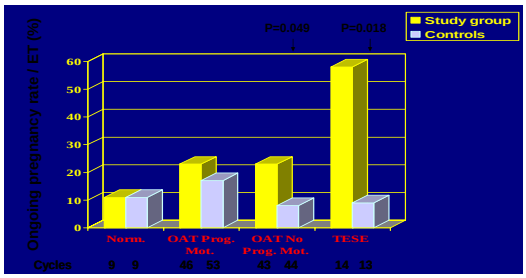
SPERMATOZOA

PROTOPLASMIC STRUCTURE

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



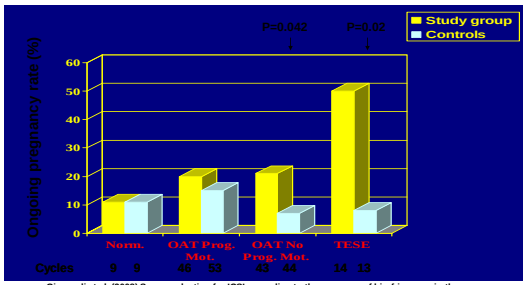
Gianaroli et al. (2008) Sperm selection for ICSI according to the presence of birefringence in the sperm head. Fertil Steril. 90, 104-112

S.I.S.ME.R. VISION 2000 09.05-07.06

SPERMATOZOA

PROTOPLASMIC STRUCTURE

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



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

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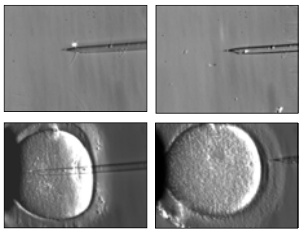
SPERMATOZOA
PROTOPLASMIC STRUCTURE

The properties of sperm birefringence have supported the application of polarization microscopy to the ICSI technique as a novel tool for sperm selection.



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. 90, 104-112

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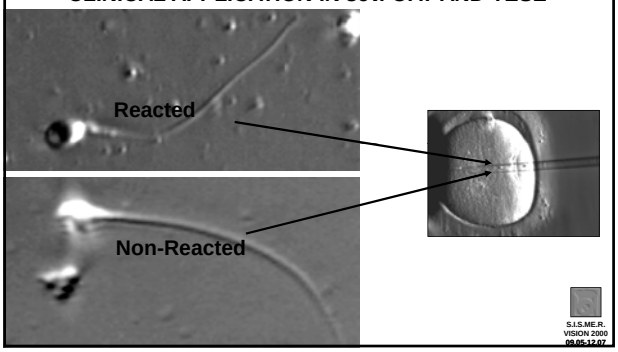
BIREFRINGENCE IN SPERM HEAD
CLINICAL APPLICATION IN sev. OAT AND TESE



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AIM OF THE STUDY

To verify the clinical outcome related to the injection of reacted vs. non reacted spermatozoa

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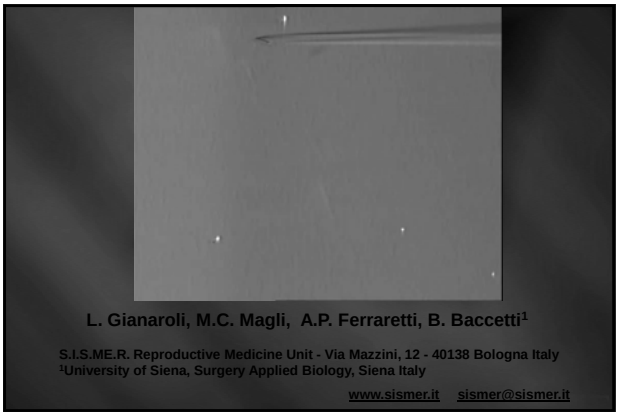

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MATERIALS AND METHODS

71 ICSI cycles
35.6±4.5 yrs

- 45 severe OAT
- 26 TESE

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. (2008) Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for ICSI. Fertil Steril. In press.

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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

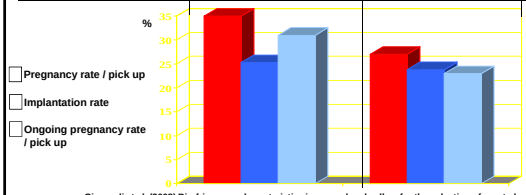
22 cycles – Mixed group: Acrosome reacted +
Acrosome non-reacted spermatozoa

Gianaroli et al. (2008) Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for ICSI. Fertil Steril. In press.

S.I.S.ME.R. VISION 2000 09.05.12.07

RESULTS

	Severe OAT	TESE	TOTAL
No. cycles	45	26	71
Age (M±SD) yrs	35.9±4.2	35.1±5.0	35.6±4.5



Gianaroli et al. (2008) Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for ICSI. Fertil Steril. In press.

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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)

Gianaroli et al. (2008) Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for ICSI. Fertil Steril. In press.

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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 [c]P=0.002 [d]P=0.048 [f]P=0.033

Gianaroli et al. (2008) Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for ICSI. Fertil Steril. In press.

S.I.S.ME.R. VISION 2000 09.05.12.07

CONCLUSIONS

The analysis of birefringence in human spermatozoa represents an accurate method of sperm selection for ICSI, which generates high pregnancy and implantation rates in patients with a severe male factor of infertility.

It provides the possibility of selecting spermatozoa on the basis of parameters that go beyond the classical characteristics of motility and morphology, including the status of their acrosome reaction.

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CONCLUSIONS

The injection of reacted spermatozoa promotes the generation of embryos with increased chances of implantation.

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The acrosome reaction could be a prerequisite for sperm incorporation to occur, not only for in vivo conception and after conventional IVF, but also after ICSI.

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THE IMPORTANCE OF SPERM QUALITY. BIREFRINGENCE: CHOOSING ACCORDING TO PROTOPLASMIC STRUCTURE

L. Gianaroli, M.C. Magli, A.P. Ferraretti, B. Baccetti[1]

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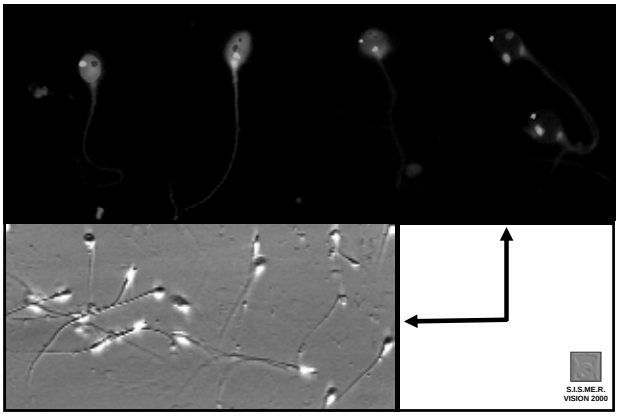
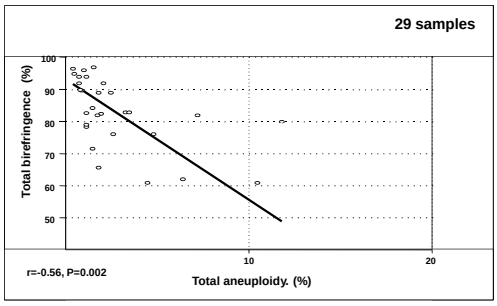


CORRELATION BETWEEN INCIDENCE OF ANEUPLOIDY AND PRESENCE OF HEAD BIREFRINGENCE



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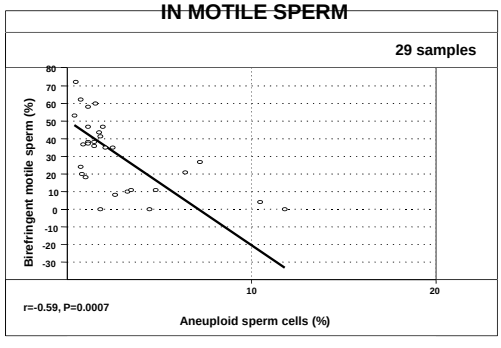
31.12.07

CORRELATION BETWEEN INCIDENCE OF ANEUPLOIDY AND PRESENCE OF HEAD BIREFRINGENCE IN MOTILE SPERM



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31.12.07

CONCLUSIONS

These data suggest that:

- **A correlation exists between the incidence of aneuploidy and the characteristics of the protoplasmic texture.**

↓

- **It can be concluded that the selection of birefringent spermatozoa increases the chances of identifying a vital sperm cell having a normal chromosomal complement.**

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THE IMPORTANCE OF SPERM QUALITY. BIREFRINGENCE: CHOOSING ACCORDING TO PROTOPLASMIC STRUCTURE

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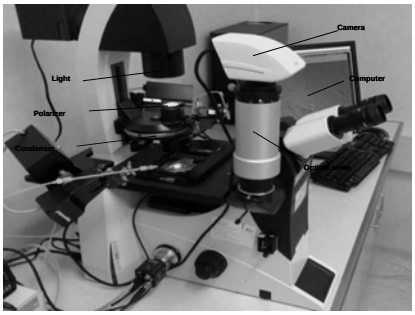
POLARIZING MICROSCOPE EQUIPPED WITH MICROMANIPULATION SYSTEM



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