Sperm gamete screening

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Sperm screening

- Standard semen analysis
- Sperm capacitation
- Oocyte activating factor (PLC_{zeta})
- Chromatin structure
- Assessment of centriole function
- FISH analysis
- Study of meiotic alterations

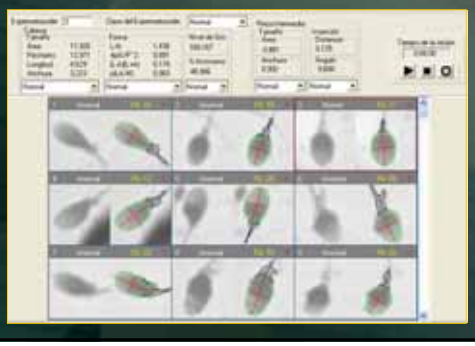
Semen analysis

- Sperm concentration
- Sperm viability
- Sperm morphology
- Kinematic parameters (VCL, ALH)
- Immunobeads test
- Isoprostanes in semen

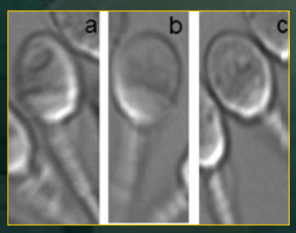
Sperm morphology

- Sperm morphometry
  - Tygerberg strict criteria (> 4% normal forms)
  - Teratozoospermic index (TZI < 1.6)
- High-magnification ICSI

Sperm morphometry

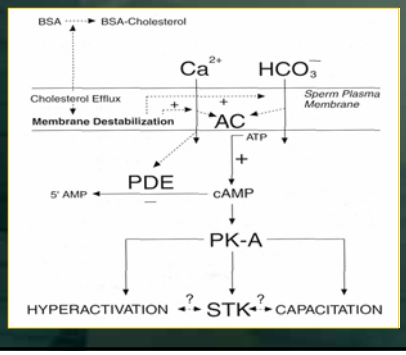


High-magnification ICSI

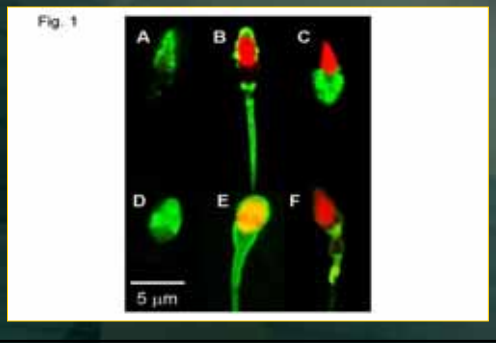


Sperm capacitation

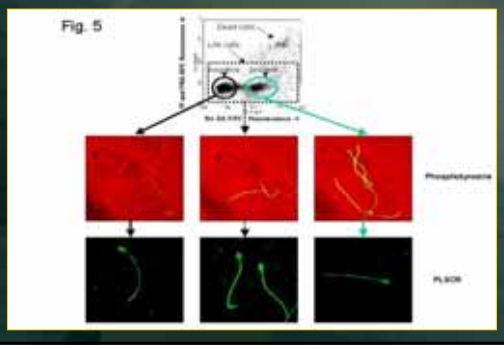
Sperm capacitation



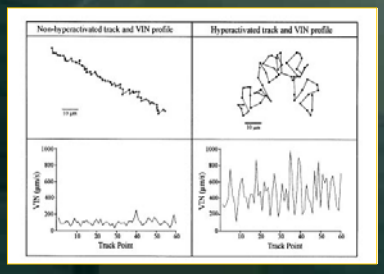
Sperm capacitation



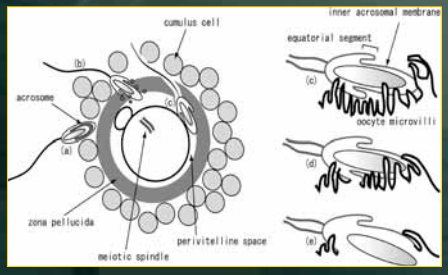
Sperm capacitation



Sperm hyperactivation



Sperm hyperactivation

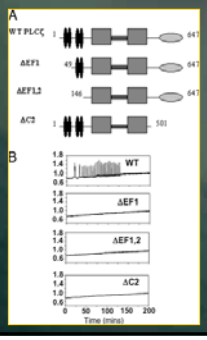


How to monitor sperm capacitation?

- Curvilinear velocity (VCL > 150 μm/sec)
- Lateral head displacement (ALH > 6μm)
- Flagellar protein phosphorylation
- Annexin V binding apical region sperm

Sperm activating factor

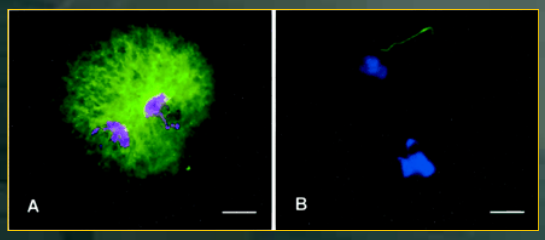
- Phospholipase C_{zeta}
- Nuclear membrane
- Calcium pulses
- Oocyte activation



Functional centrioles

- Mitotic aster formation
- Normal spermiogenesis
- Head-midpiece insertion
- Sperm morphology

Non-functional centrioles



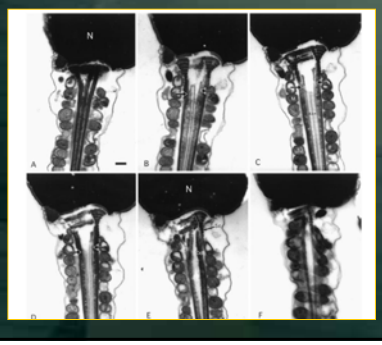
Rawe et al., Hum Reprod, 2002

How to monitor OSA and functional centrioles

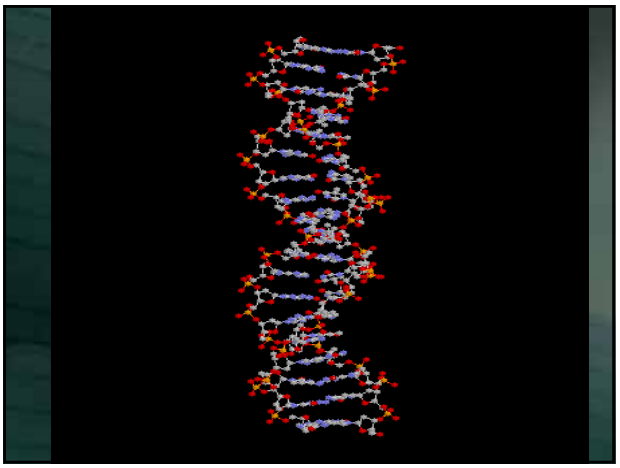
- Mouse oocyte activation test (MOAT)
- Microinjection of sperm in hamster or mouse oocytes
- The resulting embryo develops to 2-cell stage
  - oocyte activating factor
  - functional centrioles

Heyndrickx et al., Hum Reprod, 2006

Non-functional centrioles



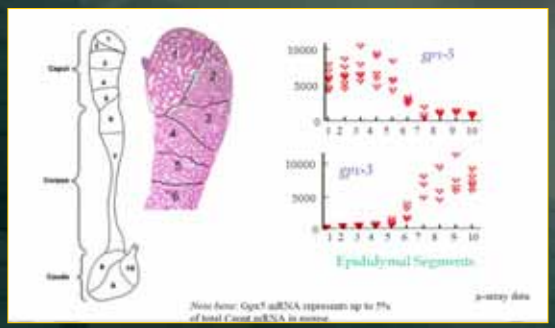
Manandhar et al., Hum Reprod, 2000



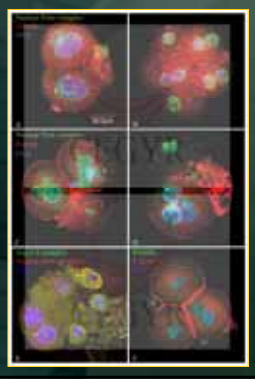
Chromatin structure

- S-S crosslinking of protamines
- DNA fragmentation
- Oocyte fertilization
- Embryo development: *late paternal effect*

Expresión de GPx en el epidídimo



Micronucleation



Micronucleation

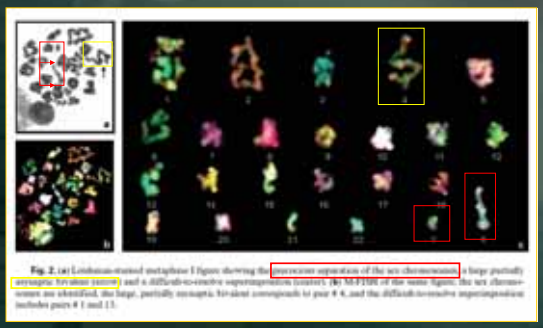
Genomic studies

- Sperm DNA fragmentation
- FISH in semen
- Study of meiotic alterations
- Study of Yq11 microdeletions
- CFTR gene mutations

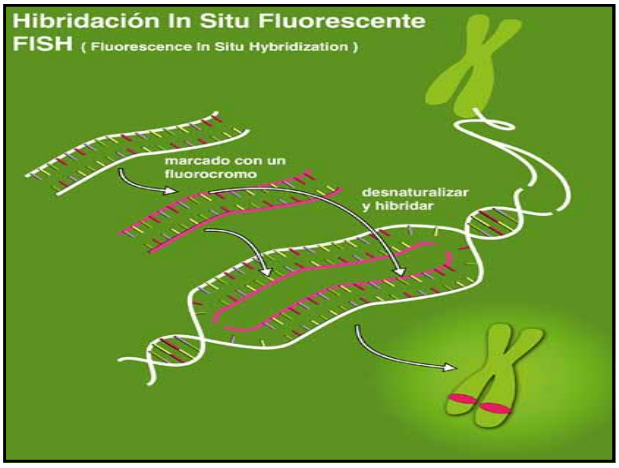
Normal chromosomal composition

- Synapsis / desynapsis during prophase of meiosis I
- Normal metaphase I and II
- Normal FISH analysis in semen
- Normal karyotype

Meiotic alterations

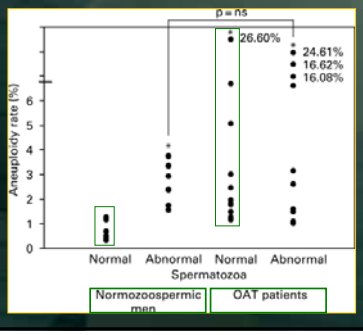


Egozcue et al., Cytogenet Genome Res, 2005



Normal sperm morphology is not always indicative of euploidy

Morfología y aneuploidías



Burrello et al., Hum Reprod, 2004

Chromatin integrity

- Testicular sperm
- Annexin V columns
- Laser scattering spectroscopy (0.1-10 μm)
- Flow cytometry

Testicular sperm and DNA fragmentation

Table I. Basic sperm parameters of the patients involved in this study and the incidence of DNA fragmentation in their ejaculated and testicular sperm samples

Patient	Basic sperm parameters			% Spermatozoa with fragmented DNA	
	Concentration ($\times 10^6$/ml)	Motility (%)	Normal forms (%)	Ejaculate	Testic
1	6	11	2	20	3
2	31	52	3	15	5
3	38	71	20	24	4
4	33	40	11	21	3
5	3	19	9	27	2
6	25	65	15	31	6
7	75	42	48	25	4
8	22	3	8	23	1
9	24	15	6	22	18
10	2	14	7	25	5
11	19	29	25	26	4
12	51	41	48	21	3
13	12	63	11	37	6
14	24	32	61	19	5
15	1	42	22	17	6
16	33	21	13	24	4
17	17	56	10	20	5
18	66	44	58	27	3
				5,6%	45%

Greco et al., Hum Reprod, 2005

Suganuma et al. (*Hum Reprod, 2005, 20:3101*)

Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI

Ryota Suganuma[1,3], Ryuzo Yanagimachi[1] and Marvin L.Meistrich[2,4]

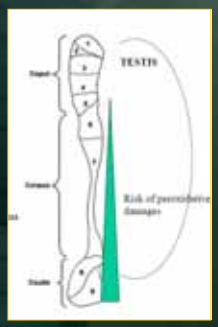
The advantage of using caput epididymal sperm for ICSI in certain situations may be considered as an approach to be tested in human assisted reproduction

Steele et al. (*Mol Hum Reprod, 1999, 5:831*)

A comparison of DNA damage in testicular and proximal epididymal spermatozoa in obstructive azoospermia

Testicular sperm DNA appears to be significantly less damaged than epididymal sperm DNA, and so testicular spermatozoa should be used in preference for ICSI to treat men with obstructive azoospermia

Risk of oxidative damage



Institut Marques study

- Couples with sperm DNA fragmentation values in semen > 20%
- Repeated IVF failure without apparent cause
- Prior egg donation cycles in 50% of the cases
- Simultaneous TESA-ICSI

García et al., RBM Online, 2007

Results

Case	Concentration (million/ml)	TUNEL	Meiosis/FISH	Pregnancy	Cycle nº	Previous cycles
1	120.0	35%	NR / NR	+	1º DO	2DO/2CT
2	40.0	30%	normal/normal	+	1º PO	2DO/2CT/2FIV
3	11.5	21%	normal/NR	+	1º DO	3DO/2CT
4	30.0	27%	NR/normal	-	1º DO	2FIV/2DO
5	1.07	32%	normal/NR	+	1º PO	2FIV
6	40.0	30%	NR / NR	+	1º DO	2FIV/1DO
7	0.60	32%	normal/NR	+	1º DO	2FIV/2DO
8	247.0	35%	NR / NR	+	1º DO	2FIV/3DO/1CT
9	60.0	37%	NP/normal	+	1º DO	2FIV/1DO/1CT
10	96.5	28%	NR/ patológico	+	1º DO/PGD	2FIV/1DO
11	20.8	52%	NR/ patológico	-	1º DO	2FIV/1DO
12	99.5	30%	NR/ NR	-	1º DO	1FIV71DO
13	48.6	22%	NR/ NR	-	1º DO	2FIV/1DO
14	92.0	36%	NR/ normal	+	1º DO	1FIV/1SPA
15	70.0	57%	NR/normal	+	1º OP	3FIV

Preliminary results

- Testicular sperm was obtained in all 30 cases
- Fertilization rate of 70%
- Pregnancy rate of 55% in first cycle
- Prevalence of pathological DNA fragmentation of 40% in couples with repeated IVF failure

García et al., RBM Online, 2007

Preliminary results Barros et al.

- 43 couples with repeated IVF failure
- Sperm DNA fragmentation was not evaluated
- All FIV/ICSI cycles were with patient's oocytes
- Pregnancy rate per cycle of 30.2%

Why recommend simultaneous TESA-ICSI?

Cryopreservation and DNA fragmentation

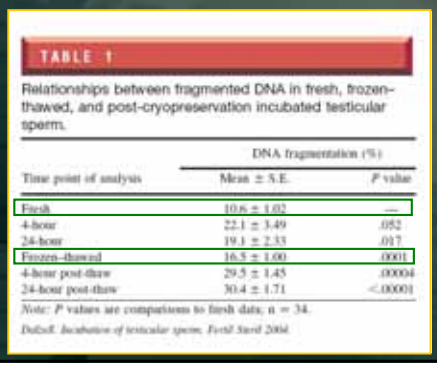


TABLE 1

Relationships between fragmented DNA in fresh, frozen-thawed, and post-cryopreservation incubated testicular sperm.

Time point of analysis	DNA fragmentation (%) Mean ± S.E.	*P* value
Fresh	10.6 ± 1.02	—
4-hour	22.1 ± 3.49	.052
24-hour	19.1 ± 2.33	.017
Frozen-thawed	16.5 ± 1.00	.0001
4-hour post-thaw	29.5 ± 1.45	.00004
24-hour post-thaw	30.4 ± 1.71	<.00001

Note: P values are comparisons to fresh data; n = 34.

Dalzell. Incubation of testicular sperm. Fertil Steril 2004.

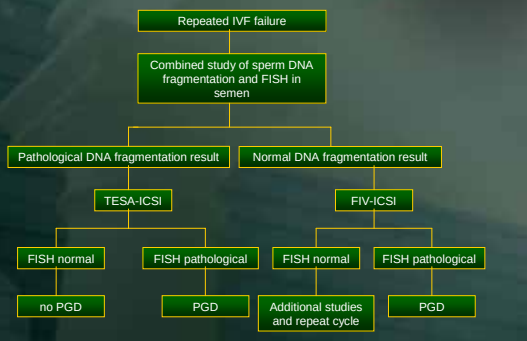
Indications DNA fragmentation analysis

- Repeated IVF failure without apparent cause
- Male age > 45 años
- Varicocele
- Episode of high fever
- Previous treatment with chymo / radiotherapy
- Recurrent abortion
- Severe necroasthenozoospermia

Indications of FISH analysis

- Repeated IVF failure without apparent cause
- Previous treatment with chymo / radiotherapy
- Recurrent abortion
- Non-conclusive results in meiosis study

Algorithm IM



Indications of Yq11 analysis

- Oligospermia < 5 million/ml (AZFc)
- Azoospermia (AZFa, AZFb, AZFc)
- Father or brother affected of microdelection
- Freezing of semen at puberty in sons of fathers with microdeletions (AZFc)

Indications of CFTR gene mutations

- Uni or bilateral absence of vas deferens
- Oligospermia / azoospermia
- Hipospermia with pH < 7.0
- Family history of CFTR gene mutations
- Kit of 34 CFTR gene mutations
- Compund heterozygotes (ΔF508/5T)

Conclusions

- Semen analysis
- Sperm morphology
- DNA fragmentation analysis
- FISH in semen
- Study of meiosis
- Microdeletions Yq11
- CFTR gene mutations
- Oxidative stress
- MOAT test
- Light scattering spectroscopy
- Flow cytometry