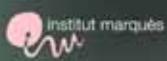


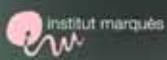
Sperm gamete screening

Juan G. Alvarez, M.D, Ph.D
Instituto Marquès, Barcelona
Harvard Medical School, Boston



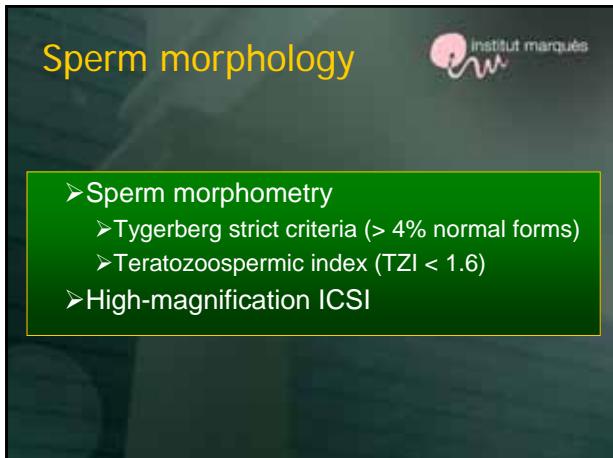
Sperm screening

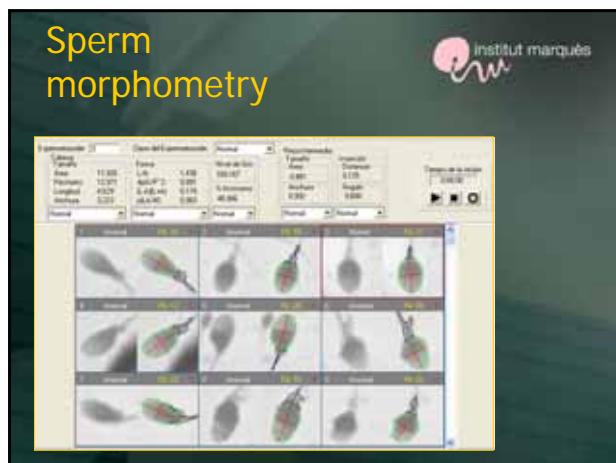
- Standard semen analysis
- Sperm capacitation
- Oocyte activating factor (PLC_{zeta})
- Chromatin structure
- Assessment of centriole function
- FISH analysis
- Study of meiotic alterations



Semen analysis

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



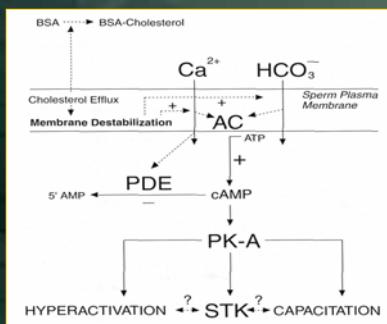




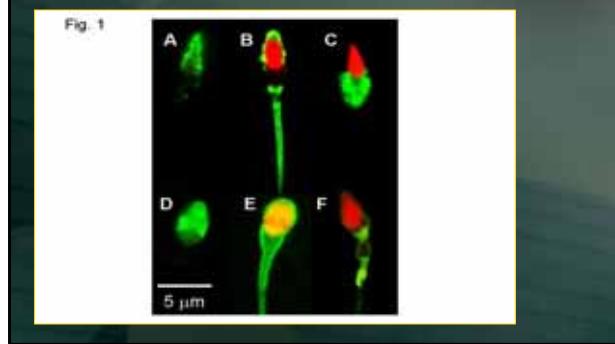
Sperm capacitation

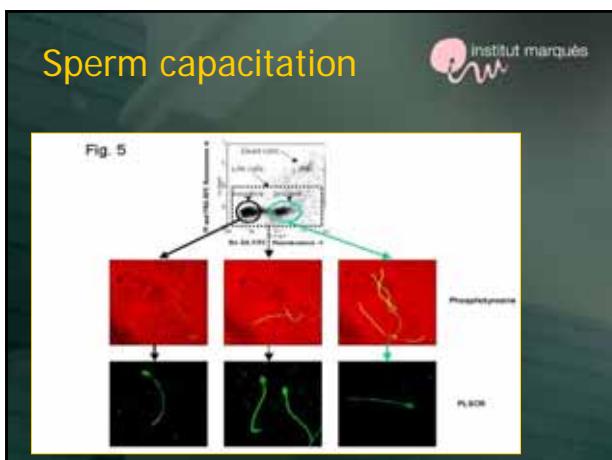


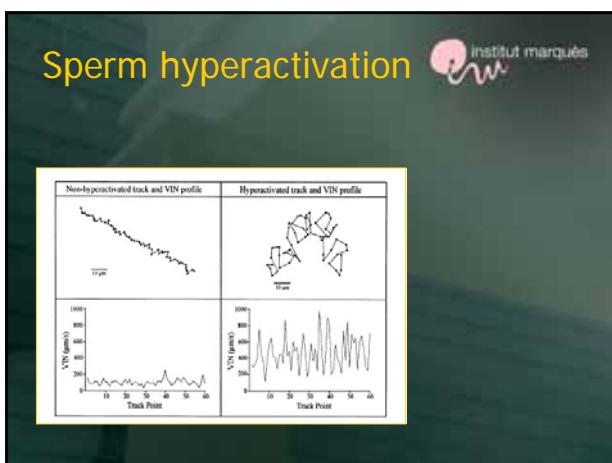
Sperm capacitation

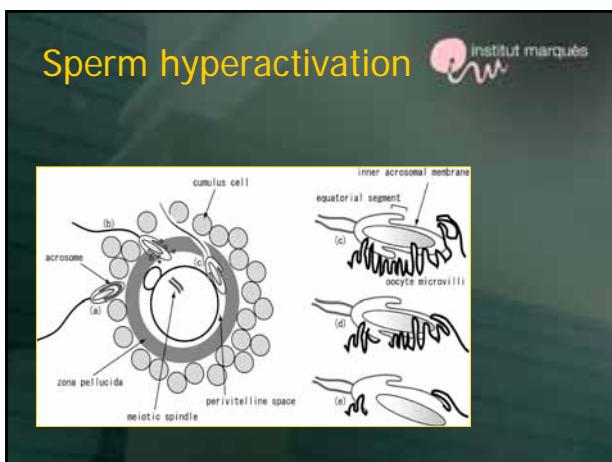


Sperm capacitation

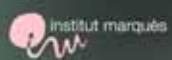








How to monitor sperm capacitation?

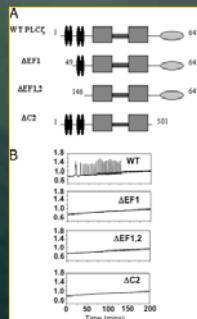


- Curvilinear velocity (VCL > 150 µm/sec)
- Lateral head displacement (ALH > 6µm)
- Flagellar protein phosphorylation
- Annexin V binding apical region sperm

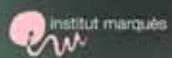
Sperm activating factor



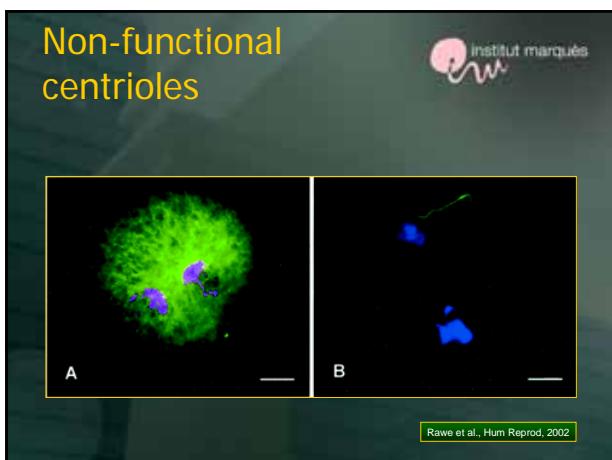
- Phospholipase C_{zeta}
- Nuclear membrane
- Calcium pulses
- Oocyte activation



Functional centrioles



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

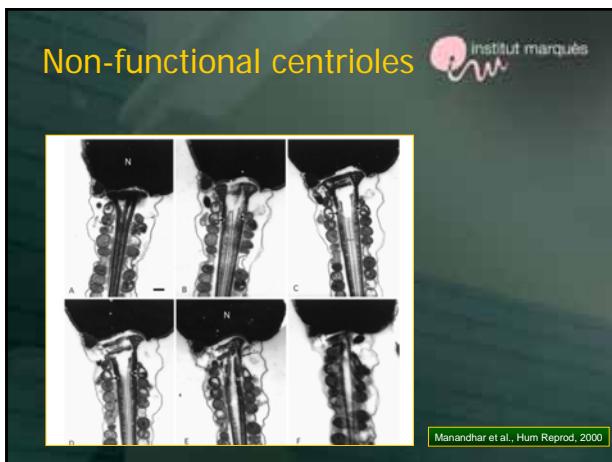


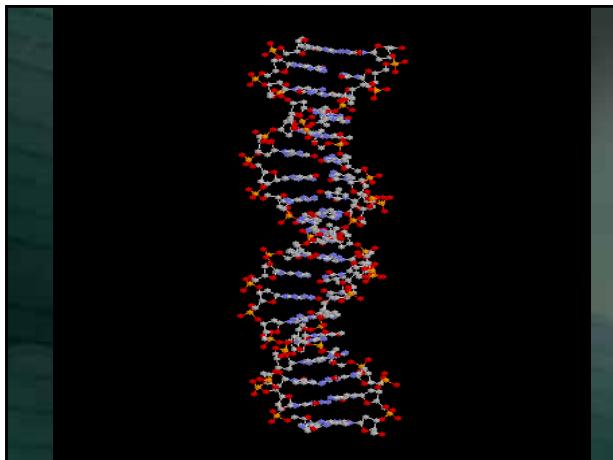
How to monitor OSA and functional centrioles

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- 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





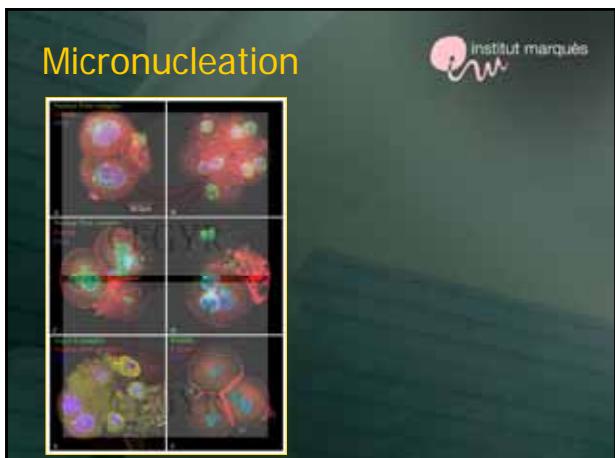
Chromatin structure

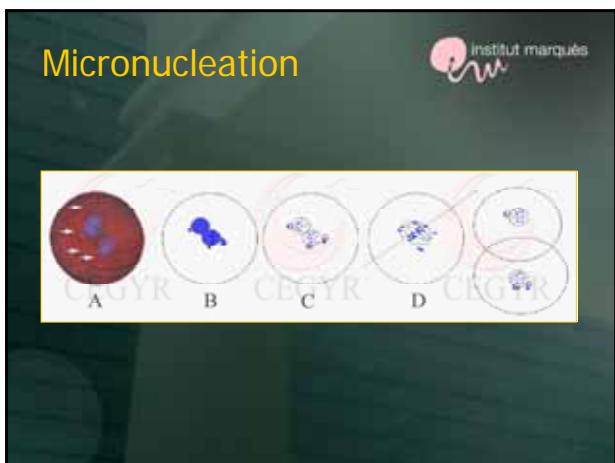
institut marqués

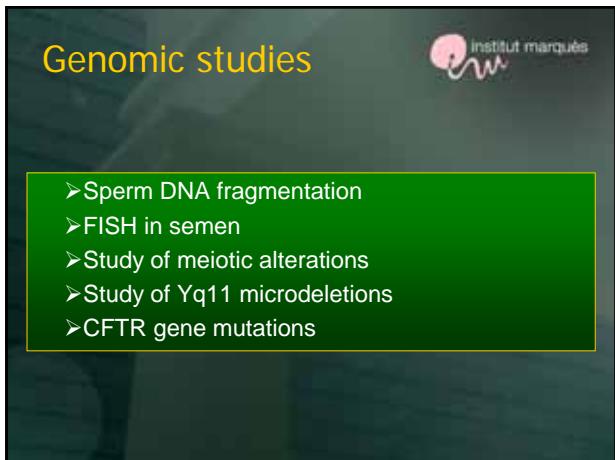
- S-S crosslinking of protamines
- DNA fragmentation
- Oocyte fertilization
- Embryo development: *late paternal effect*

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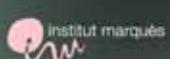








Normal chromosomal composition



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

Meiotic alterations

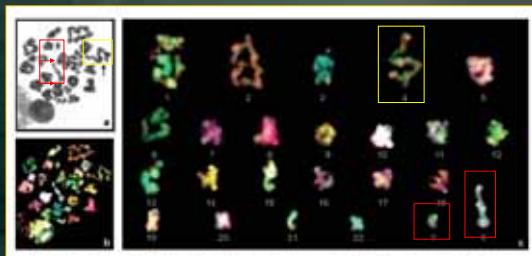
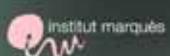
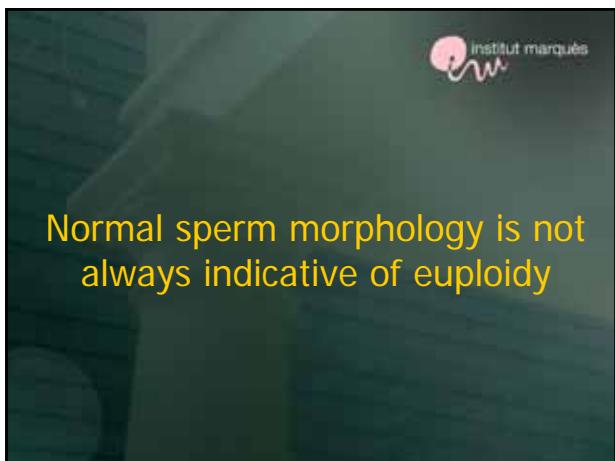


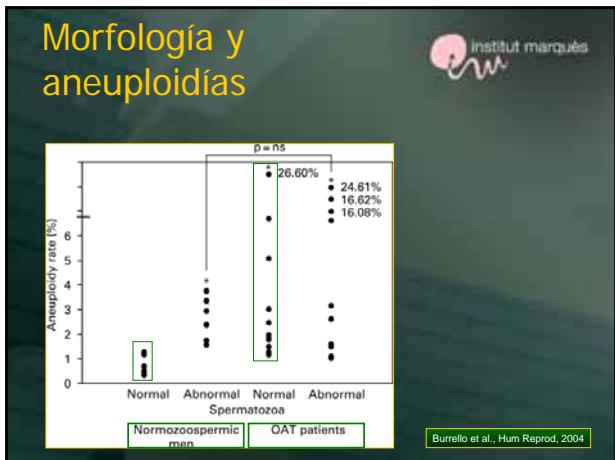
Fig. 2. (A) Leishman-stained metaphase I figure showing the incorrect separation of the sex chromosomes, a large partially synapsic bivalent (red box) and a difficult-to-score supercompaction bivalent (yellow box). (B) M-FISH of the same figure; the sex chromosomes are identified, the large, partially synapsic bivalent corresponds to pair # 4, and the difficult-to-score supercompaction includes pairs # 1 and 13.

Egozcue et al., Cytogenet Genome Res, 2005

Hibridación In Situ Fluorescente FISH (Fluorescence In Situ Hybridization)







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

Testicular sperm and DNA fragmentation

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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	Testis
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	22	2
6	25	65	15	31	6
7	75	42	48	25	4
8	27	38	8	21	3
9	18	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	18	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)

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

Ryo Suganuma^{1,2}, Ryuzo Yanagimachi¹ and Marvin L. Meistrich^{1,2}

¹Institute for Biogenesis Research, University of Hawaii Medical School, Honolulu, HI 96822 and ²Department of Experimental Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA

*To whom correspondence should be addressed. E-mail: meistrich@mdanderson.org

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

Abstract. *For many years, it has been assumed that sperm from the cauda epididymis of mutant males showed increased abnormalities. Injection of testicular or caput epididymal sperm from *Tspy*^{-/-} mice into oocytes produced lower implantation rates and yields of live born than those from wild-type mice. CONCLUSIONS: These results demonstrate that in mice with sperm abnormalities, a decline in quality of sperm after the caput epididymis. 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.*

Key words: embryo development; epididymal sperm; ICSI; testicular sperm; testis nuclear protein

Steele et al. (*Mol Hum Reprod*, 1999, 5:83)

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

E. Richard Steele^{1,2}, Neil McCausland^{1,2}, Robert J. Maxwell² and Siobhan E. M. Lenihan¹

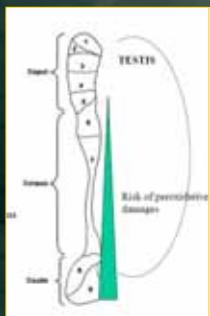
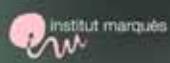
¹Department of Obstetrics & Gynaecology, The Queen's University of Belfast, Institute of Clinical Sciences, Grosvenor Road, Belfast BT7 1NN, ²Regional Fertility Units, Royal Maternity Hospital, Belfast BT7 1NN, and ³Department of Surgery, Royal Victoria Hospital, Belfast BT7 1NN, UK

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

Summary. DNA from testicular spermatozoa (DNA_{TE}) (3.2 ± 0.8 nmol 2,6-diaminopurine (2,6-DP) $\times 10^{-9}$ mol/g DNA) was significantly less damaged than DNA from proximal epididymal spermatozoa (DNA_{PE}) (4.5 ± 1.0 nmol 2,6-DP $\times 10^{-9}$ mol/g DNA). In contrast, a normal level of DNA damage was found in spermatozoa from men with non-obstructive azoospermia. There were no significant differences in undamaged DNA in these two occasions (0.9 ± 0.2 nmol 2,6-DP $\times 10^{-9}$ mol/g DNA). CONCLUSIONS: Our data suggest that 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.

Key words: DNA/proximal spermatozoa/testicular spermatozoa/testicular spermatogenesis

Risk of oxidative damage



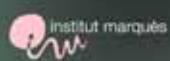
Institut Marques study



- 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

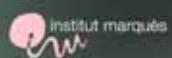
García et al. RBM Online, 2007

Results



Case	Concentration (million/ml)	TUNEL	Meiosis/FISH	Pregnancy	Cycle n°	Previous cycles
1	120.0	35%	NR / NR	+	1 ^o DO	2DO/2CT
2	40.0	30%	normal/normal	+	1 ^o PO	2DO/zCT/2FIV
3	11.5	21%	normal/NR	+	1 ^o DO	3DO/2CT
4	30.0	27%	NR/normal	-	1 ^o DO	2FIV/2DO
5	1.07	32%	normal/NR	+	1 ^o PO	2FIV
6	40.0	30%	NR / NR	+	1 ^o DO	2FIV/1DO
7	0.60	32%	normal/NR	+	1 ^o DO	2FIV/2DO
8	247.0	35%	NR / NR	+	1 ^o DO	2FIV/2DO/1CT
9	60.0	37%	NP/normal	+	1 ^o DO	2FIV/1DO/1CT
10	96.5	28%	NR/ patológico	+	1 ^o DO/PGD	2FIV/1DO
11	20.8	52%	NR/ patológico	-	1 ^o DO	2FIV/1DO
12	99.5	30%	NR/ NR	-	1 ^o DO	1FIV/7DO
13	48.6	22%	NR/ NR	-	1 ^o DO	2FIV/1DO
14	92.0	36%	NR/ normal	+	1 ^o DO	1FIV/1SPA
15	70.0	57%	NR/normal	+	1 ^o OP	3FIV

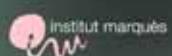
Preliminary results



- 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

Garcia et al., RBM Online, 2007

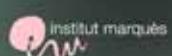
Preliminary results Barros et al.



- 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

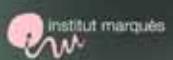


TABLE 1

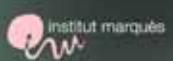
Relationships between fragmented DNA in fresh, frozen-thawed, and post-cryopreservation incubated testicular sperm.

Time point of analysis	DNA fragmentation (%)	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.

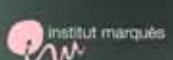
DOI:10.1016/j.fertnstert.2004.09.024

Indications DNA fragmentation analysis

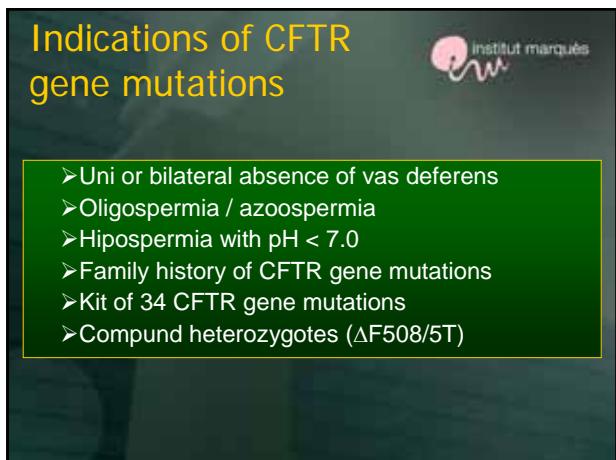
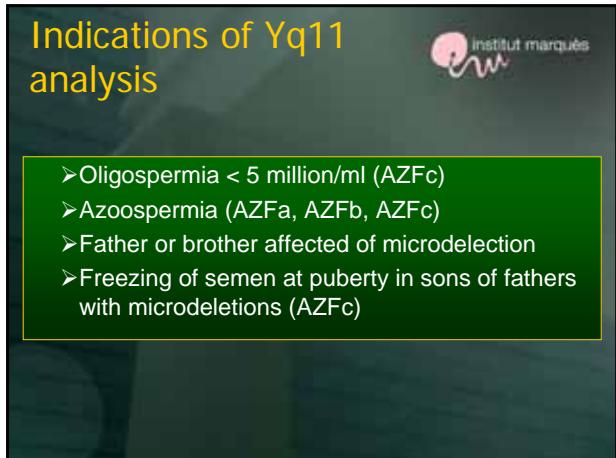
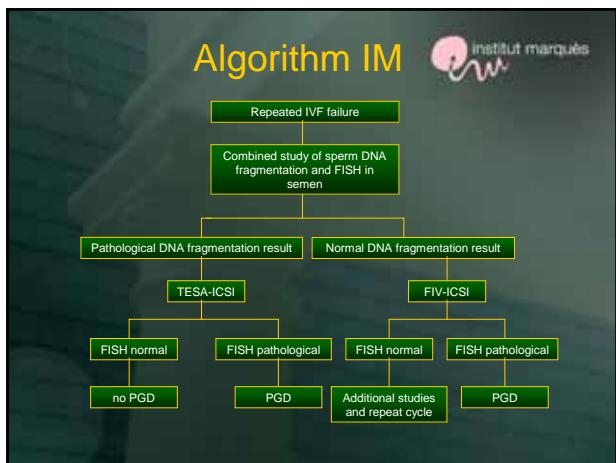


- Repeated IVF failure without apparent cause
- Male age > 45 años
- Varicocele
- Episode of high fever
- Previous treatment with chymo / radiotherapy
- Recurrent abortion
- Severe necroasthenozoospermia

Indications of FISH analysis



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



Conclusions

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- 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

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Información para profesionales
El próximo mes de octubre tendrá lugar en Zaragoza la I Reunión Nacional de Especialistas en Reproducción que organiza el Servicio de Fertilidad del Instituto Marqués.
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INSTITUTO MARQUÉS
Avda. de Ctra. de la Salud, 70, 21. 01014 BARCELONA
93.239.79.98. - 93.222.57.94
www.institutmarques.com

INSTITUTO MARQUÉS
Alfonso Ferrer, 33.
09004 ALICANTE
96.569.82.14. - 96.268.91.58
www.institutmarques.com

INSTITUTO MARQUÉS
Hospital Clínica, 10.
01015 VALLADOLID
91.379.47.26. - 91.379.47.27
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