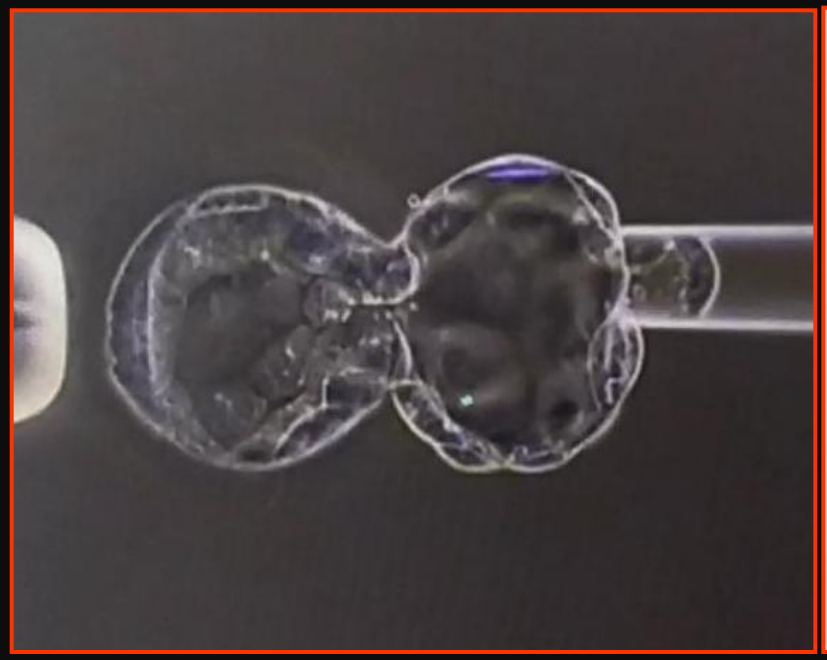
Ο Προεμφυτευτικός Έλεγχος στην Ανδρική Υπογονιμότητα



Κατερίνα Χατζημελετίου B.Sc, M.Sc, Ph.D
Αναπληρώτρια Καθηγήτρια Εμβρυολογίας- Γενετικής Α.Π.Θ

Υπεύθυνη Εργαστηρίου Εξωσωματικής Γονιμοποίησης
Α' Μαιευτική & Γυναικολογική Κλινική Α.Π.Θ
Γενικό Νοσοκομείο Θεσσαλονίκης 'Παπαγεωργίου'

SEMEN ANALYSIS

Volume
Sperm count
Motility
Morphology
pH
Liquefaction

According to WHO (Cooper et al., 2010)
Normal Volume ≥ 1.5ml
Normal count≥ 15 x10^6/ml
Linear Progression ≥ 32%
Normal Morphology ≥ 4%

Sperm DNA Fragmentation
Sperm Molecular Cytogenetic Analysis
Karyotype
Y chromosome microdeletions
CFTR mutations

Male infertility and sperm aneuploidy

1-2% of infertile males have Obstructive Azoospermia.

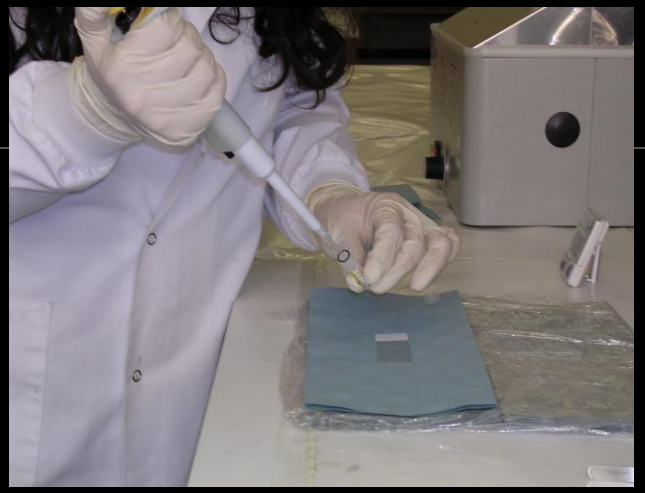
90% of cases are due to CFTR mutations. There are more than 1500 CFTR mutations worldwide ……. >100 identified in the Greek population.

In general the lower the sperm count…… the higher the incidence of sperm aneuploidy.

Men with $<5x10^6$/ml are 10x more likely to have increased sperm aneuploidy.

Men with NOA are at a high risk of having sperm with increased aneuploidy (especially of sex chromosomes). *Clementini et al 2005 & Vincent et al 2002.*

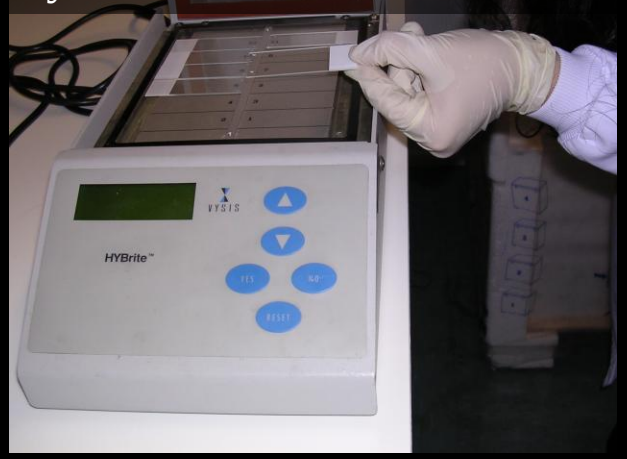
Molecular cytogenetic analysis by FISH



Addition of
Fluorescent Probe

Placement of
Coverslip

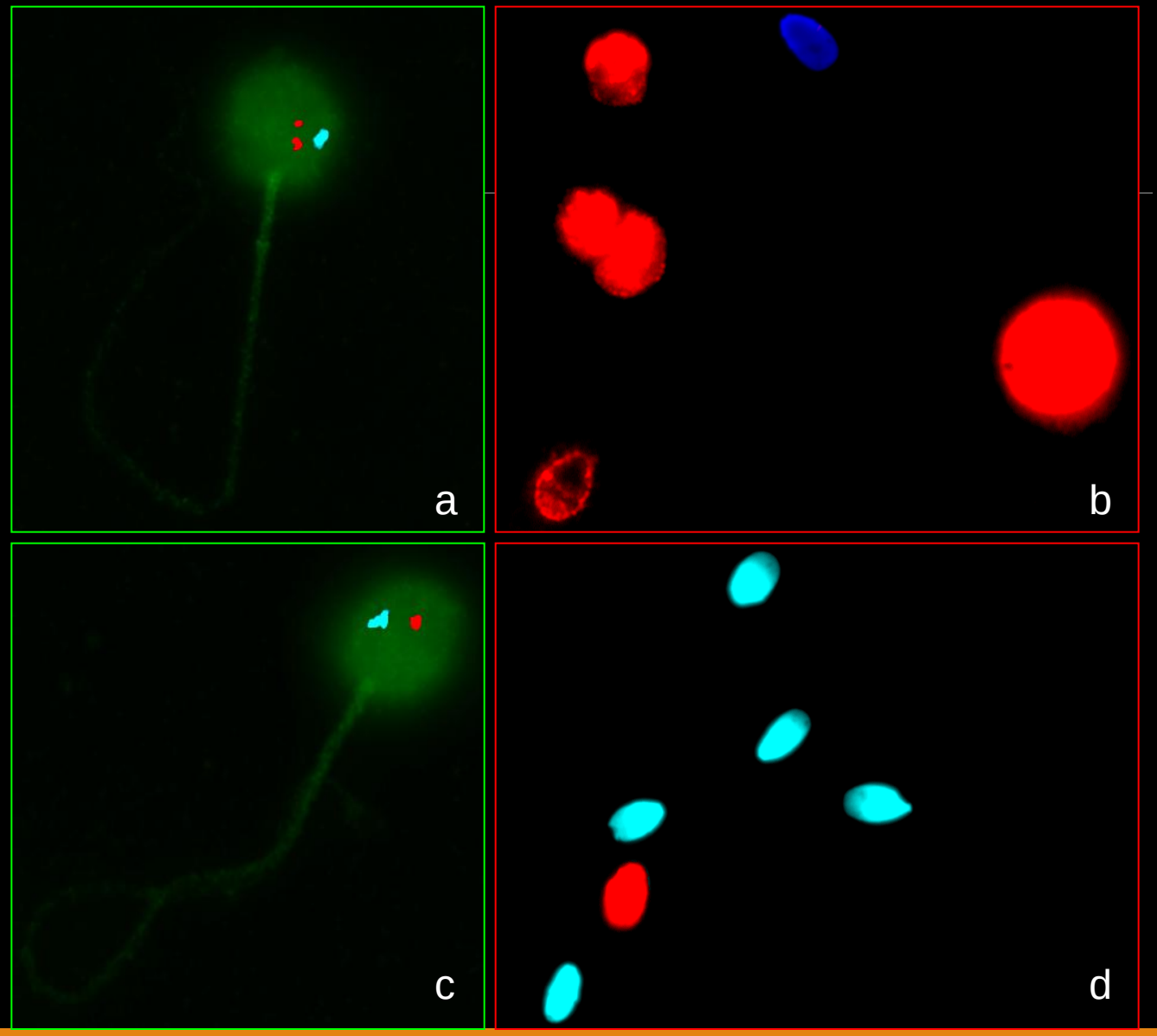
DNA Denaturation at 75°C
Hybridisation at 37°C



Washes

Fluorescence Microscopy

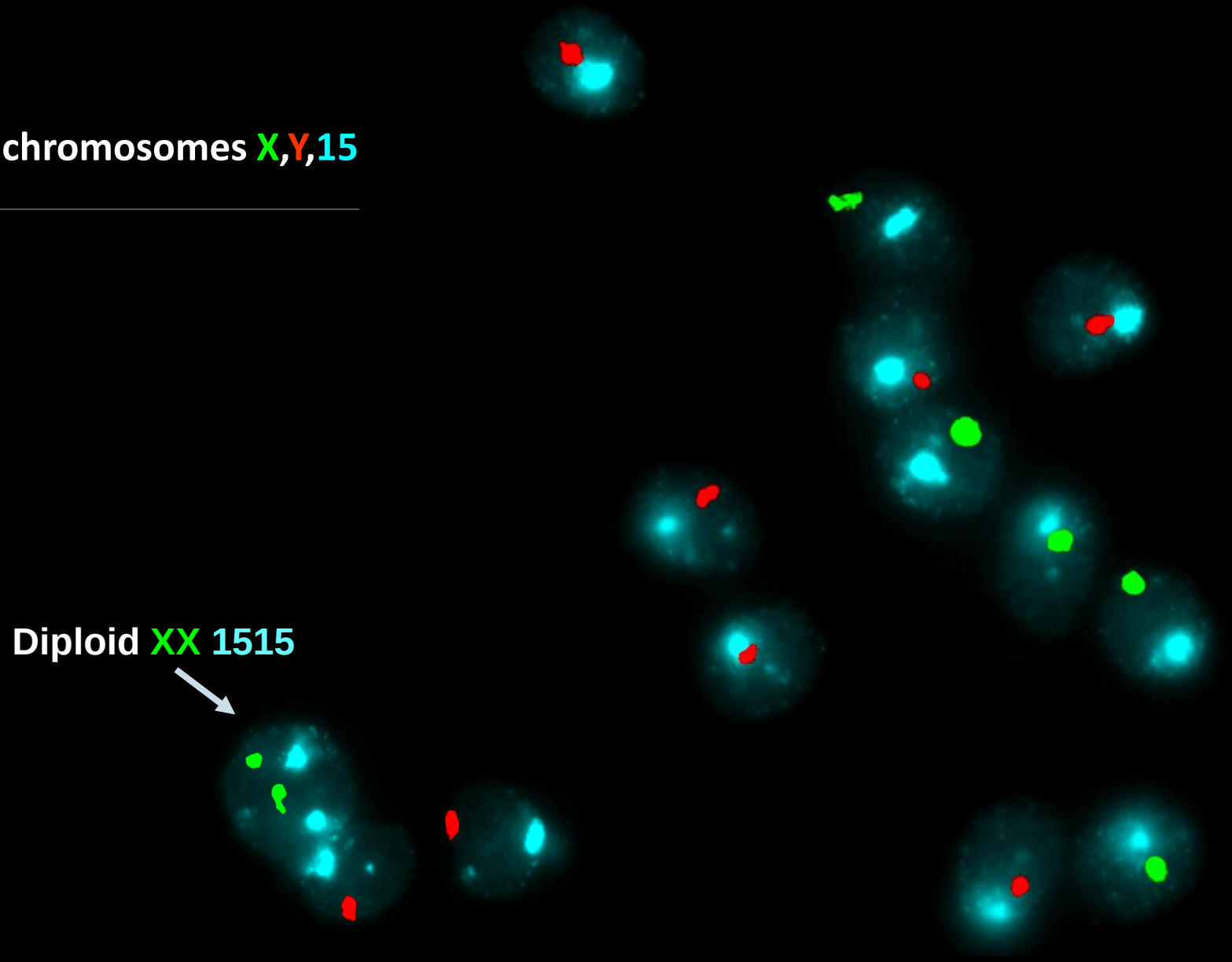
Molecular cytogenetic analysis by FISH and DFI by Tunnel



Photomicrographs showing sperm analysis by FISH and TUNNEL in a patient with OAT and RIF (a-b) and a patient with normal sperm parameters (c-d). (a,c) sperm hybridised with a probe for chromosomes X-green, Y-red, 18-aqua showing disomy for chromosome Y (a) and normal haploid chromosomal constitution (c). (b, d) Photomicrographs showing Tunel labeled sperm. Note that in (b) there is only one normal spermatozoon (blue) while all the remaining are fragmented (red). In contrast, all the sperm except one (red) are normal in (d).

Molecular cytogenetic analysis by FISH

Probe for chromosomes X,Y,15



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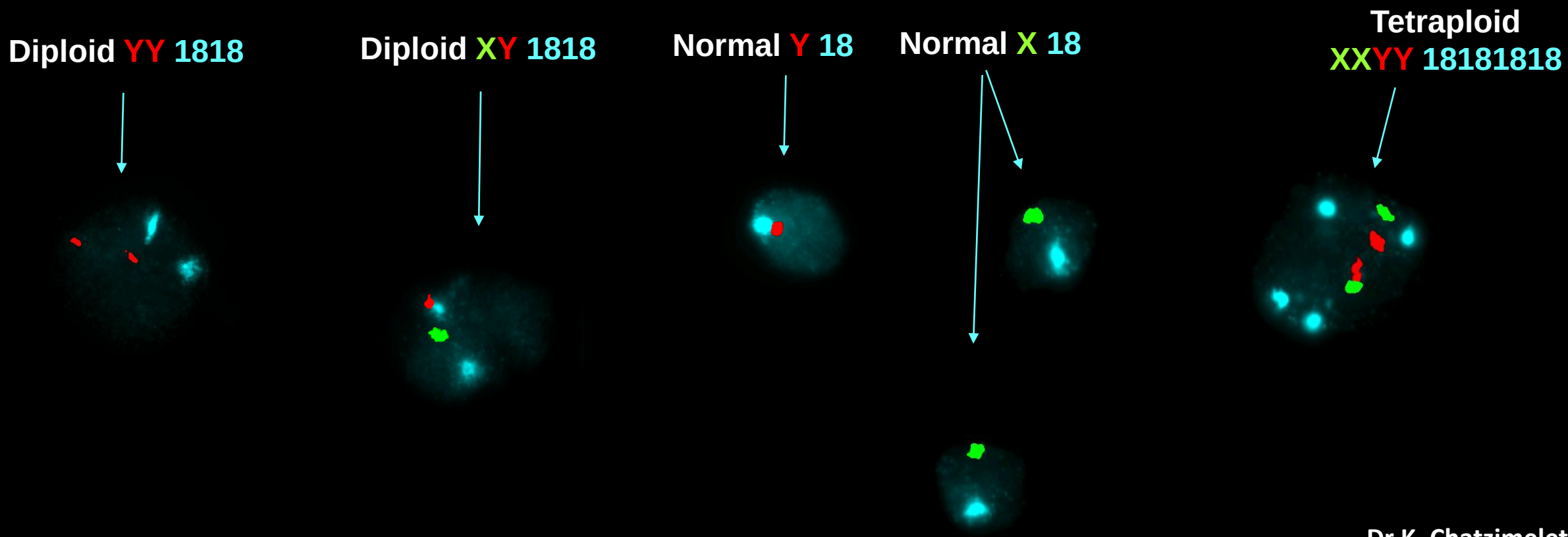
Molecular cytogenetic analysis by FISH using a triple-colour probe for chromosomes X,Y,18



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Hydatidiform Moles

Pregnancies characterized by abnormal development of extraembryonic and embryonic tissues. Subclassified on the basis of ploidy, histopathology and clinical features as complete or partial.

Complete hydatidiform moles	Partial hydatidiform moles (HM)
No foetal tissues Focal trophoblastic hyperplasia with villous hydrops	Identifiable foetal tissues Focal trophoblastic hyperplasia with villous hydrops
Diploid (46 chromosomes) but entirely androgenetic	triploid (69 chromosomes) 23 maternal + 46 paternal
They result from fertilisation of an inactivated egg by two spermatozoa, or more frequently by a single diploid sperm, or a haploid sperm which has undergone chromatid endoreduplication.	They arise usually from fertilization of a normal egg by two spermatozoa or by a diploid spermatozoon.

CASE REPORT

2430.e1 Fertility and Sterility® Vol. 95, No. 7, June 2011

Semen analysis by electron and fluorescence microscopy in a case of partial hydatidiform mole reveals a high incidence of abnormal morphology, diploidy, and tetraploidy

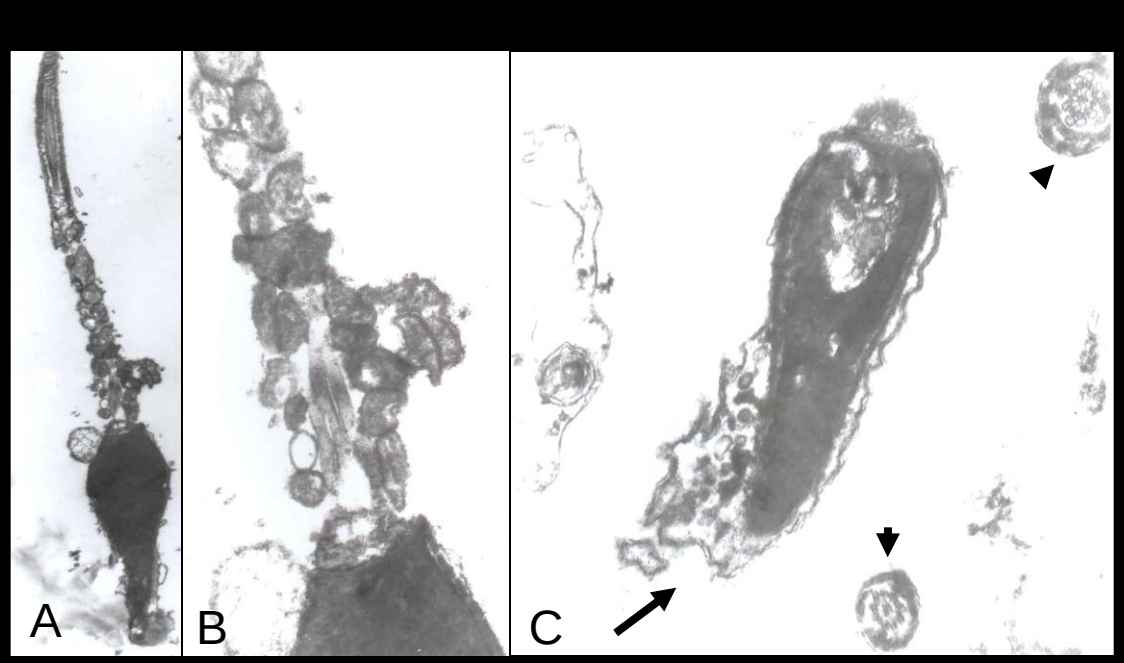
Katerina Chatzimeletiou, M.Sc., Ph.D.,[a] *Antonia Sioga, Ph.D.,*[b] *Louisa Oikonomou, M.D., Ph.D.,*[b] *Sophia Charalampidou, M.D.,*[c] *Persa Kantartzi, M.D.,*[a] *Vasiliki Zournatzi, M.D., Ph.D.,*[c] *Dimitrios Panidis, M.D., Ph.D.,*[c] *Dimitrios G. Goulis, M.D., Ph.D.,*[a] *Ioannis Papadimas, M.D., Ph.D.,*[a] *and Basil C. Tarlatzis, M.D., Ph.D.*[a]

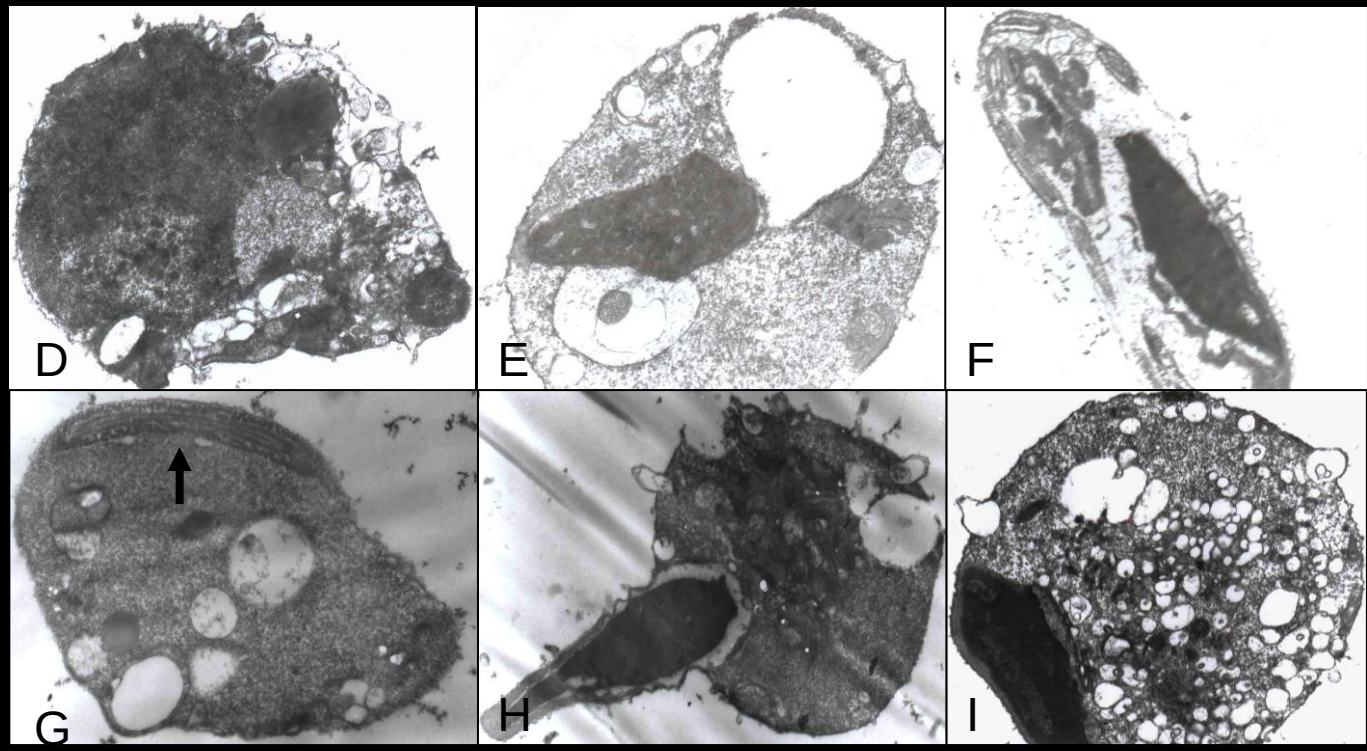
[a] Section of Reproductive Medicine, First Department of Obstetrics and Gynaecology, Aristotle University Medical School, Papageorgiou General Hospital; [b] Laboratory of Histology and Embryology, Aristotle University Medical School; and [c] Unit of Endocrinology and Human Reproduction, Second Department of Obstetrics and Gynaecology, Aristotle University Medical School, Hippokration General Hospital, Thessaloniki, Greece

Standard semen analysis.

Measurement	Value
Volume	3 mL
pH	8.5
No.	61.37 × 10^6/mL
Total number	184.125/ejaculation
Motility, %	
Linear progression	31
No progression - tail moving	30
Immotile	39
Morphology	
Normal, %	4
Abnormal, %	96
Big head	3
Small head	3
Long head	4
Pear-shaped head	27
Round head	1
Amorphous head	18
Vacuoles	36
Small acrosome	3
Short tail	1
Double tail	1
Fourchette	3
Broken tail	1
Spiral tail	9
Asymmetric tail extrusion	1
Broken neck	12
Cytoplasmic droplet	14
Thick midpiece	34
White blood cells	5%
Round spermatids	8%

SEMEN ANALYSIS BY TRANSMISSION ELECTRON MICROSCOPY



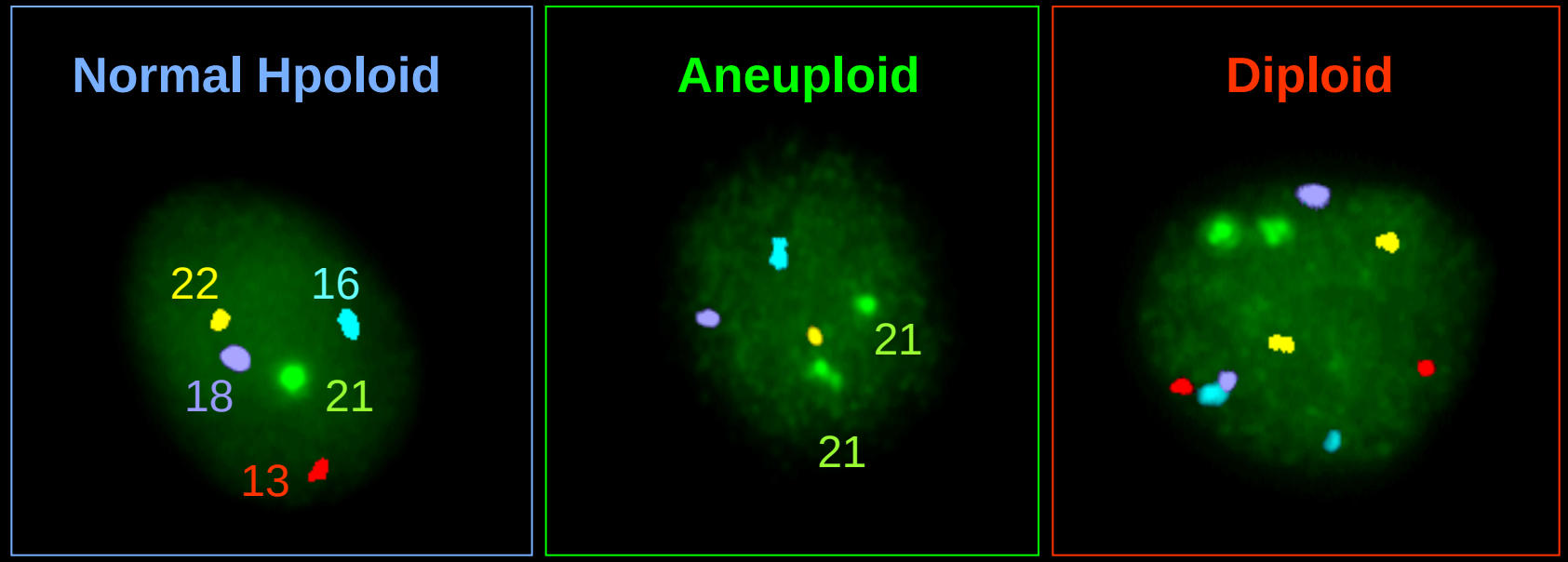


Chatzimeletiou et al 2011 Fertility and Sterility 95(7):2430.e1-5.

Incidence of chromosomal abnormalities in sperm assessed by FISH (total number of sperm analyzed in each probe set used = 400).

	Haploid, n (%)	Aneuploid, n (%)	Diploid, n (%)	Tetraploid, n (%)
Probe: Multivysion PB				
Chromosomes 13, 16, 18, 21, 22	373 (93.25)	5 (1.25)	21 (5.25)	1 (0.25)
Probe: CEPX/Y/18				
Chromosomes X, Y, 18	374 (93.5)	4 (1.0)	6 (XY), 14 (XX,YY) (5.0)	2 (0.5)
Probe: CEPX/Y/15				
Chromosomes X, Y, 15	375 (93.75)	2 (0.5)	7 (XY), 15 (XX, YY) (5.5)	1 (0.25)

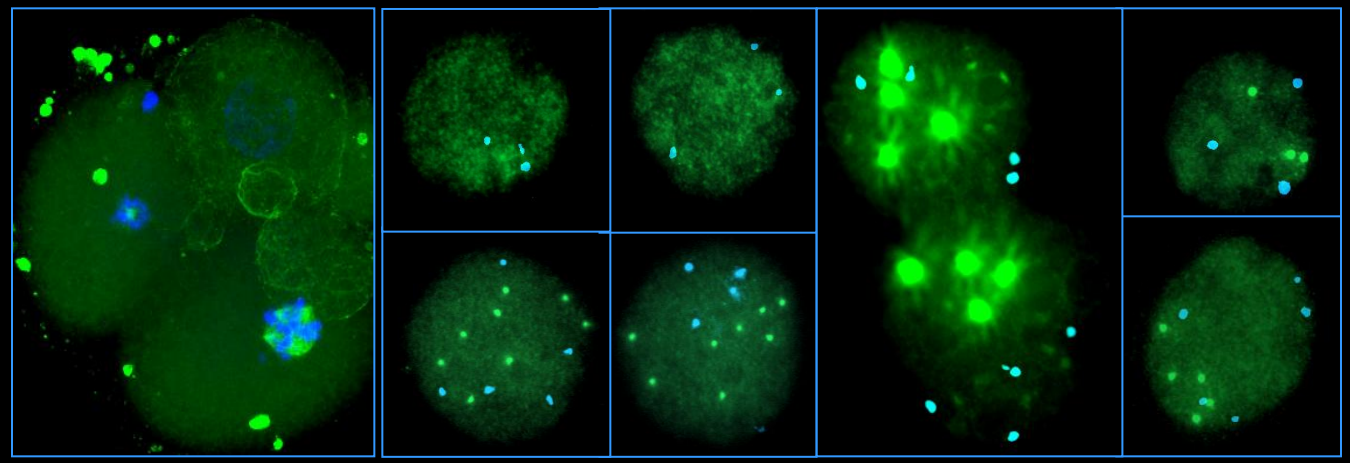
Chatzimeletiou. Semen analysis in a case of partial mole. Fertil Steril 2011.



Chatzimeletiou et al 2011 Fertility and Sterility 95(7):2430.e1-5.

Indications for Preimplantation Genetic Testing for aneuploidy (PGT-A) due to male factor

1. Previous history of aneuploid pregnancies due to increased sperm aneuploidy
2. Previous history of molar pregnancies due to increased sperm diploidy
3. Some cases of severe male infertility (OAT)

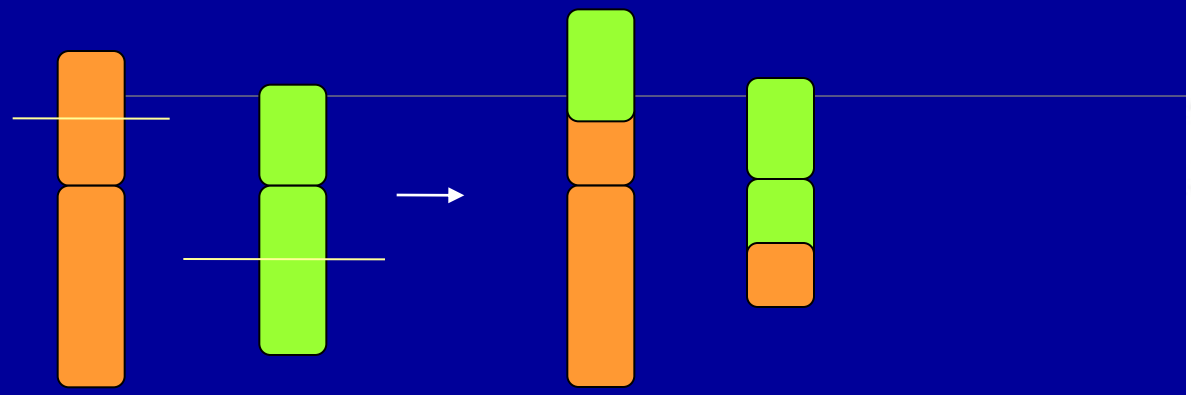


Chatzimeletiou et al., 2007 Zygote 15: 81-90
Chatzimeletiou et al., 2005 Hum. Reprod. 20 (3): 672-682

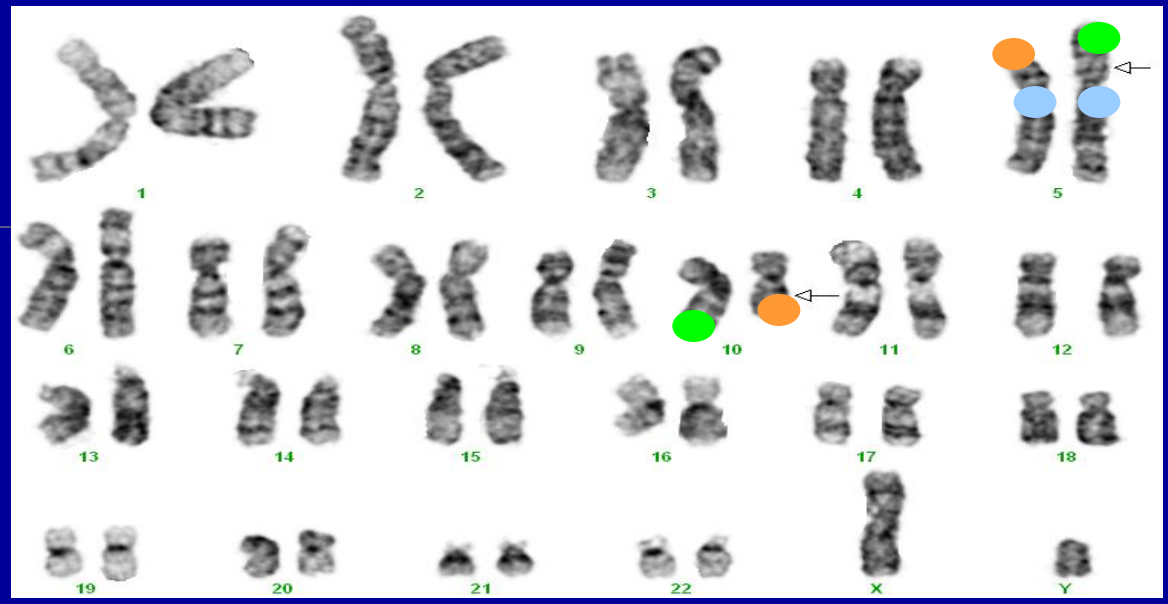
4. Males with Robertsonian or Reciprocal Translocations
5. Males with XXY or XYY Karyotype

Balanced translocations

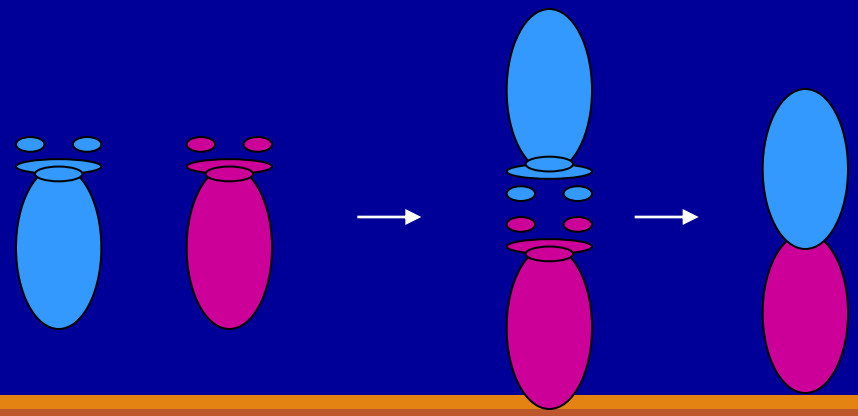
Reciprocal translocations 1:600 livebirths



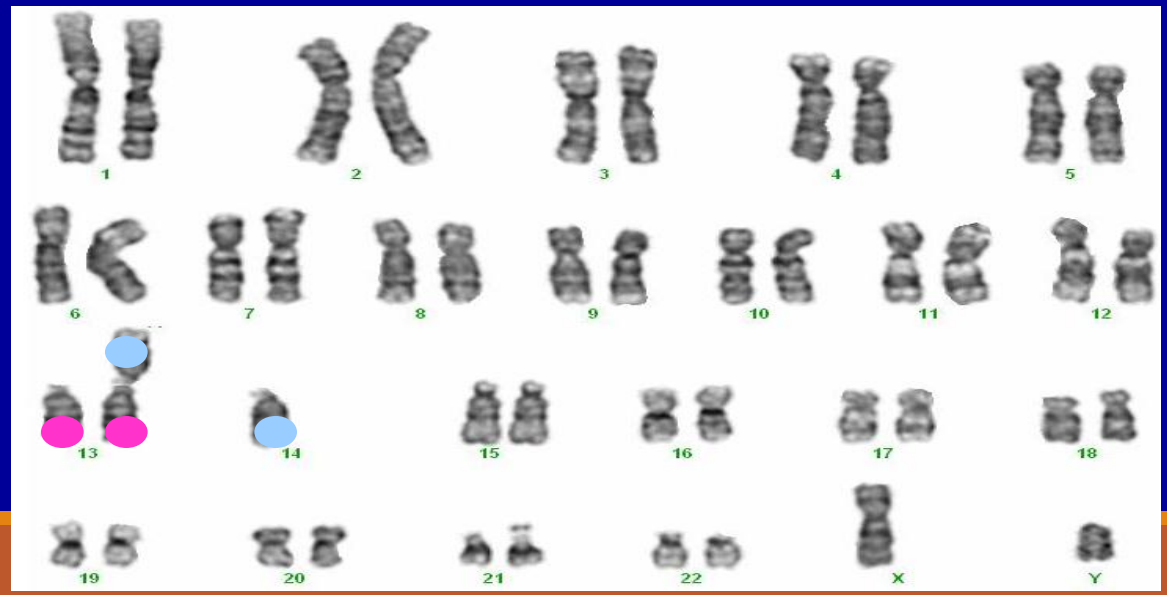
46,XY,t(5;10)(p14.2;q22.1)



Robertsonian translocations 1:1000 livebirths



45,XY der (13;14)(q10;q10)

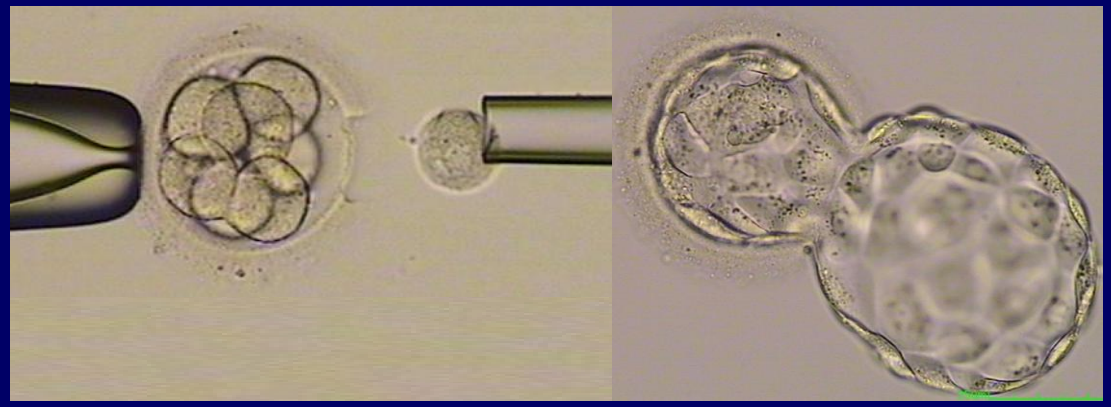


Preimplantation Genetic Testing for aneuploidy (PGT-A) due to male factor

Day 3 Embryo Biopsy

Embryo biopsied on day 3

Fresh Embryo Transfer on day 5

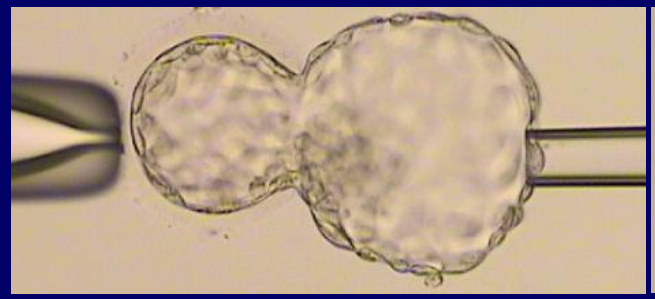


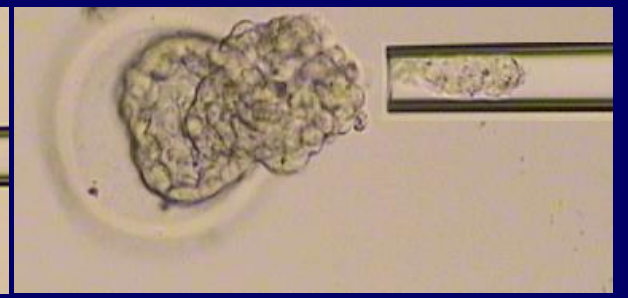
K. Chatzimeletiou Biogenesis IVF Unit

Day 5 Blastocyst Biopsy

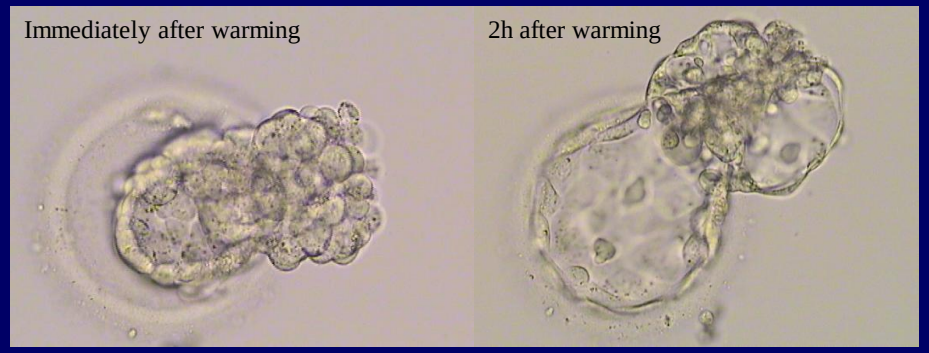
Embryo biopsied on day 5

Vitrification on day 5





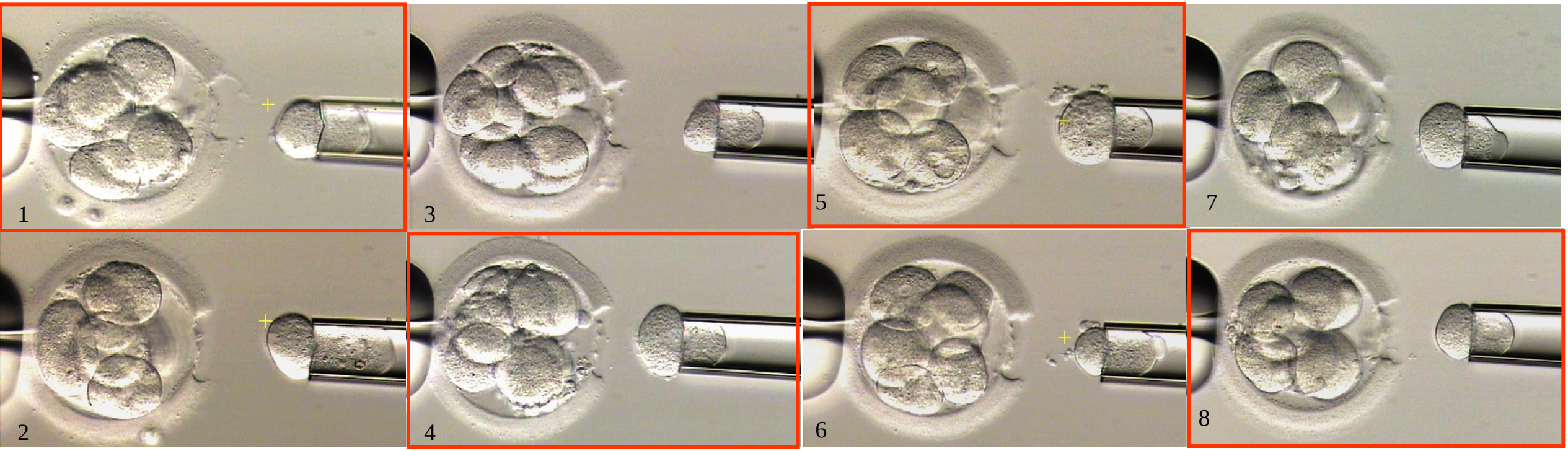
FET



K. Chatzimeletiou Biogenesis IVF Unit

Preimplantation Genetic Testing for aneuploidy (PGT-A)

For the indication of Paternal Robertsonian Translocation 45,XY der (13;14)(q10;q10)



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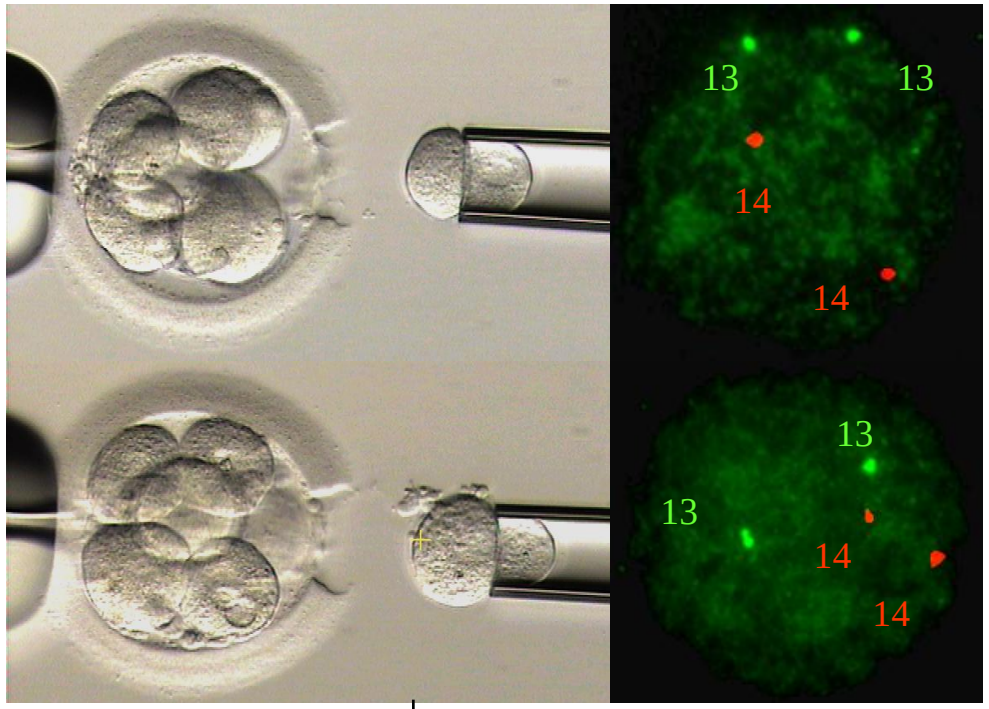
For the indication of Paternal Robertsonian Translocation 45,XY der (13;14)(q10;q10)

2 normal embryos were transferred



Twin Pregnancy

Dr K. Chatzimeletiou

For the indication of Paternal Reciprocal Translocation 46,XY t (3;16)(p28.2;p26.4)

ΓΕΝΙΚΟ ΠΕΡΙΦΕΡΕΙΑΚΟ ΝΟΣΟΚΟΜΕΙΟ ΠΑΠΑΓΕΩΡΓΙΟΥ

ΝΟΣΟΚΟΜΕΙΟ ΠΑΠΑΓΕΩΡΓΙΟΥ

ΕΡΓΑΣΤΗΡΙΟ ΠΡΟΕΜΦΥΤΕΥΤΙΚΗΣ ΓΕΝΕΤΙΚΗΣ ΔΙΑΓΝΩΣΗΣ
ΜΟΝΑΔΑ ΑΝΘΡΩΠΙΝΗΣ ΑΝΑΠΑΡΑΓΩΓΗΣ
Α' ΜΑΙΕΥΤΙΚΗ & ΓΥΝΑΙΚΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ Α.Π.Θ.
Διευθυντής Κλινικής: Β. Κ. Ταρλατζής Υπεύθυνη Εργαστηρίου: Κ. Χατζημελετίου

Τηλ. 231332 3827, 231332 3377

Προεμφυτευτική Γενετική Διάγνωση

Όνοματεπώνυμο ♀: [redacted]		No♀: [redacted]
ΗΩ: [redacted]	Ηλικία♀: 33	Ένδειξη: 46,XY t(3 ; 16)(p ; p)

Ανιχνευτές: CEP 16 (aqua), Τελομερικά 16p (green) , 3p (orange) (Abbott, Cytocell)

Ωάρια : 5 MII Ωάρια : 5 2pn Ζυγωτά : 3 Διαιρούμενα έμβρυα: 3

Τρία (3) φυσιολογικά γονιμοποιημένα (2pn) έμβρυα, διαιρέθηκαν και υποβλήθηκαν σε βιοψία την ημέρα 3 μετά τη γονιμοποίηση, στο στάδιο των 8 κυττάρων (ε2 grade 2.1, ε4 grade 2.2, ε5 grade 2.2).
Ένα βλαστομερίδιο απομονώθηκε απο κάθε έμβρυο, και πυρήνες ανιχνεύτηκαν σε όλα τα βλαστομερίδια.
Τα αποτελέσματα της μοριακής κυτταρογενετικής ανάλυσης έδειξαν ότι :
Το έμβρυο 2 ήταν ισοζυγισμένο (2x3p, 2x16cep, 2x16p).
Το έμβρυο 4 ήταν ανισόζυγο/ανευπλοειδές (2x3p, 3x16cep, 3x16p).
Το έμβρυο 5 ήταν ισοζυγισμένο (2x3p, 2x16cep, 2x16p).

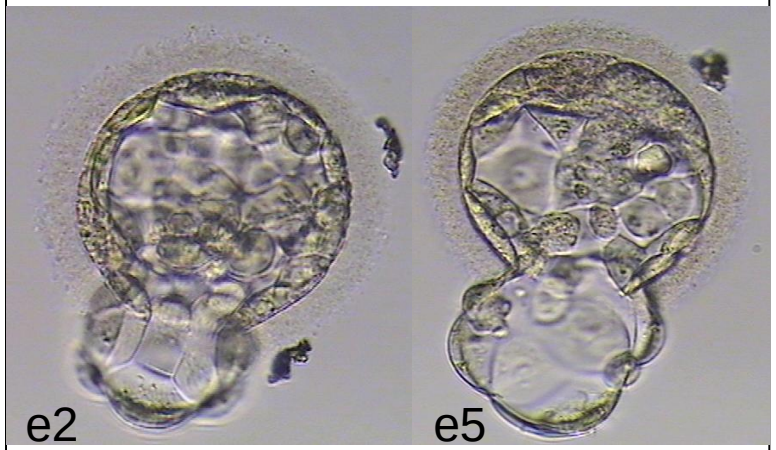
Προτείνονται τα έμβρυα 2 και 5 για μεταφορά στη μήτρα.

Έμβρυο No	ΧΡΩΜΟΣΩΜΙΚΗ ΚΑΤΑΣΤΑΣΗ (3p, 16cep, 16p)	ΕΜΒΡΥΟΜΕΤΑΦΟΡΑ	
2	Ισοζυγισμένο	ΝΑΙ	
4	Ανισόζυγο	ΟΧΙ	
5	Ισοζυγισμένο	ΝΑΙ	

Εμβρυολόγος- Γενετιστής: Κατερίνα Χατζημελετίου B.Sc. (Hons), M.Sc., Ph.D.

2/11/2012

Development of the 2 normal embryos transferred



Twin Girls born
free of translocation

Summary of PGT-A results for male factor at Papageorgiou General Hospital

Total No of eggs collected	229
No of MII eggs	188
No of 2PN zygotes	138
No of embryos biopsied	103
No of embryos analysed	100
No of normal embryos	41
No of abnormal embryos	59
+ve hCG/ ET	73.3%
Clinical pregnancy rate/ ET	60.0%

Next generation sequencing (NGS) for preimplantation genetic screening (PGS) improves pregnancy outcomes compared with array comparative genomic hybridization in single thawed euploid embryo transfer (STEET) cycles

Friedenthal et al (2018) Fertil Steril. 109(4):627-632

548 STEET cycles using NGS vs 368 STEET cycles using array-CGH

The implantation rate was significantly higher in the NGS group compared with the aCGH group (71.6% vs. 64.6%). The OP/LBR was also significantly higher in the NGS group (62% vs. 54.4%), and there were significantly more biochemical pregnancies in the aCGH group compared with the NGS group (15.1% vs. 8.7%).

ΣΥΜΠΕΡΑΣΜΑΤΑ

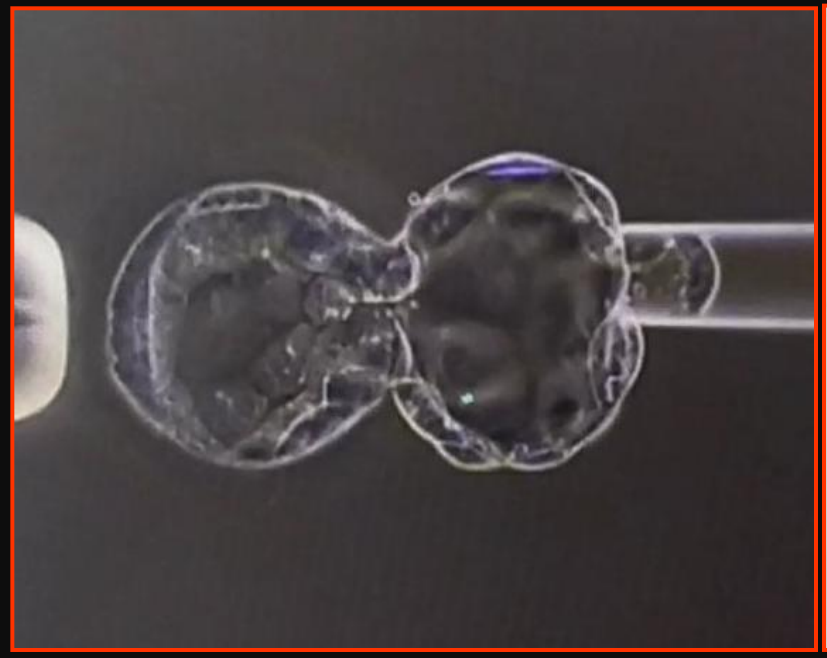
Ο Προεμφυτευτικός Γενετικός Έλεγχος χρησιμοποιείται σε συγκεκριμένες περιπτώσεις ανδρικού παράγοντα υπογονιμότητας:

1. Αυξημένη Ανευπλοειδία ή/και διπλοειδία στο σπέρμα
2. ΟΑΤ
3. Καρυότυπο με Robertsonian και Reciprocal Μεταθέσεις
4. Καρυότυπο με XXY και XYY

ΣΥΜΠΕΡΑΣΜΑΤΑ

Ο Προεμφυτευτικός Γενετικός Έλεγχος συγκαταλέγεται σήμερα στις σημαντικότερες τεχνολογικές εξελίξεις στον τομέα της ανθρώπινης αναπαραγωγής συμβάλλοντας ουσιαστικά στην πρόληψη των γενετικών και χρωμοσωμικών ασθενειών και στη γέννηση νεογνών απαλλαγμένων από χρωμοσωμικές ανωμαλίες και από το βαρύ κληρονομικό ιστορικό που φέρουν οι γονείς τους.

ΣΑΣ ΕΥΧΑΡΙΣΤΩ !



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