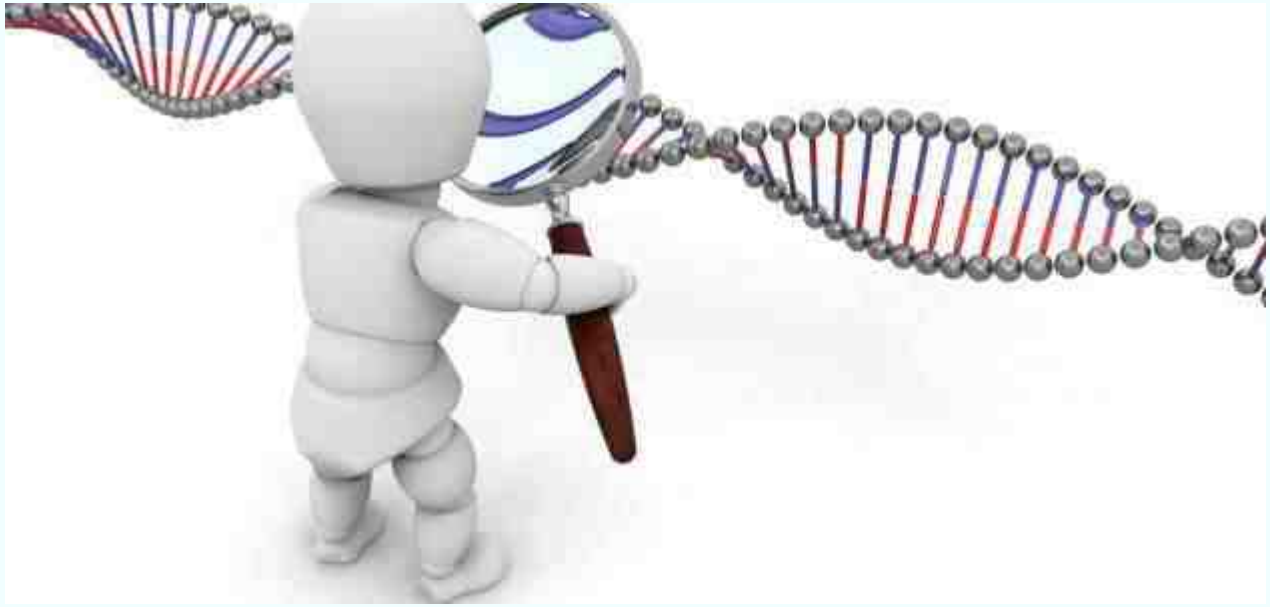
New Technologies in Genetics Diagnosis



Joep Geraedts

Maastricht, The Netherlands

Antalya

Clinical genomics

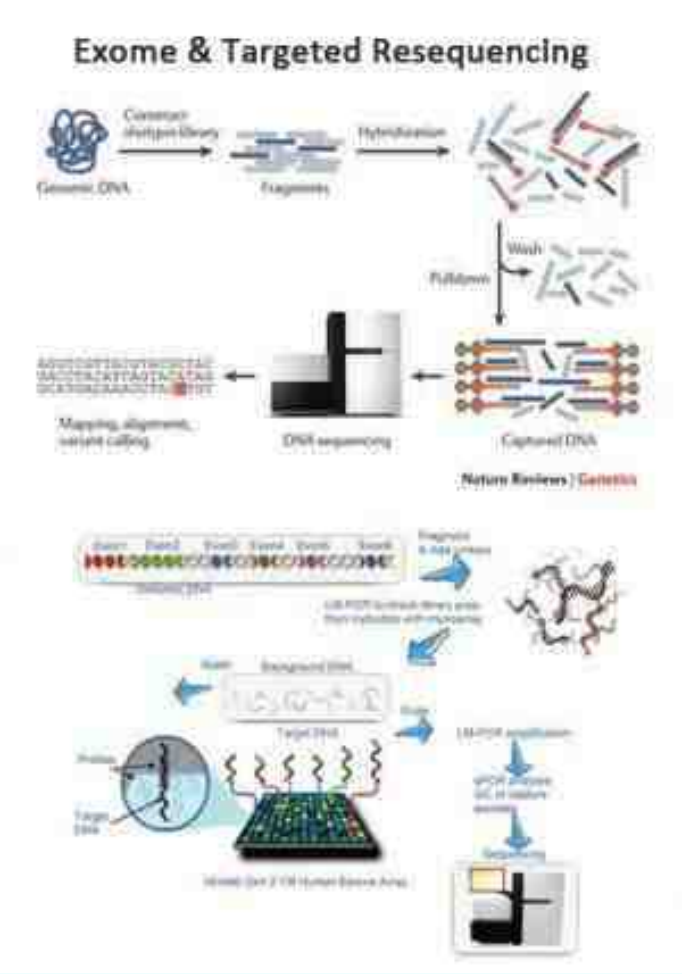
- Genome sequencing to diagnose inherited disease
- Oncogenomics: stratifying cancer for better treatment
- Pharmacogenomics: predicting drug response and reducing adverse drug reactions

Clinical genomics

- Genome sequencing to diagnose inherited disease
- Oncogenomics: stratifying cancer for better treatment
- Pharmacogenomics: predicting drug response and reducing adverse drug reactions

EXOME SEQUENCING

- Next Generation Sequencing (NGS) allows to study all exons of all (~20.000) genes of a patient in one test.
- For a number of heterogeneous disorders a disease-focused gene panel is analysed first.
- If no mutation is found within the disease-focused gene panels the whole exome will be analysed.
- This targeted next-generation sequencing (NGS) approach is comprehensive, rapid, and cost-effective.

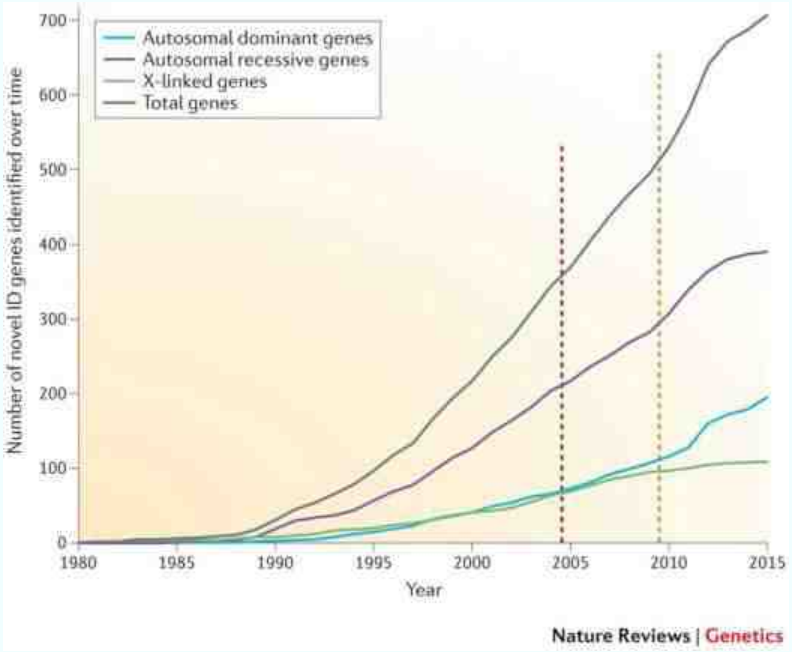


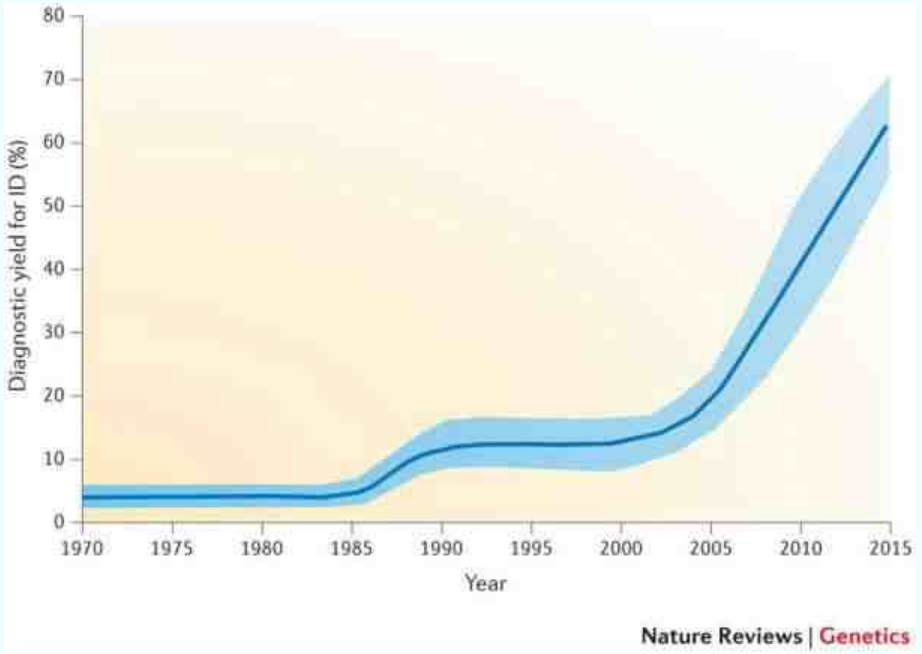
Disease-focused gene panels for exome sequencing

- Ciliopathies
- Cardiac diseases
- Craniofacial abnormalities
- Deafness
- Disorders of sexual differentiation
- Epilepsy
- Hereditary cancer
- Hypogonadotropic hypogonadism
- Intellectual disabilities
- Iron metabolism disorder
- Kidney diseases
- Metabolic disorders
- Mitochondriële aandoeningen
- Movement disorders
- Multiple congenital abnormalities
- Neuromuscular diseases
- Primary immune deficiency disorders
- Skin disorders
- Visual disturbances

Genetic studies in intellectual disability and related disorders

Lisenka E. L. M. Vissers[1], Christian Gilissen[1] and Joris A. Veltman[1,2]





Original Article

X-Linked *TEX11* Mutations, Meiotic Arrest, and Azoospermia in Infertile Men

Alexander N. Yatsenko, M.D., Ph.D., Andrew P. Georgiadis, B.A., Albrecht Röpke, Ph.D., Andrea J. Berman, Ph.D., Thomas Jaffe, M.D., Marta Olszewska, Ph.D., Birgit Westernströer, Ph.D., Joseph Sanfilippo, M.D., Maciej Kurpisz, M.D., Ph.D., Aleksandar Rajkovic, M.D., Ph.D., Svetlana A. Yatsenko, M.D., Sabine Kliesch, M.D., Stefan Schlatt, Ph.D., and Frank Tüttelmann, M.D.

The NEW ENGLAND JOURNAL of MEDICINE

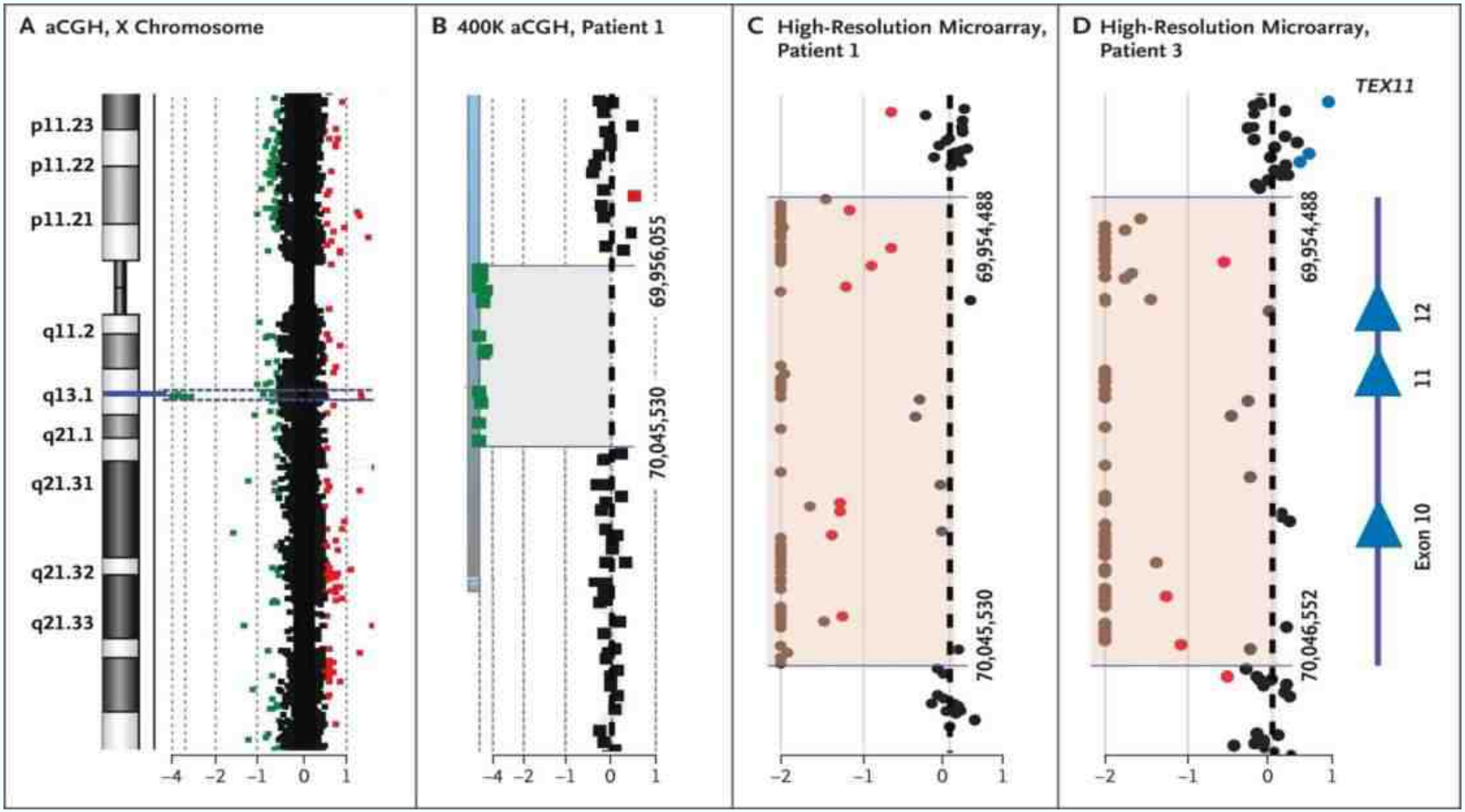
Study Overview

- Some genetic causes of male infertility have been identified, but most remain unknown.
- In this study, 7 of 289 men with azoospermia (2.4%) harbored a mutation in *TEX11,* a gene expressed in the testes that is critical to chromosomal recombination.

Volume 372(22):2097-2107

May 28, 2015

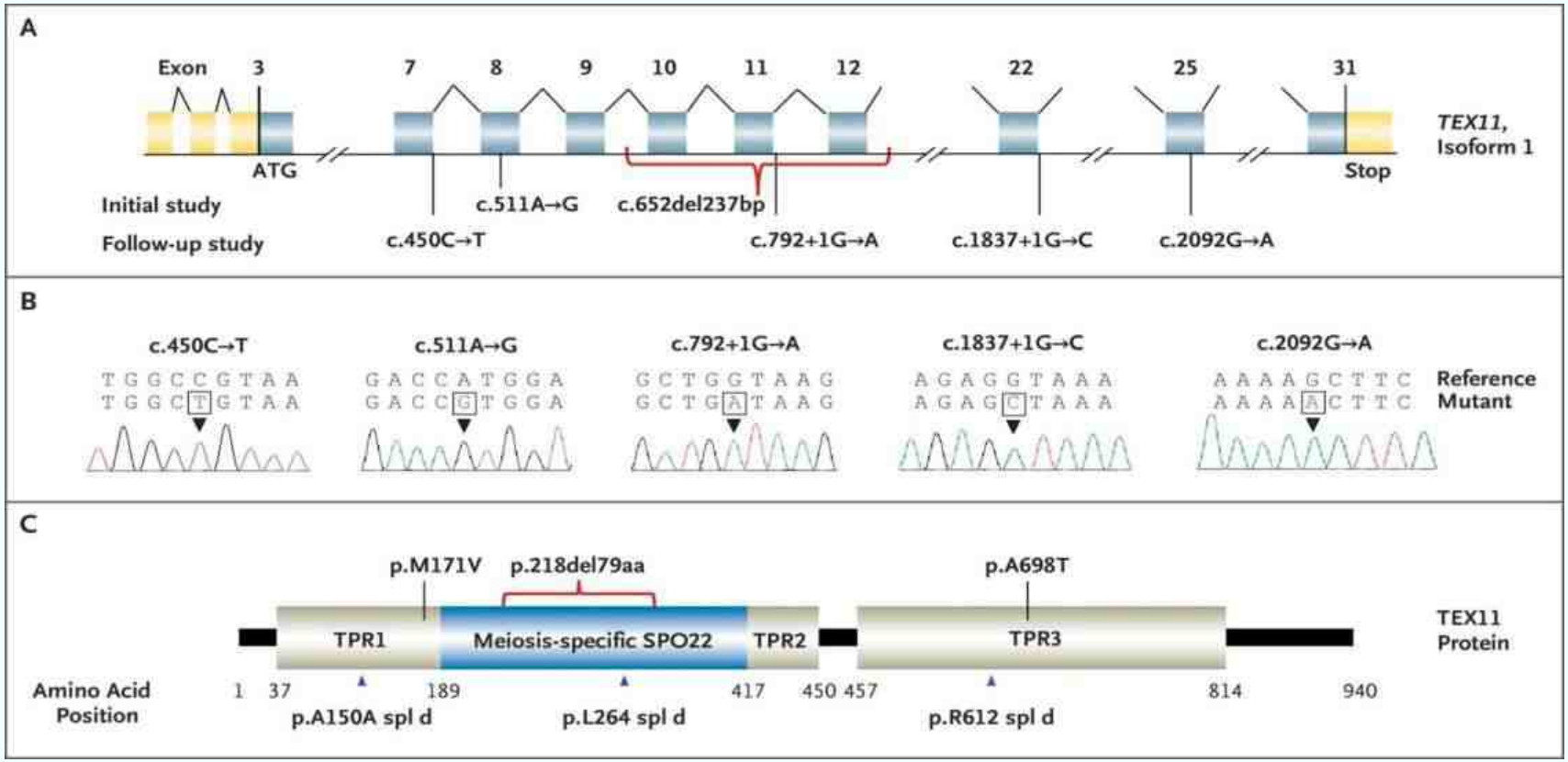
Hemizygous Deletion of *TEX11* Exons 10 to 12 and Flanking Intronic Regions in Two Men with Azoospermia.



Yatsenko AN et al. N Engl J Med 2015;372:2097-2107

The NEW ENGLAND JOURNAL of MEDICINE

TEX11 Mutations Detected in Men with Azoospermia.



Yatsenko AN et al. N Engl J Med 2015;372:2097-2107

The NEW ENGLAND JOURNAL of MEDICINE

Incidence and Frequency of the *TEX11* Mutation, According to Cohort and Phenotype of the Study Population.

Table 3. Incidence and Frequency of the *TEX11* Mutation, According to Cohort and Phenotype of the Study Population.

Cohort	Phenotype			All Men with Azoospermia	All Men with Normozoospermia
	Meiotic Arrest	Mixed Testicular Atrophy	Sertoli Cell–Only		
	no. of patients/total no. (%)			*no./total no. (%)*	
Initial study cohort	2/19 (11)	1/21 (4.8)	0/9	3/49 (6.1)	0/192
Follow-up study cohort	3/14 (21)	1/172 (0.6)	0/54	4/240 (1.7)	0/192
Total	5/33 (15)	2/193 (1.0)	0/63	7/289 (2.4)	0/384

Yatsenko AN et al. N Engl J Med 2015;372:2097-2107

PGD: Celebration first published cases: 25 years

Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification

A. H. Handyside, E. H. Kontogianni, K. Hardy & R. M. L. Winston

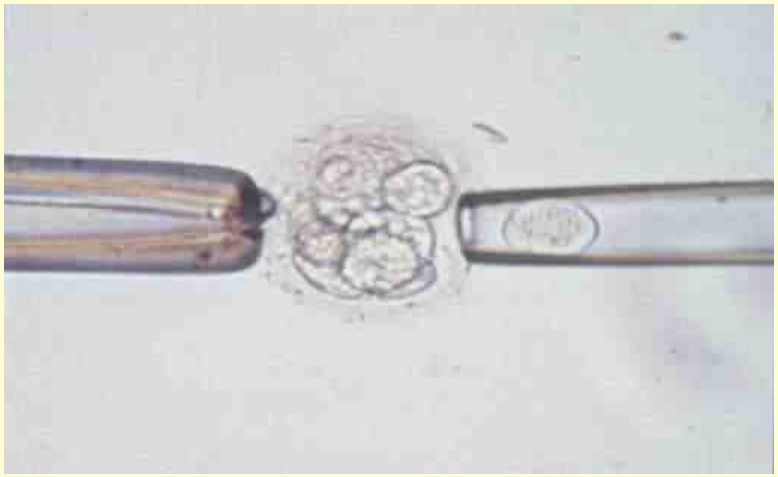
Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

...inked diseases, typically ...een identified. In many of ...y chorion villus sampling ...togenetic, biochemical or ...ed from the conceptus. In ...ine the sex of the fetus. If ...is male, abortion can be ... preimplantation embryos ...ntified and transferred to

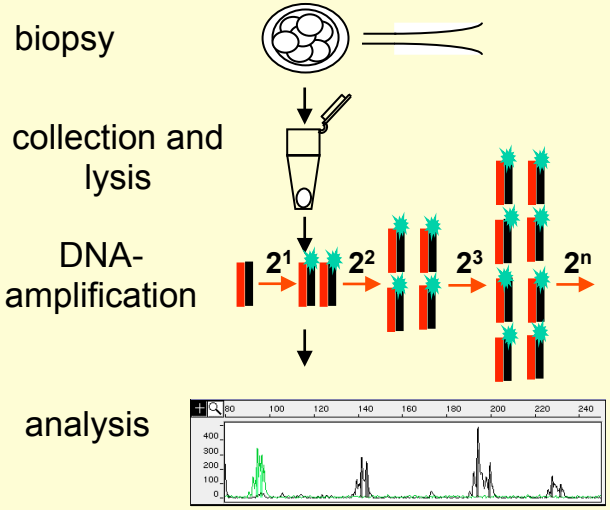
Hammersmith Hospital Londen

Prof. Alan Handyside

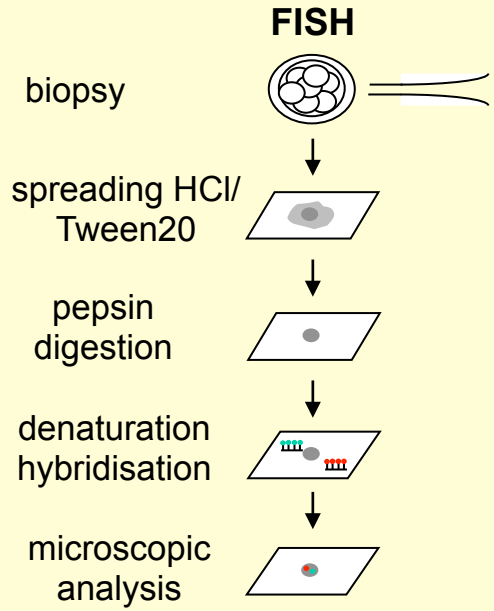
Diagnostic procedures



- **Sex determination (FISH)**
- **Structural chromosome abnormalities (FISH)**
- **Aneuploidy screening (FISH)**
- **Monogenic diseases (PCR)**
- **HLA typing (PCR)**



PCR procedure



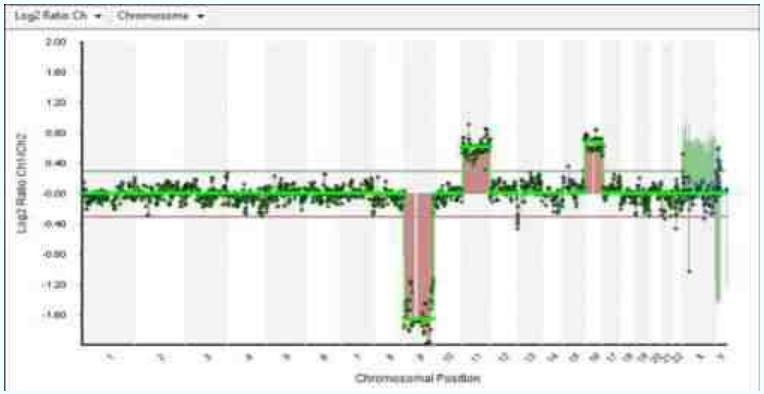
FISH procedure

Developments in genome analysis

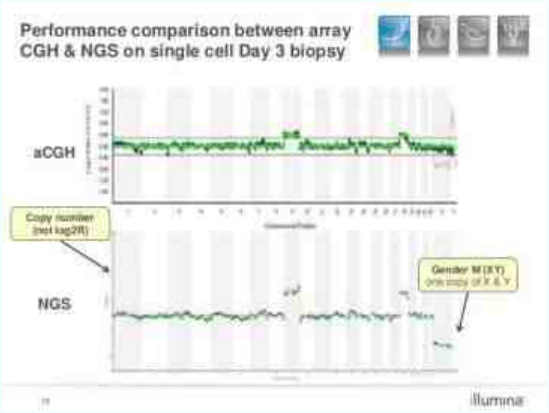
LOW

HIGH

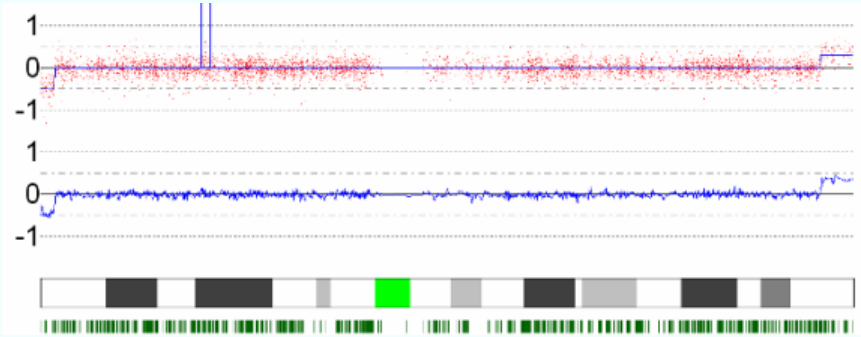
Array-CGH



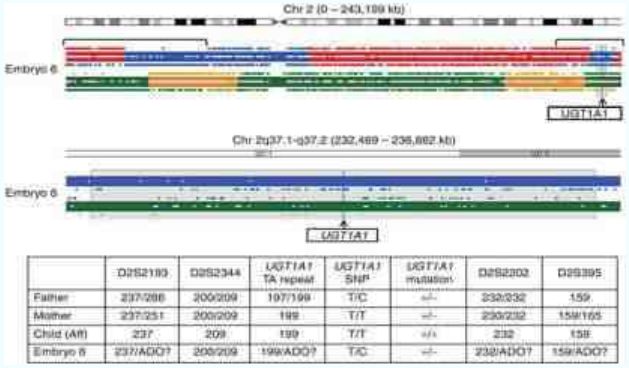
NGS



SNP-arrays

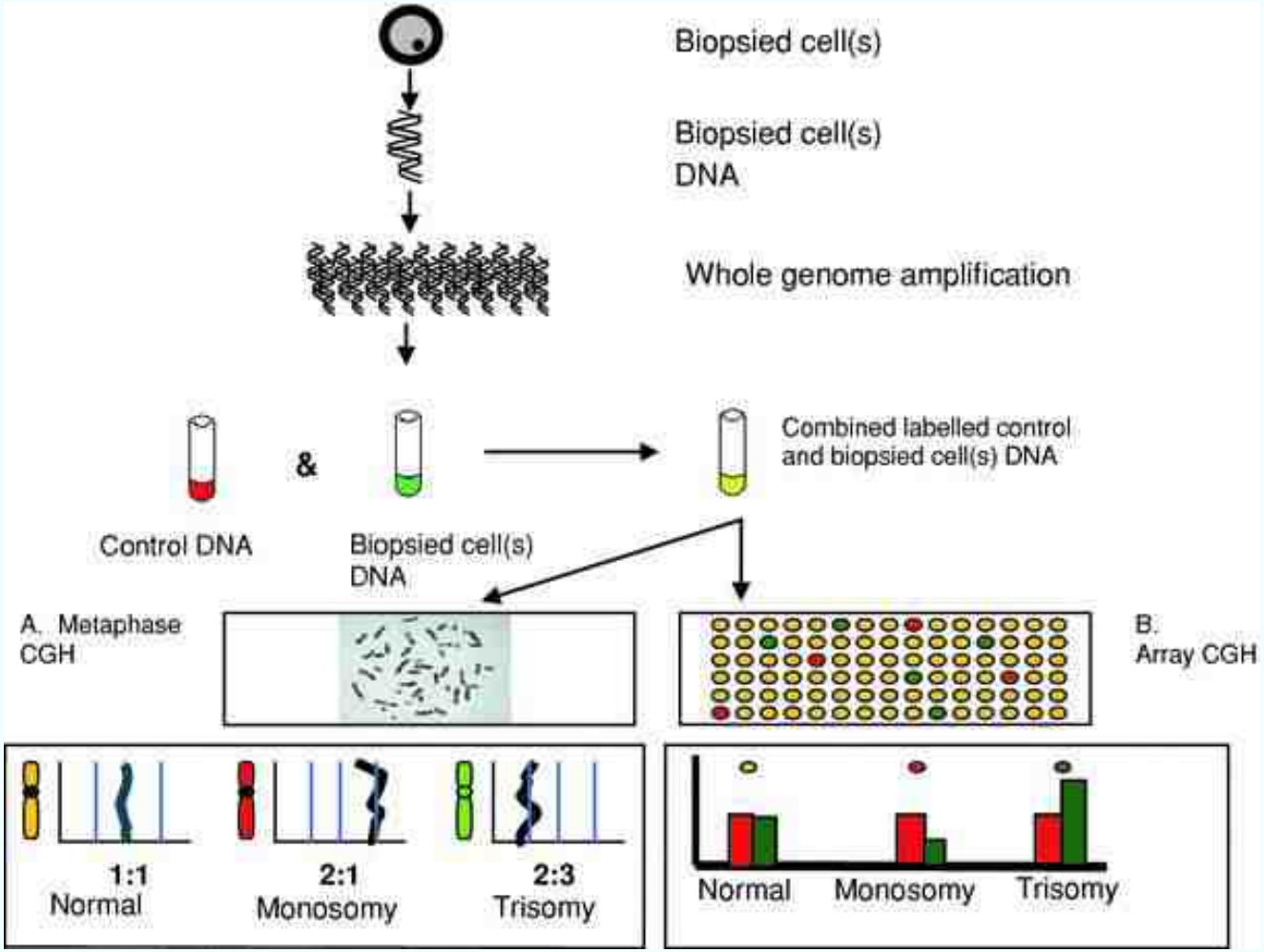


Karyomapping



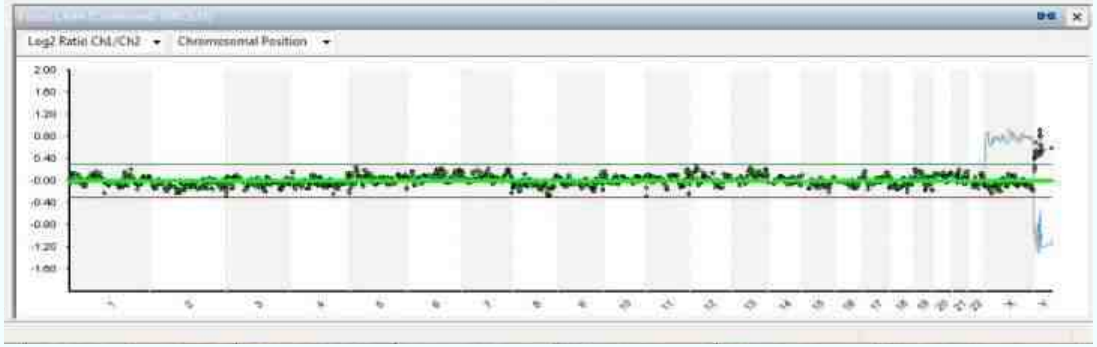
	D2S2180	D2S2344	UGT1A1 TA repeat	UGT1A1 SNP	UGT1A1 mutation	D2S2202	D2S395
Father	237/286	209/209	197/199	T/C	+/-	232/232	159
Mother	237/251	209/209	199	T/T	+/-	230/232	159/165
Child (Aff)	237	209	199	T/T	+/+	232	159
Embryo 6	237/ADO?	209/209	199/ADO?	T/C	+/-	232/ADO?	159/ADO?

Single cell CGH

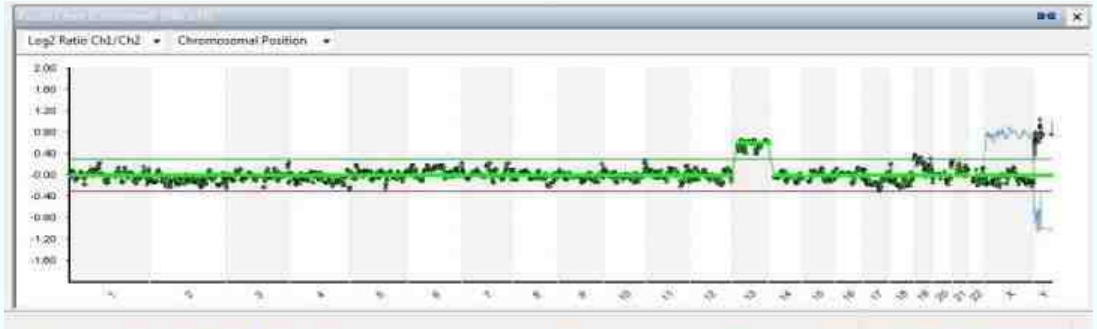


Harper and Harton, Fertil Steril, 2010

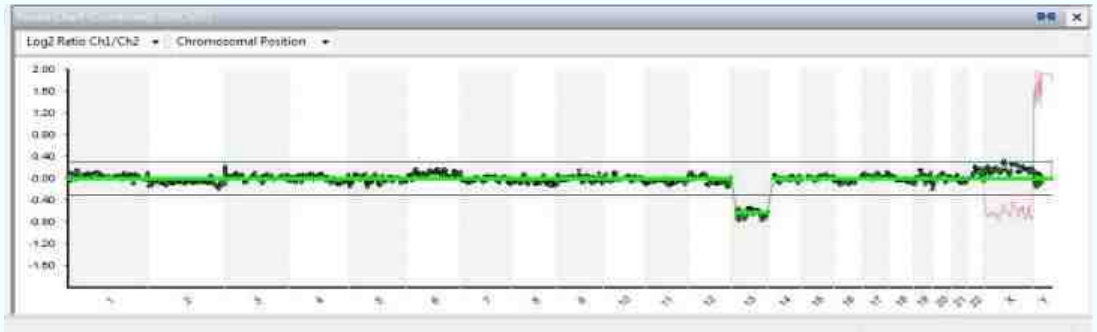
PB 1


Log2 Ratio Ch1/Ch2
Chromosomal Position

PB 2


Log2 Ratio Ch1/Ch2
Chromosomal Position

Day 3


Log2 Ratio Ch1/Ch2
Chromosomal Position

Reproductive BioMedicine Online (2015) 31, 394–403

ELSEVIER

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Live births following Karyomapping of human blastocysts: experience from clinical application of the method

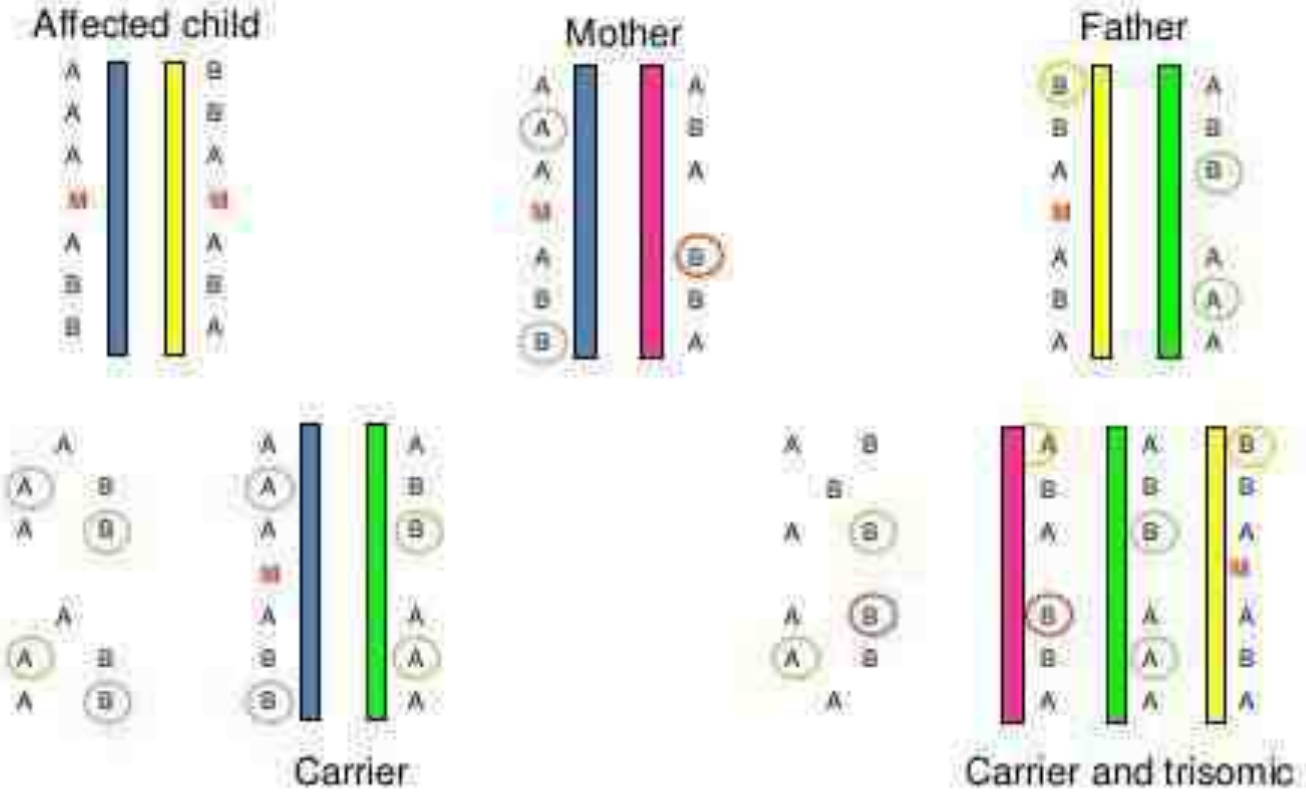
Michalis Konstantinidis [a,*,1], Renata Prates [a,1], N-Neka Goodall [a], Jill Fischer [a], Victoria Tecson [a], Tsion Lemma [a], Bo Chu [a], Amy Jordan [a], Erin Armenti [a], Dagan Wells [b,c], Santiago Munné [a]

Reducing work-up time for PGD of single gene disorders - Karyomapping

Reprogenetics

Thousands of polymorphisms on each chromosomes

Each chromosome (region) has a unique DNA fingerprint



Human Reproduction, Vol.29, No.12 pp. 2802–2813, 2014
Advanced Access publication on October 21, 2014 doi:10.1093/humrep/deu277

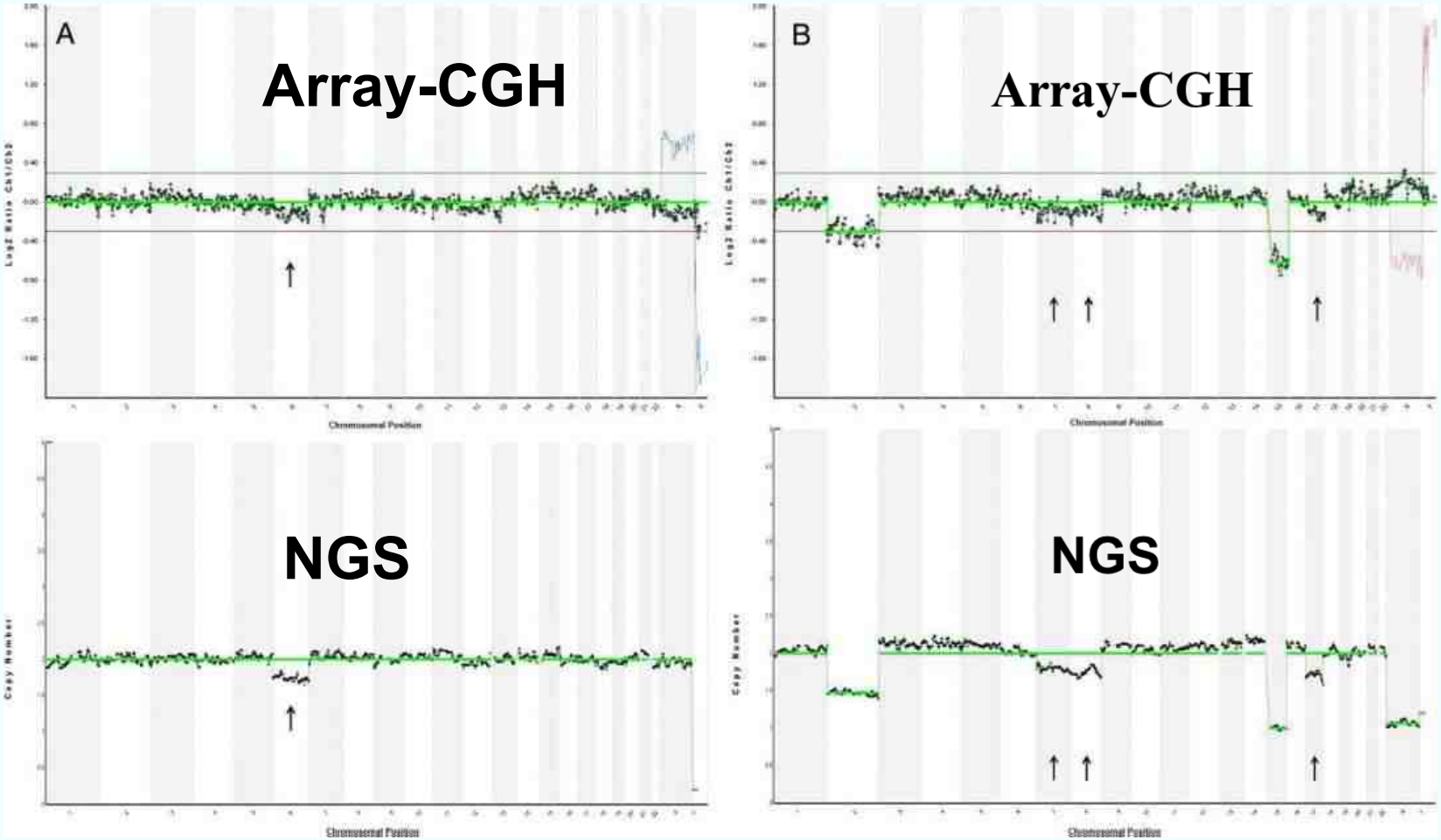
human reproduction

ORIGINAL ARTICLE *Reproductive genetics*

Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles

Francesco Fiorentino[1,*], Sara Bono[1], Anil Biricik[1], Andrea Nuccitelli[1], Ettore Cotroneo[1], Giuliano Cottone[1], Felix Kokocinski[2], Claude-Edouard Michel[2], Maria Giulia Minasi[3], and Ermanno Greco[3]

Examples of array comparative genomic hybridization (array-CGH) and next-generation sequencing (NGS) results.



Francesco Fiorentino et al. Hum. Reprod. 2014;29:2802-2813

human reproduction

Table III **Next-generation sequencing performance on blastocysts.**

Concordance analysis	No. (95% CI)
Chromosome calling comparison	4545
Euploid chromosomes (true negatives)	4334
Aneuploid chromosomes (true positives)	211
Missed chromosome calls (false negatives)	0
Extra chromosome calls (false positives)	1
Aneuploidy call performance	
Sensitivity	100% (99.25–100%)
Specificity	99.98% (99.87–100%)
Whole-embryo aneuploidy/euploidy status comparison	
Euploid embryo (true negatives)	74
Aneuploid embryo (true positives)	106
Missed aneuploid embryo calls (false negatives)	0
Extra aneuploid embryo calls (false positives)	0
Aneuploid embryo call performance	
Sensitivity	100% (96.55–100%)
Specificity	100% (95.09–100%)
Positive predictive value	100% (96.55–100%)
Negative predictive value	100% (95.09–100%)

Trends in genetic analysis – PGD Consortium survey

Data collected by

Dr. Martine De Rycke, Brussels

PGD for chromosomal indications (n=757)

- **FISH** **71%**
- **aCGH** **27%**
- **CGH/PCR/qPCR/SNP/NGS** **2%**

PGS (n=2725)

- FISH 17%
- aCGH 76%
- SNP 2%
- qPCR 4%
- NGS <<1%

PGS 2.0

In comparison to PGS 1.0

PGS 2.0 is characterized by:

1. Polar body biopsy or trophectoderm biopsy in place of day-3 embryo biopsy.

2. Aneuploidy assessments of all 24 chromosome pairs instead of FISH of a limited set of chromosomes.

Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials.

- **Three trials have been published comparing PGS 2.0 and routine IVF care.**
- **PGS 2.0 is associated with higher clinical implantation rates, and higher ongoing pregnancy rates when the same number of embryos is transferred in both PGS and control groups.**
- **Additionally, PGS 2.0 improves embryo selection in eSET practice, maintaining the same ongoing pregnancy rates between PGS and control groups, while sharply decreasing multiple pregnancy rates.**
- **These results stem from good-prognosis patients undergoing IVF.**
- **Whether these findings can be extrapolated to poor-prognosis patients with decreased ovarian reserve remains to be determined.**

Dahdouh et al. Reprod Biomed Online. 2015 Mar;30(3):281-9

Blastocentesis: a source of DNA for preimplantation genetic testing. Results from a pilot study

Luca Gianaroli, M.D., M. Cristina Magli, M.Sc., Alessandra Pomante, Ph.D., Anna M. Crivello, B.Sc., Giulia Cafueri, B.Sc., Marzia Valerio, B.Sc., and Anna P. Ferraretti, M.D.

Reproductive Medicine Unit, Società Italiana Studi di Medicina della Riproduzione, Bologna, Italy

TABLE 2

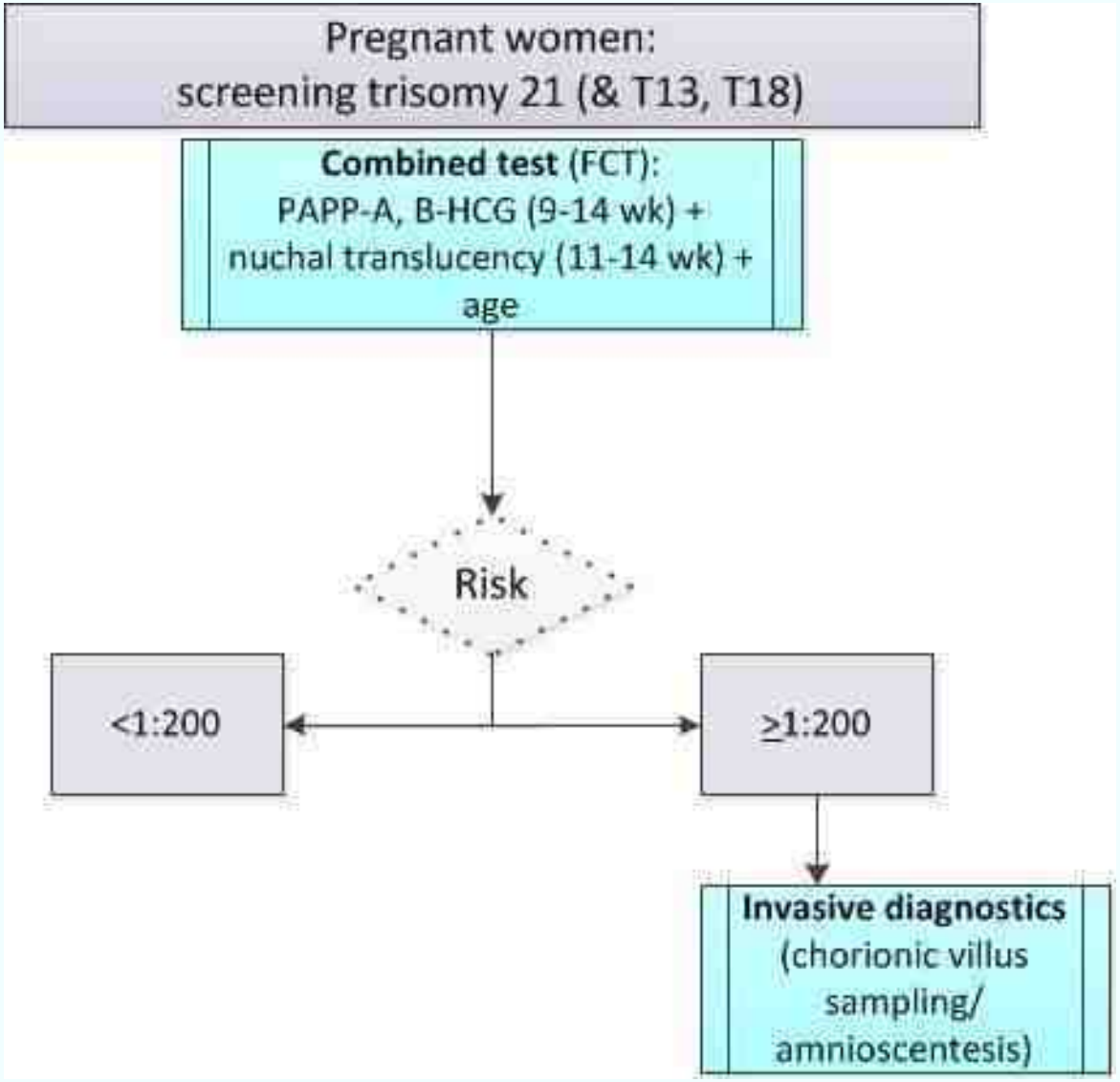
Overall chromosome concordance by stage at biopsy over the whole embryo calculated per ploidy condition (euploid vs. aneuploid) and per single chromosome.

	Concordance, n (%)			
Data collected	**Full**	**Partial**	**Null**	**Total**
Whole embryo vs. PBs				
Embryos	16 (80)	3 (15)	1 (5)	20
Chromosomes	368/368 (100)	65/69 (94.2)	22/23 (95.7)	455/460 (98.9)
Whole embryo vs. blastomere				
Embryos	6 (100)	0	0	6
Chromosomes	144/144 (100)			144/144 (100)
Whole embryo vs. TE cells				
Embryos	21 (80.8)	5 (19.2)	0	26
Chromosomes	504/504 (100)	107/120 (89.2)		611/624 (97.9)
Whole embryo vs. BF				
Embryos	22 (84.6)	4 (15.4)	0	26
Chromosomes	528/528 (100)	84/96 (87.5)		612/624 (98.1)

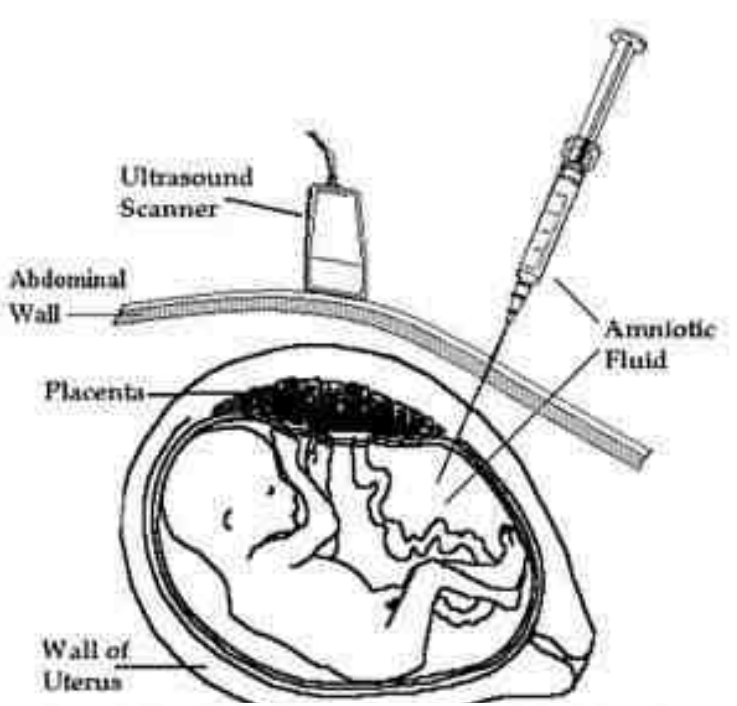
Note: Abbreviations as in Table 1.

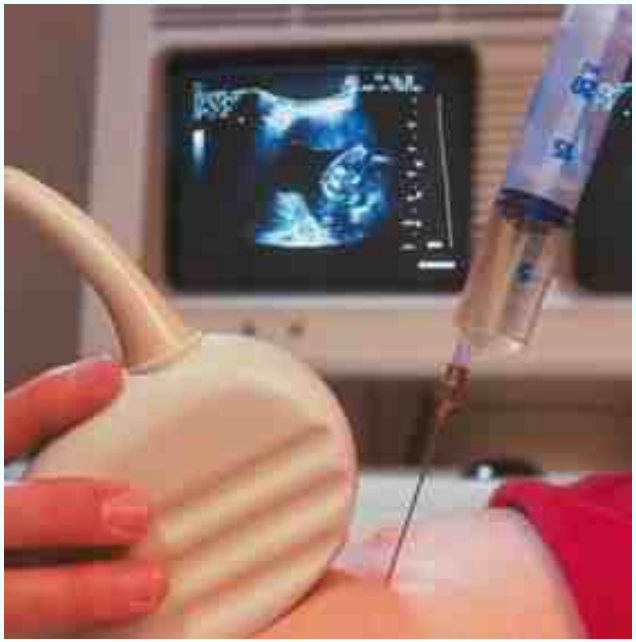
Gianaroli. Blastocentesis as a source of DNA. Fertil Steril 2014.

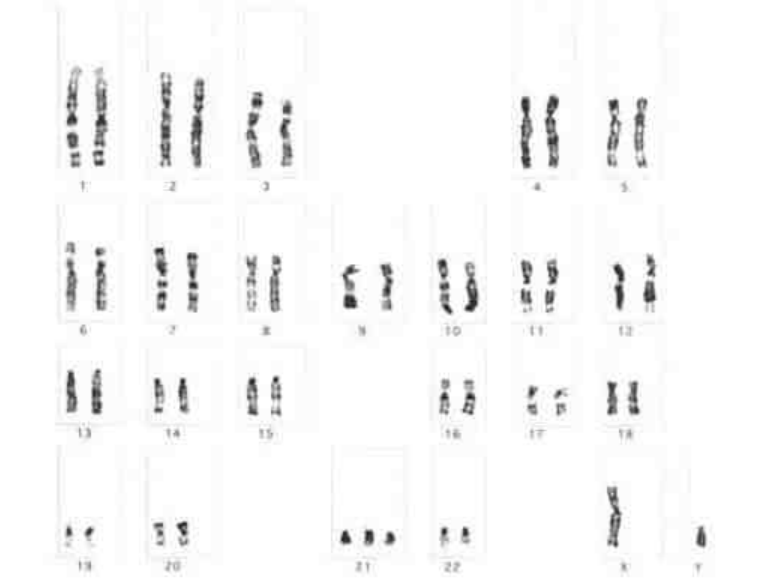
Prenatal screening 1.0



Invasive diagnostics





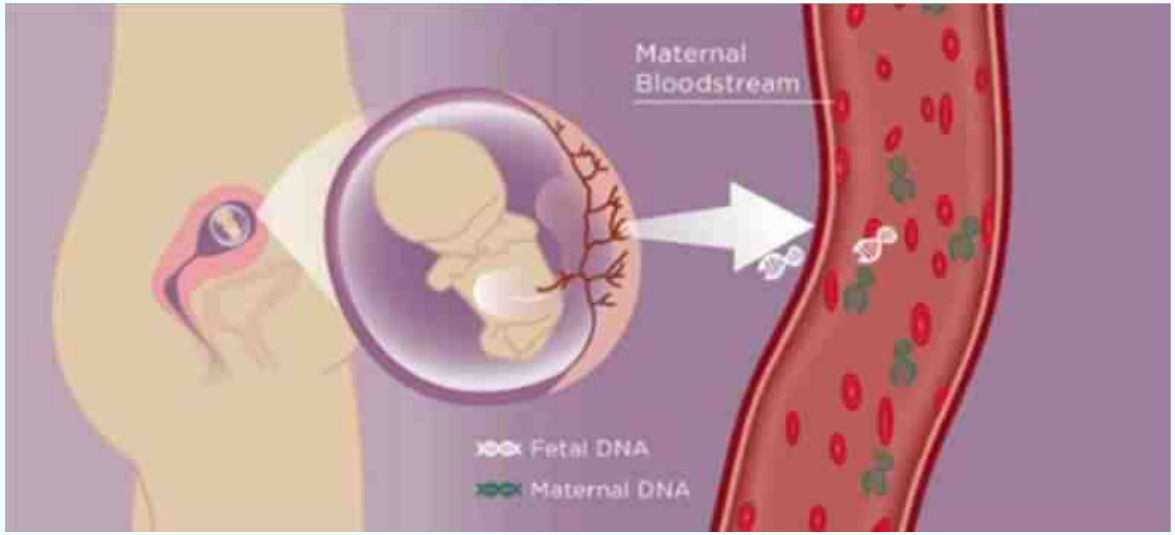


Miscarriage risk
0.5-1%

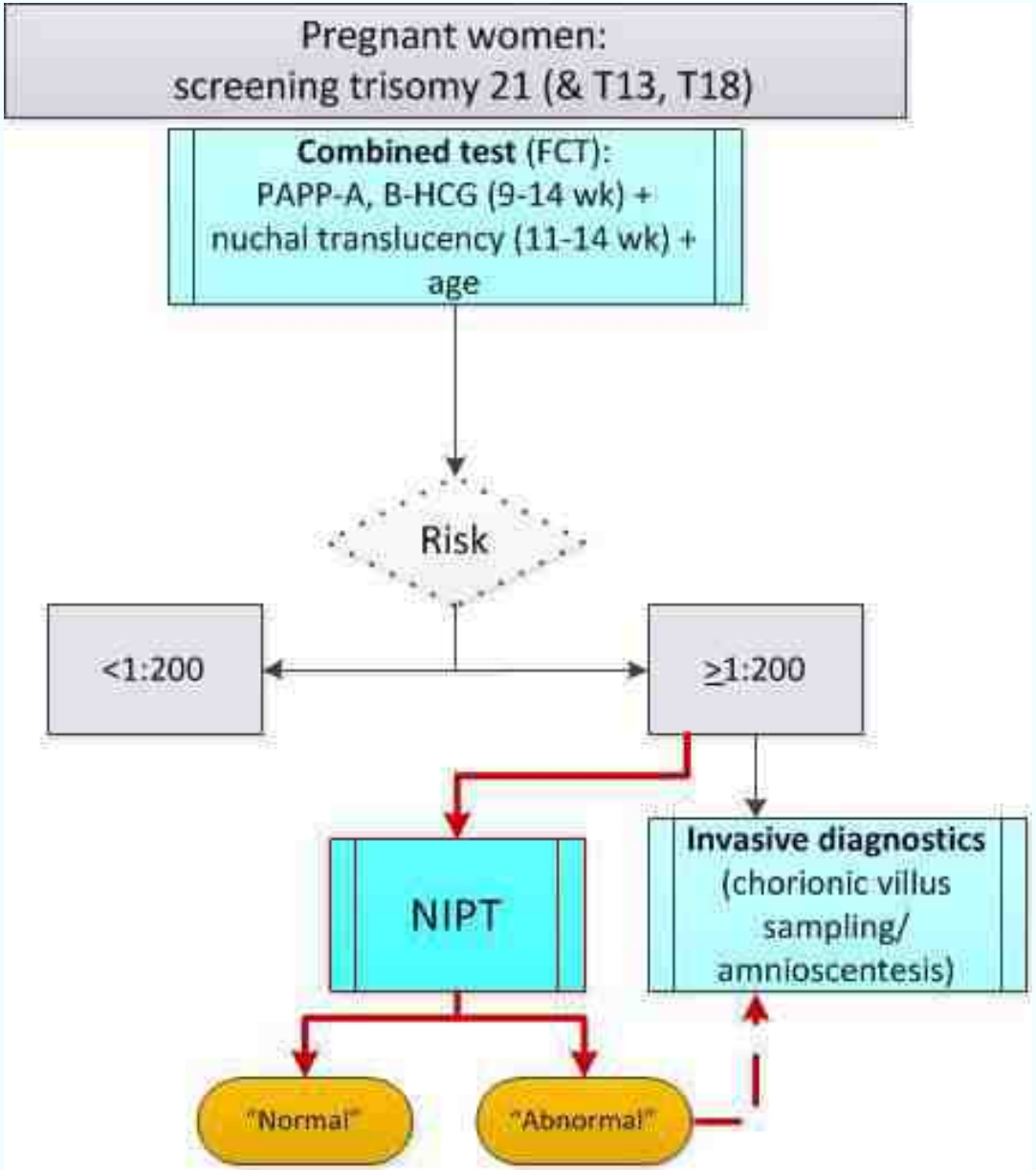
Prenatal screening 2.0
Non-invasive prenatal testing (NIPT)

Cell free foetal DNA in maternal plasma:

- **Originates from the placenta**
- **~10% of DNA fragments**
- **Detection ≥10 weeks**
- **Post-partum clearance <1 day**



NIPT for high-risk women



NIPT sensitivity:

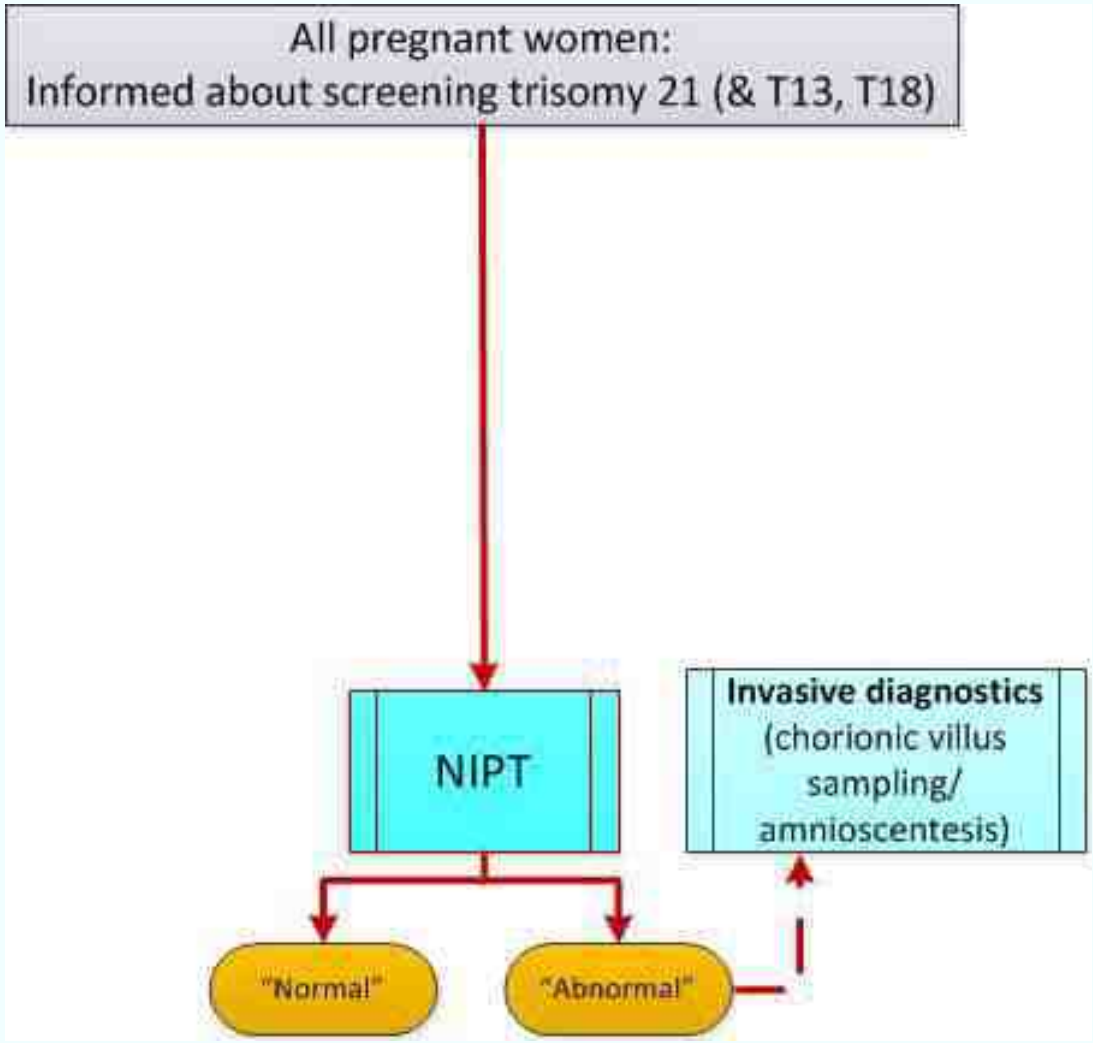
>99% T21

97% T18

92% T13

Most (high-risk) women can avoid miscarriage risk by using NIPT

NIPT as first screening test



e.g. Norton et al. NEJM 2015

Introduction of NIPT

In many countries:

NIPT commercially introduced, no governmental guidance

lifecodexx

SEQUENOM CMM
CENTER FOR MOLECULAR MEDICINE

verinata Health™

natera™
Conceive. Deliver.

Dondorp et al. Eur J Hum Genet 2015

The future of NIPT:
to test the whole foetal genome?

Potential to test for other Mendelian disorders (monogenic treatable/non-treatable)?

Lo et al. Sci Transl Med 2010; Van Dijk et al., Front Genet 2012

DOI: 10.1002/pd.4272 PRENATAL DIAGNOSIS

ORIGINAL ARTICLE

Obstetricians and gynecologists' practice and opinions of expanded carrier testing and noninvasive prenatal testing

Peter Benn[1], Audrey R. Chapman[2*], Kristine Erickson[3], Mark S. DeFrancesco[4,5], Louise Wilkins-Haug[6,7], James F. X. Egan[5] and Jay Schulkin[8]

[1]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA
[2]Department of Community Medicine and Healthcare, University of Connecticut School of Medicine, Farmington, CT, USA
[3]Department of Psychology, American University, Washington, DC, USA
[4]Women's Health Connecticut, Avon, CT, USA
[5]Department of Obstetrics and Gynecology, University of Connecticut Health Center, Farmington, CT, USA
[6]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA
[7]Department of Obstetrics and Gynecology, Harvard Medical School, Boston, MA, USA
[8]American Congress of Obstetricians and Gynecologists, Washington, DC, USA
*Correspondence to: Audrey R. Chapman. E-mail: achapman@uchc.edu

ABSTRACT

Obstetricians' opinions of appropriate use for noninvasive tests assuming they were developed and proven accurate

• All aneuploidies	193	97.5
• Severe early onset disorders	179	90.4
• Adult onset predispositions (cancer/heart disease)	59	29.8
• Other adult onset predispositions	53	26.8
• Gender identification (non medical)	31	15.7

Obstetricians' opinions of potential effects of noninvasive tests

- n (%)

Non-invasive tests would result in	
(a) Greater societal pressures on individual patients to utilize prenatal diagnosis	120 (38.1)
(b) Reduced birth incidence in Down syndrome and other disorders due to greater uptake of non-invasive prenatal diagnosis	124 (63.9)
(c) Greater numbers of pregnancy terminations for milder disorders	142 (73.2)
Altered perception of individuals with handicaps as a result of (a), (b) and/or (c)	**63 (32.5)**

Thank you

joep.geraedts@mumc.nl