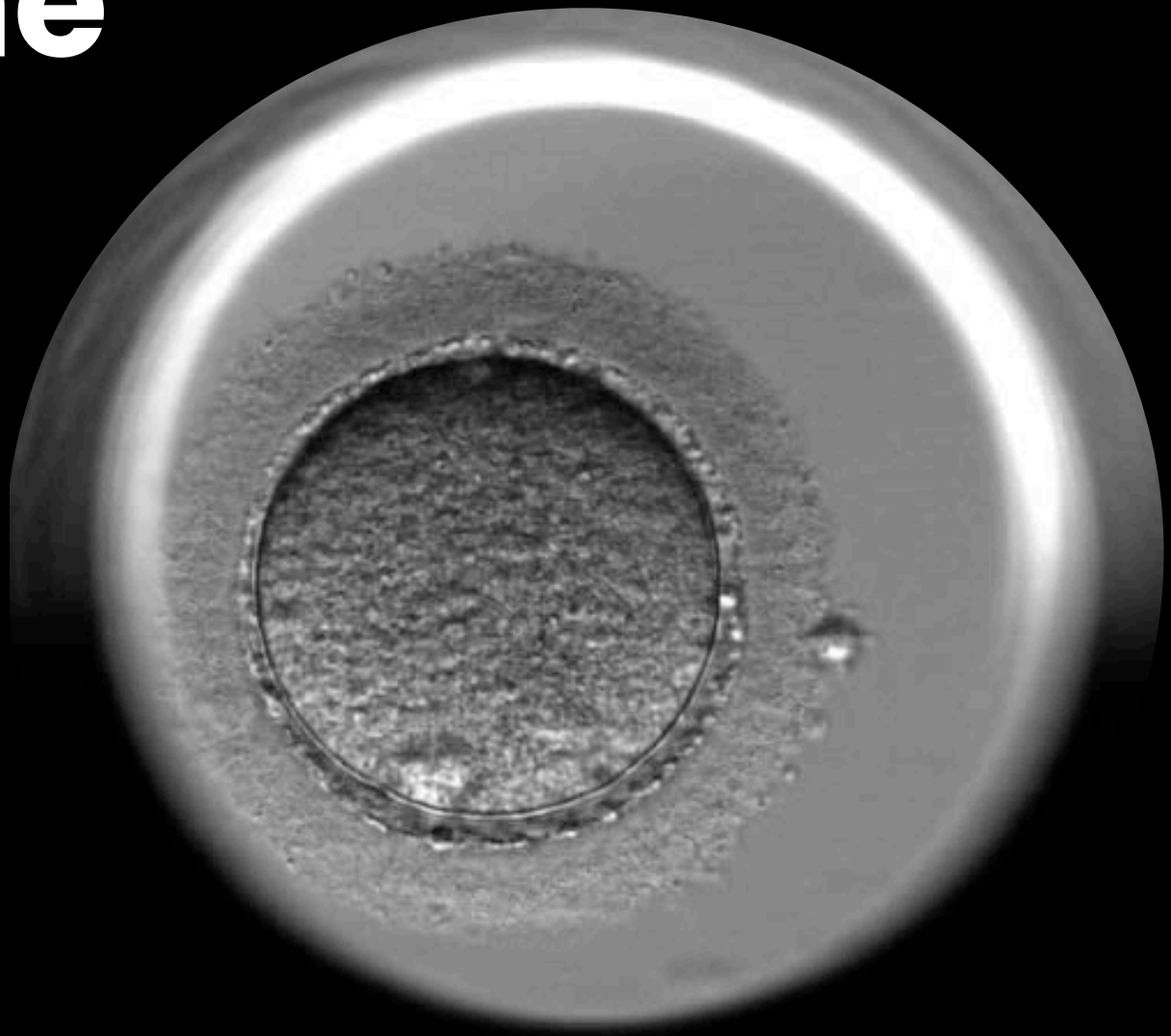
Embryo Genome Editing



Diego Marin, PhD, HCLD(ABB)
Head Global Business Dev & Scientific Affairs
Genomic Prediction

Adjunct Professor
Department of Human Genetics
Rutgers University

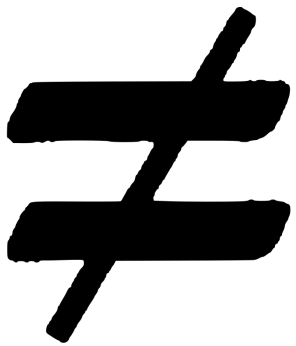
DeSciNYC 07.08.25

Courtesy IVI Panama

The Embryology Lab

Courtesy IVI Panama

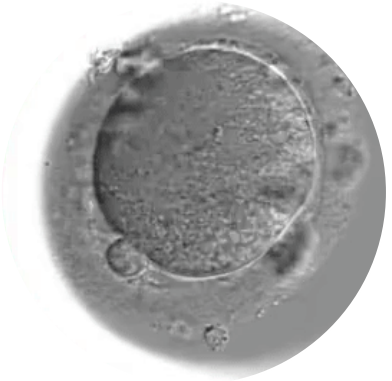
Genetic
Selection

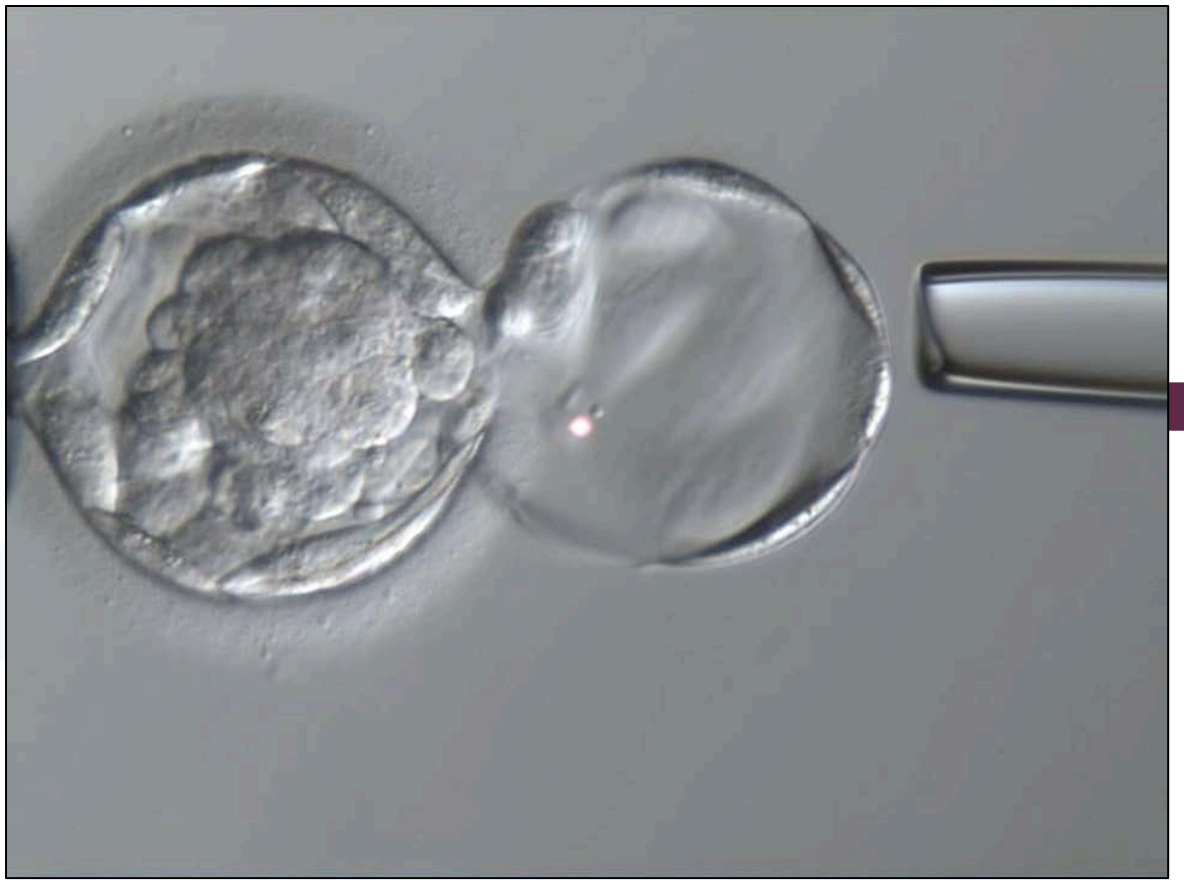


Gene
Editing

IVF and Preimplantation Genetic Testing (PGT)

1. In vitro Fertilization





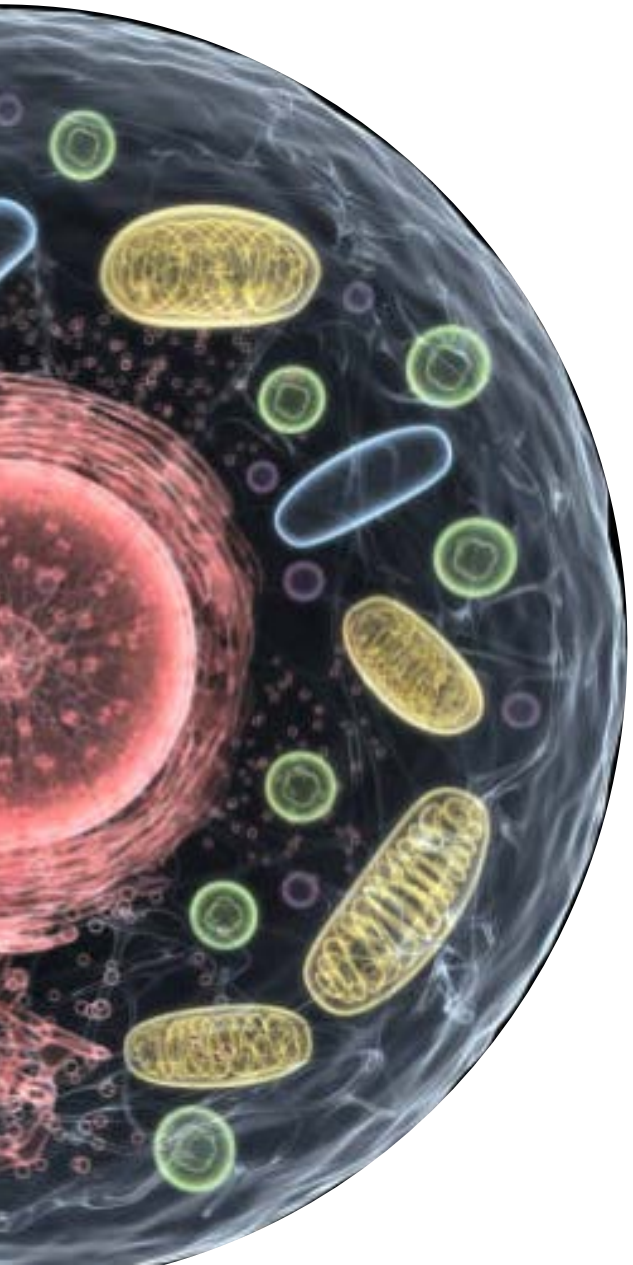
4. Report

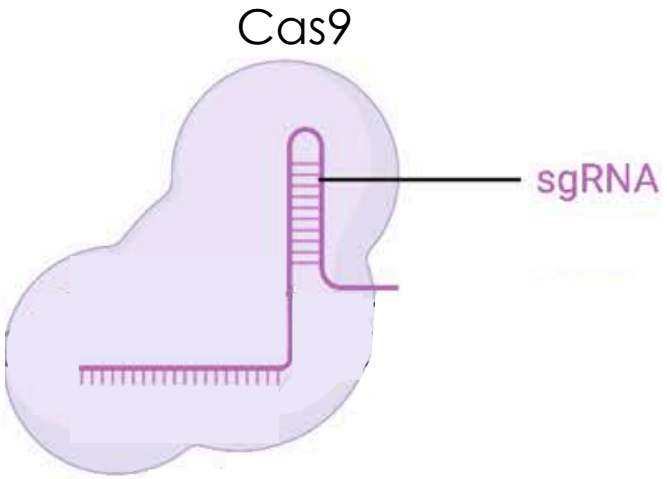
Adapted from https://gpclaboratory.com/pgt-a

Genetic
Selection

Gene
Editing

CRISPR/Cas9 as a Gene Editing Tool

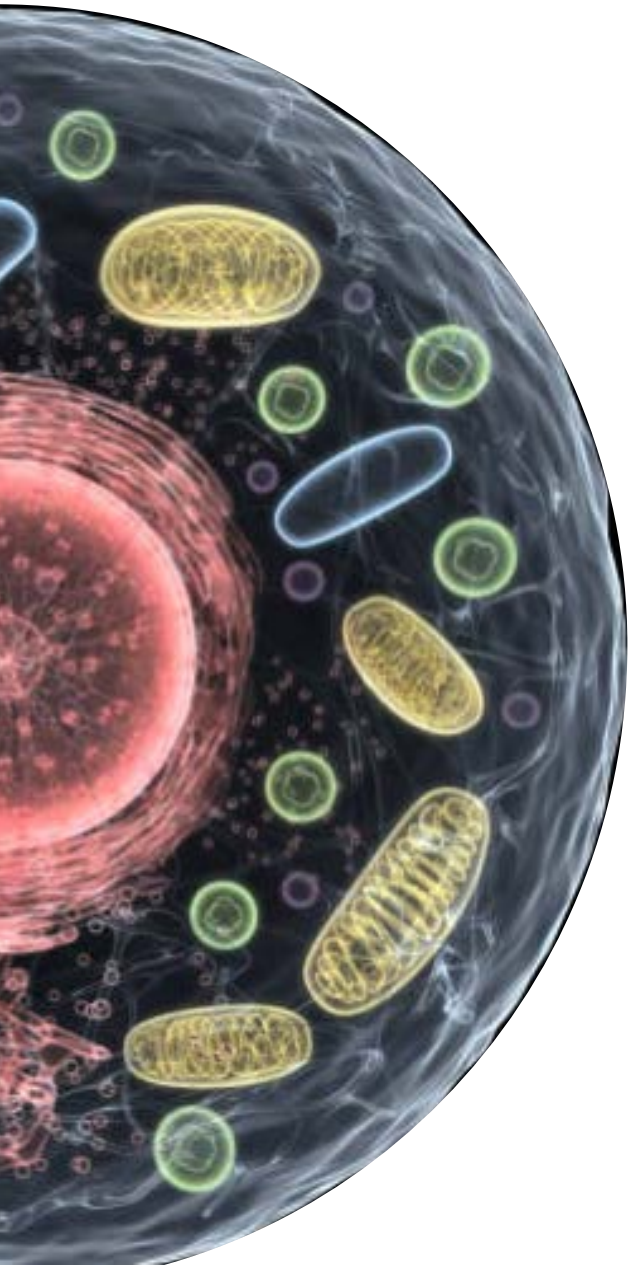


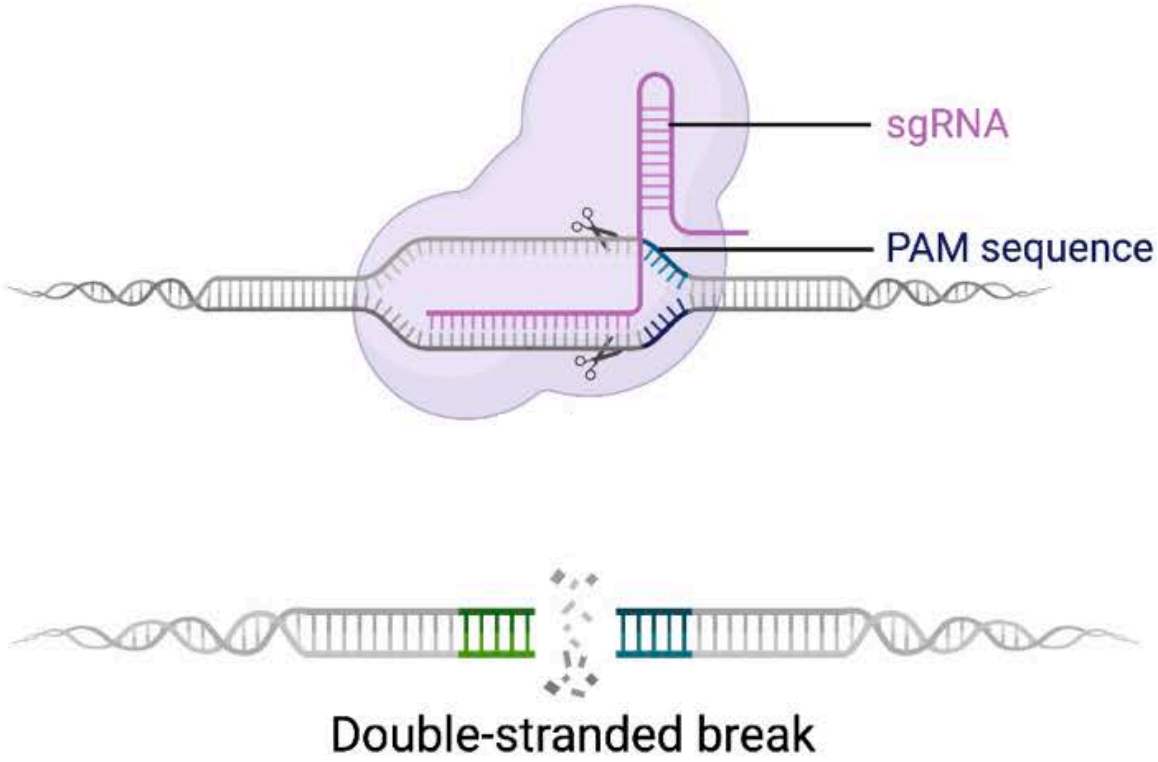


Target DNA

Adapted from Turocy et al. 2021

CRISPR/Cas9 as a Gene Editing Tool





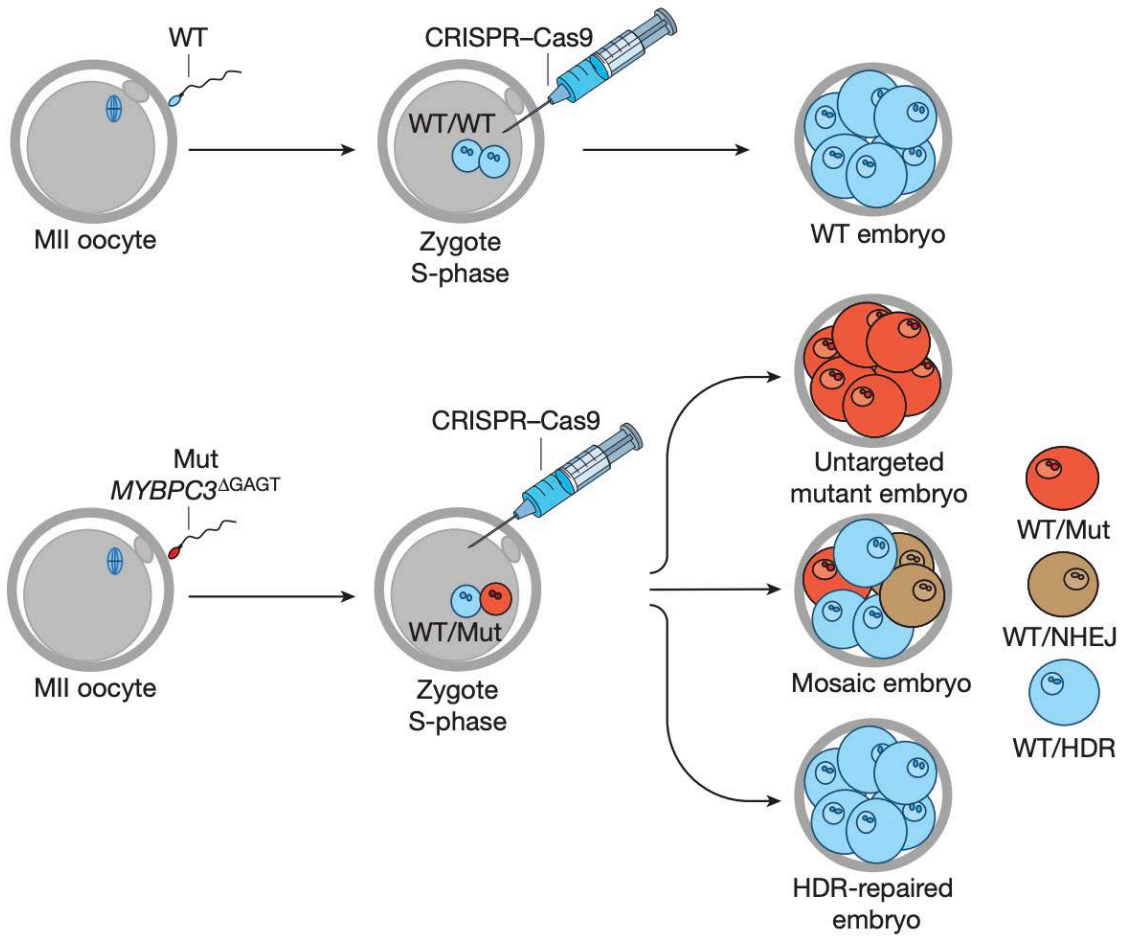
Adapted from Turocy et al. 2021

ARTICLE

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Re Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], Dav Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§ Shoukhrat Mitalipov[1,10]§



Ma et al, 2017. Nature

BRIEF COMMUNICATIONS ARISING

Inter-homologue repair in fertilized human eggs?

Dieter Egli[1]*, Michael V. Zuccaro[2], Michael Kosicki[3], George M. Church[4], Allan Bradley[3] & Maria Jasin[5]*

ARISING FROM H. Ma et al. *Nature* **548**, 413–419 (2017); https://doi.org/10.1038/nature23305

NATURE | NEWS

Doubts raised about CRISPR gene-editing study in human embryos

Alternative explanations challenge whether technique actually fixed a genetic mutation as claimed.

Ewen Callaway

31 August 2017

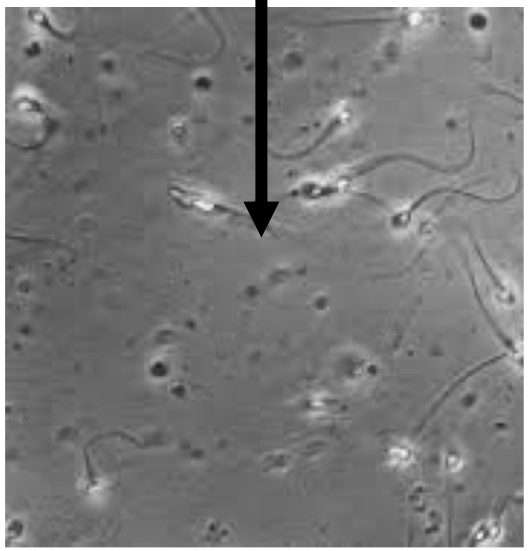
*"The critique levelled by Egli et al. offers no new results but instead relies on alternative explanations of our results based on **pure speculation**," Mitalipov said in a statement.*

EYS gene-editing

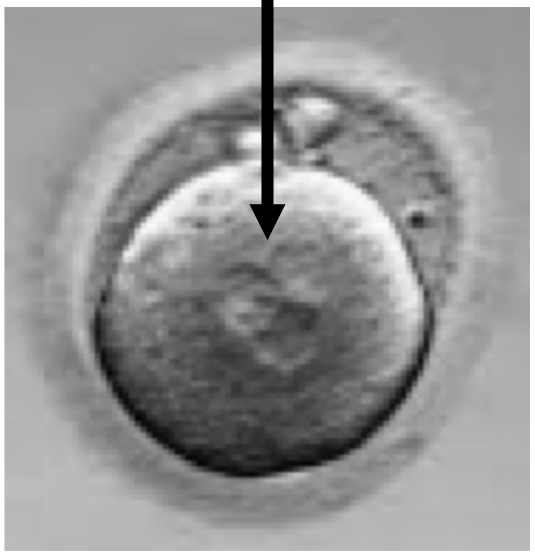
COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

Genomic Prediction Clinical Laboratory

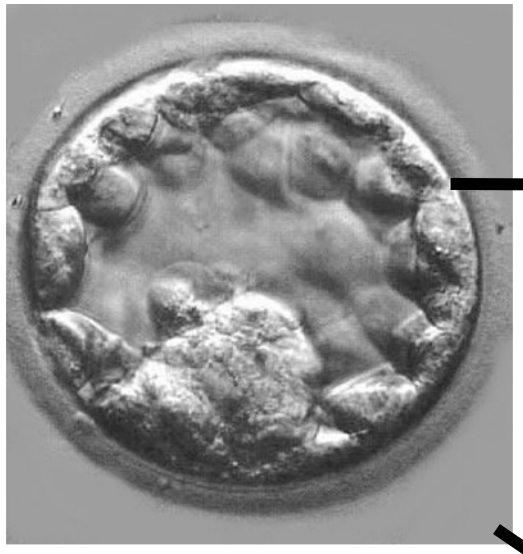
EYS hom mut



CRISPR/Cas9

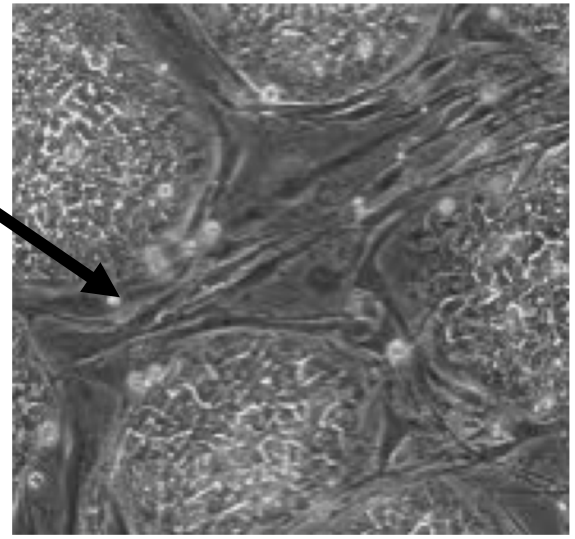


Research Use Only

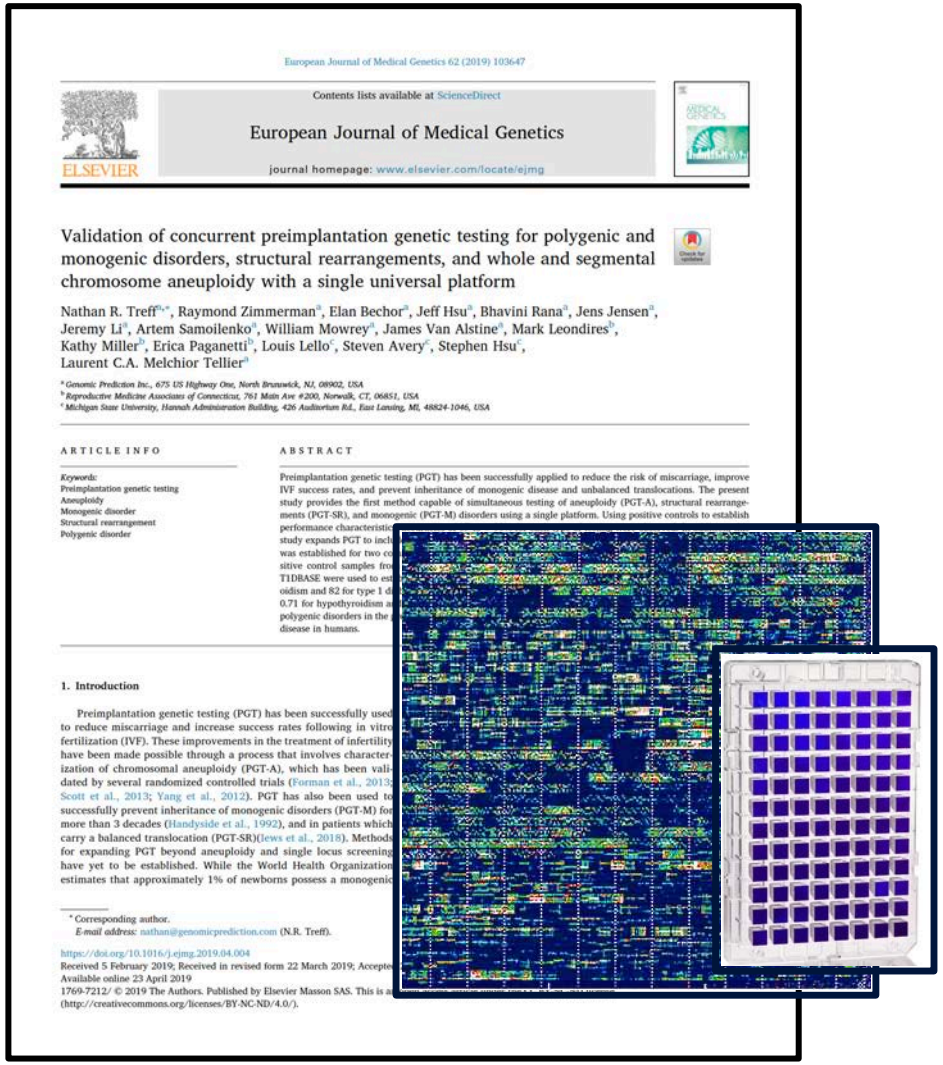


Embryo Biopsy and PGT

Embryonic Stem Cell Line Derivation and PGT



SNP Array PGT for Aneuploidy



European Journal of Medical Genetics 62 (2019) 103647

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/ejmg

Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform

Nathan R. Treff[a,*], Raymond Zimmerman[a], Elan Bechor[a], Jeff Hsu[a], Bhavini Rana[a], Jens Jensen[a], Jeremy Li[a], Artem Samoilenko[a], William Mowrey[a], James Van Alstine[a], Mark Leondires[b], Kathy Miller[b], Erica Paganetti[b], Louis Lello[c], Steven Avery[c], Stephen Hsu[c], Laurent C.A. Melchior Tellier[a]

[a] Genomic Prediction Inc., 675 US Highway One, North Brunswick, NJ, 08902, USA
[b] Reproductive Medicine Associates of Connecticut, 761 Main Ave #200, Norwalk, CT, 06851, USA
[c] Michigan State University, Hannah Administration Building, 426 Auditorium Rd., East Lansing, MI, 48824-1046, USA

ARTICLE INFO

Keywords:
Preimplantation genetic testing
Aneuploidy
Monogenic disorder
Structural rearrangement
Polygenic disorder

ABSTRACT

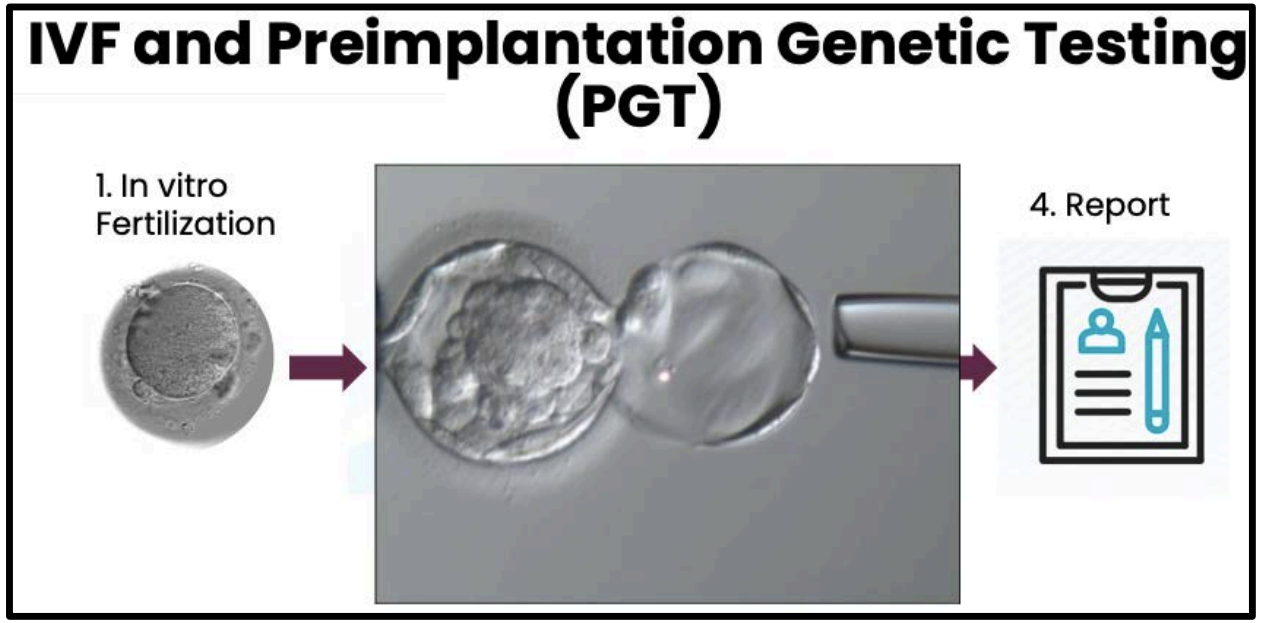
Preimplantation genetic testing (PGT) has been successfully applied to reduce the risk of miscarriage, improve IVF success rates, and prevent inheritance of monogenic disease and unbalanced translocations. The present study provides the first method capable of simultaneous testing of aneuploidy (PGT-A), structural rearrangements (PGT-SR), and monogenic (PGT-M) disorders using a single platform. Using positive controls to establish performance characteristic
study expands PGT to incl
was established for two co
sitive control samples fro
T1DBASE were used to est
oidism and 82 for type 1 di
0.71 for hypothyroidism a
polygenic disorders in the
disease in humans.

1. Introduction

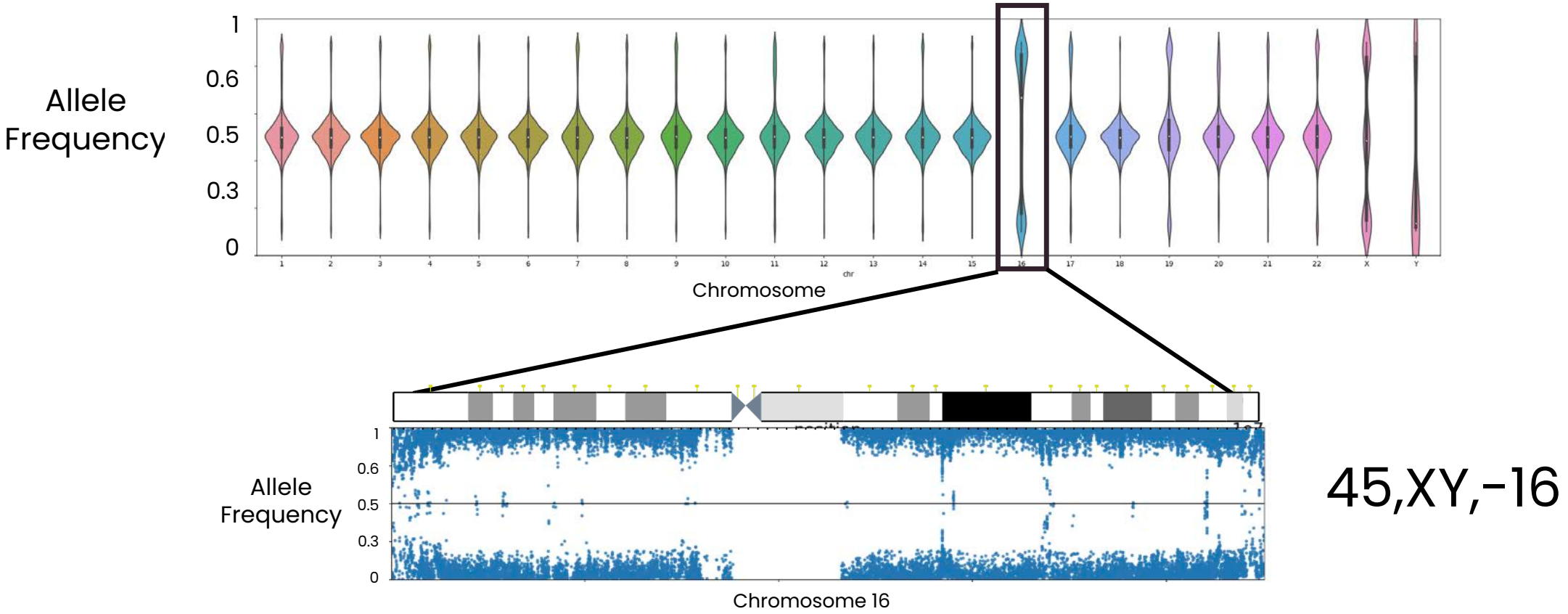
Preimplantation genetic testing (PGT) has been successfully used
to reduce miscarriage and increase success rates following in vitro
fertilization (IVF). These improvements in the treatment of infertility
have been made possible through a process that involves character-
ization of chromosomal aneuploidy (PGT-A), which has been vali-
dated by several randomized controlled trials (Forman et al., 2013;
Scott et al., 2013; Yang et al., 2012). PGT has also been used to
successfully prevent inheritance of monogenic disorders (PGT-M) for
more than 3 decades (Handyside et al., 1992), and in patients which
carry a balanced translocation (PGT-SR)(Iews et al., 2018). Methods
for expanding PGT beyond aneuploidy and single locus screening
have yet to be established. While the World Health Organization
estimates that approximately 1% of newborns possess a monogenic

* Corresponding author.
E-mail address: nathan@genomicprediction.com (N.R. Treff).

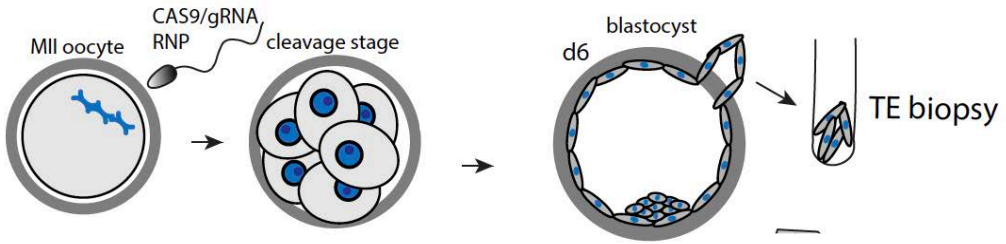
https://doi.org/10.1016/j.ejmg.2019.04.004
Received 5 February 2019; Received in revised form 22 March 2019; Accepte
Available online 23 April 2019
1769-7212/

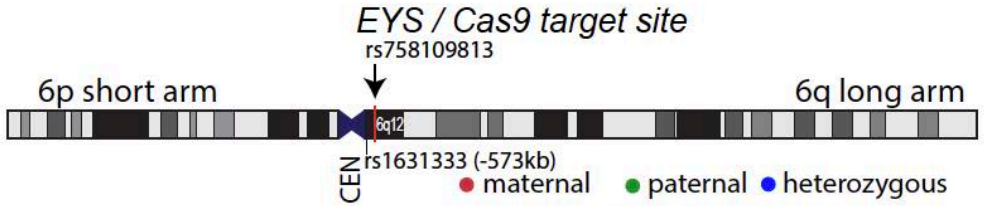


High-Throughput SNP Array PGT

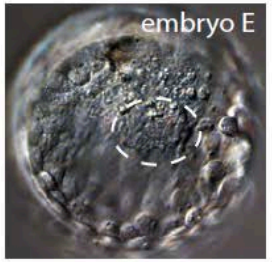


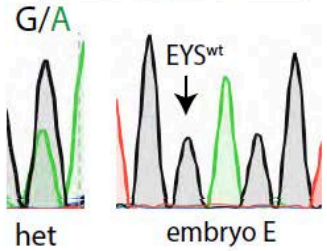
45,XY,-16

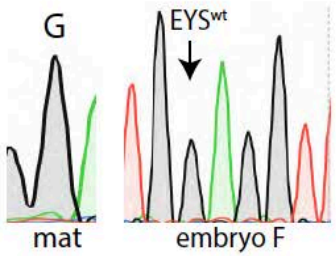


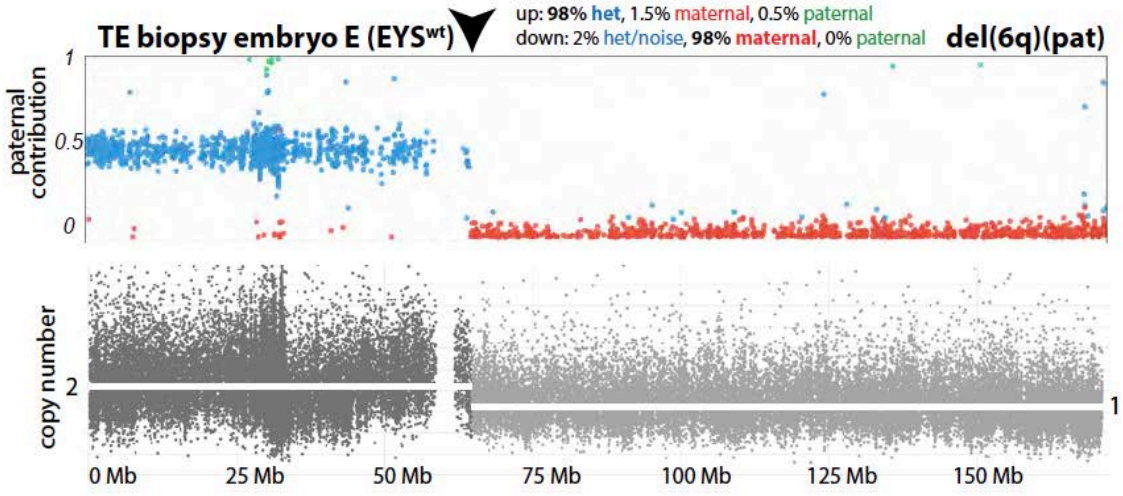


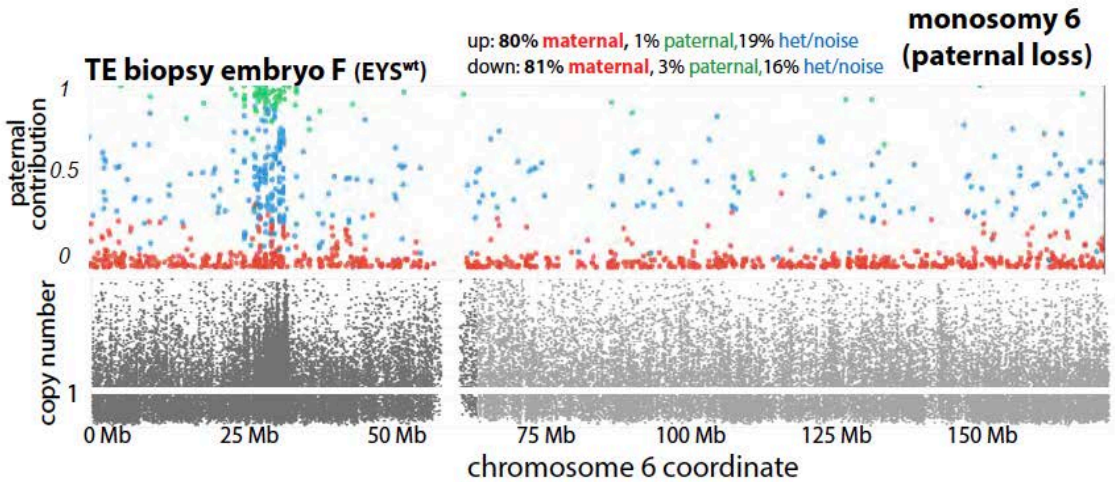
CRISPR/Cas9 system was eliminating part of or entire chromosomes!!







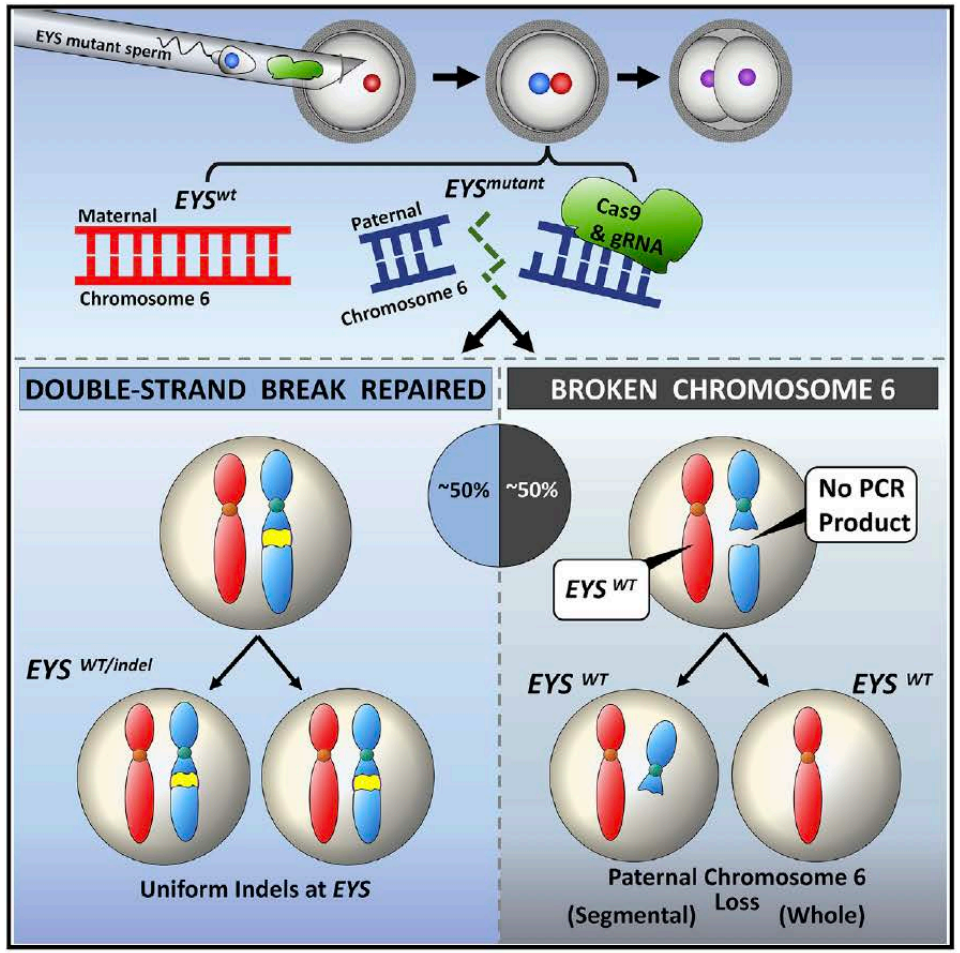




Cell

Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos

Graphical Abstract



Authors

Michael V. Zuccaro, Jia Xu, Carl Mitchell, ..., Rogerio Lobo, Nathan Treff, Dieter Egli

Correspondence

de2220@cumc.columbia.edu

In Brief

CRISPR-Cas9 gene editing in early human embryos leads to frequent loss of the targeted chromosome, indicating that human germline gene editing would pose a substantial risk for aneuploidy and other adverse genetic consequences

REPRODUCIBILITY!!!

COLLOQUIUM PAPER

Frequent loss of heterozygosity in CRISPR-Cas9–edited early human embryos

Gregorio Alanis-Lobato[a], Jasmin Zohren[b], Afshan McCarthy[a], Norah M. E. Fogarty[a,c], Nada Kubikova[d,e], Emily Hardman[a], Maria Greco[f], Dagan Wells[d,g], James M. A. Turner[b], and Kathy K. Niakan[a,h,1]

[a]Human Embryo and Stem Cell Laboratory, The Francis Crick Institute, NW1 1AT London, United Kingdom; [b]Sex Chromosome Biology Laboratory, The Francis Crick Institute, NW1 1AT London, United Kingdom; [c]Centre for Stem Cells and Regenerative Medicine, Guy's Campus, King's College London, SE1 9RT London, United Kingdom; [d]Nuffield Department of Women's and Reproductive Health, John Radcliffe Hospital, University of Oxford, OX3 9DU Oxford, United Kingdom; [e]Jesus College, University of Oxford, OX1 3DW Oxford, United Kingdom; [f]Ancient Genomics Laboratory, The Francis Crick Institute, NW1 1AT London, United Kingdom; [g]Juno Genetics, OX4 4GE Oxford, United Kingdom; and [h]The Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge, CB2 3EG Cambridge, United Kingdom

Session title: Session 26: Advances and challenges in modeling human reproduction

Session type: Selected oral communications

Presentation number: O-095

Abstract title:

Assessment of genome editing outcomes in human preimplantation embryos subjected to CRISPR-Cas9 – most loss of heterozygosity (LOH) events are caused by DNA repair deficiency

N. Kubikova[1,2], M. Savash[3,], M. Esbert[4,], S. Titus[5,], J. Fagan[3,], R. Scott[5,], D. Wells[2,3].
[1]University of Oxford, Nuffield Department of Women's and Reproductive Health and Jesus College, Oxford, United Kingdom.
[2]Juno Genetics UK, Genetics laboratory, Oxford, United Kingdom.

Genomic Prediction Technology Uncovers New CRISPR Safety Concerns in Human Embryos

Company's high-resolution preimplantation genetic testing platform enables discovery of unexpected - and unintended - CRISPR changes to human embryonic DNA.

THE WALL STREET JOURNAL.

Crispr Gene Editing Can Lead to Big Mistakes in Human Embryos

Columbia University study of Crispr technology found it made unwanted chromosomal changes in human embryos

The New York Times

Crispr Gene Editing Can Cause Unwanted Changes in Human Embryos, Study Finds

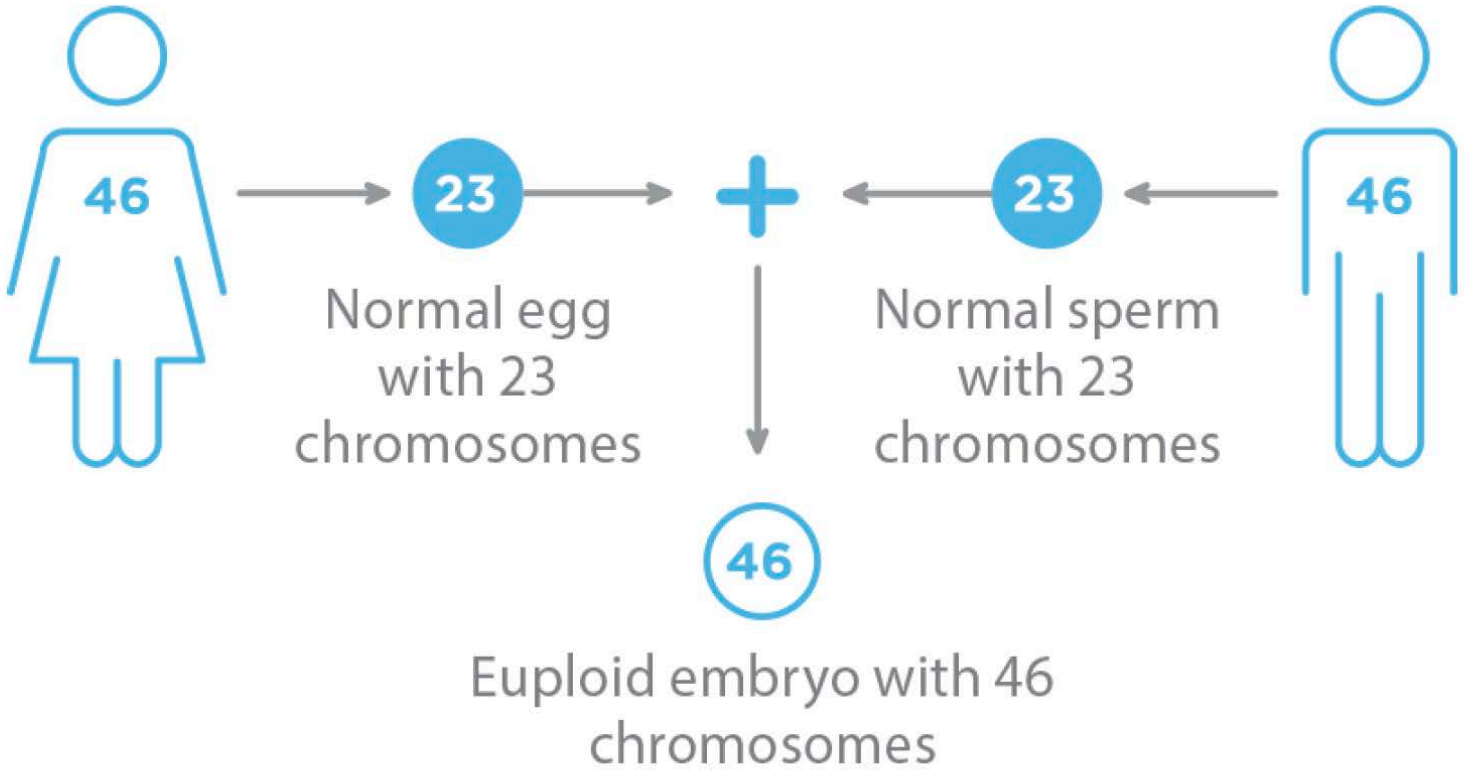
Instead of addressing genetic mutations, the Crispr machinery prompted cells to lose entire chromosomes.

WIRED

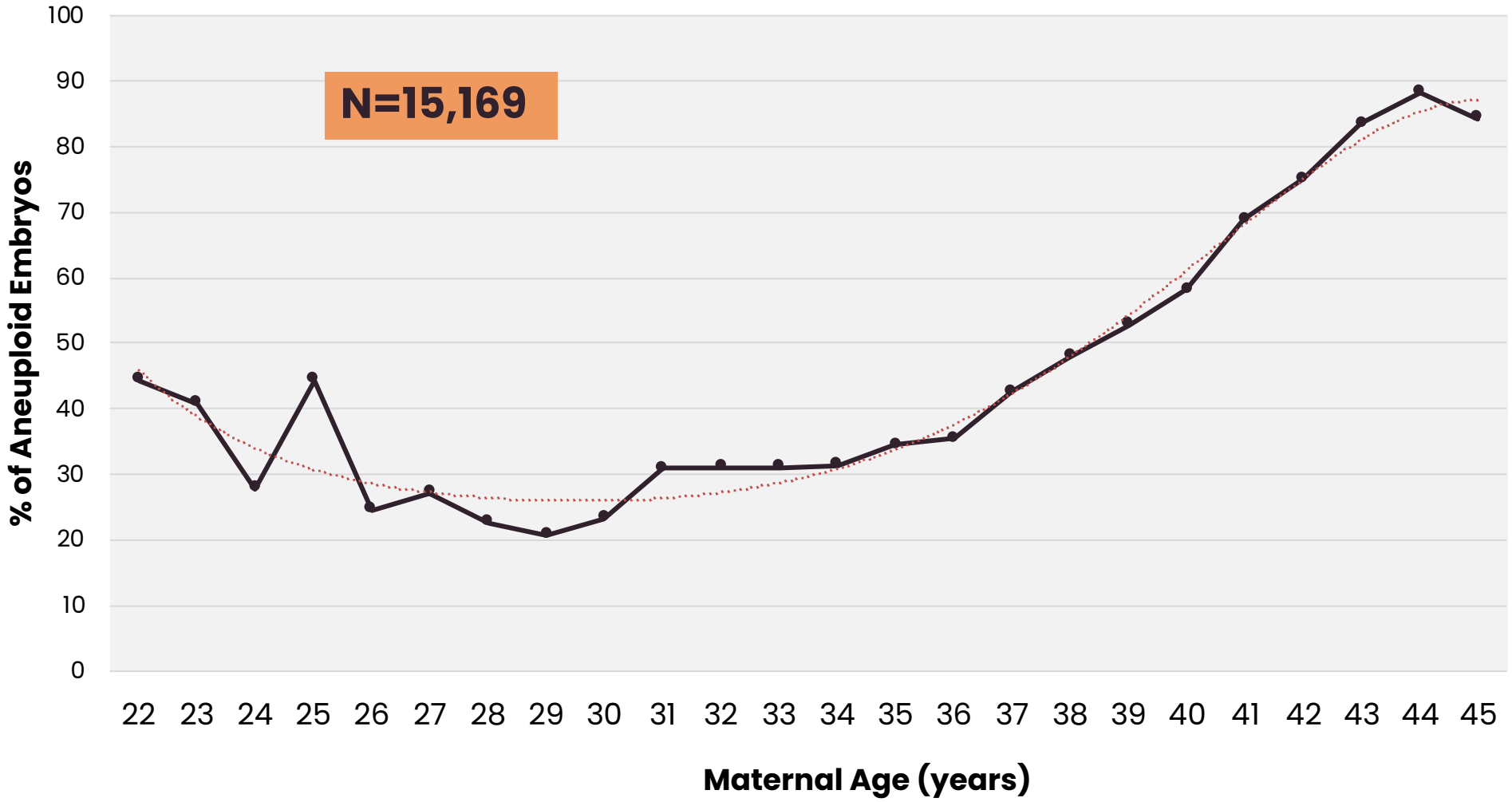
In Embryos, Crispr Can Cut Out Whole Chromosomes—That's Bad

The DNA-cutting tool has been hailed as a way to fix genetic glitches. But a new study suggests it can remove more than scientists bargained for.

Aneuploidy is the Most Common Genetic Abnormality in Humans



Aneuploidy is the Most Common Genetic Cause of Infertility



Franasiak et al. 2014. *Fertil Steril*

"New Technologies for Diagnosis and Treatment of Gynecologic Diseases"

Correction of aneuploidy

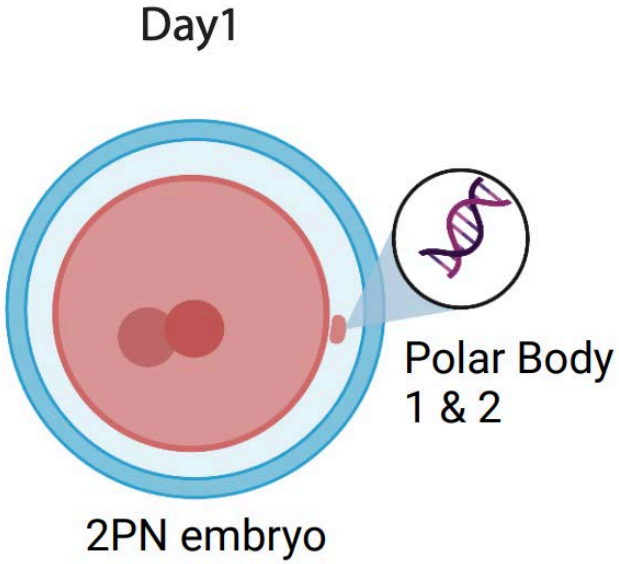
Polar Body PGT → Parental DNA WGS → Design guide RNA against one of three chromosomes (haplotype/trio based analyses)

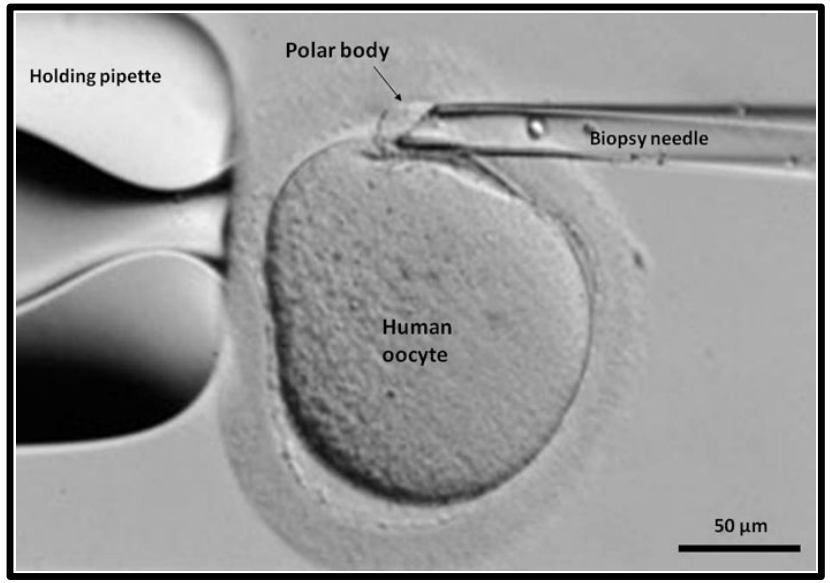
↓

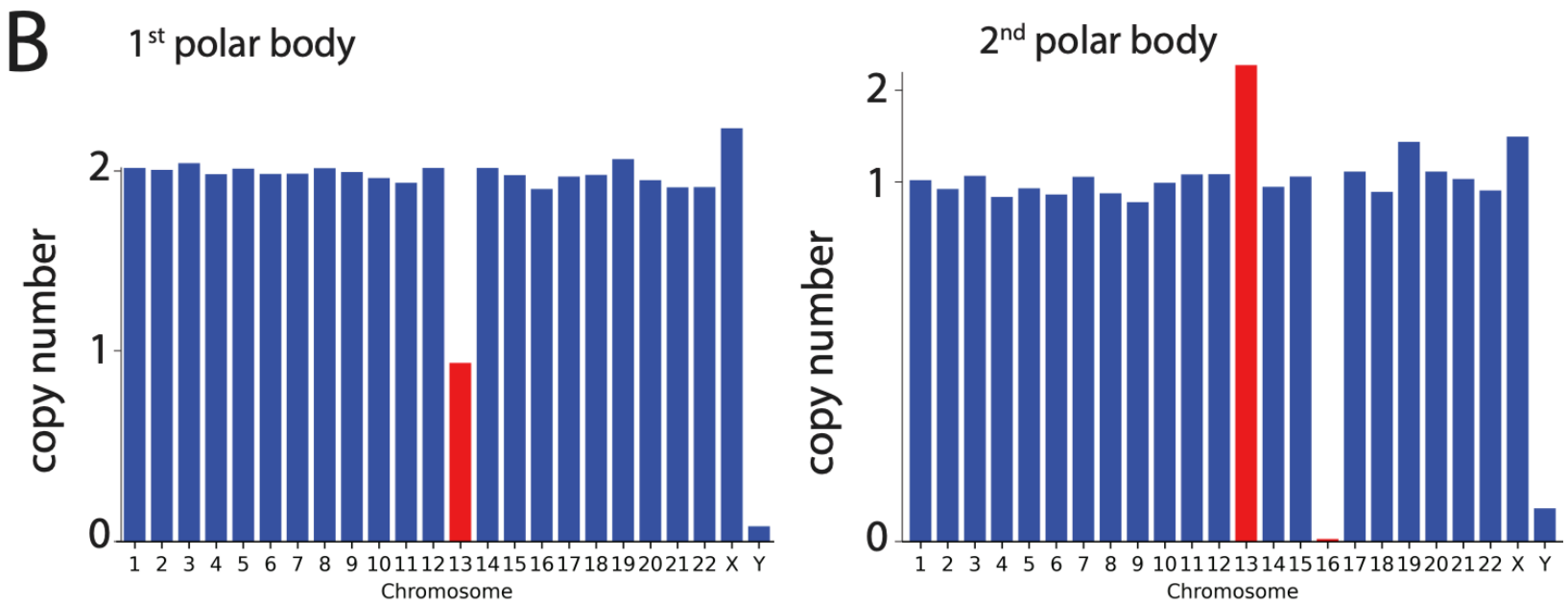
Inject 2PN embryos

↓

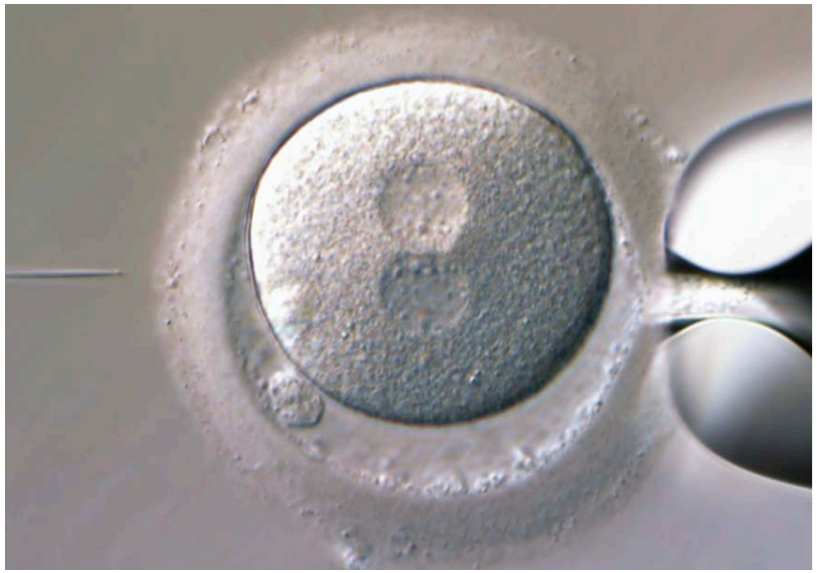
PGT on biopsies from treated blastocysts/
Embryonic stem cell line derivation

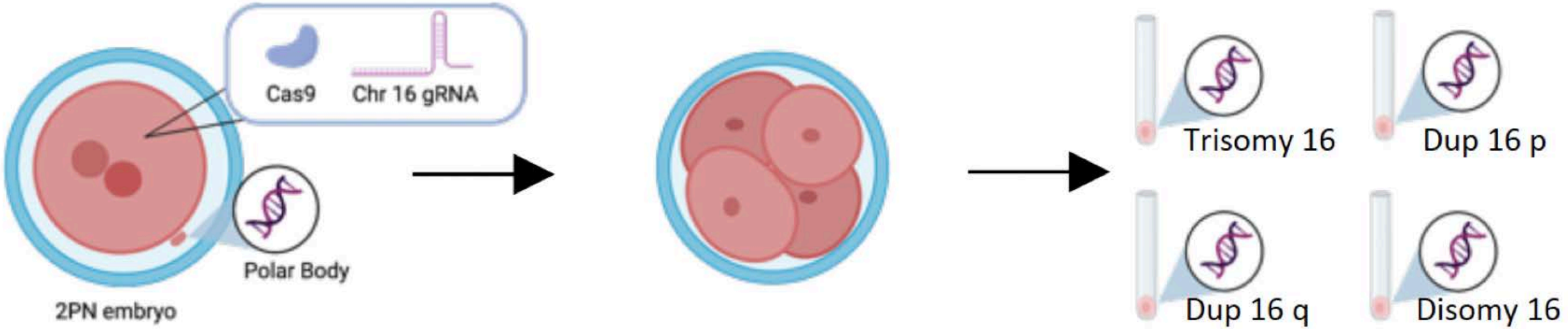






Turocy et al. 2022. BioRxiv

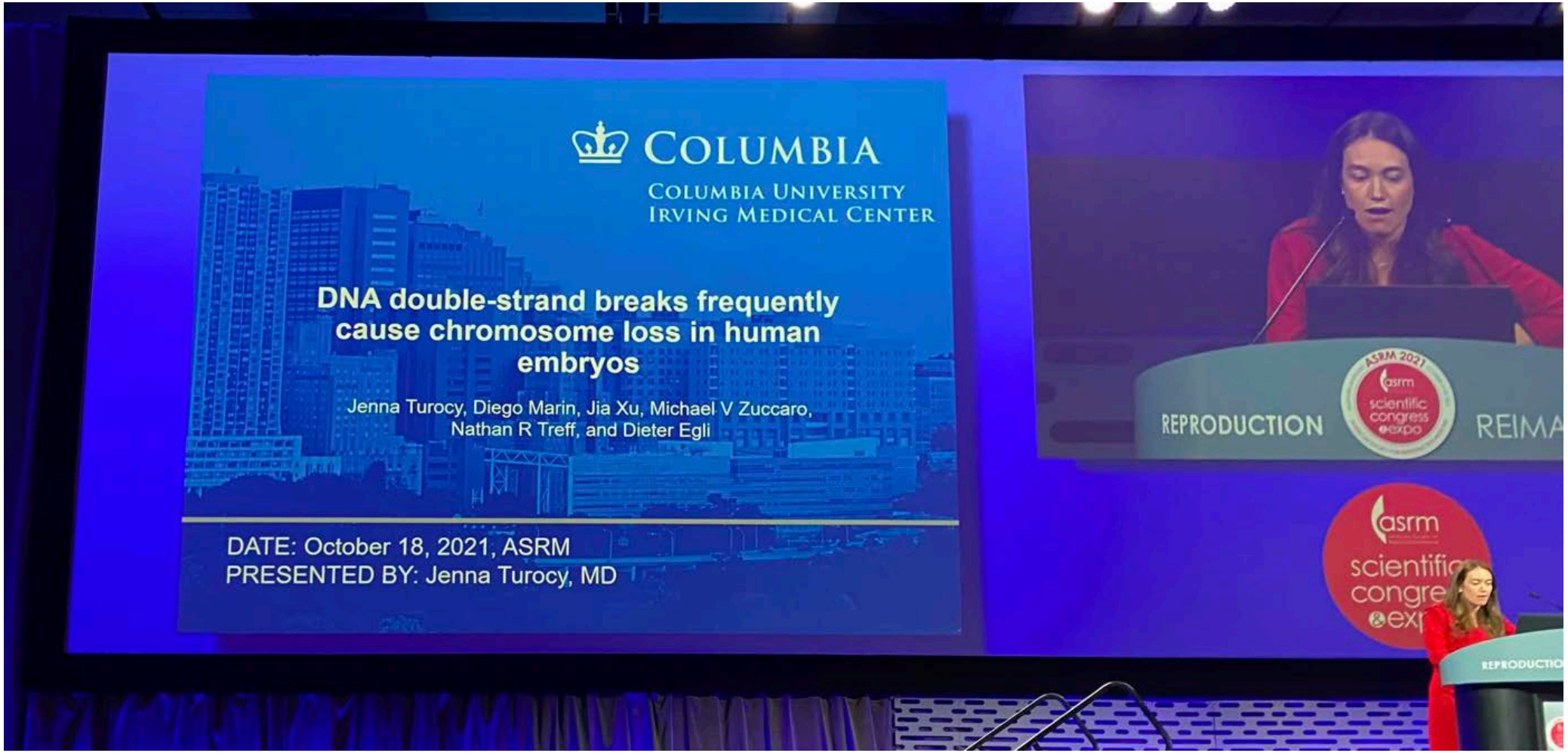




First Report of Aneuploidy Correction in a Human Embryo

Turocy et al. 2022. BioRxiv

ASRM Scientific Congress Awards 2021



New Results

DNA Double Strand Breaks cause chromosome loss through sister chromatid tethering in human embryos

Jenna Turocy, Diego Marin, Shuangyi Xu, Jia Xu, Alex Robles, Nathan Treff, Dieter Egli

doi: https://doi.org/10.1101/2022.03.10.483502

https://www.biorxiv.org/content/10.1101/2022.03.10.483502v1

AP

Lab tests show risks of using CRISPR gene editing on embryos

By MARILYNN MARCHIONE October 29, 2020

The new work suggests that gene editing might hold promise for correcting disorders caused by an extra copy of a chromosome, such as Down syndrome.

PNAS NEXUS

PNAS Nexus, 2025, **4**, pgaf022

https://doi.org/10.1093/pnasnexus/pgaf022
Advance access publication 18 February 2025
Research Report

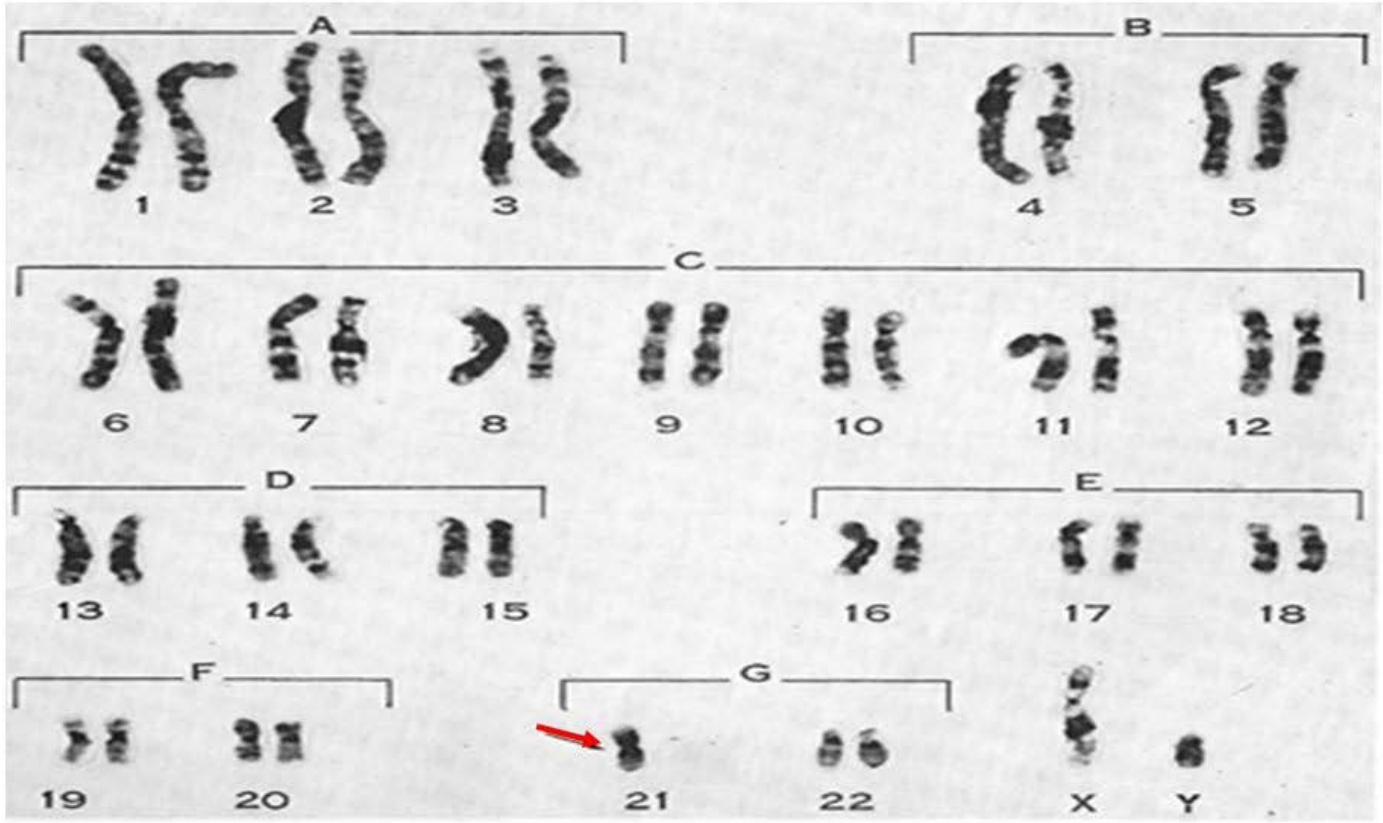
Trisomic rescue via allele-specific multiple chromosome cleavage using CRISPR-Cas9 in trisomy 21 cells

Ryotaro Hashizume[a,b,*], Sachiko Wakita[a], Hirofumi Sawada[c], Shin-ichiro Takebayashi[d], Yasuji Kitabatake[e], Yoshitaka Miyagawa[f], Yoshifumi S Hirokawa[g], Hiroshi Imai[b,h] and Hiroki Kurahashi[i]

Possible germline genome editing case

Structural abnormalities-cont'd

❖ Robertsonian translocation (centric fusion): Normal carrier

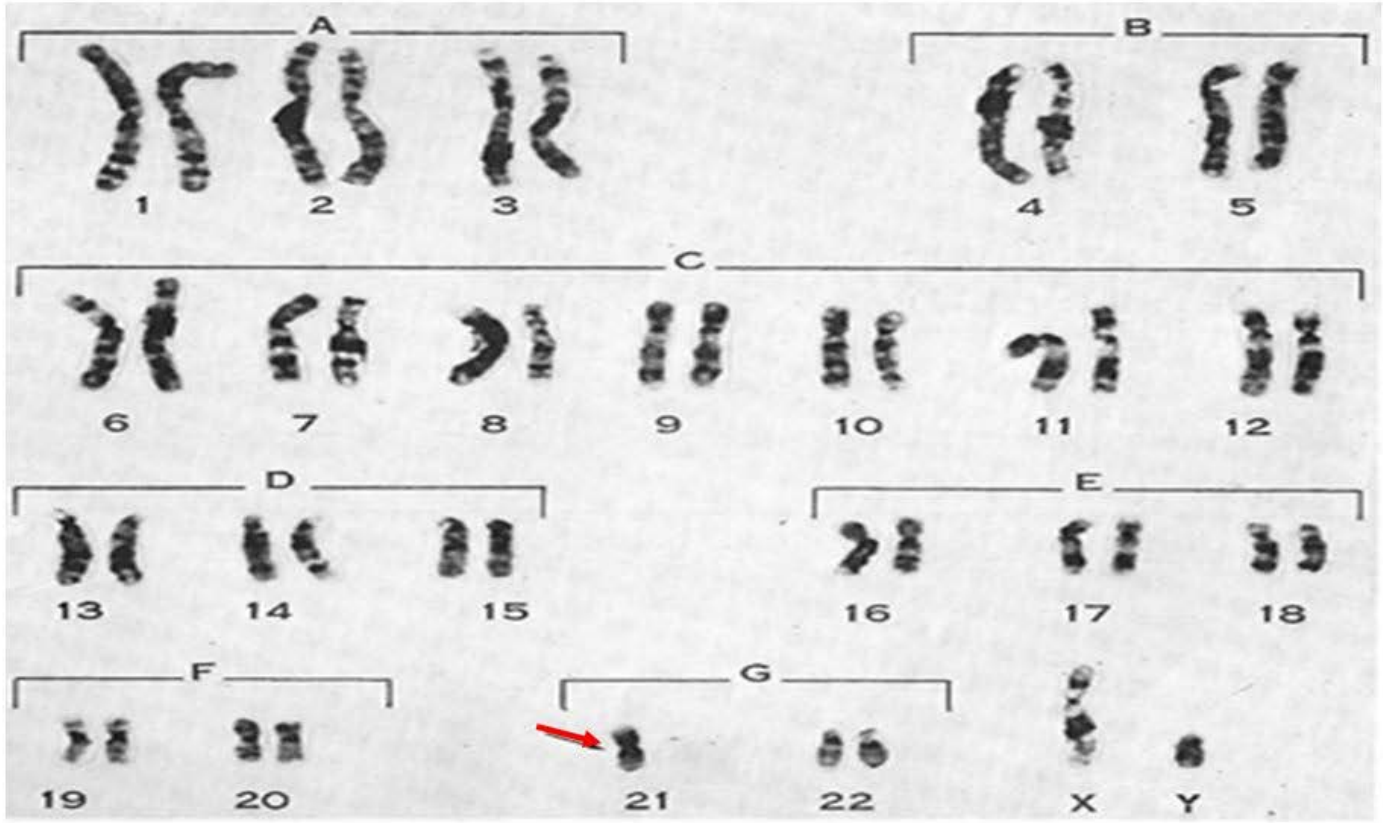


G-banding karyotype of 45,XY,-21,-21,+rob(21q21q)

Possible germline genome editing case

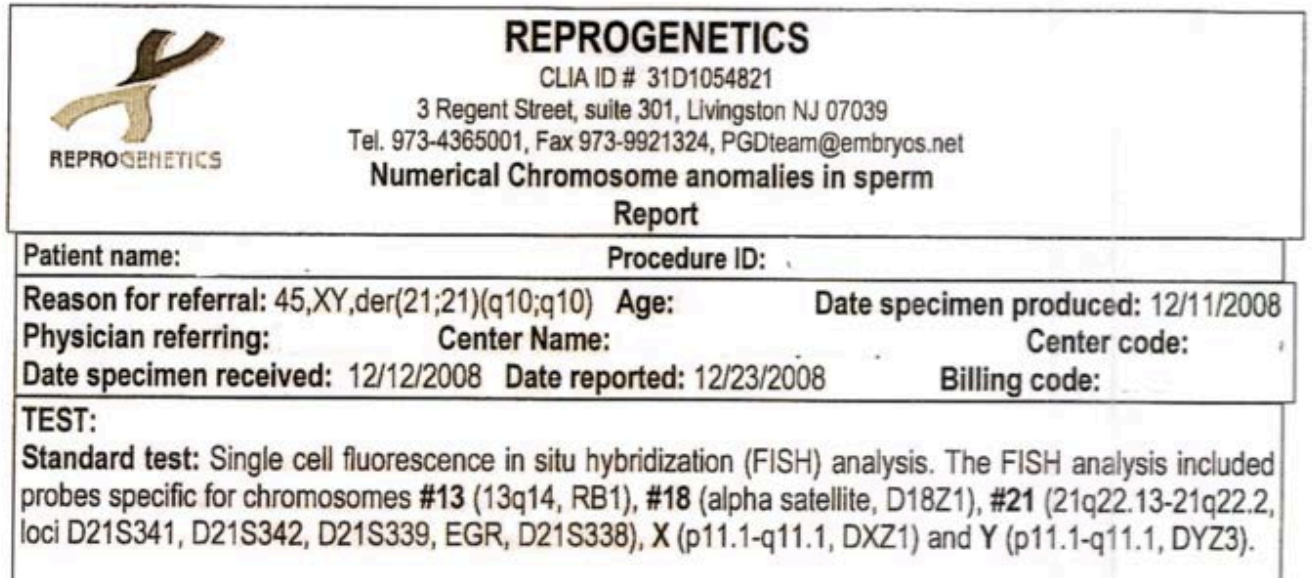
Structural abnormalities-cont'd

❖ Robertsonian translocation (centric fusion): Normal carrier



G-banding karyotype of 45,XY,-21,-21,+rob(21q21q)

t(21;21) carrier sperm analyses



REPROGENETICS
CLIA ID # 31D1054821
3 Regent Street, suite 301, Livingston NJ 07039
Tel. 973-4365001, Fax 973-9921324, PGDteam@embryos.net
Numerical Chromosome anomalies in sperm Report

Patient name: **Procedure ID:**

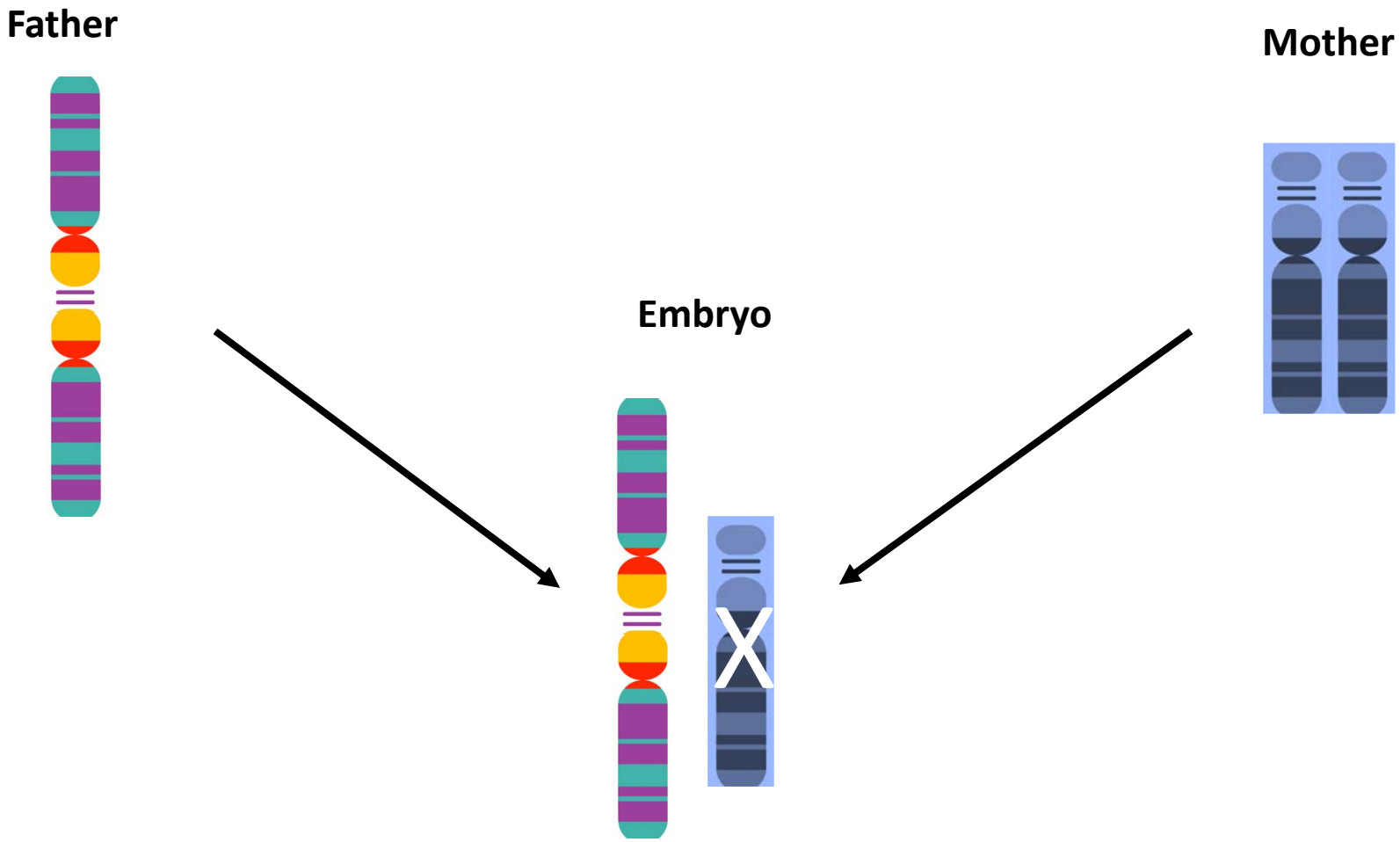
Reason for referral: 45,XY,der(21;21)(q10;q10) **Age:** **Date specimen produced:** 12/11/2008
Physician referring: **Center Name:** **Center code:**
Date specimen received: 12/12/2008 **Date reported:** 12/23/2008 **Billing code:**

TEST:
Standard test: Single cell fluorescence in situ hybridization (FISH) analysis. The FISH analysis included probes specific for chromosomes **#13** (13q14, RB1), **#18** (alpha satellite, D18Z1), **#21** (21q22.13-21q22.2, loci D21S341, D21S342, D21S339, EGR, D21S338), **X** (p11.1-q11.1, DXZ1) and **Y** (p11.1-q11.1, DYZ3).

RESULTS:

Sperm type	No of Sperm.	% of Sperm
Total Counted with results:	500	
Sperm cells with no results:	0	
X/Y Ratio *		
X-bearing sperm	250	50.2 %
Y-bearing sperm	248	49.8 %
Normal sperm	**0**	**0 %**
Abnormal sperm	**500**	**100 %**
Nullisomy X/Y	1	0.2 %
Nullisomy 13	2	0.4 %
Nullisomy 18	2	0.4 %
Nullisomy 21	252	50.4 %
Disomy 13	2	0.4 %
Disomy 18	2	0.4 %
Disomy 21	248	49.6 %
Disomy X	1	0.2 %

Chromosome 21 removal in the preimplantation embryo



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Timing of CRISPR/Cas9 activity

New Results

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Delayed indel formation after Cas9 cleavage promotes mosaicism and aneuploidy in human embryos

Alex Robles, Julie Sung, Stepan Jerabek, Jishnu Talukdar, Diego Marin, Jia Xu, Nathan Treff, Dieter Egli

doi: https://doi.org/10.1101/2025.05.07.652614

ASRM 2023
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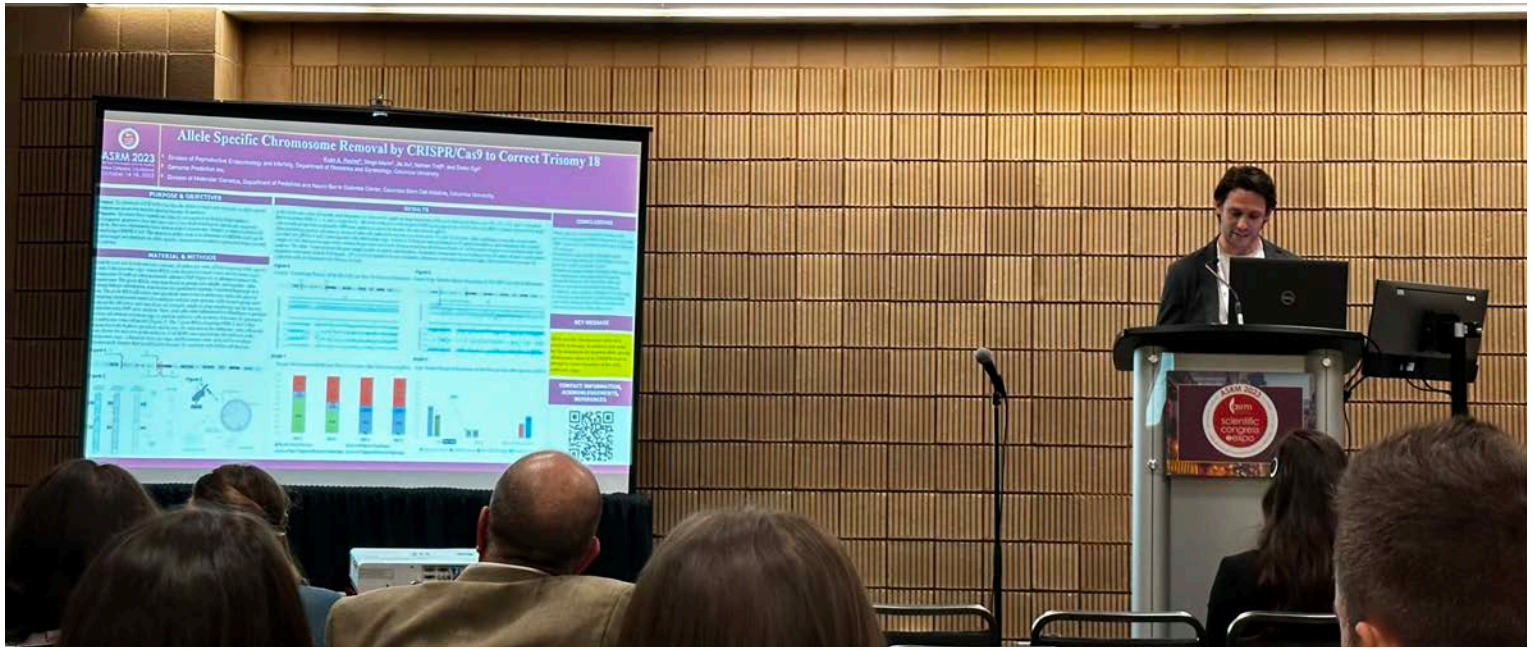
Allele Specific Chromosome Removal by CRISPR/Cas9 to Correct Trisomy 18

Evan A. Reshef[1], Diego Marin[2], Jia Xu[2], Nathan Treff[2], and Dieter Egli[3]

1 Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Columbia University. 2.Genomic Prediction Inc. 3. Division of Molecular Genetics, Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia Stem Cell Initiative, Columbia University.



Evan Rashef, MD
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The Future of IVF, Genome Editing, and Preimplantation Genetics: *Curing Disease before Pregnancy*

Whole genome sequencing and germline editing to prevent Monogenic, Polygenic, and Aneuploidy Disorders

-Nathan Treff

Thank you!

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