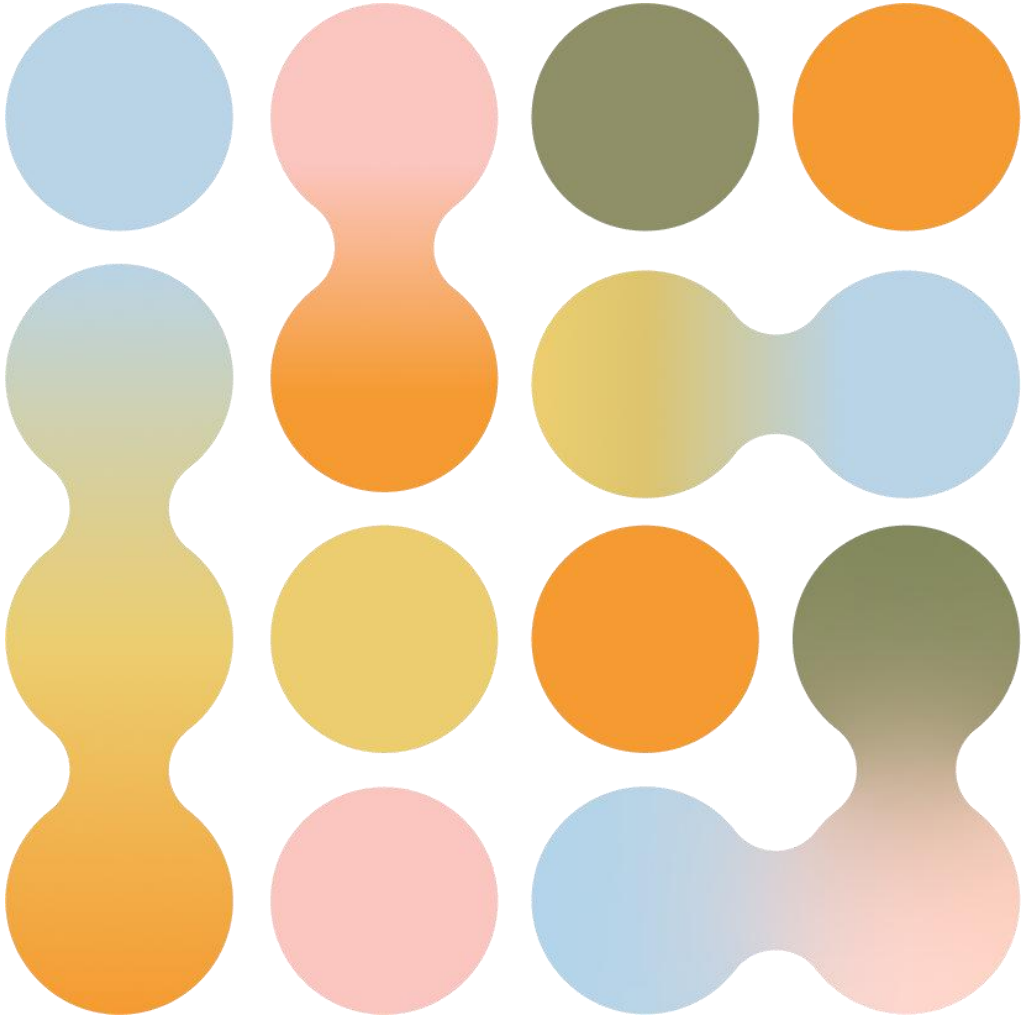
Luminary Genetics

Benefits of SNP-Based NGS

Claire Jones, MS, LCGC

Southwest Embryology Summit 2025



Introduction to Luminary Genetics

- PGT company utilizing Next Generation Sequencing (NGS)
  - Located in Santa Clara, CA
  - CAP/CLIA certified
- Cengiz Cinnioglu, Ph.D.
  - Founder, Luminary Genetics (formerly known as NextGen Genetics)
- Team of laboratory personnel, genetic counselors, molecular biologist, software engineer, sales, operations, billing and more
  - Claire Jones, MS, LCGC
    - 7 years in the field of genetic counseling
    - 3 years as a practicing genetic counselor
    - 1 year specializing in IVF/PGT

Current Products and Offerings

- Standard products
  - PGT-A (Pre-implantation Genetic Testing for Aneuploidy)
  - PGT-SR (Pre-implantation Genetic Testing for Segmental Rearrangements)
  - PGT-M (Pre-implantation Genetic Testing for Monogenic Disorders)
  - POC (Product of Conception)
- New products
  - PGT-A Link™ (cohort QC) via SNP-NGS
  - PGT-A LinkPlus™ (cohort QC and gamete confirmation) via SNP-NGS
  - PGT-M with SNP-NGS
- Other offerings
  - Genetic counseling
  - Research

Agenda

- Moving from next generation sequencing (NGS) to single nucleotide polymorphism with next generation sequencing (SNP-NGS)
  - Comparison of NGS vs SNP-NGS
  - Using SNP-NGS to determine ploidy status of 0PN/1PN embryos
    - Current literature
    - Survey
    - Internal studies using STR for 0PN/1PN embryos
  - Other benefits of SNP-NGS
    - Contamination detection

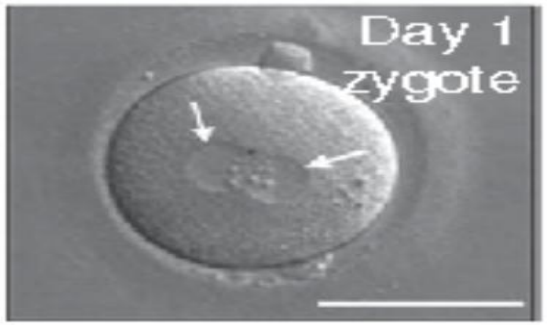
Comparison: NGS vs SNP-NGS

Detection	NGS	SNP-NGS
Aneuploidy	✓	✓
Structural abnormalities	✓	✓
Mosaicism	✓	✓
Polyploidy	✓ (69 XXY only)	✓ (69 XXX and XXY)
Haploidy	✗	✓
Cohort QC	✗	✓
Monogenic disorder	✗	✓
Contamination detection (cumulus cells)*	✗	✓
Gamete confirmation**	✗	✓

* Biological maternal samples required.
** Both biological maternal and paternal samples required.

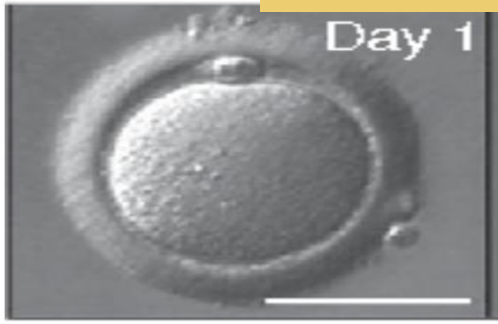
Fertilization Check (0PN, 1PN, 2PN, 2.1PN, and 3PN)

Normal fertilization

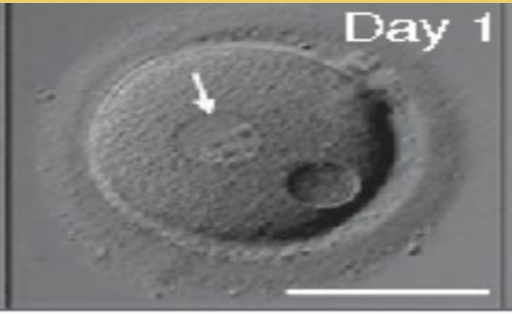


2PN

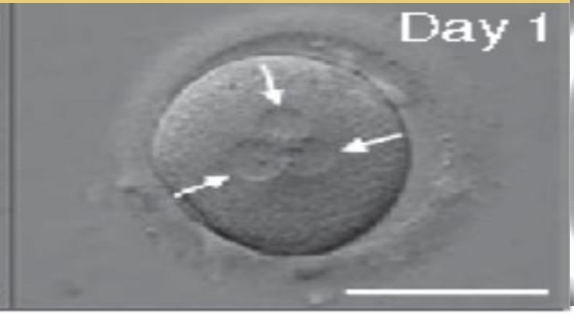
Abnormal fertilization



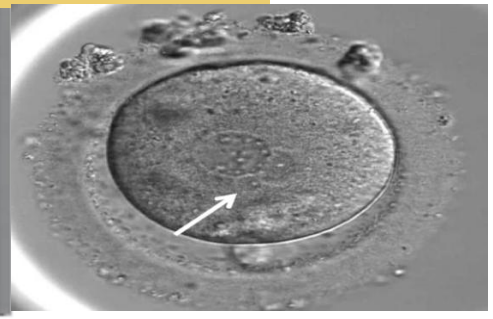
0PN



1PN



3PN



2.1PN

Paula Faduola, *Transl Biomed* 6:1, 2015.

Abnormally Fertilized Oocytes Can Result in Healthy Live Births

- 5,026 metaphase II oocytes injected
- 5.2% 1PN
- 0.7% 2.1PN
- 1PN-derived blastocysts were mostly diploid
  - Diploid: 9/13 (69.2%)
  - Haploid: 3/13 (23.1%)
  - Triploid: 1/13 (7.7%)
- The 2.1PN-derived blastocysts were mostly diploid (12/14; 85.7%); remainder were triploid
- Most 1PN embryos **arrest** their development even before the first cellular division

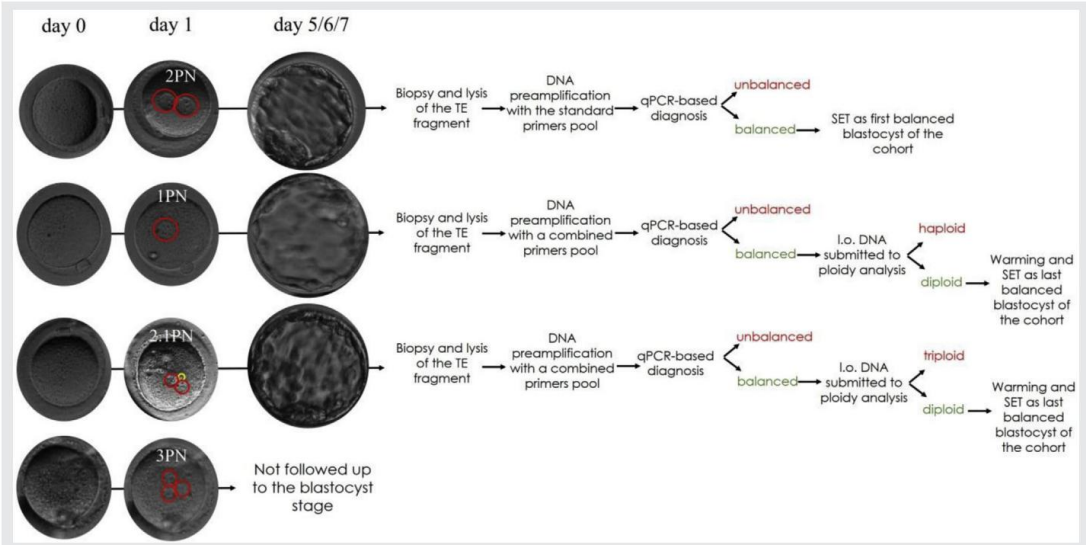
CrossMark

Abnormally fertilized oocytes can result in healthy live births: improved genetic technologies for preimplantation genetic testing can be used to rescue viable embryos in in vitro fertilization cycles

Antonio Capalbo, Ph.D.,[a,b] Nathan Treff, Ph.D.,[c] Danilo Cimadomo, M.Sc.,[a,d] Xin Tao, Ph.D.,[c] Susanna Ferrero, M.D.,[a] Alberto Vaiarelli, M.D.,[a] Silvia Colamaria, M.D.,[a] Roberta Maggiulli, Ph.D.,[a] Giovanna Orlando, M.Sc.,[a] Catello Scarica, Ph.D.,[a] Richard Scott, M.D.,[c] Filippo Maria Ubaldi, M.D.,[a,b] and Laura Rienzi, M.Sc.[a,b]

[a] Genera, Centers for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy; [b] Genetyx, Molecular Genetics Laboratory, Marostica, Italy; [c] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey; and [d] Dipartimento di Scienze Anatomiche, Istologiche, Medico Legali e dell'Apparato Locomotore, Università degli Studi di Roma "Sapienza," Rome, Italy

Objective: To test whether abnormally fertilized oocyte (AFO)–derived blastocysts are diploid and can be rescued for clinical use.
Design: Longitudinal-cohort study from January 2015 to September 2016 involving IVF cycles with preimplantation genetic testing for aneuploidy (PGT-A). Ploidy assessment was incorporated whenever a blastocyst from a monopronuclear (1PN) or tripronuclear zygote (2PN + 1 smaller PN; 2.1 PN) was obtained.



Antonio Capalbo et al., *Fertility and Sterility* Vol. 108, No. 6, December 2017.

& More Recent Papers...

Ploidy results distribution in blastocysts derived from normally and abnormally fertilized oocytes and the correlation with time-lapse assessment

L. Girardi [1], C. Patassini [1], J. Miravet Valenciano [2], Y. Sato [3], N. Fagundes Cagnin [4], J.A. Castellón [5], D. Blesa [2], J. Jimenez Almazan [2], A. Akinwole [6], B. Coprerski [4], C. Rubio [2]

Developmental potential of non- and mono-pronuclear zygotes and associated clinical outcomes in IVF cycles

Mengyi Zhu†, Qiyin Dong†, Yurong Zhu, Yun Le, Tengfei Wang, Yuanping Zhou, Sheng Yang*

Clinical outcomes of frozen–thawed blastocysts from zygotes with no or one pronucleus for in vitro fertilization and intracytoplasmic sperm injection cycles

Xiaomei Tong [1 2], Jiamin Jin [1 2], Yamei Xue [1 2], Lu Fang [1 2], Haiyan Zhu [1 2], Lingying Jiang [3 4], Songying Zhang [5 6]

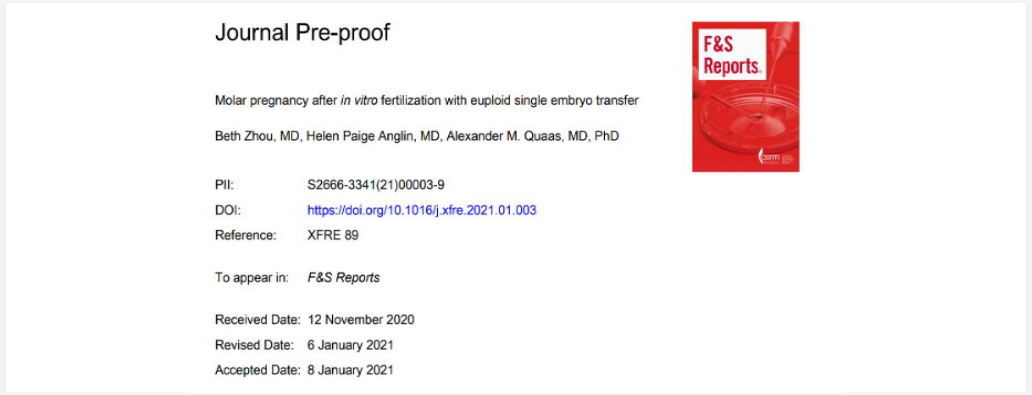
Article

The Identification of Molecular Ploidy Status of Abnormal Pronuclear Zygotes Reveals a Significant Number of Euploid Blastocysts Available for Conception

Blair R. McCallie *, Mary E. Haywood, Lauren N. Henry, Rachel M. Lee, William B. Schoolcraft and Mandy G. Katz-Jaffe

Case Report: Molar Pregnancy After In Vitro Fertilization with Euploid Single Embryo Transfer

- 42 years old, 1PN resulted in 46,XX (with NGS)
- Limitation: Haploidy and triploidy cannot be detected with NGS platform. Single-nucleotide polymorphism (SNP) analysis was not performed
- The patient was counseled regarding outcomes from transfers of embryos arising from monopronuclear zygotes, in particular a remote risk of triploidy, and desired to proceed with the embryo transfer
- 1PN embryo was transferred and resulted in miscarriage
- POC results with NGS/STR showed 46,XX complete molar pregnancy with a single set of paternally derived alleles confirmed with the use of short tandem repeats (STRs)



Journal Pre-proof

Molar pregnancy after *in vitro* fertilization with euploid single embryo transfer

Beth Zhou, MD, Helen Paige Anglin, MD, Alexander M. Quaas, MD, PhD

PII: S2666-3341(21)00003-9
DOI: https://doi.org/10.1016/j.xfre.2021.01.003
Reference: XFRE 89

To appear in: *F&S Reports*

Received Date: 12 November 2020
Revised Date: 6 January 2021
Accepted Date: 8 January 2021

Molar pregnancy

Complete mole: A complete mole is caused by a single (90%) or two (10%) sperm combining with an egg that has lost its DNA (the sperm then reduplicates forming a "complete" 46 chromosome set). The genotype is typically 46,XX (diploid) due to subsequent mitosis of the fertilizing sperm, but can also be 46,XY (diploid). 46,YY (diploid) is not observed.

Complete moles have a risk of up to 15–20% of degenerating into a choriocarcinoma.

Partial mole: It occurs when an egg is fertilized by two sperm or by one sperm that reduplicates itself yielding the genotypes of 69,XXY (triploid) or 92,XXXY (tetraploid). Complete hydatidiform moles have a higher risk of developing into choriocarcinoma—a malignant tumor of trophoblast cells—than do partial moles.

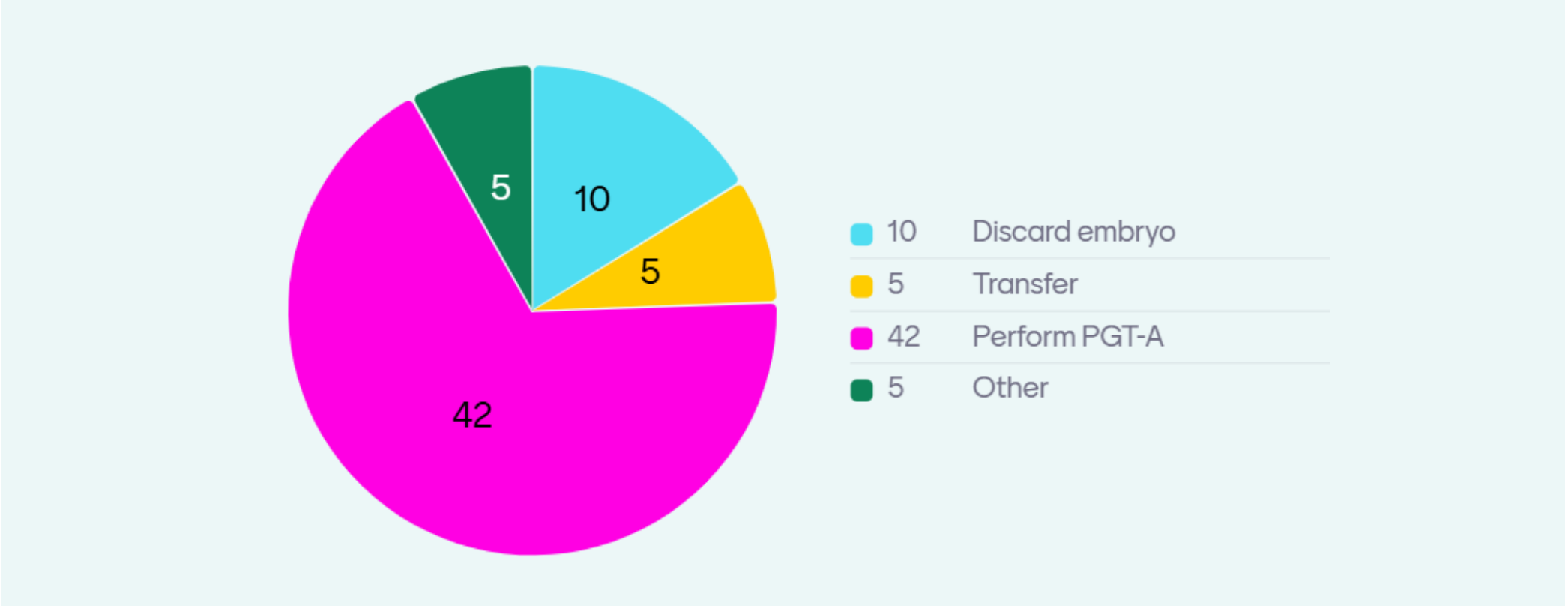
ASRM Guidelines (2022)

Fertilization Check

Written procedures for performing fertilization check should include a defined period of time that fertilization check is to occur and the time and technician performing the fertilization check, the status of each oocyte should be recorded (number of pronuclei and if not fertilized the maturity and number of polar bodies as applicable), and there should be a written procedure that for the immediate disposition of oocytes with an abnormal number of pronuclei. This may include disposal, continued culture, freezing, training, or institutional review board-approved research.

SWES Survey 2025:
What do you currently do with 0PN and 1PN embryos that continue to develop?

Review of Survey from 2024



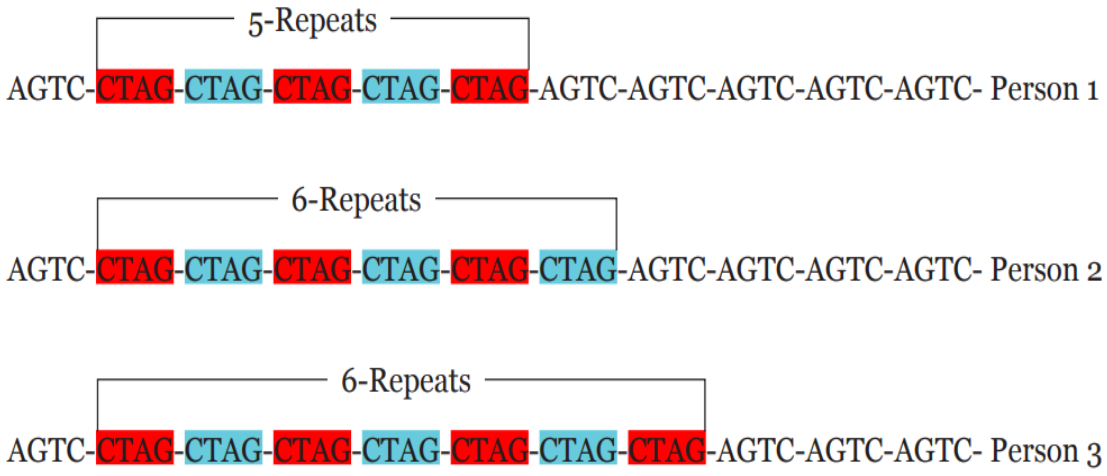
SWES Survey 2025:
Has your clinic removed fertilization checks altogether?

Join at menti.com | use code 4382 8481

STR

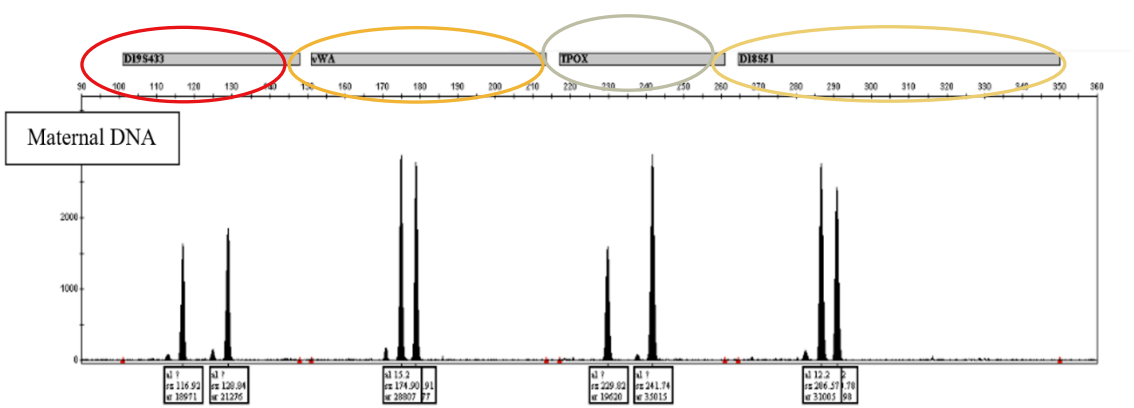
Clinical Application of Short Tandem Repeat (STR)

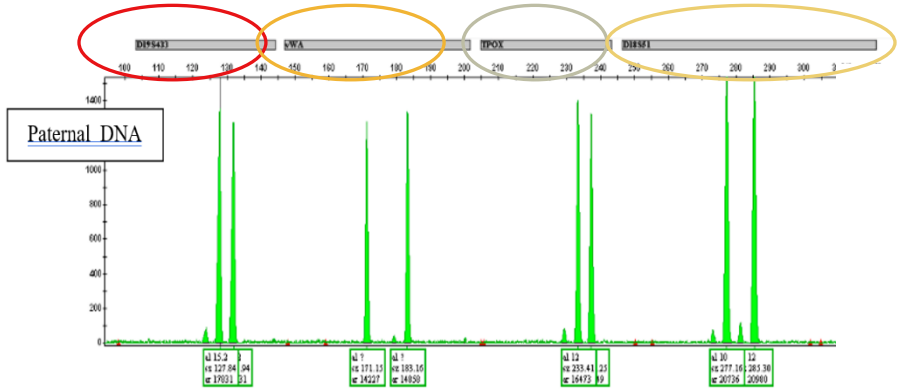
- Parentage
- Human identification/fingerprinting
- Product of conception testing (POC)
  - Detect or rule out maternal cell contamination (MCC)
  - Detecting molar pregnancy (partial/complete mole)
- Embryo
  - Detecting haploidy (23,X/46,XX)
  - Single-gene disorders (PGT-M)
  - niPGT: detecting possible cumulus cell contamination for niPGT-A

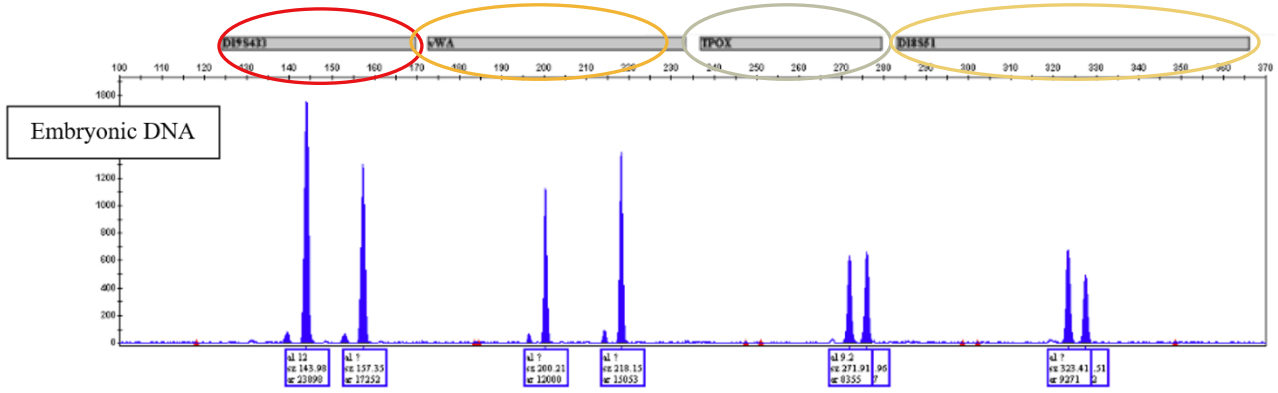


Embryo DNA Fingerprinting (Parentage)

Forensic application—short tandem repeat (STR)





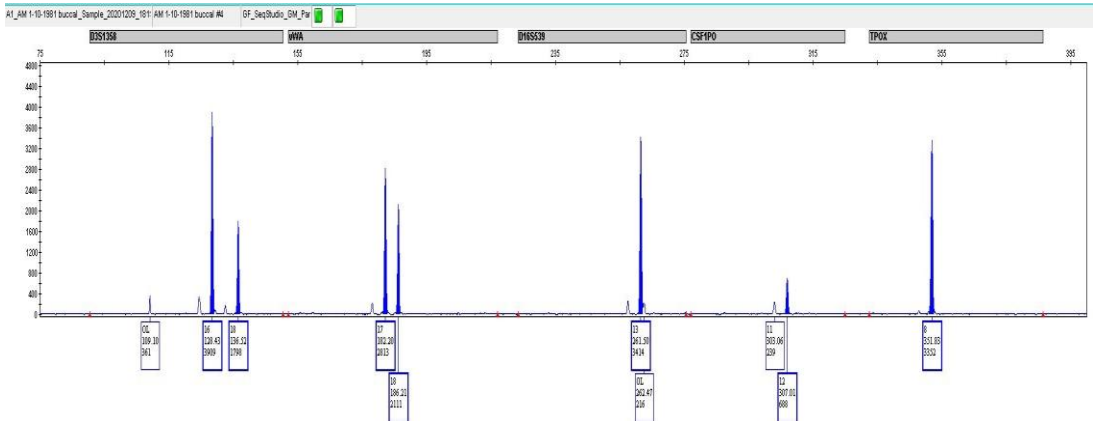


Capillary electrophoresis

Validation: Haploidy and Diploidy Detection for Embryo Analysis with STR

Maternal DNA (STR profile)

Paternal DNA (STR profile)



Embryonic DNA (STR profile)

Validation: Haploidy and Diploidy Detection for Embryo Analysis with STR with Parental Sampling

Sample / Marker	FATHER (BF)		MOTHER (LF)		EMBRYO LF1161986-1	
D3S1358	16	17	16	18	17	18
VWA	15	18	14	17	15	17
D16S539	10	13	11	13	11	13
CSF1PO	10	12	10	12	10	12
TPOX	8	11	8	11	NO RESULT	
Y indel	2		-		-	
Amel	X	Y	X	X	X	X
D8S1179	14	15	11	15	14	15
D21S11	28	31	30	31	31	31
D18S51	11	15	15	16	11	15
DYS391	NO RESULT		NO RESULT		NO RESULT	
D2S441	11	14	10	14	10	NO RESULT
D19S433	13	14	12	15	12	14
TH01	6	6	9	9.3	6	9.3
FGA	21	21	21	24	21	24
D22S1045	16	16	16	17	NO RESULT	
D5S818	12	12	10	11	11	12
D13S317	9	10	11	12	9	12
D7S820	11	12	10	11	10	11
SE33	17	17	NO RESULT		NO RESULT	
D10S1248	14	14	13	14	13	14
D1S1656	17	17.3	15	16.3	16.3	17
D12S391	15	20	21	22	20	21
D2S1338	17	18	19	25	17	25
CONCLUSION					Normal Euploid Embryo (heterozygous)	

Results interpretation

STR analysis is consistent with both parental contributions, indicative of a euploid embryo with the support of NGS analysis.

Validation: Haploidy and Diploidy Detection for Embryo Analysis with STR with Parental Sampling

Sample / Marker	FATHER (AM) 1-10-1981		MOTHER (LM) 7-11-1984		EMBRYO 1		EMBRYO 3	
D3S1358	16	18	15	18	18	18	15	
VWA	17	18	17	17	17	18	17	
D16S539	13	13	12	12	12	13	12	
CSF1PO	NA	NA	11	12	11	12	12	
TPOX	8	8	8	12	NA		NA	
Y indel	2	2	-	-	2	2		
Amel	X	Y	X	X	X	Y	X	
D8S1179	14	16	11	14	11	16	11	
D21S11	32	33.2	29	29	29	32.2	29	
D18S51	12	15	11	14	11	12	14	
DYS391	10	11	NA		10	11	NA	
D2S441	11	11.3	13	15	11	13	15	
D19S433	13	14	16	16.2	13	16.2	16	
TH01	8	9.3	6	9	6	8	9	
FGA	21	24	23	25	24	25	23	
D22S1045	11	15	16	16	NA		NA	
D5S818	11	12	11	11	11	NA	11	
D13S317	8	12	11	11	11	12	11	
D7S820	9	12	10	11	11	12	10	
SE33	21	22.2	23.2	23.2	22.2	23.2	23.2	
D10S1248	14	14	13	16	14	16	13	
D1S1656	12	15.3	15	17	15	15.3	15	
D12S391	17	19	17	19	17	19	19	
D2S1338	18	21	18	24	18	21	24	
CONCLUSION					Euploid 2 PN		1PN	

Results interpretation

Embryo 1: STR analysis is consistent with both parental contributions, indicative of a euploid embryo with the support of NGS analysis.

Embryo 3: STR profiles that were investigated showed only maternally derived alleles, indicative of a haploid embryo.

Haploidy and Diploidy Detection for Embryo Analysis with STR without Parental Sampling

Sample / Marker	EMBRYO 1		EMBRYO 3
D3S1358	18	18	15
VWA	17	18	17
D16S539	12	13	12
CSF1PO	11	12	12
TPOX	NA		NA
Y indel	2	2	
Amel	X	Y	X
D8S1179	11	16	11
D21S11	29	32.2	29
D18S51	11	12	14
DYS391	10	11	NA
D2S441	11	13	15
D19S433	13	16.2	16
TH01	6	8	9
FGA	24	25	23
D22S1045	NA		NA
D5S818	11	NA	11
D13S317	11	12	11
D7S820	11	12	10
SE33	22.2	23.2	23.2
D10S1248	14	16	13
D1S1656	15	15.3	15
D12S391	17	19	19
D2S1338	18	21	24
CONCLUSION	Euploid 2 PN		1PN

Results interpretation

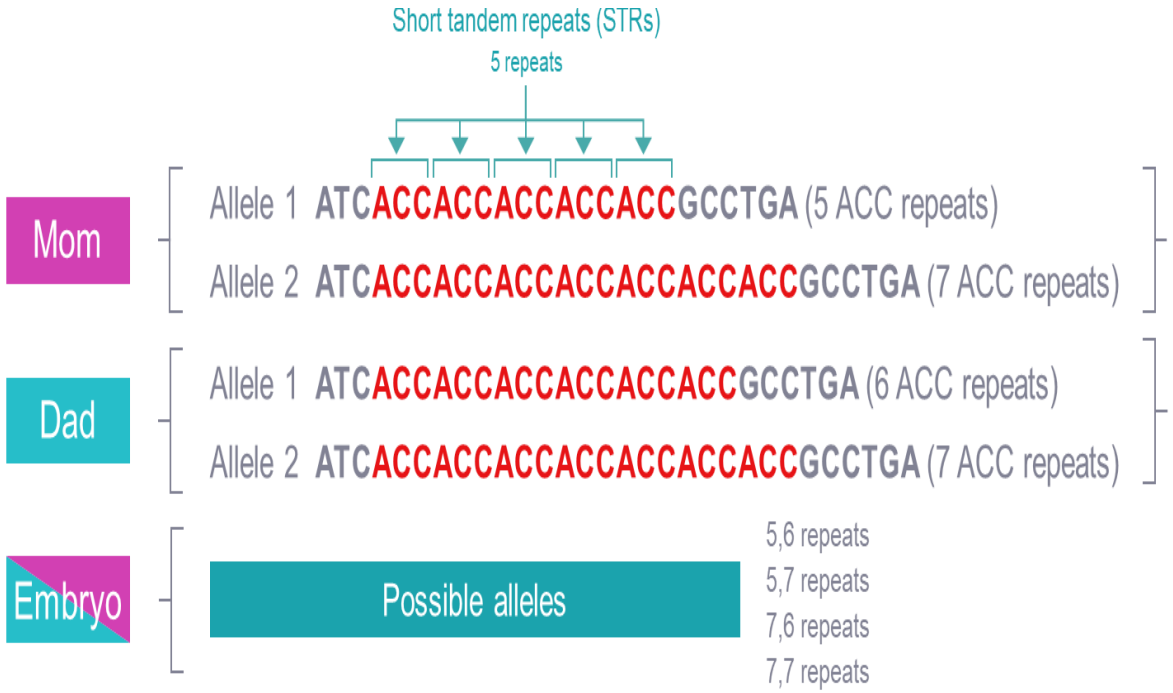
Embryo 1: All STR profiles that were investigated showed two alleles. This is indicative of a contribution from both parents and **suggests diploidy**.

Embryo 3: All STR profiles that were investigated showed only one allele. This is indicative of a contribution from only one parent and **suggests haploidy**.

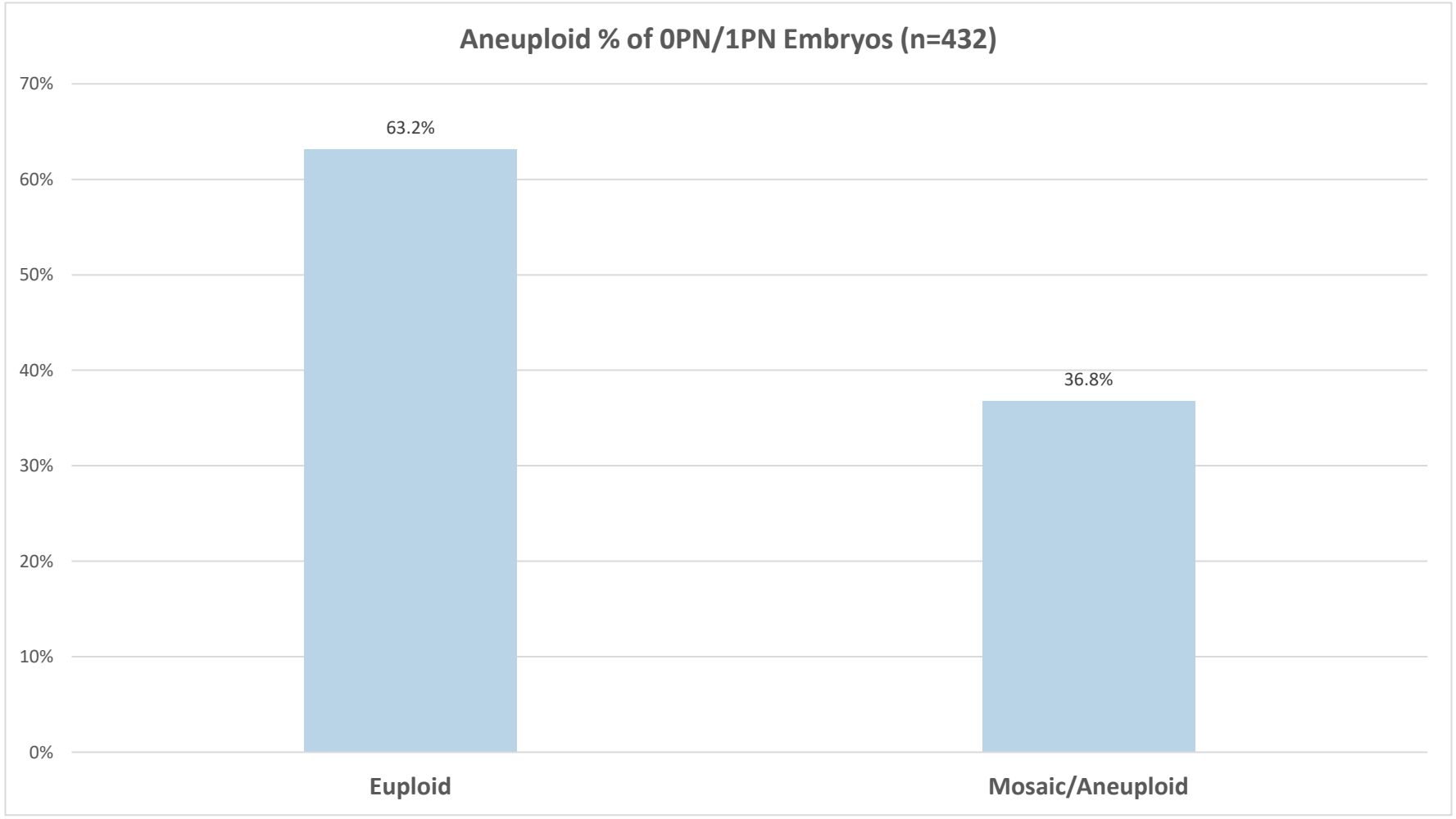
Luminary Genetics Study:
Chromosomal Analysis of Blastocysts Derived From 0 and 1PN Zygotes

- 4 IVF clinics
- N: 432 biopsy
- Fertilization check
  - 0 Pro Nuclear
  - 1 Pro Nuclear
- Initial testing with NGS
  - Further testing with STR for all Euploid results to Detect haploidy (23,X) vs Diploid (46,XX)

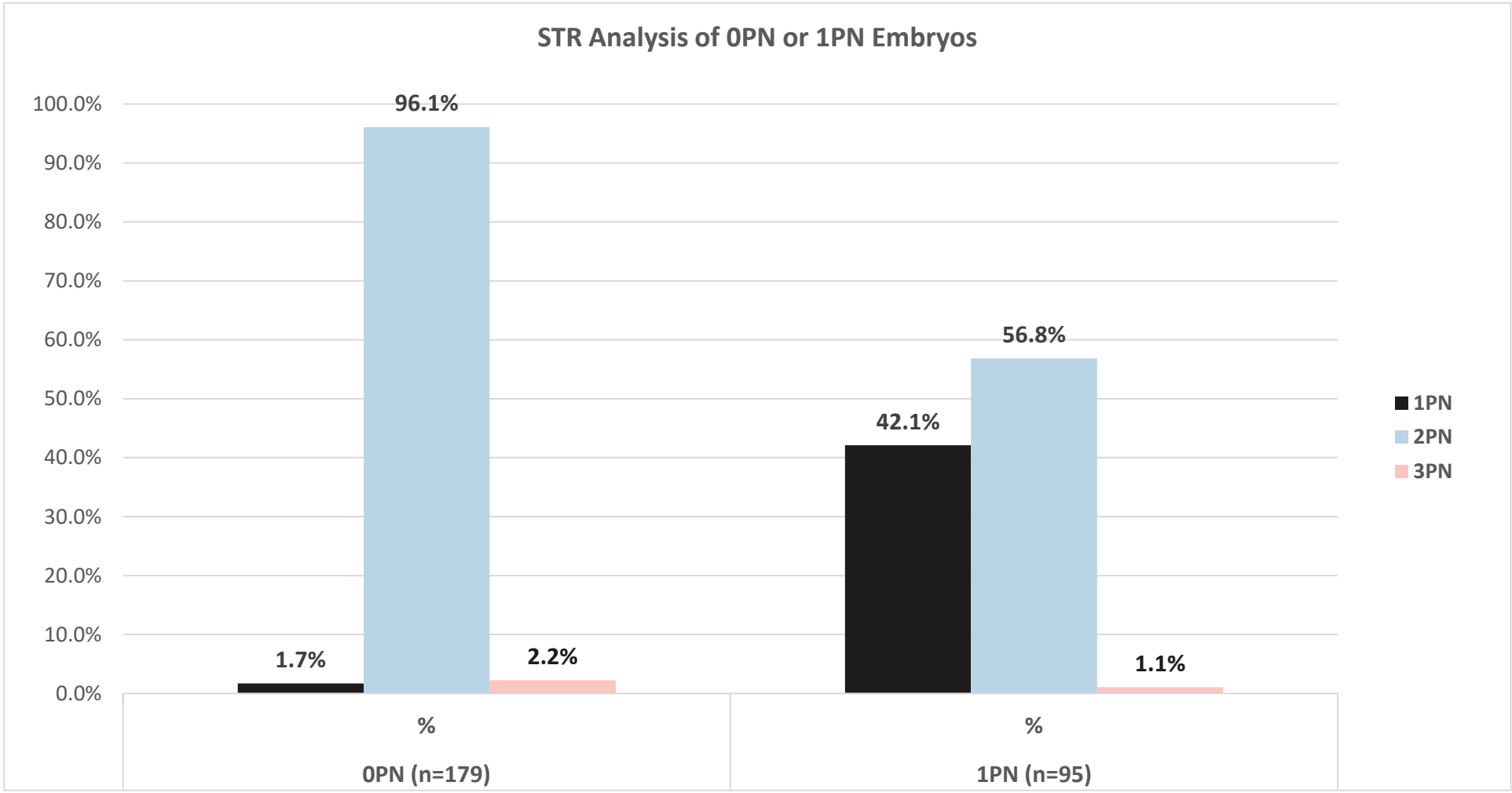
Short tandem repeat (STR)



Luminary Genetics Outcome Data:
Aneuploidy Rate Among 0PN and 1PN Embryos with NGS Only



Luminary Genetics Result Data:
STR Analysis on 0PN and 1PN Embryos



Case Example

EMBRYOS | TESTS | REPORTS | INVOICES

ALTER EMBRYO ID GENERATION

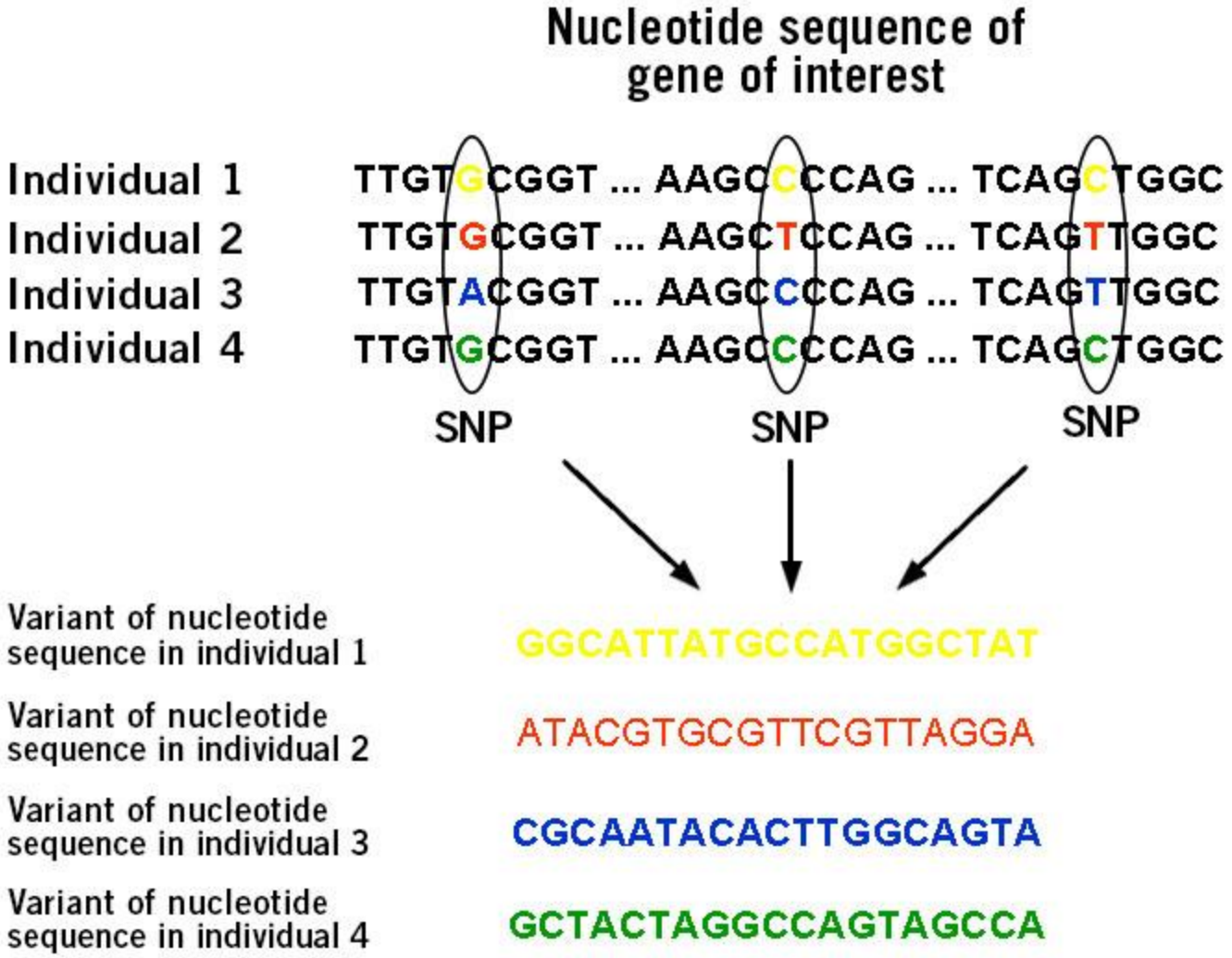
* Sample ID	Comments	Grade	Biopsy Stage	Rebiopsy (Previous No DNA Detected)	Arrested Embryo	Received	Cycle ID
[redacted]-1	0pn	4BB	6	☐	☐	☑	

Test Results Summary

Cycle ID	Embryo ID	Embryo Grade	Biopsy Day	Interpretation	Sex
	[redacted]-1	4BB	6	Euploid	XX

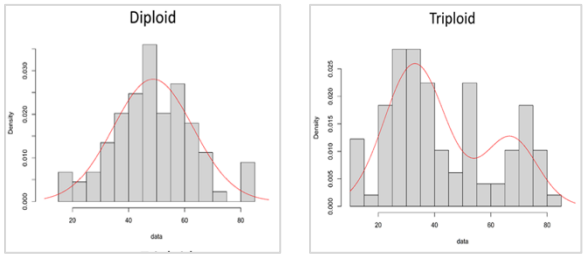
SNP-NGS

Targeted SNP-Based NGS

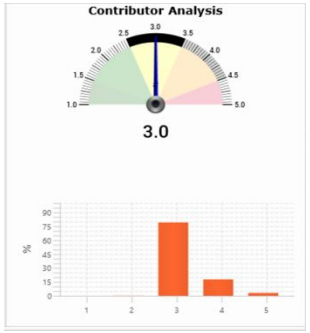


Targeted SNP-Based NGS

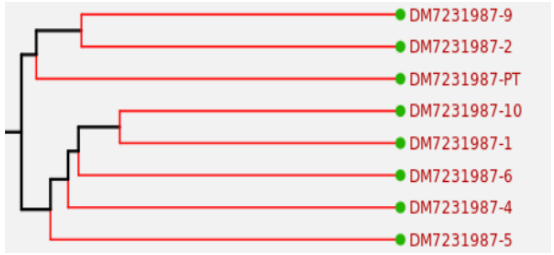
PGT-A Link



- Triploid detection
- 69,XXX females appears identical to 46,XX for PGT-A plots

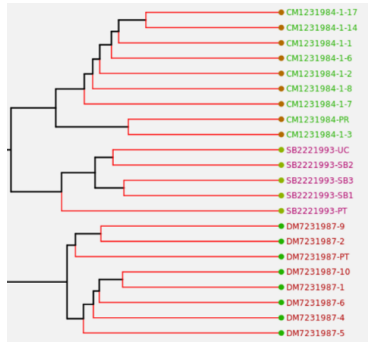


- External DNA contamination
- Cumulus cells not stripped from the oocyte skew PGT-A results



- Cohort QC
- Helps identify sample mix-ups

PGT-A Link*Plus*



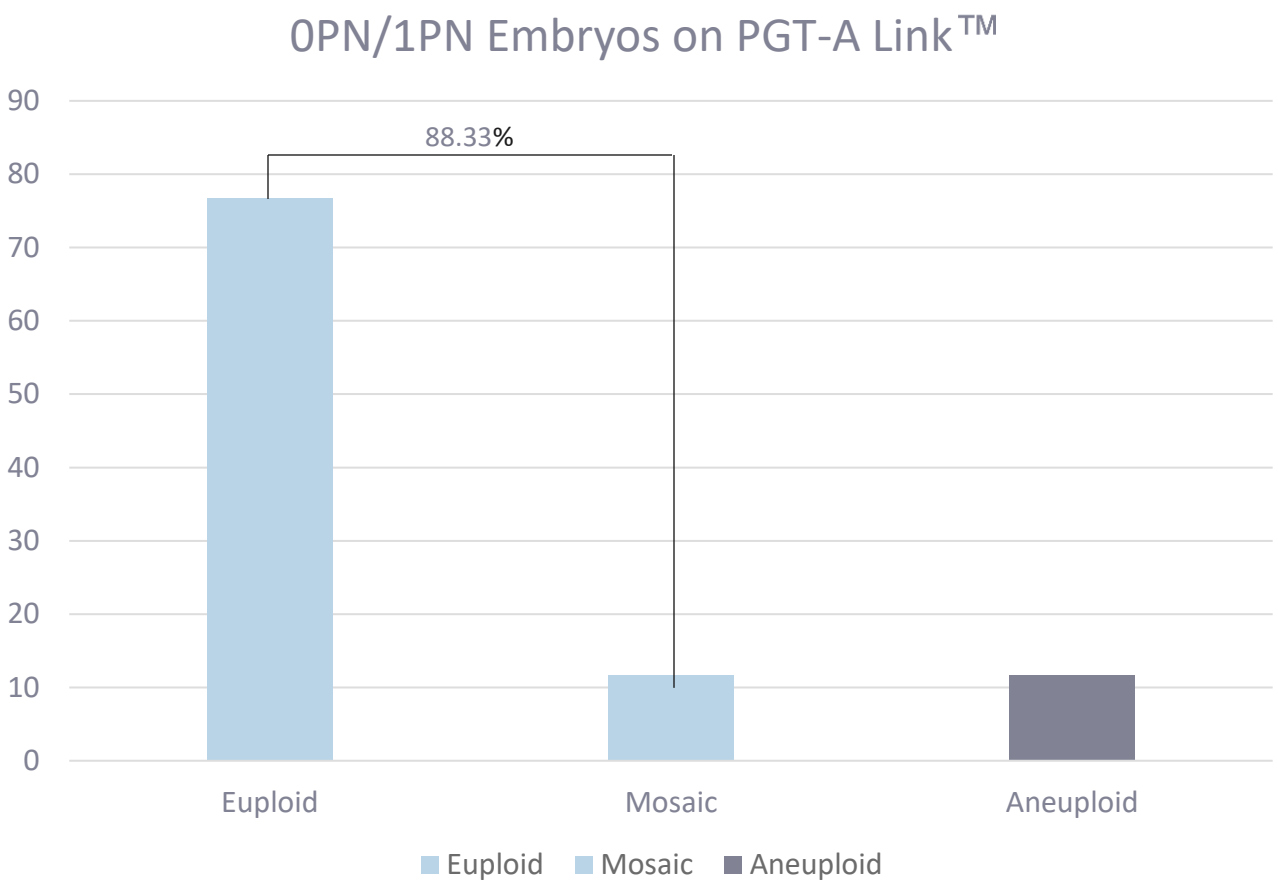
- Gamete source confirmation
- Helps identify sample mix-ups

- 74 microhaplotype amplicons selected for human identification
  - 222 SNP sites
  - 2–5 SNPs per amplicon, mean = 3
- 368 single SNP amplicons
  - Chosen to maximize MAFs in CEPH + five “super populations” based on 1000 Genomes data
    - African, Admixed American, East Asian, European, South Asian

Luminary Genetics Result Data:
PGT-A Link™ (SNP-NGS)

- ~15% of all embryos analyzed on SNP-NGS had a comment of 0PN or 1PN
- Haploidy rate <1%
- Triploidy rate ~1-2%
- Opportunity to rescue these embryos that may have been otherwise discarded or not transferred

Luminary Genetics Result Data: PGT-A Link™ (SNP-NGS)



Embryo Rescue

- <u>88.33% were found to be transferable</u> (euploid/low mosaic)*
- External contamination rate is <1%
- Cohort QC mismatch is 0%
- External contamination and cohort QC rates show embryologists are working at a high standard

*current and subject to change

Case Example

EMBRYOS | TESTS | REPORTS | INVOICES

ALTER EMBRYO ID GENERATION

* Sample ID	Comments	Grade	Biopsy Stage	Rebiopsy (Previous No DNA Detected)	Arrested Embryo	Received	Cycle ID
[redacted]-1	from 1PN	4AA	5	☐	☐	☑	
[redacted]-2		5AB	6	☐	☐	☑	
[redacted]-3		5BC	6	☐	☐	☑	

Test Results Summary

Cycle ID Embryo ID	Embryo Grade	Biopsy Day	Embryo Summary	Sex	Interpretation	Link™ (Cohort QC)
[redacted] 1	**4AA**	**5**	**Euploid**	**XX**	**Euploid**	**Match**
[redacted] 2	5AB	6	Aneuploid	XX	del(7)(p22.3p11.1) (57.77 Mb)	Match
[redacted] 3	5BC	6	Aneuploid	XX	Monosomy 15, 18	Match

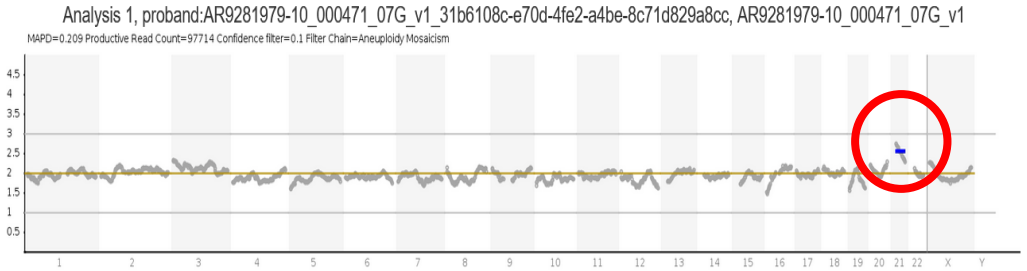
HMT: High Mosaic Trisomy. **LMT:** Low Mosaic Trisomy. **HMM:** High Mosaic Monosomy. **LMM:** Low Mosaic Monosomy. **HMdel:** High Mosaic Deletion. **LMdel:** Low Mosaic Deletion. **HMdup:** High Mosaic Duplication. **LMdup:** Low Mosaic Duplication

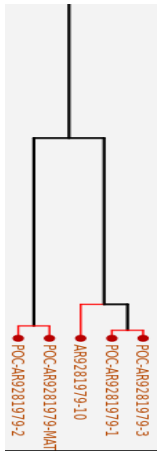
Outcome Data Coming – See Us at PCRS!

PREIMPLANTATION GENETIC TESTING (PGT) AND VIABILITY OF BLASTOCYST STAGE EMBRYOS DEVELOPED FROM ABNORMALLY FERTILIZED (0PN OR 1PN) OOCYTES

Case Report

Partial cumulus cell contamination detection; normalizing PGT-A with SNP-NGS





STR Marker	AR Maternal DNA			POC Dissection 1				POC Dissection 2			POC Dissection 3		
D3S1358	14	17		14	15	17		14	17		14	15	17
VWA	15	19		15	16	19		15	19		15	16	19
D16S539	12	12		12		12		12	12		12		12
CSF1PO	10	12		10		12		10	12		10		12
TPOX	8	8		8		10		8	8		8		10
Y indel													
Amel	X	X		X		X		X	X		X		X
D8S1179	14	15		14		15		14	15		14		15
D21S11	30	31		30	31	32		30	31		30	31	32
D18S51	14	22		15		22		14	22		15		22
DYS391	NO RESULT			NO RESULT				NO RESULT			NO RESULT		
D2S441	11	11		11		11		11	11		11		11
D19S433	12	16		12		14		12	16		12		14
TH01	6	9		9		9		6	9		9		9
FGA	21	22		21	22	23		21	22		21	22	23
D22S1045	11	15		11		15		11	15		11		15
D5S818	11	12		11		11		11	12		11		11
D13S317	13	14		11	13	14		13	14		11	13	14
D7S820	10	10		8		10		10	10		8		10
SE33	21.2	28.2		19		28.2		21.2	28.2		19		28.2
D10S1248	7	13		13		14		7	13		13		14
D1S1656	15	16		13		15		15	16		13		15
D12S391	18	20		17	18	20		18	20		17	18	20
D2S1338	19	20		19	20	22		19	20		19	20	22
CONCLUSION				Partial MCC				Complete MCC			Partial MCC		

Agenda

- Moving from next generation sequencing (NGS) to single nucleotide polymorphism with next generation sequencing (SNP-NGS)
  - Comparison of NGS vs SNP-NGS
  - Using SNP-NGS to determine ploidy status of 0PN/1PN embryos
    - Current literature
    - Survey
    - Internal studies using STR for 0PN/1PN embryos
  - Other benefits of SNP-NGS
    - Contamination detection

Luminary Genetics

Thank you!

Claire Jones, MS, LCGC
Genetic Counselor
claire.jones@luminarygenetics.com

Luminary Genetics
Genetic Counseling Team
(408) 503-6484
gc@luminarygenetics.com