Preimplantation Genetic testing (PGT) in Assisted Reproduction (ART), 2019

Changing the Paradigm of Clinical Practice

Fayek Nicholas Shamma MD FACOG
President, and CEO
IVF Michigan PC and TFC LLC
With seven locations, the physicians of IVF Michigan Fertility Center are available to help couples throughout the midwest and abroad.

**Ann Arbor**
3145 W. Clark Rd.,
Suite 301
Ypsilanti, MI 48197
(734) 434-4766

**Bloomfield Hills**
37000 Woodward Ave.,
Suite 350
Bloomfield Hills, MI 48304
(248) 952-9600

**Dearborn**
5728 Schaefer Rd.,
Suite 203
Dearborn, MI 48126
(313) 582-4333

**East Lansing**
2601 Coolidge Rd.,
Suite B
East Lansing, MI 48823
(517) 679-1410

**Macomb**
15959 Hall Rd.,
Suite 401
Macomb, MI 48044
(586) 997-8700

**Petoskey**
Bay View
2810 Charlevoix Ave.
Petoskey, MI 49770
(231) 487-0970

**Saginaw**
5400 Mackinaw Rd.,
Suite 4100
Saginaw, MI 48604
(989) 792-8771

**Toledo**
6711 Monroe St.
Bldg. 3, Suite A
Sylvania, OH 43560
(419) 885-8080
Steps of ART: controlled ovarian hyper-stimulation, followed by oocyte retrieval, laboratory embryo culture, and uterine embryo transfer. Embryos can be transferred fresh or cryopreserved and then eventually thawed, and subsequently transferred.

Factors that affect success include:

- EMBRYO QUALITY AND NUMBER
  - THAT DEPENDS ON:
    - THE PATIENT’S CHARACTERISTICS (namely age, and ovarian reserve, therefore genetics)
    - TREATMENT PROTOCOL
    - LABORATORY TECHNOLOGY AND PROFICIENCY

- UTERINE RECEPTIVITY

- TRANSFER EFFICIENCY
  - THAT DEPENDS ON PHYSICIAN’S EXPERTISE
Success of IVF

- **Multiple embryos** were traditionally transferred in situations where the implantation rate is low, occasionally because the laboratory technology is poor, or the patient reproductive potential is suboptimal.

- The process of embryo transfer involving more than one embryo often results in multiple gestations.

- We consider a multi-fetal pregnancy a medical complication.
Multiple Gestation is “The most serious complication of ART”

<table>
<thead>
<tr>
<th></th>
<th><strong>Triplets</strong></th>
<th><strong>Twins</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>20X risk</td>
<td>20X relative risk of delivery before 37 weeks</td>
<td>5.5X relative risk of delivery before 37 weeks</td>
</tr>
<tr>
<td>Mean birth</td>
<td>Mean birth weight 1735 grams</td>
<td>Mean birth weight 2389 grams</td>
</tr>
<tr>
<td>weight</td>
<td>Mean gestation 32.5 weeks</td>
<td>Mean gestation 35.8 weeks</td>
</tr>
<tr>
<td></td>
<td>Low birth weight in 90%</td>
<td>Low birth weight in 53% (9X singleton risk)</td>
</tr>
<tr>
<td></td>
<td>RR of a major handicap is 20 times</td>
<td>RR of a major handicap is NS increased</td>
</tr>
<tr>
<td></td>
<td>RR of CP is 50 times</td>
<td>RR of CP is 10 times</td>
</tr>
</tbody>
</table>
Elevated Health Risks & Gestational Number

Risks rise, still single digits, but the issues are serious

<table>
<thead>
<tr>
<th>Condition</th>
<th>Singleton</th>
<th>Twins</th>
<th>Triplets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant Mortality</td>
<td>0</td>
<td>4x</td>
<td>12x</td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td></td>
<td>10x</td>
<td>50x</td>
</tr>
<tr>
<td>Excessive L&amp;D Bleeding</td>
<td>2x</td>
<td></td>
<td>3x</td>
</tr>
</tbody>
</table>

Source: Luke, Pharoah
The New paradigm in ART

- A Single embryo per transfer.
- Almost 65% of transfers at IVF Michigan Fertility Centers in 2019 involve Pre-implantation genetic testing
- A euploid embryo is transferred
- Maximizing pregnancy rates, and minimizing multiple pregnancy
- Decreasing miscarriage rates
- (As most miscarriages happen because of aneuploidy)
Preimplantation genetic testing (PGT)

- Preimplantation genetic testing (PGT) is a test to determine if an embryo’s cells contain the appropriate number of chromosomes and therefore is fit to transfer.

- The **GOAL OF PGT** is to help us determine which embryos will lead to a live, healthy birth and to avoid transferring an embryo that will fail to implant, lead to a chemical pregnancy, a miscarriage, or the birth of an unhealthy child.

- PGT is in the midst of massive growth, and according to our data it is being used in upwards of 65% of all IVF cases.

- And this is despite **PGT’s hefty price tag** of $3,000 - $7,000, which is paid entirely by the patient, even when a patient’s insurance covers the cost of IVF treatment.
Technologies for 24 Chromosome PGT

- Metaphase spreads (1980’s)
- Spectral Karyotyping (1997)
- CGH (metaphase spreads)
- SNP arrays
- qPCR

Next Gen Sequencing
Technologies for 24 Chromosome PGT

- aCGH is dependent upon successful whole genome amplification (WGA) of metaphase DNA from the biopsied cell(s) in order to generate enough DNA for analysis.

- Following WGA, the embryonic DNA is fluorescently labelled, denatured, and hybridized to an array platform containing thousands of DNA probes that are specific to each of the human chromosomes.

- Unbound or non-specifically bound DNA is removed by washing, and a scanning device is used to measure the fluorescence intensity at each of the probes on the array.

- By comparing the fluorescence intensity of the embryonic sample with that of a control male sample (± control female sample), it is possible to determine the copy number of each chromosome in the biopsied cell(s).
Technologies for 24 Chromosome PGT: SNP

- Requires successful WGA
- Following WGA, the embryonic DNA is fragmented and hybridized to a SNP array platform, which contains probes for more than 300,000 different SNP sites across the genome
- Following hybridization, an extension and staining step is performed. A/T nucleotides at the SNP site are labelled with a red fluorochrome, and G/C nucleotides at the SNP site are labelled with a green fluorochrome
- By measuring the intensity of red-to-green fluorescence at each SNP site on the array, it is possible to simultaneously genotype more than 300,000 SNPs in each sample
- In some cases, parental DNA samples are also assessed. This parental SNP information can be used to track the inheritance of chromosomal material from the parents to the embryos. This helps to ‘clean up’ the noisy single-cell microarray data. In this way, many of the errors that are introduced during the WGA procedure (e.g. allele dropout, preferential amplification and amplification failure) can be detected
- High resolution, but expensive
Technologies for 24 Chromosome PGT: qPCR

- Involves the pre-amplification of embryonic DNA, followed by a high-order multiplex PCR reaction designed to amplify several loci from each chromosome.
- With the use of real-time qPCR, each product is quantitated, allowing a comparison across the genome.
- Only a few loci are assessed for each chromosome, resulting in significantly lower resolution.
Technologies for 24 Chromosome PGT: Next Generation Sequencing, NGS

- Requires **WGA** of the DNA from the biopsied cells
- Once amplified, the embryonic DNA is fragmented and tagged with a specific **barcode** to enable sample tracking. Hundreds of thousands of these small barcoded embryonic DNA fragments (from multiple embryo biopsy samples) are mixed together and **sequenced in parallel**
- Following sequencing, specialized computer software is used to differentiate the unique sample tracking barcodes, thereby enabling the results to be segregated according to embryo biopsy sample. Once segregated, each sequenced fragment from each sample is compared against the reference human genome and aligned with its corresponding chromosome region
- The number of aligned sequences along the length of each chromosome is then calculated
- Because the number of aligned sequences should be proportional to the copy number present in the original sample, trisomy or monosomy can be confidently identified
Embryo biopsy
Preimplantation Genetic Testing for Aneuploidies

PGT-A
<table>
<thead>
<tr>
<th></th>
<th>PGT-A</th>
<th>PGT-M</th>
<th>PGT-SR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Screens for chromosome abnormalities</strong></td>
<td>Screens for a specific single-gene disorder</td>
<td></td>
<td>Screens for inherited chromosome rearrangements</td>
</tr>
<tr>
<td><strong>Option for all IVF patients</strong></td>
<td>For couples at high-risk of having a child with a specific genetic disease</td>
<td></td>
<td>For carriers of translocations, inversions, and copy number variants</td>
</tr>
<tr>
<td><strong>Improves IVF success rates</strong></td>
<td>Reduces risk of genetic disease</td>
<td></td>
<td>Reduces risk of pregnancy loss/developmental disorders</td>
</tr>
<tr>
<td><strong>Does not require personalized test prep</strong></td>
<td>Requires personalized test prep and design</td>
<td></td>
<td>Sometimes requires personalized test prep</td>
</tr>
</tbody>
</table>
PGT-A with Preimplantation Genetic Testing for Monogenetic Disorders (PGT-M)

- Preimplantation genetic testing for monogenic disorders (PGT-M) predates PGT-A for embryo aneuploidy.
- With improvements in deoxyribonucleic acid (DNA) amplification techniques, it became possible to perform simultaneous PGT-M/PGT-A.
- Patients undergoing PGT-M/PGT-A ultimately will have fewer embryos remaining for transfer after testing.
The PGT-A process

**IVF**
In vitro fertilization is performed and the resulting embryos are incubated. ICSI is not required for PGT-A.

**EMBRYO TRANSFER**
If available, a chromosomally normal embryo is selected for transfer. Additional euploid embryos can remain frozen for future use.

**EMBRYO BIOPSY**
An embryologist carefully removes a small cell sample from each embryo.

**PGT-A**
Samples are sent to the PGT laboratory, testing is performed, and results are released to the IVF center.
Indications for PGT-A/M/SR

- Advancing maternal age
- Recurrent pregnancy loss
- Previous IVF failure
- Single embryo transfer
- Unexplained infertility
- Monogenic disorders
- Structural parental chromosomal concerns

Chromosomal health of embryos

Euploid Embryos

- Correct number of chromosomes
- Higher likelihood of successful pregnancy
Chromosomal health of embryos

Aneuploid Embryos

- Incorrect number of chromosomes
- Aneuploidy is found in embryos of IVF patient of any age, but shows a maternal age-dependent increase
- Result in:
  - Failed implantation
  - Miscarriage
  - Genetic disease
All couples are at risk

Maternal age

- Egg donors (N=7304)
  - Euploid: 54%
  - Clinical relevant mosaic: 32%
  - Aneuploid: 14%
- <35 (N=17329)
  - Euploid: 50%
  - Clinical relevant mosaic: 36%
  - Aneuploid: 14%
- 35-37 (N=11541)
  - Euploid: 43%
  - Clinical relevant mosaic: 45%
  - Aneuploid: 12%
- 38-40 (N=11328)
  - Euploid: 31%
  - Clinical relevant mosaic: 10%
  - Aneuploid: 8%
- 41-42 (N=5325)
  - Euploid: 20%
  - Clinical relevant mosaic: 8%
  - Aneuploid: 15%
- >42 (N=2653)
  - Euploid: 8%
  - Clinical relevant mosaic: 6%
  - Aneuploid: 14%

CooperGenomics, including IVF Michigan internal data on file
PGTai 2.0
At IVF Michigan and Cooper Genomics

- The PGTai\textsuperscript{SM} 2.0 Technology Platform is here.
- Best-in-class technology platform is about to get even better...
- By introducing single nucleotide polymorphism (SNP) analysis and paired-end sequencing, PGTai 2.0 will provide the following added benefits for you and your patients:
  - Female/male triploidy, or haploidy detection
  - Parent of origin of abnormality (optional)
  - 2PN validation
PGTai 2.0 platform – Technology overview

Built on basics of the PGTai platform
- Built on embryo biopsy data (>1000) resulting in live births and sustained pregnancy outcomes
- Validated using sequencing data from >10,000 embryos

Now utilizing on average >10x more data to provide:
- More accurate sequencing (paired-end sequencing)
- Global SNP coverage (world first for PGT-A)
- Two independent methods for aneuploidy detection (world first for PGT-A)
Not all whole chromosome aneuploidy is maternally derived

- Variability among aneuploidy inheritance exists
  - Meiotic whole chromosome aneuploidy are maternally derived ~90% of the time\(^1,2\)
  - Meiotic segmental aneuploidy is most often of paternal origin (~70%)\(^2\)
- Parent of origin aneuploidy assessment can provide direct assessment of gametic contribution to embryo aneuploidy
  - Enabling more confident donor gamete decisions
  - Providing patient piece of mind

1. Hassold et al. 1992
2. Kubicek et al. 2019
Clinical benefits of the PGTai 2.0 platform

Most robust assessment of embryos in the market; providing greater confidence in embryo transfers

- Two independent methods for confirming abnormality *(unique to CooperSurgical)*
- Detection of all forms of ploidy
  - Haploidy, male and female triploidy
- Parent of origin of abnormality *(optional)*
Single nucleotide polymorphisms (SNP) are a common form of genetic variation

- Each SNP is a difference in a single DNA building block, within a specific stretch of DNA
- They result from natural variation in human evolution and reproduction
- Most SNPs have no effect on health or development
Two independent methods for aneuploidy detection

- **Primary** (CNV) **AND secondary** (SNP) assessment of aneuploidy
  - Removal of artifacts
  - 5Mbp resolution
  - Best-in-class mosaic calling

- NGS copy number (chromosome) counting
- Global SNP (chromosome) counting

World first in PGT!
1300+ extra euploid embryos
(Net change observed in ploidy classification)
More patients with at least one euploid embryo

Observed a 4% increase in patients with ≥1 euploid available for transfer (p<0.0001)
Providing patients of all age ranges with a greater chance at a euploid embryo transfer
SNPs patterns can provide secondary aneuploidy assessment and aid in identifying genomic inheritance

- Each individual has a unique pattern of SNPs in their genome
- Act as biological markers
  - >99.8% inherited directly from parents
- Established method of assessing unique parental allelic contributions

*Identical twins the exception to this.
PGTai 2.0 analysis: Taking the best-in-class PGTai technology platform to the next level

- Provides two independent assessments of ploidy status
- Generates more data enabling deeper analysis
- Detects all forms of triploidy
- Allows for parent origin testing (optional)
- Includes 2PN validation

More euploids, more confidence, more transfers
Published benefits of PGT-A

- Mitigates the effect of maternal age$^{1,2,3}$
- Increases implantation success$^{2,3,4}$
- Reduces rates of spontaneous abortion/miscarriages$^{2,4}$
- Increases ongoing pregnancy rates$^{3,4}$
- Enables more efficient single embryo transfers to reduce rates of high-risk multiple pregnancies$^{2,5}$

Clinical outcome in good prognosis patients

Single euploid embryo vs. double morphologically good embryos, (Forman, 2012)

The primary outcome of ongoing pregnancy beyond 20 weeks was similar between the study and control groups (60.7% [54/89] vs. 65.1% [56/86]).

The multiple pregnancy rate for patients in the study group was significantly lower than in the control group (0% [0/54] vs. 53.4% [31/56]).

The authors concluded that transfer of a single euploid blastocyst was non-inferior in terms of ongoing pregnancy rates compared with transfer of two blastocysts with an unknown chromosome status.
PGT Randomized Pilot Study Results, Improvement in Pregnancy rates ≥20 wks

Cohort for analysis

Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial

Santiago Munné, Ph.D., Brian Kaplan, M.D., John L. Frattarelli, M.D., H.C.I.D., Tim Child, M.D., Gary Nakhuda, M.D., F. Nicholas Shamma, M.D., Kaylen Silverberg, M.D., Tasha Kalista, M.A., Alan H. Handside, Ph.D., Mandy Katz-Jaffe, M.D., Dagan Wells, Ph.D., Tony Gordon, Ph.D., Sharyn Stock-Meyer, Ph.D., and Susan Willman, M.D., on behalf of the STAR Study Group

Objective: To evaluate the benefit of next-generation sequencing (NGS)-based preimplantation genetic testing for aneuploidy (PGT-A) for embryo selection in frozen-thawed embryo transfer.

Design: Randomized controlled trial.

Setting: Not applicable.

Patient(s): Women aged 25–40 years undergoing IVF with at least two blastocysts that could be biopsied.

Intervention(s): Randomization for single frozen-thawed embryo transfer with embryo selection based on PGT-A euploid status versus morphology.

Main Outcome Measure(s): Ongoing pregnancy rate (OPR) at 20 weeks' gestation per embryo transfer.

Result(s): A total of 661 women (average age 33.7 ± 3.6 years) were randomized to PGT-A (n = 331) or morphology alone (n = 331). The OPR was equivalent between the two arms, with no significant difference per embryo transfer (50% [117/234] vs. 49% [110/218]) or per infestation to treat (ITT) at randomization (41.4% [134/326] vs. 41.5% [130/313]). Post hoc analysis of women aged 35–40 years showed a significant increase in OPR per embryo transfer (51% [62/122] vs. 37% [54/145]) but not per ITT.

Conclusion(s): PGT-A did not improve overall pregnancy outcomes in all women, as analyzed per embryo transfer or per ITT. There was a significant increase in OPR per embryo transfer with the use of PGT-A in the subgroup of women aged 35–40 years who had two or more embryos that could be biopsied, but this was not significant when analyzed per ITT.

Received March 12, 2019; revised July 25, 2019; accepted July 29, 2019.

S.M. reports consulting fees from Cooper Genomics. B.K. has nothing to disclose. J.L.F. has nothing to disclose. T.C. has nothing to disclose. G.N. reports financial assistance to his institution from Illumina, the study sponsor, to support work relating to subject recruitment and enrollment, costs related to ethical approval for the study, and data entry relating to the study. I.N.S. has nothing to disclose. K.S. has nothing to disclose. T.K. is an employee of Illumina. A.H. was principal scientist (part time) for Illumina, San Diego, California 2013–2019, and is scientific advisor (part time) for Vittorelli Sweden, Gothenburg, Sweden. M.K. has nothing to disclose. D.W. was an employee of Reprogenetics (Cooper Genomics) at the time of the study. Illumina undertook data monitoring and coordinated study design at its own expense. T.C. is a paid employee and shareholder of Cooper Surgical. S.S.M reports nonfinancial support from Illumina financially assisted with payment to a staff member to help with enrollment of subjects for this study, and provided reagents needed for sequencing for this study. S.W. served on the Medical Advisory Board for Natara, which involved two clinical meetings (Feb 2016 and 2017) to review advances in technology and give feedback on applications of preimplantation genetic testing and noninvasive prenatal testing. Supported by Illumina.

A complete list of members of the Single-Embryo Transfer of Euploid Embryo (STAR) Study Group is provided in the Acknowledgments. Reprint requests: Susan Willman, M.D., Reproductive Science Center 601 Ross Street, Oakland, CA 94618 (E-mail: susan@susanwillman.com).
Rationale of STAR study, Illumina.

- All patients with 2 viable blastocysts are randomized.
- In group A, all blastocysts are biopsied, genetically tested, and frozen.
- In group B, all blastocysts except one are biopsied, genetically tested, and all are frozen.
- In group A, one PGS-NGS normal embryo is thawed and transferred.
- In group B, one best looking blastocyst is thawed and transferred.
- Pregnancy rates are compared.
When evaluated as a proportion of theITT population, the frequency of an erupted embryo increased with maternal age: 8.8% (14/170) in women 25-34 years of age versus 17.2% (24/141) in women aged 35-48 years.

The demographics for the ITT population are presented in Table 1. Characteristics were similar for the two arms. Embryo characteristics are presented in Table 2. An average of 7.6 days of culture was obtained per patient in both arms. Of the 1,376 embryos treated by means of PGT-A, 3,331 (24.7%) were reported as euploid and 1,441 (10.3%) as aneuploid. When analyzed within maternal age ranges, the percentage of euploid embryos decreased with increasing maternal age, from 18.0% for women <35 years of age to 35.5% in women aged 35-48 years (Supplementary Table 2, available online at www.fertility.org). The distribution of chromosomal abnormalities observed is shown in Supplementary Figure 1 (available online at www.fertility.org). Of note, of the 1,441 chromosomally abnormal embryos, 144 (10.0%; 10.0% of all embryos) were reported to have a whole or partial chromosome mosaic aneuploidy for one or more chromosomes (Table 3). A detailed breakdown of the chromosome abnormalities observed in embryos classified as mosaic is shown in Supplementary Figure 2 (available online at www.fertility.org). A majority (96%) had one to three chromosome abnormalities classified as mosaic aneuploid, with no other chromosome abnormalities.

Clinical outcomes for transferred embryos are presented in Table 3. An increase in patients with an ongoing pregnancy at 6 weeks’ gestation continued to a live birth. Thus, the OR of a live birth reflects the live birth rate in this study cohort. The overall ORs per transfer at 6 weeks gestation did not significantly differ between the PGT-A and control arms for either the embryo transfer population (OR: 1.15 [95% CI: 0.80; 1.68]; P = 0.32) or the ITT population (OR: 1.15 [95% CI: 0.80; 1.68]; P = 0.32). Finally, the rates of negative β-HCG (P = 0.80), biochemical pregnancy (P = 0.32)
Screening results by chromosome in the preimplantation genetic testing for aneuploidy (PGT-A) arm. (A) Autosomal monosomies and trisomies in patients 25–34 years of age. (B) Autosomal monosomies and trisomies in patients 35–40 years of age. (C) Autosomal mosaics aneuploidies and segmental copy number changes in patients 25–34 years of age. (D) Autosomal mosaics aneuploidies and segmental copy number changes in patients 35–40 years of age. (E) Sex chromosomes for patients of all ages.

SUPPLEMENTAL FIGURE 3

(A) Frequency distribution of Gardner scores for transferred embryos. (B) Comparison of morphologic quality ratings for transferred embryos. NGS = next-generation sequencing; PGS = preimplantation genetic screening.

Clinical site and laboratory variables: (A) Maternal age distribution by clinical site for sites with 20 or more transfers. (B) Percentage of euploid embryos in the preimplantation genetic testing for aneuploidy (PGT-A) arm for sites with 20 or more transfers. (C) Maternal age distribution by genetic testing laboratory. (D) Percentage of euploid embryos in the PGT-A arm per genetic testing laboratory by age group. Ongoing pregnancy rate in each arm per genetic testing laboratory.

Figure 4: Comparison of clinical outcomes across genetic testing laboratories and age groups.
A total of 588 eligible women with a mean age of 34 years had an embryo transfer—274 in the PGT arm and 314 in the control arm. The 20 week OPR was 49.6% (136/274) in the PGT arm and 45.9% (144/314; P=0.3369) in the control arm.

A post-hoc subgroup analysis revealed that women aged 35-40 had an OPR of 50.8% (62/122) in the PGS arm vs 37.2% (54/145) in the control arm (p=0.0349), with miscarriage rates of 8.2% (10/122) and 11.0% (16/145), respectively.
STAR STUDY

- This multicenter study, which predominantly enrolled women aged 25 to 34 years, did not replicate earlier, more tightly controlled, single-center studies which showed a benefit of PGS in all patients.

- These results suggest that standardization of clinical and laboratory protocols is essential for future studies.

- A benefit with PGT in women 35 years and older, despite the low miscarriage rate in the control arm, is consistent with recent SART data.
IVF Michigan
January-December 2017
Euploid/Aneuploid Rate by Maternal Age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Euploid Rate</th>
<th>Aneuploid Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35 (112 Patients)</td>
<td>49%</td>
<td>49%</td>
</tr>
<tr>
<td>35-37 (64 Patients)</td>
<td>42%</td>
<td>57%</td>
</tr>
<tr>
<td>38-40 (54 Patients)</td>
<td>33%</td>
<td>65%</td>
</tr>
<tr>
<td>41-42 (21 Patients)</td>
<td>16%</td>
<td>82%</td>
</tr>
<tr>
<td>&gt;42 (16 Patients)</td>
<td>12%</td>
<td>86%</td>
</tr>
</tbody>
</table>
2017 Average Cohort Size by Maternal Age

- <35: 6.9
- 35-37: 6.4
- 38-40: 4.1
- 41-42: 3.5
- >42: 2.7
2016 - 2017 (Jan-Oct) Preg. rate comparison between SET & DET (PGS cases only)

AGE | SET | DET | Age group average
--- | --- | --- | ---
<35 | 63% | 76% | 67%
35-37 | 63% | 100% | 71%
38-40 | 74% | 60% | 71%
>=41 | 73% | 60% | 69%
AVG | 68% | 74% |

AGE | # of SET | # of DET | Total # of ET
--- | --- | --- | ---
<35 | 84 | 41 | 125
35-37 | 35 | 10 | 45
38-40 | 19 | 5 | 24
>=41 | 11 | 5 | 16
## PGT-A Results

<table>
<thead>
<tr>
<th>Euploid</th>
<th>Low-level mosaic</th>
<th>High-level mosaic</th>
<th>Aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Mixed (some normal &amp; some abnormal)</td>
<td>Mixed (some normal &amp; some abnormal)</td>
<td>Abnormal</td>
</tr>
<tr>
<td>High</td>
<td>Lower</td>
<td>Low, unlikely</td>
<td>Very low, very unlikely</td>
</tr>
</tbody>
</table>

PGT-A improves IVF outcomes

PGT-A reduces miscarriage rates

- <35: 11.2% (IVF with PGT-A), 12.0% (IVF without PGT-A)
- 35-37: 13.0% (IVF with PGT-A), 13.6% (IVF without PGT-A)
- 38-40: 16.3% (IVF with PGT-A), 25.0% (IVF without PGT-A)
- 41-42: 37.9% (IVF with PGT-A), 17.2% (IVF without PGT-A)
- >42: 58.8% (IVF with PGT-A), 17.2% (IVF without PGT-A)

PGT-A increases live birth rates

- <35: 56.6% (IVF with PGT-A), 48.1% (IVF without PGT-A)
- 35-37: 54.2% (IVF with PGT-A), 39.2% (IVF without PGT-A)
- 38-40: 52.2% (IVF with PGT-A), 27.6% (IVF without PGT-A)
- 41-42: 46.7% (IVF with PGT-A), 15.3% (IVF without PGT-A)
- >42: 42.4% (IVF with PGT-A), 5.0% (IVF without PGT-A)

Data from SART.org. 2015.
In vitro fertilization (IVF) with (PGT) is more cost effective to achieve a live birth compared to IVF alone. 

IVF Michigan Fertility centers/Toledo Fertility Center

- At IVF Michigan Fertility Centers/Toledo Fertility Center, with a delivery rate of 68% with the first euploid frozen embryo transfer attempt, and 65% after the second, (2018 DATA):
  - To achieve a 90% efficiency, the patient does:
    - spend $27,000
    - And do maybe just one RETRIEVAL cycle AND 2 FEET
    - The number of embryos transferred per cycle is ONE
Intention to treat: how many initiated PGT cycles produce blastocysts for biopsy as maternal age increases?

- PGT is a selection method to determine which embryos have the highest potential of implanting. Obviously, PGT cannot help the 21% of cycles producing one or no embryos, although embryo banking can be considered for poor prognosis patients.
- Additionally, over 30% of the 396 cycles in women >40 produced no embryos for biopsy.
- Finally, over 80% of cycles with only one blastocyst decided to cancel the PGT, while, only 10% of cycles with ≥2 blastocysts cancelled the PGT.
- The current data is helpful to manage patient expectations before cycle start about their odds of producing blastocysts for biopsy, and combined with already published data on aneuploidy rates per age group, many more cycles are needed to obtain euploid embryos in the older age group, Munne 2014.
An embryo cohort which contains all aneuploid embryos is not indicative of future embryo cohort aneuploidy

- In 2012-2014, 316 patients had complete embryonic aneuploidy and 128 of these patients pursued a subsequent cycle. Of all embryos screened in a subsequent cycle, 43.8% were euploid. For all patients undergoing a subsequent cycle, 59.4% eventually had at least 1 euploid embryo. Of those patients that had complete embryonic aneuploidy in the 2nd consecutive cycle, 20 pursued an additional cycle for an overall embryonic euploid rate of 39.9%. Within this group, 60% had at least 1 euploid embryo in a subsequent cycle. Of those with 3 consecutive cycles with complete embryonic aneuploidy, only 3 patients completed a subsequent cycle with an overall euploid rate of 20%.

- An embryo cohort which contains all aneuploid embryos is not indicative of future embryo cohort aneuploidy, Scott 2014
Can we rely on only morphokinetic parameters to detect embryo aneuploidy?

- Our results here revealed that aneuploidy cannot be predicted using the morphokinetic parameters tested by time-lapse microscopy without PGT screening.

- Patient’s age in the PGT cases should also be considered as a parameter for further time-lapse studies, Shamma 2014.
Paternal Factors and Aneuploidy, PGT results

- **Paternal age** does not have a significant effect on the rate of aneuploidy when oocyte factors are held constant in a donor oocyte model, Griffo 2015

- Neither severe sperm morphology concerns, Stevens 2016, nor DNA fragmented sperm seem to affect success rates following PGT, Rodriguez-Purata 2016
WHY DO SOME EUPLOID EMBRYOS NOT IMPLANT OR EVEN WORSE MISCARRY!

1. EMBRYO SEGMENTAL ANEUPLOIDY, PGT cannot detect changes at less than 5 MB, or single gene abnormality
2. EMBRYO MOSAICISM
3. EMBRYO MITOCHONDRIAL GENOMIC ABNORMALITY (16K bp in mitochondria c/w 3 billion bp in nucleus)
4. LABORATORY CONCERNS
5. UTERINE FACTORS
6. PHYSICIAN TRANSFER EFFICIENCY
Mosaicism

The presence of two or more populations of cells with different genotypes in one individual embryo

Mosaicism

- Euploid cell
- Aneuploid cell

Mosaic Embryos
- Implant less
- Miscarry more
- Can sometimes lead to live birth

Selection against mosaic embryos improves IVF success rates
APPROACHING MOSAIC RESULTS

When no euploid embryos are available, current studies and guidelines suggest prioritizing mosaic embryos based on the percent aneuploidy in the biopsied sample and number of chromosomes involved, as shown below.\(^1\,^5\) Prioritization based on which chromosomes are impacted may also be considered, but the direction of the change (monosomy v. trisomy) is of less importance.\(^1\,^3\,^5\,^7\)

LOW-LEVEL MOSAICS (20-40% abnormal cells)

have been shown to more frequently have euploid ICMs and result in a 50% ongoing pregnancy rate (n=102).\(^*\) Samples with low-level mosaicism involving a single chromosome may be prioritized when no euploid embryos are available.

HIGH-LEVEL MOSAICS (>40-80% abnormal cells)

have been shown to result in a 30% ongoing pregnancy rate (n=44).\(^*\) Samples with high-level mosaicism, as well as samples with low-level mosaicism involving two chromosomes, may be given lower priority.

COMPLEX MOSAICS\(^\dagger\) (Mosaicism in ≥3 chromosomes)

have been shown to result in a 6% ongoing pregnancy rate (n=32).\(^*\) Complex mosaics may be given lowest priority.

Appropriate counseling by a physician and/or genetic counselor is recommended for all PGS cases. Prenatal diagnosis by amniocentesis should be offered for all resulting pregnancies.
They Have Theories – We Have Data
Theory: Mosaic Embryos Make Healthy Babies
(and we are discarding them by testing)

<table>
<thead>
<tr>
<th>Patient</th>
<th>SET #</th>
<th>Embryo diagnosis</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Mosaic Partial Monosomy 18pter-p11.21, Mosaic Trisomy 19</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>Mosaic Monosomy 22</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>Mosaic Partial Monosomy 7q11.22-qter, 9q21.11-qter</td>
<td>Delivery (reported healthy; no confirmatory testing)</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>Mosaic Partial Trisomy 6q22.2-qter</td>
<td>Miscarriage, sac only</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>Mosaic Partial Trisomy 2q24.1-qter</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>Mosaic Trisomy 19</td>
<td>Miscarriage, sac only</td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td>Mosaic Trisomy 22</td>
<td>Miscarriage, sac and heartbeat</td>
</tr>
<tr>
<td>H</td>
<td>8</td>
<td>Mosaic Monosomy 16</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>I</td>
<td>9</td>
<td>Mosaic Partial Monosomy 3pter-p12.2</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>J</td>
<td>10</td>
<td>Mosaic Partial Monosomy 4pter-p11</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>K</td>
<td>11</td>
<td>Mosaic Partial Trisomy 4pter-p15.2</td>
<td>Biochemical pregnancy</td>
</tr>
<tr>
<td>L</td>
<td>12</td>
<td>Mosaic Partial Trisomy 17pter-p11.2</td>
<td>Ongoing pregnancy</td>
</tr>
<tr>
<td>M</td>
<td>13</td>
<td>Mosaic Partial Monosomy 7q11.21-qter</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>Mosaic Partial Trisomy 10pter-p11.21</td>
<td>Miscarriage, sac only</td>
</tr>
<tr>
<td>O</td>
<td>15</td>
<td>Mosaic Partial Trisomy 7pter-p21.1</td>
<td>Ongoing pregnancy</td>
</tr>
<tr>
<td>P</td>
<td>16</td>
<td>Mosaic Partial Monosomy 11pter-p13</td>
<td>Ongoing pregnancy</td>
</tr>
<tr>
<td>Q</td>
<td>17</td>
<td>Mosaic Partial Monosomy 1q21.2-qter, Mosaic Monosomy 22</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>R</td>
<td>18</td>
<td>Mosaic Partial Monosomy 6pter-p21.1</td>
<td>Miscarriage, sac and heartbeat (normal microarray on products of conception)</td>
</tr>
</tbody>
</table>
IVF Michigan Fertility Centers
Mosaicism Data

- All embryos prior to 7/1/2018 with low grade mosaicism (20-40%) were reported from Cooper Genomics to us as normal.
- Of 12 SET cases that I transferred with presumably euploid embryos, but in fact were low grade mosaic embryos, 6 delivered normal children.
- Thus a 50% of low mosaic embryos resulted in babies.

- We at IVF Michigan analyzed 81 patients with **RECURRENT PREGNANCY LOSS** who desired IVF with PGT and had at least one euploid embryo.

- For the 81 IVF/PGT cycles in which a euploid embryo was available to transfer, the live birth rate was **77% (63 of 81)**, which was significantly better than the 34% live birth rate after 6 months of expectant management.

- The miscarriage rate in the group that had a euploid embryo transfer was **4.7%**
Summary of PGT-A and RPL

- In women with RPL:
  - When euploid embryos are identified in women after PGT the live birth rate is improved.
  - IVF with PGT-A probably improves miscarriage rates compared with expectant management, although current published data is inconclusive.
PREGNANCY OUTCOMES FOLLOWING IN VITRO FERTILIZATION FROZEN EMBRYO TRANSFER (IVF-FET) WITH OR WITHOUT PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A) IN WOMEN WITH RECURRENT PREGNANCY LOSS SART-CORS STUDY. Morelli et al. 2019

- 24,007 IVF-FET SART CORS cycles from the PGT-A group and 43,811 cycles from the control group were included in the analysis.

- The adjusted odds ratio (OR) comparing IVF-FET with PGT-A versus without PGT-A for live birth outcome was 1.30 (95% CI: 1.24, 1.37) for age<35 and 2.01 (95% CI: 1.92, 2.11) for age more than 35.
Neonatal and Childhood Outcomes

- Obstetric, neonatal, and early childhood outcome data seem reassuring thus far, though much has focused on PGT-M (single gene) rather than PGT-A (aneuploidy).

- The PGT-M vs PGT-A parental groups are often inherently different in that most patients undergoing PGT-M do not have concomitant infertility.

- Nonetheless, kindergarten-aged PGT-M offspring perform as well as their IVF/ICSI and naturally conceived peers on measures of **cognition** (Wechsler Preschool and Primary Scale of IntelligenceTM), **motor skills** (Movement ABC) and **psychosocial development** (Child Behavior Checklist [CBCL] and Caregiver-Teacher Report Form [C/TRF]), Winter, 2015.
THE FUTURE

- CELL FREE EMBRYONIC DNA
- CRISPR
- ROUTINE POPULATION GENETIC SCREENING
EMBRYONIC CELL FREE DNA (ECF-DNA) AS A TOOL FOR NON-INVASIVE PREIMPLANTATION GENETIC SCREENING AND DIAGNOSIS. M. Surdo, a A. Biricik, a S. Bono, a M. Minasi, b E. Cursio, b E. Greco, b E. Cotroneo, a F. Fiorentino, a F. Spinella 2016

- A high-quality WGA product was obtained from all genomic DNA and from 8/10 cfDNA samples. A full STR screening concordance between analyzed embryos versus culture medium was obtained were observed amplification, confirming the embryonic origin of each related cfDNA samples. NGS-based 24-chromosomal analysis of blastocysts revealed 6/8 euploid and 2 aneuploid embryos, of these 1 had whole chromosome trisomies and segmental aneuploidies and 1 showed a single trisomy. All euploid and chromosomally abnormal samples were correctly diagnosed by cfDNA based PGS analysis, demonstrating 100% concordance between matching cfDNA and TE samples.

- CfDNA from blastocysts culture media can be amplified and characterized by 24-chromosome comprehensive screening NGS. This study provides the first evidence that embryonic cfDNA could represent a potential source of DNA for non-invasive detection of whole and partial chromosome abnormality, analogous to what can be detected by PGS/PGD analysis in preimplantation embryos following invasive procedures.

- Currently a study is being put together at IVF Michigan using ECF-DNA using high resolution NGS.
CRISPR (the future)
Clustered regularly-interspaced short palindromic repeats
He Jiankui had used CRISPR technology to create gene-edited babies.

November 25, 2018: The bombshell: a team led by He at the Southern University of Science and Technology, in Shenzhen, has been recruiting couples to create the first gene-edited babies. Specifically, they removed the gene CCR5, in the hope of rendering the offspring resistant to HIV, smallpox, and cholera.

He claims to have altered embryos for seven couples, resulting in one successful pregnancy and subsequent birth of twin girls.

Two girls Lulu and Nana in 2018 are born using CRISPR.

He’s attempt to delete the CCR5 gene could inadvertently have changed the girls’ brains in ways that affect cognition and memory.
CRISPR (the future)
Clustered regularly-interspaced short palindromic repeats

Can CRISPR-Cas9 Boost Intelligence?

A letter was recently published in Nature on 329,000 young people identifying 74 genetic variants—spelling mistakes in single nucleotides in the six billion letter human genome—which can be used to predict nearly 20 percent of the variation in school years completed, a quantitative trait of fortitude which is correlated to general intelligence, and which you can learn about by sequencing your own genome.

“In my opinion, CRISPR could in principle be used to boost the expected intelligence of an embryo by a considerable amount,” said James J. Lee, a researcher at University of Minnesota, one of the authors of that study.
CRISPR (the future)
Clustered regularly-interspaced short palindromic repeats

- The safety and accuracy of CRISPR-Cas9 is fast improving. New proteins are being discovered and selected which make the tool more accurate and less likely to cause “off target effects.”

- The bioethicists Julian Savulescu and John Harris have argued that it was not only a right but a duty to manipulate genetic code of our future children, a concept termed “procreative beneficence,” and extending the term parental neglect to “genetic neglect,” if we don’t gene engineer. The bio-ethicist Hille Haker, has by comparison, noted there is more to being a human than genetics. Others, including the University of New Mexico academic David Correia, have envisioned dystopian outcomes, suggesting the wealthy might use genetic engineering to translate power from the social sphere into the enduring code of the genome, effectively as “legacy genetics,” establishing “permanent capitalist social relations.”

- But, whatever changes we code into our genomes will end up getting thrown up against different genetic backgrounds in future generations, due to random rearrangements in chromosomes, so it’s unlikely to fix any permanent relations.
Routine genomic screening in healthcare promising

Similar to population screening of newborns for over 30 genetic conditions, routine genomic screening for all patients is becoming increasingly viable.
Routine genomic screening in healthcare promising

- Conservatively at least 1% of the U.S. population has an identifiable genetic risk for cancer or heart disease.
- All patients undergoing ART at IVF Michigan are offered pre-conception carrier screening for 210 recessive disorders (1/27 couples are at risk), and Fragile X.
- It is reasonable to expect that in the 21st century, the actionable gene list will enlarge to include most, if not all, of a human’s 20,000 genes.
- If everyone’s goal is to achieve better outcomes, then functioning implementation models could to be developed. IS PGT-M THE ANSWER?
CRISPR/UNIVERSAL GENETIC SCREENING/PGT
Preimplantation Genetic Testing and In vitro fertilization.

The new Paradigm:

The transfer of ONE euploid embryo that would result in one healthy baby at term.
Thank God for medicine and technology. More importantly, thank God for not needing it in most instances.
THANK YOU
Overall implantation rates

27% (mean maternal age 32) reported by Gutierrez-Mateo, C., et al. *Fertility and sterility* 92, 1544-1556 (2009)

In my opinion, day 3 biopsy is of historic interest only
PGTai 2.0 platform – Technology overview

Built on basics of the PGTai platform

- Built on embryo biopsy data (>1000) resulting in live births and sustained pregnancy outcomes
- Validated using sequencing data from >10,000 embryos

Now utilizing on average >10x more data to provide:

- More accurate sequencing (paired-end sequencing)
- Global SNP coverage (world first for PGT-A)
- Two independent methods for aneuploidy detection (world first for PGT-A)