The incidence of euploid blastocyst and implantation potential as a function of age and ovarian stimulation

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Disclosures

- Member of the Board of Directors of a Not-For-Profits which provides embryonic aneuploidy screening

- NO compensation of any kind for service as a board member

- No compensation of any kind from any aspect of the evaluation of embryo biopsies

- Not-For-Profits (Foundation for Embryonic Competence; FEC) uses funds to sponsor research
Efficiency of Human Reproduction through IVF

N=132,874 Mature Follicles

What might explain the high failure rate?

Retrospective Eval.
Proportion leading to delivery:
- 7% of Follicles
- 13% of Embryos
- 21% of 8 Cells
- 42% of Blastocysts
Why do Embryos Deemed Suitable for Transfer Arrest in Their Development Prior to Delivery?

Is the interpretation of PGD objective?

Hybridization for 13, 18, 21, X and Y

FISH for Aneuploidy Screening
Novel Comprehensive Chromosome Screening Technologies

aCGH (Lab 1)
45, XY, -8

qPCR (Lab 2)
46, XY

SNP array (Lab 3)
46, XY

NextGen
Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study

Zhihong Yang\(^1\), Jiaen Liu\(^2\), Gary S Collins\(^3\), Shala A Salem\(^1\), Xiaohong Liu\(^2\), Sarah S Lyle\(^1\), Alison C Peck\(^1\), E Scott Silis\(^4\) and Rifaa D Salem\(^1\)

Table 3 Comparison of laboratory findings and clinical outcome among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>p</th>
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<tbody>
<tr>
<td>Fresh blastocyst transfer according to morphology assessment:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 5/6</td>
<td>31 (56.4)</td>
<td>28 (58.3)</td>
<td>0.677(^a)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>21 (38.2)</td>
<td>19 (39.6)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (5.4)</td>
<td>1 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>39 (70.9)</td>
<td>22 (45.8)</td>
<td>0.017(^a)</td>
</tr>
<tr>
<td>Ongoing pregnancy (≥20wks GA)</td>
<td>38 (69.1)</td>
<td>20 (41.7)</td>
<td>0.009(^a)</td>
</tr>
<tr>
<td>Missed abortion</td>
<td>1 (2.6)</td>
<td>2 (9.1)</td>
<td>0.597(^b)</td>
</tr>
</tbody>
</table>

Notes: All data reported as n (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age. \(^a\) by Chi-squared test, \(^b\) by Fisher’s exact test.

Monosomy:Trisomy Ratio of 2

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aCGH enhances delivery rates – an RCT

- RCT
- Age
  - All < 35
  - Mean age of 31
- Sample Size
  - 55 aCGH
  - 48 control
- Significant improvement in outcomes
- Answers one of the four critical validation questions
Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial

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Comprehensive chromosome screening alters traditional morphology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer

Eric J. Forman, M.D. a, b, c Kathleen M. Upham, B.S. a Michael Cheng, B.S. a Tian Zhao, B.S. a Kathleen H. Hong, M.D. a, b Nathan R. Treff, Ph.D. a, b and Richard T. Scott Jr., M.D. a, b

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Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial

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Implantation rates in RCT using PGS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PGS</th>
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<tbody>
<tr>
<td>Yang et al. 2012</td>
<td>46%</td>
<td>69%</td>
</tr>
<tr>
<td>Scott et al. 2013</td>
<td>63%</td>
<td>80%</td>
</tr>
<tr>
<td>Forman et al. 2013</td>
<td>40%</td>
<td>58%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>53%</td>
<td>73%</td>
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</table>

P<0.001
Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study

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With greater experience, the actual negative predictive value is ~98.8%

Clinical Experience

**Misdiagnoses**

- 4974 embryos
- 2976 gestations (62.1%)
- 10 errors
  - 1 tetraploid
  - 2 monosomies
  - 7 trisomies
- 3168 transfers
- 2354 ongoing / delivered (72.1%)
- Mean age 38.4 years
- 10 errors
  - 7 found in losses
  - 3 found in ongoing preg.

**Clinical Error Rate**

<table>
<thead>
<tr>
<th></th>
<th>Per embryo</th>
<th>Per transfer</th>
<th>Per ongoing pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Error Rate</td>
<td>0.2%</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Mosaicism evaluated in 4 samples – 100% mosaic
Aneuploidy Rate by Chromosome

N=15,169

Capture Rate
• 5 Chromosome FISH – 21.8%
• 9 Chromosome FISH – 59.8%

Fransasiak et al – in review
Contemporary Understanding of Maternal Age and Human Embryonic Aneuploidy

Franasiak et al – Fertil Steril 2014
Embryonic Aneuploidy Rate Relative to Maternal Age (aCGH)

Harton GL et al. Fertil Steril 2013 100:1695-1703

Sample Size
Day 3: 3,412
Blast: 2,467
The No Transfer Rate with CCS

No-Euploid Blasts Rate (%)

Age (yrs)

N=15,169

Franasiak et al – Fertil Steril 2014
How Many Embryos Do Patient Undergoing CCS Have?

- **Proportion of Cases with this number of blastocysts available for Biopsy (%):**
  - 52% of cases had 3 or fewer evaluable embryos.

- **Cumulative Proportion of Cases with this number of blastocysts available for Biopsy (%):**
  - N=15,169

**References:**
Franasiak et al – Fertil Steril 2014
Number of Embryos Evaluated per Cycle Relative to Maternal Age

Franasiak et al 2014
Trisomy:Monosomy Ratio by Age

N=15,169

Ratios consistent across nine programs

Key Indicator for QA of your assay

Franasiak et al – Fertil Steril 2014
Changes in Aneuploidy Rates by Chromosome with Increasing Maternal Age

Franasiak 2014
Chromosome Structure, Embryonic Aneuploidy, and Maternal Age

Mean Error Rate per Chromosome in Each Structural Class

Acrocentric: 13, 14, 15, 21, 22
Metacentric: 1, 3, 16, 19, 20

Franasiak 2014
All fifteen possibilities have been observed.
CHARACTERIZATION OF THE SOURCE OF HUMAN EMBRYONIC ANEUPLOIDY

The Sequential Bx Study

- Population
  - 1104 aneuploid chromosomes
  - 786 embryos
  - 198 patients

- Microarray PGD on
  - 1\textsuperscript{st} polar body
  - 2\textsuperscript{nd} polar body
  - Blastomere or Trophectoderm

- Fingerprinting done on aneuploidy chromosomes at embryonic level to determine if mitotic or paternal

Northrop et al and Treff et al 2010
PSSC is the principal maternal error in meiosis I.

This provides an opportunity for reciprocal errors.
Developmental Stage of Error Versus Maternal Age

Proportion of Errors (%)

Maternal Age (yrs)

< 35  |  35-37  |  38-40  |  41-42  

N=327  

P=NS

MI error  |  MII error

P=NS  

N=327
Will Chromosomal Instability Make PGS Unreliable?

Mosaicism of cleavage stage embryos (FISH analysis)

<table>
<thead>
<tr>
<th></th>
<th>All embryos (n = 615)</th>
<th>Developing, cleavage-stage embryos analysed for ≥8 chromosomes (n = 107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid</td>
<td>177 (22%)</td>
<td>15 (14%)</td>
</tr>
<tr>
<td>Mosaic</td>
<td>599 (73%)</td>
<td>77 (72%)</td>
</tr>
<tr>
<td>Diploid–aneuploid mosaic</td>
<td>480 (59%)</td>
<td>49 (46%)</td>
</tr>
<tr>
<td>% Diploid cells</td>
<td>(101/135/14115) (72%)</td>
<td>(101/324) (47%)</td>
</tr>
<tr>
<td>Aneuploid mosaic</td>
<td>119 (15%)</td>
<td>28 (26%)</td>
</tr>
</tbody>
</table>

Summary of the findings of 36 studies on the chromosomal makeup of human preimplantation embryos.


„Chaos in the embryo“

Vanneste et al., Nature Medicine 15, 577-583 (2009)
Origins of UPD

Monosomy → Rescue → Uniparental Isodisomy
Uniparental Isodisomy – Does self correction exist?

Characterization of single cells from established cell lines
Origins of UPD

Trisomy

Rescue

Normal

Uniparental disomy
Uniparental disomy in the human blastocyst is exceedingly rare

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Initial Implantation Rates Amongst Euploid Embryos with Increasing Maternal Age - aCGH

Harton GL et al Fertil Steril 2013 100:1695-1703

Sample Size
Day 3:  508
Blast:   543
PGS Improves but Does Not Normalize Implantation and Delivery Rates in Older Women

N=18,567
Why do 30-40% of high-quality embryos still fail to implant?

• Embryonic factors
  – Non-chromosomal
    • indels, methylation
  – Mosaicism
  – Mitochondrial heteroplasmy

• Uterine factors
  – Endometrial receptivity
    • Timing
    • Infection/Inflammation, Microbiome
    • Endometrial contractility
Mosaicism in Human Embryos

• Only evaluable in arrested embryos (upward bias)

• Cleavage stage
  – Embryo biopsied in its entirety into individual cells
  – Each cell analyzed

• Blastocyst
  – Three widely separated biopsies from the trophectoderm
  – One biopsy from the inner cell mass

• N=614 embryos

![Graph showing embryos with any evidence of mosaicism (%)](image-url)
Outcomes from Proven Mosaic Embryos Which Were Transferred

- Identified by sequential biopsy
- N=98
- Results were unknown at time of transfer
- Follow up with DNA fingerprinting to assure accurate assignment of outcome
How much effort to attain the deliveries of Louise Brown & Elizabeth Carr?

40th attempt was ectopic

104th attempt Louise was born

41 natural cycles

54th attempt Elizabeth was born

13th attempt with hMG
Does Superovulation Impact Aneuploidy Risk?

47,XY,+16
Natural Conceptions: Incidence of Trisomy

Incidence of Trisomy (% of clinically recognized pregnancies) vs. Age

Adapted from Hassold T & Hunt P, Nat Rev Genet, 2001
Rate of Aneuploidy in *Natural* Conceptions and Embryos from *Stimulated* Cycles Stratified by Age

![Graph showing the rate of aneuploidy in natural and stimulated cycles stratified by age.](image_url)
Rate of Aneuploidy in *Natural* Conceptions and Embryos from *Stimulated* Cycles Stratified by Age

- **Natural Conceptions:** Incidence of Trisomy in Miscarriage Specimens
- **Stimulated Cycles:** Incidence of embryonic aneuploidy
Superovulation versus Spontaneous Conception: Risk of Aneuploidy in the Products of Conception Following Loss

Percentage of chromosomal anomalies

- no fertility medications
- 1 cycle after hMG or CC
- hMG or CC taken in conception cycle
- ≥2 cycles after hMG or CC

Boué JG and Boué A, Lancet, 1973
Superovulation vs. Mild Stimulation: Embryonic Aneuploidy Risk

• Prospective RCT

• Mild Stimulation (N=67)
  – GnRH Antagonist with 150 FSH
  – 45% aneuploidy

• Conventional Stimulation (N=44)
  – GnRH agonist down regulation with 225 FSH
  – 63% aneuploidy

• PGD: Blastomere Biopsy and FISH analysis

Baart EB et al, Hum Reprod, 2007
Minimal Stimulation: Miscarriage Risk

- Retrospective Review

- Minimal Stimulation
  - Clomid, day 3
  - HMG/FSH, day 8 qod
  - Oocyte Maturation: GnRHa

- 6,549 pregnancies conceived after minimal stimulation

- Rate of miscarriage may reflect age-related increase in aneuploidy

Teramoto & Kato, RBMO, 2007
• **Natural Cycle IVF**
  – Spontaneous cycle, no additional medications
  – Aim for single oocyte

• **Modified Natural Cycle IVF**
  – Spontaneous cycle with additional medications
    - hCG, GnRH antagonist +/- FSH or HMG add-back, NSAIDs
  – Aim for single oocyte with decreased risk of cancellation

• **Mild IVF**
  – Lower doses and/or shorter duration of FSH or HMG with GnRH antagonist co-treatment or:
    – Oral compounds +/- FSH or HMG
  – Aim for 2-7 oocytes

Nargand G et al, Hum Reprod, 2007
Natural Cycle IVF Study

• Inclusion Criteria
  – Age 18-49 with regular ovulatory cycles
  – No medical contraindications to treatment

• Spontaneous cycle
  – **No** exogenous medications to induced ovulation utilized
  – (i.e. no LH, FSH, GnRH antagonist or NSAID)

  – Final Oocyte Maturation
    • induced when follicle ≥15mm (mean of ~18 mm)
    • Dual Trigger: hCG and GnRH agonist
Conventional IVF: Control Population

- Retrospective Cohort
  - 15,169 embryos
  - Franasiak 2014

- Inclusion Criteria: Infertile patients undergoing IVF cycles with CCS

- Age Range: 22-49 years old
Results: Natural Cycle IVF

- 173 embryos obtained from natural IVF cycles
  - 99 Usable Blastocysts + 74 Arrested Embryos
  - 53.2% (N=90) of all embryos were aneuploid

- 99 Usable Blastocysts
  - 40.4% (N=40) were aneuploid
Aneuploidy rate by maternal age:

\[ n=173 \text{ embryos: usable blasts & arrested} \]

Patient age (years):
- <31
- 31-34
- 35-37
- 38-40
- 41-42
- >42

Proportion of embryos with aneuploidy (%):

- Natural
- Superovulation

\[ P=NS \]
Aneuploidy rate by maternal age:

\[ n=99 \text{ usable blasts} \]
Embryonic Aneuploidy in Stimulated and Natural Cycles

- Embryonic aneuploidy rates in natural and stimulated IVF cycles do not differ.

- These data do not support a causative role for gonadotropin superovulation in embryonic aneuploidy.
Rate of Aneuploidy in **Natural** Conceptions and Embryos from **Stimulated** Cycles Stratified by Age

[Graph showing the rate of aneuploidy in natural and stimulated cycles stratified by age.]