Should Every Embryo be Screened or Frozen?
What does the evidence say?

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- aCGH (Lab 1)
- SNP array (Lab 3)
- qPCR (Lab 2)
Contemporary Understanding of Maternal Age and Human Embryonic Aneuploidy

Franasiak et al – Fertil Steril 2014

N=15,169
Is transferring an aneuploid embryo clinically useful?
What are the “Burdens” of CCS

Thus the real questions are:

1. Safely attaining embryonic DNA
2. Predictive values of the techniques
3. Proportion of euploid embryos that will fail
4. Cost effectiveness
Preimplantation genetic screening: back to the future

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Submitted on April 1, 2014; resubmitted on May 9, 2014; accepted on June 3, 2014
• All embryo selection techniques are detrimental
• Inappropriate to use “Implantation Rates” as an endpoint
• “it can be questioned whether all patients will ever be able to understand all of the complexities concerning PGS”
• “cost-effectiveness is being forgotten”
• “evidence is now accumulating that all embryos in an IVF cycle can be cryopreserved and transferred in subsequent cycles without impairing, and maybe even improving, the cumulative pregnancy rate of that IVF cycle”
• Embryo selection should therefore not be used to select out embryos, but only to determine the order in which the embryos will be transferred, as the time to pregnancy can be improved by embryo selection, if embryos with the highest implantation potential are transferred first.
• Culturing to the blastocyst may be harmful
Does Embryo Biopsy Impact the Developmental Potential of the Oocyte

Routine IVF Care through Retrieval

Identify mature oocytes

ICSI, culture, and select 2 best embryos for transfer

One embryo randomized to undergo biopsy

Cell submitted for eventual aneuploidy screening and fingerprinting

Transfer the embryos

Implantation, Maternal serum sampling for free fetal DNA and Fingerprinting

N=113 pairs; 226 embryos
Overall implantation rates

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

In our opinion, day 3 biopsy will soon be of historic interest only.

39% reduction

Insignificant
Is knowing the predictive value of a normal result sufficient?

If they were the same it would likely be a rare coincidence...

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

Richard T. Scott Jr., M.D., a, b Kathleen Ferry, B.S., a Jing Su, M.S., a Xin Tao, M.S., a Katherine Scott, M.S., a and Nathan R. Treff, Ph.D. a, b

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

To have the opportunity for meaningful improvement, when you select for one criteria you most commonly deselect for another.

Transfer Based on Embryo Morphology

Abnl Abnl NI Abnl NI

Transfer Based on Aneuploidy Screening and Embryo Morphology

CCS changes the embryo selected 40% of the time
Forman et al ASRM 2012
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 Yang1, Jiaen Liu2, Gary S Collins3, Shala A Salem1, Xiaohong Liu4, Sarah S Lyle1, Alison C Peck1, E Scott Sils1 and Rifaat D Salem1

**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>
</tr>
</thead>
<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>0.677a</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>0.017a</td>
</tr>
<tr>
<td>Ongoing pregnancy (≥20wks GA)</td>
<td>38 (69.1)</td>
<td>20 (41.7)</td>
<td>0.009a</td>
</tr>
<tr>
<td>Missed abortion</td>
<td>1 (2.6)</td>
<td>2 (9.1)</td>
<td>0.597b</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**

- **RCT**
  - 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

Richard T. Scott Jr., M.D.,* Kathleen M. Upham, B.S.,* Eric J. Forman, M.D.,† Tian Zhao, M.S.,* and Nathan R. Treff, Ph.D.‡,§,∥

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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


* Reproductive Medicine Associates of New Jersey, Department of Reproductive Endocrinology, Basking Ridge; and ‡ University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Department of Obstetrics, Gynecology & Reproductive Sciences, New Brunswick, New Jersey

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


* Reproductive Medicine Associates of New Jersey, Morristown, New Jersey; † Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology, and Reproductive Science, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey; and ‡ Atlantic Reproductive Medicine Specialists, Raleigh, North Carolina

Scott et al Fertility and Sterility 2013; 100:697-703
The No Transfer Rate with CCS

Franasiak et al – Fertil Steril 2014

N=15,169
How Many Embryos Do Patient Undergoing CCS Have?

52% of cases had 3 or fewer evaluable embryos

N=15,169
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
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.

Mosaicism evaluated in 4 samples – 100% mosaic

Clinical Error Rate

<table>
<thead>
<tr>
<th>Per embryo</th>
<th>0.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per transfer</td>
<td>0.3%</td>
</tr>
<tr>
<td>Per ongoing pregnancy</td>
<td>0.1%</td>
</tr>
</tbody>
</table>
Consolidated Pregnancy Outcomes

Proportion of All Pregnancies

N=4,754 pregnancies

Scott KL et al – RMA
PGS Improves but Does Not Normalize Implantation and Delivery Rates in Older Women

N=28,567
Fertility Treatments and Multiple Births in the United States

A Twin Births

B Triplet and Higher-Order Births

Fertility Treatments and Multiple Births in the United States

Singleton Term Delivery: The Ideal IVF Outcome

- IVF twin pregnancies are at an increased risk of:
  - Preeclampsia (2-fold risk increase)\(^1\)
  - Extreme prematurity (7.4-fold increase delivery <32 wks)\(^2\)
  - NICU admission (3.8-fold increased risk)\(^2\)
  - Perinatal Death (2-fold increase)\(^2\)

- Two IVF singleton deliveries have better obstetrical outcomes than one IVF twin delivery\(^3\)

Sources:
The Perception that Patients Love Multiples if They are Born Healthy is not Well Validated

Provided by a patient...
With >2 blastocysts, even patients at high aneuploidy risk are very likely to have at least one euploid blastocyst.

**Blastocyst Aneuploidy Rate by Age Group**

- **<35**
  - 21%
  - 34%
  - 55%
  - 64%

- **35-37**
  - 21%
  - 34%
  - 55%
  - 64%

- **38-40**
  - 21%
  - 34%
  - 55%
  - 64%

- **41-42**
  - 21%
  - 34%
  - 55%
  - 64%

Forman EJ et al., O-161
FRESH SET RESULTS IN LOWER DELIVERY RATES THAN DOUBLE EMBRYO TRANSFER (DET)

- Cochrane Review of 6 randomized trials from 1999-2006 (N = 1,257)
- Young, good prognosis patients with “top quality” embryos available
- Slightly more singletons after DET

Pandian Z et al., Cochrane Database Syst Rev, 2009. PMID: 19370588
The Dropout Rate from IVF is Significant

Source: Schroder AK: Cumulative pregnancy rates and drop out rates of a German IVF programme: 4, 102 cycles in 2,130 patients. RBM Online (2004) 8:600-606
Can $1 \geq 2$?
CCS Results in Higher Implantation Rates

Implantation = cardiac activity at time of discharge to obstetrical care (~9 weeks)

Euploid SET: 66.3% (N=83)

Traditional DET: 51.2% (N=170)

P=0.02
Same Delivery Rate: Randomized Controlled Trial

Delivery Rate Per Patient (n=175)

- Single euploid blastocyst transfer (N=89) - 61%
- Untested 2-blastocyst transfer (N=86) - 65%

P=0.5

Eliminates Multiples

- Single euploid blastocyst transfer: 100%
- Untested 2-blastocyst transfer: 52% Singletons, 48% Multiples

P < 0.001

Better Obstetrical Outcomes are Attained CCS/eSET than Conventional Two Embryo Transfer

- **Mean Birthweight:**
  - Single euploid: $3408 \pm 562g$
  - 2-Blastocyst: $2745 \pm 743g$  
    - (P<0.001)

- **Low birthweight (<2,500g):**
  - Single Euploid: 4.4% (2/45)
  - 2-Blastocyst: 31.9% (22/69)  
    - (P<0.001)

- **Very low birthweight (<1,500g):**
  - Single Euploid: 0% (0/45)
  - 2-Blastocyst: 7.2% (5/69)  
    - (P=0.06)
Ongoing Pregnancy Rates
Fresh vs. Frozen Transfers

Control SET
- Fresh ET: 37%
- Frozen ET: 43%
P = 0.4

CCS SET
- Fresh ET: 57%
- Frozen ET: 54%
P = 0.7

66% of transfers
Obstetrical Costs for 100 Patients
Current Standard Of Care

<table>
<thead>
<tr>
<th>Costs per Delivery*</th>
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<tbody>
<tr>
<td>Singleton</td>
<td>$21,458</td>
</tr>
<tr>
<td>Twins</td>
<td>$104,831</td>
</tr>
<tr>
<td>Triplets</td>
<td>$407,199</td>
</tr>
</tbody>
</table>

Does not include:
- Pediatric costs after 28 days of age
- Disability costs during bed rest
- Loss of productivity in the work place

Overall Cost to Provide Care

CCS with SET versus Conventional Treatment

- Use actual cost data
- Inclusive of all IVF costs including
  - IVF cycle costs
  - CCS costs
  - Medication costs
- Delivery costs and subsequent hospital stay through 28 days of life
Do we ever recommend two embryo transfers?

Yes – but with caution...
CLINICAL RESEARCH OPPORTUNITY

SOLAIRE

SCREENING OF LOW RESPONDERS FOR ANEUPLOIDY TO IMPROVE REPRODUCTIVE EFFICIENCY
Time Lapse Observations in the Embryology Laboratory

And others.....
**Time Lapse and Aneuploidy**

**Traditional Markers**

Hong KH et al – in review

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**ICSI to start of 1st cytokinesis (p=0.61)**

![Graph showing ICSI to start of 1st cytokinesis](image1)

**Duration of the 2 cell stage (p=0.88)**

![Graph showing Duration of the 2 cell stage](image2)

**Duration of the 3 cell stage (p=0.12)**

![Graph showing Duration of the 3 cell stage](image3)
Rate of euploid embryos (%)

**Time from start of 1st cytokinesis to start of cavitation (p=0.01)**

- 1st Quartile: 90%
- 2nd Quartile: 80%
- 3rd Quartile: 70%
- 4th Quartile: 60%

**Time from start of 5 cell stage to start of cavitation (p=0.0076)**

- 1st Quartile: 80%
- 2nd Quartile: 70%
- 3rd Quartile: 60%
- 4th Quartile: 50%

Hong KH et al in review
Can Time Lapse Help Distinguish Which Euploid Blasts will Deliver from those Destined to Fail?

Temporal data evaluated:
- 5 conventional endpoints through cleavage stage
- Additional temporal endpoints from extended culture:
  - First compaction
  - Morula formation
  - First cavitation
  - Blastocyst Expansion
  - First contraction

NO: None of the 5 traditional parameters or 5 additional blast related parameters prognosticate outcome
Next Generation Sequencing

Aligned Results

Reference sequence from human genome database

Multiple Reads (depth)
The Economics of NextGen
A Major Factor for Accuracy

NextGen Sequencing Chip

$$$$$$$
NextGen Molecular Barcoding → Reduced Costs

combine samples for a single sequencing chip

Barcode 1  CTAAGGTAAC
Barcode 2  TAAGGAGAAC
The Economics of NextGen
A Major Factor for Accuracy

NextGen Sequencing Chip

$\frac{\text{\$\$\$\$\$\$\$\$\$/2}}{}$
The Economics of NextGen
A Major Factor for Accuracy

NextGen Sequencing Chip

$\text{$$$$$$$/4}$
The Economics of NextGen
A Major Factor for Accuracy

NextGen Sequencing Chip

$\ldots/48$
The Economics of NextGen
A Major Factor for Accuracy

NextGen Sequencing Chip

96 or more...

$\text{\$\$\$\$\$}/96$
WGS
(16 per chip)

known trisomy

known monosomy

unpublished data
WGS
(48 per chip)

unpublished data
Targeted NGS
(96 per chip)

unpublished data
Embryo calibration results

unpublished data
Chromosome specific cutoffs

NGS based copy number on chr16

unpublished data
Embryonic Endometriatal Synchrony

It take two.....
Embryonic-Endometrial Asynchrony Increases with Maternal Age

- Retrospective
- 1,341 IVF cycles
- Thresholds for Asynchrony (either)
  - $P > 1.5$ mg/mL on day of hCG
  - No blastulation prior to day 6
- Risk for asynchrony increases with maternal age
- Live birth predicted
  - Day 5 blastulation ($P < 0.0001$)
  - $P < 1.5$ ng/mL ($P = 0.0002$)

Is it asynchrony or an intrinsic diminution in quality?

Shapiro BS et al Fertil Steril 2013 100:S287
Late follicular rise in progesterone

- Retrospective study
- 4032 patients
- $P_4 \geq 1.5 \text{ng/mL}$ associated with lower ongoing pregnancy rates

Progesterone and the Endometrial Transcriptome

Adapted from S. Young, MD, PhD
Progesterone Pharmacokinetics

Usadi RS, et al. JCEM 2008 & Young Lab, Unpublished
Progesterone and the Endometrial Transcriptome

<table>
<thead>
<tr>
<th>Number of genes differentially expressed vs. 40 mg P</th>
<th>Natural Cycle</th>
<th>10 mg P</th>
<th>5 mg P</th>
<th>2.5 mg P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 2-fold change</td>
<td>0</td>
<td>0</td>
<td>70</td>
<td>236</td>
</tr>
<tr>
<td>≥ 1.5-fold change</td>
<td>0</td>
<td>0</td>
<td>605</td>
<td>1186</td>
</tr>
</tbody>
</table>

Young Lab, Unpublished

Adapted from S. Young, MD, PhD
Progesterone and Impaired Implantation: A Pilot Study of Euploid Embryos

All patients had normal P levels prior to the administration of hCG
Beware of Interference in your P Assay

- Patients receiving DHEA have elevated DHEA-SO\(_4\) levels
- These levels may falsely elevate P levels
- Assay dependent

Forman - RMANJ
Natural Cycle

Ovulation

hCG administration

Progesterone Rise

Embryonic Window of Implantation

Endometrial Window of Implantation

Franasiak et al ASRM 2013
embryo and endometrium synchrony - revisited

hCG administration

Ovulation

Progesterone Rise

24h

Endometrial Window of Implantation

Franasiak et al ASRM 2013
Fresh day 5 embryo transfer

Franasiak et al ASRM 2013
Fresh day 6 embryo transfer

Franasiak et al ASRM 2013
Frozen synchronous cycle

Endometrial Window of Implantation

Progesterone Start

(time)
Older patients are more likely to have “slow” embryos.
Frozen day 6 embryo transfer

Franasiak et al ASRM 2013
Obstetrical Outcomes Following Fresh versus Cryopreserved Embryo Transfer

- Fresh embryos at increased risk for
  - Preterm birth
  - Low birth weight
  - Small for gestational age

Wennerholm et al Hum Reprod 2013 28:2545-53
The supraphysiologic milieu which accompanies superovulation impact low birth weight risk

- Retrospective review of SART data
- 2004-2006
- 56,792 neonates
- Fresh embryo transfer at increased risk for LBW
The RMA New Jersey Team

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- Maria Costantini
- Michael Drews
- Eric Forman
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