Extended embryo culture in the era of Time-Lapse, PGS and Vitrification

Joe Conaghan, PhD, Pacific Fertility Center, San Francisco
Question: How often are you culturing embryos to D7 for your patients?

a. Never (0%)
b. Occasional patients depending on circumstances
c. Most patients
d. All (100%)
Procedure stats 2010-2016

Total Retrievals-numbers by year
Procedure stats 2010-2016

Fertility Preservation-number of cases by year
Procedure stats 2010-2016

Retrievals less FP cases-numbers by year
Percentage of retrievals with ET

Year | % Retriivals with ET
--- | ---
2005 | 100
2006 | 90
2007 | 80
2008 | 70
2009 | 60
2010 | 50
2011 | 40
2012 | 30
2013 | 20
2014 | 10
2015 | 0
Question: What percentage of your patients have a fresh ET?

a. <25
b. 26-50
c. 51-74
d. >75
Clinical application of comprehensive chromosomal screening at the blastocyst stage.

Schoolcraft WB¹, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D.

Abstract

OBJECTIVE: To evaluate a new strategy for comprehensive chromosome screening at the blastocyst stage.

DESIGN: Clinical research study.

SETTING: An IVF clinic and a specialist preimplantation genetic diagnosis laboratory.

PATIENT(S): Forty-five infertile couples participated in the study. The mean maternal age was 37.7 years, and most couples had at least one previous unsuccessful IVF treatment cycle (mean 2.4).

INTERVENTION(S): This study used a novel chromosome screening approach, combining biopsy of several trophectoderm cells on day 5 after fertilization and detailed analysis of all 24 types of chromosome using comparative genomic hybridization.

MAIN OUTCOME MEASURE(S): Proportion of embryos yielding a diagnostic result, aneuploidy rate, implantation rate, and pregnancy rate.

RESULT(S): A diagnosis was obtained from 93.7% of embryos tested. The aneuploidy rate was 51.3%. The probability of an individual transferred embryo forming a pregnancy reaching the third trimester/birth was 68.9%, an implantation rate 50% higher than contemporary cycles from the same clinic. The pregnancy rate was 82.2%.

CONCLUSION(S): The comprehensive chromosome screening method described overcomes many of the problems that limited earlier aneuploidy screening techniques and may finally allow preimplantation genetic screening to achieve the benefits predicted by theory. The high embryo implantation rate achieved is particularly encouraging and, if confirmed in subsequent studies, will be of great significance for IVF clinics attempting to reduce the number of embryos transferred or to implement single embryo transfer.
The changing face of IVF

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

Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR.

Abstract

OBJECTIVE: To determine whether blastocyst biopsy and rapid quantitative real-time polymerase chain reaction (qPCR)-based comprehensive chromosome screening (CCS) improves in vitro fertilization (IVF) implantation and delivery rates.

DESIGN: Randomized controlled trial.

SETTING: Academic reproductive medicine center.

PATIENT(S): Infertile couples in whom the female partner (or oocyte donor) is between the ages of 21 and 42 years who are attempting conception through IVF.

INTERVENTION(S): Embryonic aneuploidy screening.

MAIN OUTCOME MEASURE(S): Sustained implantation and delivery rates.

RESULT(S): We transferred 134 blastocysts to 72 patients in the study (CCS) group and 163 blastocysts to 83 patients in the routine care (control) group. Sustained implantation rates (probability that an embryo will implant and progress to delivery) were statistically significantly higher in the CCS group (89 of 134; 66.4%) compared with those from the control group (78 of 163; 47.9%). Delivery rates per cycle were also statistically significantly higher in the CCS group. Sixty one of 72 treatment cycles using CCS led to delivery (84.7%), and 56 of 83 (67.5%) control cycles ultimately delivered. Outcomes were excellent in both groups, but use of CCS clearly improved patient outcomes.

CONCLUSION(S): Blastocyst biopsy with rapid qPCR-based comprehensive chromosomal screening results in statistically significantly improved IVF outcomes, as evidenced by meaningful increases in sustained implantation and delivery rates.
Multiple Pregnancies

Multiple pregnancies are still the largest complication in IVF

The New England Journal of Medicine

Fertility Treatments and Multiple Births in the United States

Aniket D. Kulkarni, M.B., B.S., M.P.H., Denise J. Jamieson, M.D., M.P.H., Howard W. Jones, Jr., M.D., Dmitry M. Kissin, M.D., M.P.H., Maria F. Gallo, Ph.D., Maurizio Macaluso, M.D., Dr.P.H., and Eli Y. Adashi, M.D.
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There Really Are So Many More Twins Now

A million more, roughly, when compared to the pre-1980 twin rates. So what changed?

Computed from CDC data (Alexis Madrigal)
Number of FET’s
Procedure stats 2010-2016

PGS Cases-numbers by year

- 2010: [Value]
- 2011: [Value]
- 2012: [Value]
- 2013: [Value]
- 2014: [Value]
- 2015: [Value]
- 2016: [Value]
Procedure stats 2010-2016

PGS cases as % of (non-FP) retrievals

- 2010: 10%
- 2011: 10%
- 2012: 20%
- 2013: 30%
- 2014: 40%
- 2015: 50%
- 2016: 80%
### Procedure stats 2010-2016

<table>
<thead>
<tr>
<th>Year</th>
<th>Retrievals</th>
<th>Fertility Preservation</th>
<th>Retrievals less FP</th>
<th>PGS Cases</th>
<th>PGS cases as %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>763</td>
<td>21</td>
<td>742</td>
<td>77</td>
<td>10%</td>
</tr>
<tr>
<td>2011</td>
<td>862</td>
<td>40</td>
<td>822</td>
<td>80</td>
<td>10%</td>
</tr>
<tr>
<td>2012</td>
<td>833</td>
<td>89</td>
<td>744</td>
<td>167</td>
<td>22%</td>
</tr>
<tr>
<td>2013</td>
<td>882</td>
<td>144</td>
<td>738</td>
<td>365</td>
<td>49%</td>
</tr>
<tr>
<td>2014</td>
<td>1006</td>
<td>171</td>
<td>835</td>
<td>541</td>
<td>65%</td>
</tr>
<tr>
<td>2015</td>
<td>1088</td>
<td>228</td>
<td>860</td>
<td>668</td>
<td>78%</td>
</tr>
<tr>
<td>2016</td>
<td>1082</td>
<td>258</td>
<td>824</td>
<td>677</td>
<td>82%</td>
</tr>
</tbody>
</table>
Fresh transfers: Jan 2015-August 2016

1373 cycles inseminated
277 fresh transfers (15%)

<table>
<thead>
<tr>
<th>Day of transfer</th>
<th>Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>D3</td>
<td>91</td>
<td>33</td>
</tr>
<tr>
<td>D5</td>
<td>148</td>
<td>53</td>
</tr>
<tr>
<td>D6</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

45% 54%
Day of transfer and pregnancy outcome (277 patients)

<table>
<thead>
<tr>
<th>Number of embryos transferred</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>&gt;2</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleton</td>
<td>89</td>
</tr>
<tr>
<td>Twin</td>
<td>11</td>
</tr>
<tr>
<td>High order</td>
<td>0</td>
</tr>
</tbody>
</table>
**Freezing: Jan 2015-August 2016**

<table>
<thead>
<tr>
<th>Day of freezing</th>
<th>Embryos</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>D3</td>
<td>23</td>
<td>&lt;1</td>
</tr>
<tr>
<td>D5</td>
<td>1860</td>
<td>27</td>
</tr>
<tr>
<td>D6</td>
<td>4181</td>
<td>61</td>
</tr>
<tr>
<td>D7</td>
<td>861</td>
<td>12</td>
</tr>
</tbody>
</table>

- **<1%**
- **>99%**
Frozen transfers: Jan 2015-August 2016

Transfer and pregnancy outcome (1399 patients)

<table>
<thead>
<tr>
<th>Number of embryos transferred</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91*</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>&gt;2</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleton</td>
<td>92</td>
</tr>
<tr>
<td>Twin</td>
<td>8</td>
</tr>
<tr>
<td>High order</td>
<td>0</td>
</tr>
</tbody>
</table>

*1249 patients had 1 embryo transferred. 899 (72%) were true eSET’s 13 patients (1%) had no ET (6 no survival + 7 PGS)
We’re doing a lot of freezing

Jan 2015-August 2016

<table>
<thead>
<tr>
<th>Cycle type</th>
<th>Number of cycles</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte freezing</td>
<td>438</td>
<td>24</td>
</tr>
<tr>
<td>Fresh ET</td>
<td>277</td>
<td>15</td>
</tr>
<tr>
<td>PGS</td>
<td>1009</td>
<td>73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oocytes vitrified</th>
<th>6386</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos vitrified</td>
<td>6926</td>
</tr>
</tbody>
</table>
Note: All blastocysts are collapsed before vitrification.
More PGS means more blastocyst culture

Number of patients having blastocyst culture

- 2012
- 2013
- 2014
- 2015
We’re culturing all embryos to D7!

Should embryos developing to blastocysts on day 7 be cryopreserved?
Started D7 PGS January 2014

Total PGS cases (2.5 months)

<table>
<thead>
<tr>
<th>PGS cases</th>
<th>D5 Biopsy</th>
<th>D6 Biopsy</th>
<th>D7 Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>21 (32%)</td>
<td>62 (94%)</td>
<td>27 (41%)</td>
</tr>
</tbody>
</table>

Euploid embryos

<table>
<thead>
<tr>
<th>Embryos*</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>157 (5.8/pt.)</td>
<td>17/27 (62%)</td>
<td>52/87 (60%)</td>
<td>19/43 (44%)</td>
</tr>
</tbody>
</table>

*D7 Biopsy patients only
• We don’t freeze early blastocysts
• D7 blastocysts need to be really nice
We are generating more embryos

% used from 2PN

<table>
<thead>
<tr>
<th>Year</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average 2PN/Patient</td>
<td>14.6</td>
<td>16.7</td>
<td>16</td>
<td>16.7</td>
<td>18</td>
</tr>
<tr>
<td>% used from 2PN</td>
<td>43</td>
<td>46</td>
<td>51</td>
<td>56</td>
<td>67</td>
</tr>
<tr>
<td>Average used/retrieval</td>
<td>6.3</td>
<td>7.6</td>
<td>8.2</td>
<td>9.4</td>
<td>12.1</td>
</tr>
</tbody>
</table>
Changing Landscape

- More patients for blastocyst culture
- More PGS/CCS
- Increased volume of vitrification
- Increasing volume of FET’s
- More D3 AH
- Increased dish prep
- Higher equipment usage
Goal: Can we simplify the culture system

Number of patients having blastocyst culture

- 2012: 500
- 2013: 600
- 2014: 700
- 2015: 800
2015 Changes

2014 Sequential culture system

Fertilization medium D0-1 ➔ Cleavage medium D1-3 ➔ Blastocyst medium D3-5 ➔ Blastocyst medium D5-7

2015 Continuous culture system

Fertilization medium D0-1 ➔ Continuous culture D1-7
Incubator Questions

• Ideal number of embryos/drop?
• Medium/embryo/day?
• Embryo sorting?
• Is changing medium necessary?
Moving to dry benchtop incubators

- Verify gas concentrations
- Measure pH and osmolality
- Blood gas analyzer
- Be aware that chamber is dry
Media pH in a dry benchtop incubator

- 60μl of medium under oil
- Equilibrated overnight (16 hours)
- pH is average of 4 measurements
Does osmolality of media change after 7 days in the incubator?

Osmometer calibration
- 100 mOsmol/Kg
- 290 mOsmol/Kg
- 1000 mOsmol/Kg

Manufacturer suggested range

![Graph showing 1-day and 7-day osmolality for Benchtop and Big Box models.](image)
Suggested system

- Six embryos per 60µl droplet CSCM + 10% SSS
- Culture from D1 up to D7 (2PN to Blastocyst)
- Undisturbed culture (no D3 check)
Established system

- **Five embryos per 50µl droplet CSCM + 10% SSS**
- Culture from D1 up to D7 (2PN to Blastocyst)
- Undisturbed culture (no D3 check)
Established system

Example 1: Patient has 10 x 2PN
Question: Patient has 6 x 2PN. How do we divide embryos among culture drops?

A

B

C

D
Established system

Example 2: Patient has 6 x 2PN
Established system

Example 3: Patient has 12 x 2PN
Tiny Incubators

- Group
- Autocrine effects
- Nutrient depletion
- Waste build-up

- Single
- Less waste
- More nutrients
- Loss of synergistic effects
Are we making more blastocysts?

% of 2PN’s that made usable blastocysts
How many patients have no blastocysts to biopsy?

Percentage of patients/year with no blastocysts biopsied

2012-2014: 202/1805

2015: 69/796
How we made more blastocysts

- Media change
- Continuous culture from D1-D7
- Undisturbed culture*
- Group culture. 5 embryos/50µl droplet

*Embryos may have AH on D3
Can development be too good?
Are there benefits

- Increased pregnancy rate
- Decreased miscarriage rate
- Faster turnaround for patients
- Lower numbers of embryos transferred
- Reduced costs
- Increased efficiency
## Clinical PGS outcomes 2014

<table>
<thead>
<tr>
<th>Age</th>
<th>ET's</th>
<th># transferred</th>
<th>Clinical</th>
<th>Sacs</th>
<th>Live Birth cycles</th>
<th># babies born</th>
<th>Clinical Preg. Rate</th>
<th>Implantation Rate</th>
<th>Live Birth Rate/cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>83</td>
<td>88</td>
<td>56</td>
<td>60</td>
<td>52</td>
<td>55</td>
<td>67.5%</td>
<td>68.2%</td>
<td>62.7%</td>
</tr>
<tr>
<td>35-37</td>
<td>99</td>
<td>103</td>
<td>60</td>
<td>63</td>
<td>53</td>
<td>56</td>
<td>60.6%</td>
<td>61.2%</td>
<td>53.5%</td>
</tr>
<tr>
<td>38-40</td>
<td>89</td>
<td>89</td>
<td>59</td>
<td>61</td>
<td>48</td>
<td>49</td>
<td>66.3%</td>
<td>68.5%</td>
<td>53.9%</td>
</tr>
<tr>
<td>41-42</td>
<td>29</td>
<td>30</td>
<td>17</td>
<td>18</td>
<td>14</td>
<td>15</td>
<td>58.6%</td>
<td>60.0%</td>
<td>48.3%</td>
</tr>
<tr>
<td>&gt;42</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>100.0%</td>
<td>100.0%</td>
<td>66.7%</td>
</tr>
<tr>
<td>All own eggs</td>
<td>306</td>
<td>316</td>
<td>198</td>
<td>208</td>
<td>171</td>
<td>179</td>
<td>64.7%</td>
<td>65.8%</td>
<td>55.9%</td>
</tr>
</tbody>
</table>
How many embryos have we vitrified (2014-6)?

<table>
<thead>
<tr>
<th>Year</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>870</td>
<td>1911</td>
<td>466</td>
</tr>
<tr>
<td>2015</td>
<td>1102</td>
<td>2183</td>
<td>534</td>
</tr>
<tr>
<td>2016</td>
<td>1132</td>
<td>2879</td>
<td>487</td>
</tr>
<tr>
<td>Total</td>
<td>3104</td>
<td>6973</td>
<td>1487</td>
</tr>
</tbody>
</table>

- Total D5: 27%
- Total D6: 60%
- Total D7: 13%
How many times are we vitrifying per cycle?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>446</td>
<td></td>
<td></td>
<td></td>
<td>2587</td>
</tr>
<tr>
<td>1</td>
<td>811</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1030</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>
## Day 7 PGS FET’s

<table>
<thead>
<tr>
<th>D7 embryos only</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>FET’s</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Average transferred</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>25</td>
<td>30.9</td>
</tr>
<tr>
<td>Sacs</td>
<td>27</td>
<td>31.4</td>
</tr>
<tr>
<td>Live births</td>
<td>21</td>
<td>25.9</td>
</tr>
<tr>
<td>Babies born</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>
### 2016 PGS Cases (n = 633)

<table>
<thead>
<tr>
<th>Age group</th>
<th>&lt;35</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>43+</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrievals</td>
<td>121</td>
<td>114</td>
<td>187</td>
<td>100</td>
<td>49</td>
<td>92</td>
</tr>
<tr>
<td># blastocysts biopsied</td>
<td>960</td>
<td>634</td>
<td>881</td>
<td>303</td>
<td>108</td>
<td>898</td>
</tr>
<tr>
<td>% of retrievals with embryo biopsy</td>
<td>98</td>
<td>95</td>
<td>94</td>
<td>82</td>
<td>39</td>
<td>99</td>
</tr>
<tr>
<td>% of retrievals with euploid embryo(s)</td>
<td>93</td>
<td>84</td>
<td>66</td>
<td>38</td>
<td>22</td>
<td>98</td>
</tr>
</tbody>
</table>
Euploidy rate in D5/6 blastocysts

% D5/6 Euploid %

Oocyte age group

<35 35-37 38-40 41-42 43+ OD
Euploidy Rate in D7 blastocysts
No call rate by day of Biopsy (2016)

% No call

D5 biopsy: 1005
D6 biopsy: 2620
D7 biopsy: 434
Average: 1201.5
Is D7 worth the effort: 3 years of outcomes

• 2,707 patients cultured 1 more day
• 818 patients with D7 vitrification
• 89 patients with only D7 vitrification
• 1487 more embryos vitrified
• 451 more normal embryos
• 818 extra freeze events
• 76 new FET’s
• 22 children born
Summary

• Effort to make more blastocysts has paid off
• Increased use of PGS/PGS
• Culturing all embryos to D7
• Simplified group culture system
• Making the transition to benchtop incubators
• Is culture to D7 worth the effort?

joe@pacificfertility.com
Question: Is embryo culture to D7 worth the effort?

a. No
b. For a small subset of patients
c. For all patients