The Future of ART: Looking into the Crystal Ball

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Yale University Fertility Center
New Haven, CT-USA
FUTURE IN ART

- Stimulation Protocols/Cost reduction
- Reduce IVF inefficiency and Multiple Pregnancies
- Cryopreservation
- Basic Science Studies
- Reappraisal
- Ethical Debates
- Miscellaneous
Stimulation Protocols

- Dose reduction (minimal stimulation)
- Natural cycle IVF/M
- Oral gonadotropins/Inhalers
- Different PK’s (long/short half-life)
- Other agents (DHEA, letrozole)
- “Resurrection” of Clomiphene?
Declining IVF success with increasing dose

[1,372 non-donor fresh IVF cycles – Hungary]

Daily amp IU        CP (%)   IR (%)
---   ---   ---
Lowest tertile   1128 (187) 1.8 (0.37)     1688 (187) 2.3 (0.48)
Mid tertile      2929 (761) 3.5 (0.86)

Kovacs P & Pal L, 2010

\[a p=0.012 \quad b p=0.003\]
Effect of Gonadotropin dose in Donor Egg IVF (n=22)

229 ± 101 IU/day in standard vs. 145 ± 100 in low dose regimen

Gentle induction of Follicle Growth
INCUBATION SYSTEMS

Water bath culture

A gas mixture is injected into the bag

Bag seal

2° seal

After 2 days

Water bath at 37°C
Natural IVF/IVM protocol

- Women with normal ovaries;
- > 7 antral follicles at baseline ultrasound and FSH < 10 mIU/ml;
- When leading follicle reaches 12-14 mm in diameter and endometrial thickness at least 6.0 mm, hCG (10,000 IU) given;
- Egg retrieval is scheduled 36 hrs later;
Table 1. Results of mature and immature oocyte retrieval and in-vitro maturation and fertilization, followed by embryo transfer in women with normal ovaries and regular menstrual cycles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>82</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.9 ± 2.6</td>
</tr>
<tr>
<td>Mature oocytes retrieved</td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>99</td>
</tr>
<tr>
<td>Mean</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Immature oocytes retrieved</td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>619</td>
</tr>
<tr>
<td>Mean</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>No. of oocytes matured in vitro (%)</td>
<td>495 (80)</td>
</tr>
<tr>
<td>No. of oocytes fertilized (%)</td>
<td>371 (75)</td>
</tr>
<tr>
<td>No. of embryos cleaved (%)</td>
<td>356 (96)</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>205</td>
</tr>
<tr>
<td>Mean</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>No. of clinical pregnancies (%)</td>
<td>29 (35)</td>
</tr>
<tr>
<td>No. of implantation (%)</td>
<td>39 (19)</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation unless otherwise stated.
Must Reduce

- IVF inefficiency (too many embryos do not implant and too many oocytes go “wasted”)

- Multiple Pregnancies
Must Reduce IVF inefficiency and Multiple Pregnancies

[Selection of Competent Embryos]

- **eSET** (favor blastocyst transfers (the “economical” PGS)/ education for no ET’s) [help with embryo imaging?]
- **PGS** for every embryo (aCGH and SNP)
- **Microarray** to assess oocytes by cumulus cells gene profiling (non invasive oocyte selection)
- Markers embryo quality (**proteomics**, **metabolomics** in spent culture media)
Which Embryo to select for eSET?
New PGS-Detecting Aneuploidy

Microarray-CGH (array-CGH)

Data obtained for all chromosomes

Results obtained in about 24 hrs
Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage

Connie C Wong¹,²,⁷, Kevin E Loewke¹–³,⁶,⁷, Nancy L Bossert⁴, Barry Behr², Christopher J De Jonge⁴, Thomas M Baer⁵ & Renee A Reijo Pera¹,²

Figure 6. Proposed model for human embryo development. Human embryos begin life with a set of oocyte RNAs inherited from the mother. After fertilization, a subset of maternal RNAs specific to the egg (ESSP1) must be degraded as the transition from oocyte to embryo begins. As development continues, other RNAs are partitioned equally to each blastomere (ESSP4). At EGA, ESSP2 genes are transcribed in a cell-autonomous manner. During the cleavage divisions, embryonic blastomeres may arrest or progress independently. ‘Feature extraction’ indicates the three imaging parameters for predicting successful development to the blastocyst stage: cytokinesis, the time between 1st and 2nd mitoses, and the time between 2nd and 3rd mitoses.
Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage


First cytokinesis: 14.3 ± 6.0 min
Time between first and second mitosis: 11.1 ± 2.2 h
Time between second and third mitosis: 1.0 ± 1.6 h

Predicting blastocyst stage (sensitivity and specificity of 94% and 93%), if embryos having a first cytokinesis of 0–33 min, a time between first and second mitoses of 7.8–14.3 h and a time between second and third mitoses of 0–5.8 h
The Future of Embryo Selection

The “omics”

DNA → mRNA → Proteins → Metabolites

- Gene Expression
- Gene Translation
- Functional End Products

- Genome
- Transcriptome
- Proteome
- Metabolome

Integration with Bioinformatics

Functional Phenotype

Modified from Katz-Jaffe and Behr
Protein Secretome Correlates with Aneuploidy

- 9 proteins identified as biomarker candidates to distinguish between euploid and aneuploid blastocyst secretome.
- Lipocalin-1 showed increased expression in aneuploid blastocyst secretome samples.
- LCN1 inhibits cysteine proteinases (Wojnar et al., 2001).
- Cysteine proteininases are important in embryo hatching (Sireesha et al., 2008) and implantation (Nakanishi et al., 2005).

McReynolds et al, (2011) Fertility & Sterility
Preimplantation Factor (PIF)

15 Amino Acids, 1612 Daltons
MVRIKPGSANKPSDD

- Initial Discovery of PIF’s Presence: Roussev 1996, Barnea 1999
- Identification, Sequence, Purification Source: Viable Mouse Embryo Culture Media, Barnea 2007
- Synthetic version with same properties as native peptide, >95% pure (Applied Biosystems)

Isolated from conditioned media by affinity chromatography & HPLC
Sequence by mass spec Fmoc method
A genomic and proteomic investigation of the impact of preimplantation factor on human decidual cells

Michael J. Paidas, MD; Graciela Krikun, PhD; S. Joseph Huang, MD, PhD; Richard Jones, PhD; Michael Romano; Jack Annunziato; Eytan R. Barnea, MD

OBJECTIVE: Preimplantation factor (PIF) is a novel, 15 amino acid peptide, secreted by viable embryos. This study aims to elucidate PIF’s effects in human endometrial stromal cells (HESC) decidualized by estrogen and progesterin, which mimics the preimplantation milieu, and in first-trimester decidua cultures (FTDC).

STUDY DESIGN: HESC or FTDC were incubated with 100 nmol/L synthetic PIF or vehicle control. Global gene expression was analyzed using microarray and pathway analysis. Proteins were analyzed using quantitative mass spectrometry, and PIF binding by protein array.

RESULTS: Gene and proteomic analysis demonstrate that PIF affects immune, adhesion, and apoptotic pathways. Significant up-regulation in HESC (fold change) include: nuclear factor-k-β activation via interleukin-1 receptor-associated kinase binding protein 1 (53); Toll-like receptor 5 (9); FK506 binding protein 15, 133kDa protein (2.3); and Down syndrome cell adhesion molecule like 1 (16). B-cell lymphoma protein 2 was down-regulated in HESC (21.1) and FTDC (27.1). Protein array demonstrates PIF interaction with intracellular targets insulin-degrading enzyme and beta-K+ channels.

CONCLUSION: PIF displays essential multitargeted effects, of regulating immunity, promoting embryo-decidual adhesion, and regulating adaptive apoptotic processes.

Key words: decidual cells, genomics, implantation, preimplantation factor, proteomics

HYPOTHESIS
PIF creates a favorable environment for implantation

PIF is produced by embryo and binds to immune cells to create tolerance
PIF also binds to maternal endometrium to enhance receptivity and maintain pregnancy
Cryopreservation

- Oocyte
- Blastocysts
  - Single spermatozoon
  - Ovarian tissue
  - Testicular tissue
  - Whole organ cryo

Vitrification
- Slow freezing
- Lyophilization

For re-transplantation or in vitro maturation and folliculogenesis
Egg Freezing for “Non-medical” Reasons Will Increase
Percentage of married, childless American women who experienced infertility problems for a yearlong period.

- 15-29 years: 11%
- 30-34 years: 17%
- 35-39 years: 23%
- 40-44 years: 27%

Source: Center for Disease Control and Prevention/National Center for Health Statistics, 2002
## Fresh (non-donor) IVF Cycles 1999-2008

<table>
<thead>
<tr>
<th>Year</th>
<th>&lt;35</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>&gt;42</th>
<th>Total Cycles</th>
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<tbody>
<tr>
<td>1999</td>
<td>29,682</td>
<td>15,291</td>
<td>12,848</td>
<td>5,302</td>
<td>2,628</td>
<td>65,751</td>
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<tr>
<td>2000</td>
<td>33,453</td>
<td>17,284</td>
<td>14,701</td>
<td>6,118</td>
<td>3,401</td>
<td>74,957</td>
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<tr>
<td>2001</td>
<td>35,984</td>
<td>17,791</td>
<td>16,283</td>
<td>7,044</td>
<td>3,762</td>
<td>80,864</td>
</tr>
<tr>
<td>2002</td>
<td>37,591</td>
<td>19,110</td>
<td>17,454</td>
<td>7,733</td>
<td>3,938</td>
<td>91,032</td>
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<td>2003</td>
<td>39,852</td>
<td>20,056</td>
<td>18,660</td>
<td>8,185</td>
<td>4,279</td>
<td>91,032</td>
</tr>
<tr>
<td>2004</td>
<td>40,853</td>
<td>21,019</td>
<td>19,174</td>
<td>8,487</td>
<td>4,709</td>
<td>94,242</td>
</tr>
</tbody>
</table>

| % Change | +45.8 | +52.5 | +69.6 | +83.0 | +86.2 | +56.6 |


Risks of Postponing Fertility

• Older women have more trouble naturally becoming pregnant

• Even with reproductive technologies older women have a reduced chance of becoming pregnant
  – Only 8.8% of women over the age of 42 who use IVF will become pregnant
  – Only 4.1% of them will actually give birth to a child
Reasons for Postponement

• The most common reason women give for their decision to postpone pregnancy is an uncertainty about the stability of their relationships

• Another common reason for delaying fertility postponement are future goals and aspirations
  – Women wait until reaching certain academic and career achievements
  – Women do not want to fall behind in the workplace
  – Desire to be financially secure when having a child
Misperceptions about Fertility Postponement
(Fertil.Steril.2012)

• Most women are unsure about what age infertility begins to take effect and how quickly it advances
  – Estimates suggest that as few as 75% of women understand that fertility decreases between ages 30-40

• It is not until they experience infertility themselves do women begin to learn the truth

• Women mistakenly believe that ART can overcome all age-related infertility issue
Moving Forward

• General practitioners and gynecologists who see women at an early age should have a discussion with their patients about:
  – The risks of fertility postponement
  – Alternative resources such as Oocyte and Embryo cryopreservation

• Societal practices that encourage women to postpone fertility need to be addressed

• We must not think of age-related infertility as a disease but rather a social harm
ONGOING IMPLANTATIONS and BABY RATE per VITRIFIED OOCYTE during THIRD PARTY REPRODUCTION using GAMETES from an EGG BANK

P. Patrizio, M.D., MBE\textsuperscript{1}, P.D. Bernal, DVM\textsuperscript{2}, J. Kahn, Bsc\textsuperscript{2}, C.C. Chang, Ph.D\textsuperscript{2}, D. Shapiro, M.D.\textsuperscript{2} and P. Z. Nagy, M.D., Ph.D\textsuperscript{2}.

\textsuperscript{1}Yale University Fertility Center, New Haven, CT, USA
\textsuperscript{2}Reproductive Biology Associates, Atlanta, GA, USA
Materials and Methods

- Time Period: July 2008 – February 2011
- Donation cycles: 88 donors (age 26.1) - 112 cycles - 2078 MII oocytes banked and 1772 rewarmed
- Recipients: 290 (mean age 43.1)
- 4 to 8 eggs / Recipient
## Summary Results

<table>
<thead>
<tr>
<th></th>
<th>Total oocytes</th>
<th>Total Cryo MII</th>
<th>Warmed</th>
<th>Survived Fertiliz.</th>
<th>Usable Embryo</th>
<th>Embryo Transf.</th>
<th>Ong/Del</th>
<th>LBB Oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient (n=290)</td>
<td>2078</td>
<td>1772</td>
<td>1559 (88%)</td>
<td>1169 (75%)</td>
<td>914 (59%)</td>
<td>519 (29%)</td>
<td>203/193</td>
<td>11% MII 8% Tot.</td>
</tr>
<tr>
<td>Age 41.3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean - SD</td>
<td>6.1</td>
<td>5.3</td>
<td>3.1</td>
<td>1.8</td>
<td>1.4</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donors (n=88) (GV,MI)</td>
<td>2494</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 26.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrieval (112)</td>
<td>Mean 22.2</td>
<td>Mean 18.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
# Oocyte to Baby Rate (OBR)-Fresh

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Oocyte</th>
<th>E.T.</th>
<th>Frozen</th>
<th>L.B. Fresh</th>
<th>L.B. Frozen</th>
<th>L.B. Oocyte, %</th>
<th>E.T. (IR), %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>87</td>
<td>1705</td>
<td>193</td>
<td>372</td>
<td>80</td>
<td>37</td>
<td>6.8</td>
<td>22.0</td>
</tr>
<tr>
<td>&lt;35 y</td>
<td>206</td>
<td>2917</td>
<td>546</td>
<td>295</td>
<td>105</td>
<td>20</td>
<td>4.3</td>
<td>15.6</td>
</tr>
<tr>
<td>35–37 y</td>
<td>116</td>
<td>1235</td>
<td>299</td>
<td>70</td>
<td>49</td>
<td>7</td>
<td>4.5</td>
<td>15.5</td>
</tr>
<tr>
<td>38–40 y</td>
<td>97</td>
<td>843</td>
<td>268</td>
<td>14</td>
<td>24</td>
<td>1</td>
<td>3.1</td>
<td>8.9</td>
</tr>
<tr>
<td>41–42 y</td>
<td>45</td>
<td>383</td>
<td>134</td>
<td>12</td>
<td>4</td>
<td>0</td>
<td>1.0</td>
<td>2.7</td>
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<tr>
<td><strong>Total</strong></td>
<td>464</td>
<td>5378</td>
<td>1247</td>
<td>391</td>
<td>182</td>
<td>28</td>
<td>4.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Conclusions

- Oocyte vitrification does not diminish the chances of pregnancy in a donor program.
- When corrected for the total number of oocytes retrieved (20% correction factor for MI and GV), the OBR is similar to those reported using FRESH donor oocytes (~10%).
- Oocyte banking is the future of Oocyte donation and for Fertility Postponement.
“Non-Medical” Freezing (Yale)

- Total of 231 oocytes cryopreserved
  - 134 by slow freezing
  - 97 by vitrification

- So far only one patient utilized oocytes (41 years old minister- now 43, with 13 oocytes slow freezing-9 (69%) survived-2 fertilized (22%)-no pregnancy)
## NYU cycle data stratified by age
(mean age: 38; range: 23-42 y).
\[ n = 499 \] (2005-2010)

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>( \leq 34 ) (n = 41)</th>
<th>35 - 37 (n = 129)</th>
<th>( \geq 38 ) (n = 329)</th>
<th>P (anova)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 day of OT (pg/ml ± SD)</td>
<td>2612 ± 1285</td>
<td>2416 ± 1424</td>
<td>2248 ± 1291</td>
<td>.07</td>
</tr>
<tr>
<td>Number oocytes retrieved n (range)</td>
<td>21 (4-59)</td>
<td>17 (3-47)</td>
<td>14 (2-74)</td>
<td>.0001</td>
</tr>
<tr>
<td>Number MII oocytes retrieved and frozen (range)</td>
<td>15 (2-35) 615</td>
<td>12 (1-36) 1548</td>
<td>10 (1-55) 3290</td>
<td>.0001</td>
</tr>
<tr>
<td>Number MII per total number of oocytes</td>
<td>73%</td>
<td>74%</td>
<td>71%</td>
<td>NS</td>
</tr>
<tr>
<td>Peak E2 per retrieved oocyte (pg/ml ± SD)</td>
<td>153 ± 81</td>
<td>162 ± 83</td>
<td>196 ± 118</td>
<td>.001</td>
</tr>
</tbody>
</table>

About 5,500 Total oocytes

Werner, Knopman, Arslan, Noyes, ISFP, 2011
Some Reflections on FP

• The majority of patients are older than 35 yrs
  • Too early to assess utilization rates
    • The number of women that are using oocyte cryopreservation for fertility postponement is still low
    • Since ASRM consider oocyte cryopreservation EXPERIMENTAL (!!), it cannot be advertised, patients are confused and insurances do not pay for it
  • Time for ASRM to remove the label
Ovarian Cortical Strips
Cortical Strips
Ovarian Strips-vitrification
Questions and Answers about Ovarian freezing

- **What to freeze?** [Ovarian Cortex]
- **How to freeze?** [Vitrification]
- **Is it Safe?** (so far.....no reseeding cancer)
- **Does it Work?** [Yes, Pregnancies and 19 Babies!!]
- **Where to Re-transplant?** [Orthotopic]

**BUT**

What to do with Systemic Cancers?
Work in Progress.........

- In vitro Folliculogenesis on strips
- Follicular engineering
- In vitro perfusion of Whole Ovary
In vitro Folliculogenesis

Follicle culture performed using fresh cortical strips (two-step slices) (IRB-approved protocol)
[in collaboration with E. Telfer, Univ. of Edinburgh]

Hypothesis: Manipulation of the Target of Rapamycin (TOR) kinase allows control of follicle survival and growth: improved likelihood of generating fertilizable mature eggs
Two-step Human Strip/Follicle Culture
We have shown that mTOR kinase controls granulosa proliferation and thus follicle growth (Yaba et al., 2008).

PTEN/Akt/mTOR pathway has also been shown to be a key regulator of the rate of primordial follicle growth activation in mice (Liu group) and humans (Hsueh group).

Can this pathway by manipulated to maximize the growth activation, survival, and oocyte maturation in human cortical strip cultures?
Bovine Follicles Encapsulated in ALGINATE 1.5%

[Courtesy of Talevi R.]
In vitro perfusion apparatus
[Brannstrom M]
Freeze-drying of gametes and stem cells

Pasquale Patrizio, M.D., MBE - Yale University
Amir Arav DVM PhD amir@coredynamics.com
Lessons from Nature: Tardigrada means "slow walker" and this name was given by Lazzaro Spallanzani in 1777.
A nearly universal feature of anhydrobiotic organisms is that they synthesize large quantities of disaccharides, the most common are **Sucrose** or **Trehalose**.
Freeze drying of human sperm

RESULTS

• ICSI into human donor egg resulted with the first 2PN embryo fertilized with freeze dried sperm
Freeze drying of MII ovine oocytes

Delivered to Prof. Loi in Italy in regular post

Loi and Arav unpublished data 2012
Freeze Drying of MII Oocytes

RESULTS

Loi and Arav unpublished data 2012
Remove MII nucleus from freeze dried oocytes

Loi and Arav unpublished data 2012
First 2-cell embryo obtained from injection of a metaphase plate from a freeze dried oocyte into a fresh enucleated oocyte

Loi and Arav unpublished data 2012
Dried STEM CELLS
Freeze Dried Red Blood Cells

Sterile Water  Dried Blood
Basic Science Studies

- Implantation (gene expression profiling)
- Genes in POF, endometriosis, PCO, and RPL
- Genetics in male infertility
- Mitochondrial DNA mutations
- Stem cells therapy (in vitro derivation of gametes; hESC’s)
Reappraisal

• Cancer and fertility drugs
• Follow-up IVF and ICSI-babies (many in their teens and young adults)
• Babies from Oocyte cryopreservation and Blastocysts (imprinting?? wait 2nd generation)
• Preservation of Cortical ovarian strips for non medical reasons (more than just few oocytes)
• Safety of ovarian re-transplants (MRD’s)
Ethical Debates

- Time limit on frozen embryos
- Fate of abandoned embryos
- Donor disclosure policy and payments
- Age and Reproduction
- Post-humous reproduction
- PGS x family balancing-designer babies
- Cross Borders Reproductive Care
- Therapeutic cloning
Cross-border Reproductive Care
(aka Reproductive Tourism, Fertility Tourism, Procreative Tourism)
Global “Reproflows”

• Flow of technologies between countries
• Flow of expertise between countries
• Flow of embryos between countries
• Flow of men and women seeking reproductive “assistance”
• Flow of reproductive “assistors”
• Flow of capital
• Flow of media
Miscellaneous

• Insurance coverage for IVF
  – More couples access to treatment
  – Low cost IVF
• Spread info on Age and Reproduction
  – Quicker referral lines
• Preconception genetic testing (saliva tests) for Mendelian traits
# Insurance and IVF Results

<table>
<thead>
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<th></th>
<th>IVF Insured States</th>
<th>Non Insured States</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Cycles</td>
<td>27,565</td>
<td>64,188</td>
<td></td>
</tr>
<tr>
<td>Pregnancy Rate (%)</td>
<td>35.0</td>
<td>38.8*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Live Birth Rate (%)</td>
<td>29.1</td>
<td>32.2*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Live Birth Trans (%)</td>
<td>35.4</td>
<td>37.9*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cancellation Rate (%)</td>
<td>11.0</td>
<td>10.9</td>
<td>.66</td>
</tr>
<tr>
<td>Embryos Trans</td>
<td>2.4</td>
<td>2.7*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Twin Rate (%)</td>
<td>26.0</td>
<td>28.1*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triplet Rate (%)</td>
<td>3.4</td>
<td>3.9*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Multiple Rate (%)</td>
<td>27.3</td>
<td>29.8*</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Martin JR et al. 2011 FS
Preconception Genetic Testing on Saliva

A universal carrier test for the long tail of Mendelian disease

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At the end......getting closer to

The Ultimate goal: a Single, Healthy and Happy Baby
THANK YOU!!!!