PGS and prevention of recurrent miscarriage: facts and fiction

Tanya Milachich, PhD

ESHRE Campus symposium
Effects of ART and endometriosis on pregnancy outcome

SIGs Early Pregnancy and Endometriosis and Endometrial disorders

27 - 28 January 2017, Sofia, Bulgaria
Disclosure

Conflicts of interest: none
Recurrent pregnancy loss (RPL), also referred to as recurrent miscarriage or habitual abortion, is historically defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period.

Ford and Shust, Rev. Obstet. and Gynecol. 2009
**RPL**

Second World Congress of RPL, Cannes, France 19-22 Jan, 2017

**Reasons:**
Endocrine aspects, BMI, Vit D3, Prevalence of Vitamin D;
Imm.proceses: at Feto-maternal interphase, Decidual Th1/Th2&NK1/NK2 phenotyping, Anti-P Allo-Antibodies, APLA Syndrome, Elevated peripheral NK cells, Hereditary Thrombophilia;
Mol.and Chrom. level: Trombophilia gene mutation, Deficiency of Placental Copy Number Variations, Trophoblast Growth Pathways, RNA-Seq Analysis of Chorionic Villi, Chromosomal Abnormalities e.g. Balanced Structural Chromosomal Anomalies;
Infections: Gardnerella Vaginalis, toxoplasmosis, rubella, CMV, endometritis;
Role of Oxidative Stress, Anatomical Causes, Cervical Incompetence;

**Treatment:** G-CSF Treatment, Lymphocyte Immunization, Anti-Oxidants Treatment, Endoscopic Septectomy, HS, Aspirin, Medformin, Low Molecular Weight Heparin;

**Methods:** Time-lapse, Endometrial biopsy, Embryo morphology, Role of Sperm Selection (IMSI), PGS;

**Other side effects of RPL:** Depression and Anxiety
Implantation and ART

NIHCE Guidance, 2013
The pregnancy loss iceberg
An overview of the outcome of spontaneous human conceptions

75% of embryos are lost before delivery
Most of those losses are in the period BEFORE implantation

Larsen et al. BMC Medicine 2013 11:154
Less than 5% of oocytes, collected after controlled ovarian stimulation, can lead to a pregnancy and then to the birth of a child.

How many frozen eggs does everyone need?

How many MII are needed to achieve a pregnancy?

Outcomes compared between patients 30-36 and 37-39

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>The outcome comparison between young age versus advanced age patients' oocytes after vitrification.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Young age group 30-36 y (n = 11)</th>
<th>Advanced age group 37-39 y (n = 11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (mean y ± SD)*</td>
<td>32.91 ± 1.97</td>
<td>37.90 ± 0.83</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Mean basal FSH (mean mlU/mL ± SD)</td>
<td>6.20 ± 2.26</td>
<td>6.20 ± 0.92</td>
<td>NS</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>80/97 (82.5)</td>
<td>68/89 (76.4)</td>
<td>&gt;.9999</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>68/97 (70.1)</td>
<td>56/89 (62.9)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of good-quality embryos on day 3 (%)*</td>
<td>54/97 (55.6)</td>
<td>36/89 (40.4)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>No. of embryos transferred (mean ± SD)**</td>
<td>24 (2.18 ± 0.6)</td>
<td>29 (2.64 ± 1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of clinical pregnancies (%)</td>
<td>7/11 (63.6)</td>
<td>3/11 (27.3)</td>
<td>&lt;.1984</td>
</tr>
<tr>
<td>No. of implantations (%)</td>
<td>10/24 (41.7)</td>
<td>6/29 (20.7)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of take home babies (%)</td>
<td>6/11 (54.5)</td>
<td>2/11 (18.2)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of live births</td>
<td>8</td>
<td>3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Percentage of oocyte to achieve a live birth (%)

|                      | 8/97 (8.2) | 3/89 (3.3) |

<table>
<thead>
<tr>
<th></th>
<th>Nb of oocytes to obtain a live birth</th>
<th>N=12</th>
<th>N=29</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>How many OPU ??????</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chang et al., Fertil Steril 2013
1.3% of oocytes give a baby

Example of some results

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocytes retrieved</td>
<td>9751</td>
</tr>
<tr>
<td>Embryos biopsied</td>
<td>5552</td>
</tr>
<tr>
<td>Embryos diagnosed</td>
<td>4392</td>
</tr>
<tr>
<td>Transferable embryos</td>
<td>644</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>598</td>
</tr>
<tr>
<td>Pregnancies</td>
<td>163</td>
</tr>
<tr>
<td>Babies born</td>
<td>127</td>
</tr>
<tr>
<td>Pregnancy rate/cycle initiated</td>
<td>23.2%</td>
</tr>
<tr>
<td>Pregnancy rate/embryo transfer</td>
<td>41.1%</td>
</tr>
<tr>
<td>Fetal heart beat (FHB)/cycle initiated</td>
<td>22.0%</td>
</tr>
<tr>
<td>FHB/embryo transfer</td>
<td>39.0%</td>
</tr>
<tr>
<td>FHB/embryos transferred</td>
<td>25.9%</td>
</tr>
<tr>
<td>Live birth rate (LBR)/cycle initiated</td>
<td>18.0%</td>
</tr>
<tr>
<td>Live birth rate/embryo transfer</td>
<td>32.0%</td>
</tr>
</tbody>
</table>

ESHRE PGD consortium meeting, Helsinki 2016
Still no consensus of which stage to biopsy

- 50-70%
- 60%-70%
- 60-70%
- 70-90% FR
- 80-85% M2
Palini et al., 2013; Gianaroli et al., 2014

Cell-free DNA in spent culture media

Galluzzi et al., 2015; Shamonki et al., 2016
46,XX,t(12;17)(p13;p13)
Evolution of PGD for translocations

- **Fluorescence In Situ Hybridisation (FISH)**

- **PCR-based STR Analysis** (Fiorentino et al., 2010)

- **Array Comparative Genomic Hybridisation (aCGH)** (Fiorentino et al., 2011)

- **Next Generation Sequencing (NGS)** (Bono et al., 2015)

### NGS performance for <5Mb size fragments

<table>
<thead>
<tr>
<th>TRANSLOCATION</th>
<th>Chromosome A</th>
<th>Chromosome B</th>
<th>NGS</th>
<th>aCGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>46, XY, t(3;10)(p13;q26)</td>
<td>125.5</td>
<td>74.0</td>
<td>134.4</td>
<td>1.0</td>
</tr>
<tr>
<td>46, XY, t(3;18)(p26;q12)</td>
<td>197.0</td>
<td>2.8</td>
<td>33.7</td>
<td>42.4</td>
</tr>
<tr>
<td>46, XY, t(14;15)(q32;q11)</td>
<td>104.6</td>
<td>1.8</td>
<td>39.0</td>
<td>61.3</td>
</tr>
<tr>
<td>46, XY, t(2;11)(q37;p10)</td>
<td>239.8</td>
<td>3.2</td>
<td>88.4</td>
<td>46.1</td>
</tr>
</tbody>
</table>

*Slides, used with permission of A. Biricik, Laboratorio Genoma*
PGS and prevention of recurrent miscarriage: facts

• There is still risk of miscarriage 16-30% after PGS
• We can not completely avoid the miscarriages
• PGS improve pregnancy rate in those patients
• Combined with AMA there is a very small chance for euploid embryo and for deliver a healthy baby

De Rycke et al., 2015 - ESHRE PGD Data collection XII
% aneuploidy in embryos according to indication for PGS

Data from 7000 blastocysts tested by array-CGH or NGS
Table Yb
Cycles performed for PGS, data collection XIII.

<table>
<thead>
<tr>
<th>Indication</th>
<th>AMA</th>
<th>AMA + misc</th>
<th>AMA + RIF</th>
<th>Rec.misc</th>
<th>RIF</th>
<th>SMF</th>
<th>Prev abn preg</th>
<th>AMA + Rec mis Preb</th>
<th>Num Abnor</th>
<th>AMA + Num abno</th>
<th>No indication</th>
<th>Ovum donation</th>
<th>AMA + Ovum donation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles to OR</td>
<td>1083</td>
<td>265</td>
<td>312</td>
<td>415</td>
<td>456</td>
<td>278</td>
<td>44</td>
<td>2</td>
<td>33</td>
<td>1</td>
<td>51</td>
<td>37</td>
<td>2</td>
<td>2979</td>
</tr>
<tr>
<td>Number infertile</td>
<td>688</td>
<td>162</td>
<td>297</td>
<td>145</td>
<td>405</td>
<td>245</td>
<td>14</td>
<td>0</td>
<td>25</td>
<td>1</td>
<td>43</td>
<td>36</td>
<td>2</td>
<td>2063</td>
</tr>
<tr>
<td>Female age (years)</td>
<td>39</td>
<td>37</td>
<td>40</td>
<td>36</td>
<td>32</td>
<td>37</td>
<td>36</td>
<td>42</td>
<td>36</td>
<td>41</td>
<td>37</td>
<td>43</td>
<td>44</td>
<td>39</td>
</tr>
</tbody>
</table>

**ART method**

<table>
<thead>
<tr>
<th>Delivery rate (% per OR/% per ET)</th>
<th>10/17</th>
<th>15/25</th>
<th>5/8</th>
<th>22/28</th>
<th>16/20</th>
<th>22/26</th>
<th>34/42</th>
<th>0</th>
<th>27/32</th>
<th>0</th>
<th>29/38</th>
<th>16/18</th>
<th>50/50</th>
<th>14/21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscarriages</td>
<td></td>
<td>33</td>
<td>6</td>
<td>7</td>
<td>18</td>
<td>19</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Miscarriage rate (% per clinical preg - preg lost to FU)</td>
<td>23</td>
<td>13</td>
<td>30</td>
<td>16</td>
<td>19</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancies lost to FU</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>14</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

София 1330, жк. Разсадника, ул. Христо Благоев 25-31, тел: +359 2 920 09 01, факс: +359 2 920 18 27, e-mail: contact@shterevhospital.com
Genetic consultation before ART

Y-microdeletions
Lachapelle
Morris syndrome
Teacher Collins syndrome
Genetic counseling / TIME

• Medical history – family members
• Evaluation of severity of the genetic problem
• Evaluation of all risks
• Give a realistic expectations
• Information about all processes, including confirmation of results
• Additional testing if needed
• Discussion of other options – pro and cons
• Inform consent
Seven reasons to be concerned about the use of the new - PGS

1. Do not forget evidence-based medicine
The patients should not be randomized by the number of embryos. Usually, in the new-PGS there are a minimum number of viable blastocysts as a rule to initiate patient randomization.

2. An adverse past and an uncertain future
A minimum of 6 to 8 embryos available for biopsy. AMA? DOR?

3. The trophectoderm is an area of chromosomal variability
The trophectoderm blastocyst biopsy: The aneuploidy rate can be around 70% in day 3 versus approximately 20%-50% in the blastocyst with significant degree of embryo self-correction.

4. Data are missing for several indications as RIF
At the moment, there is no proper RCT was carried out with the PGS-new in populations RIF.

5. Indicating PGS-new for infrequent populations
In 2011, 3 RCTs were published about the use of PGS in patients labeled as good prognosis (Staessen et al., 2008; Jansen et al., 2008; Meyer et al., 2009). Back then, they did not find significant differences in terms of live birth rate among patients with or without PGS.

The original intent of this study was to improve IVF pregnancy rates. As this failed, their original intent was replaced by the listed secondary goal of this study: reduction of twin pregnancies at elective single transfer (Gleicher et al., 2014).

Jose Franco, JBRA, 2015
6. Concerns about **extended culture to the blastocyst stage**

- Blastocyst stage in vitro cultures risks: 1- prolonged embryo culture has been related to significant *epigenetic changes* (Lonergan et al., 2003; Calle et al., 2012); 2- blastocyst stage culture are associated with increased *risk of premature delivery* in comparison to embryos transferred on days 2 or 3 (Maheshwari et al., 2013; Dar et al., 2014).

7. **Non-maleficence**

- In 2008, ASRM, ESHRE and the British Fertility Society declared that PGS (day 3 biopsy + FISH technique) is *ineffective* in improving IVF pregnancy rates and reducing miscarriage. Seven years later, these societies have not yet settled for or against PGS-new (day 5-6 biopsy + CGH or qPCR or NGS), as well as the Brazilian Society of Assisted Reproduction (SBRA).

*Jose Franco, JBRA, 2015*
Mosaics embryos: 55% до 73% [Munne et al., 2006; Bielanska et al, 2002];

Mosaic embryos can develop into healthy newborns

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ET</td>
<td>49</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>50</td>
</tr>
<tr>
<td>No. of +βhCG pregnancies</td>
<td>24</td>
</tr>
<tr>
<td>No. of biochemical pregnancies</td>
<td>5</td>
</tr>
<tr>
<td>No. of early miscarriages</td>
<td>4</td>
</tr>
<tr>
<td>No. of ongoing clinical pregnancies per ET</td>
<td>15 (30.6%)</td>
</tr>
<tr>
<td>No. of pregnancies went to term</td>
<td>15 (30.6%)</td>
</tr>
<tr>
<td>No. of babies born</td>
<td>16</td>
</tr>
</tbody>
</table>


Slide, used with permission of A. Biricik, Laboratorio Genoma
Why are (most of us) chromosomally normal?

Survival of the fittest cell (Robberecht, 2010);
20-30% of de novo unbalanced translocations: postzygotic origin;
PGS and prevention of recurrent miscarriage: facts and myths

Facts:

• Many embryos have ‘self-correct’ mechanism reaching the blastocyst stage and also after this stage (up to 4%);
• Near 70% of embryos in patient with RM are aneuploid;
• 28% Delivery Rate per ET in young female patients;
• RM – there is 16-18% risk of miscarriage;
• 0% Delivery Rate in patients with RM, when is combined with AMA and previous abnormal pregnancy;

Myths:

• We can completely avoid the miscarriage risk by using PGS in couples with RM
• There is a high chance for euploid embryos after PGS
• There is a high chance for delivery in AMA cases

Johnson et al., 2010; Baart et al, 2007; Munne et al., 2005; De Rycke et al., 2015 - ESHRE PGD Data collection XII
Still nearly 30% of patients with RM have chance for take-home baby after PGS if there is no issue of AMA
But with sense of humour and life expectancy 101 years!
Conclusions:
1. In the future: Are we be able to find the perfect embryo with the fast development of NGS?
2. How many embryos per couple we will need?
3. Is there a perfect human being at all?
4. For now we are capable to chose the less affected embryo among all others?
5. New data shows that mosaic embryos can develop into a healthy newborn in 32% of all cases.
6. Every case must be well discussed and genetically consulted prior and after PGS.

_The complexity and the wonder of life.....A simple friendship, a sunny day, our life_
ART team - gynaecologists, embryologists, biologists:

- A. Shterev
- T. Timeva
- D. Savova
- P. Andreeva
- G. Ganeva
- M. Yunakova
- M. Konovalova
- T. Arabadji
- R. Milcheva
- N. Magunska
- M. Alexandrov
- R. Bilchev
- S. Kyurkchiev
- D. Barov
- T. Milachich
- L. Valkova
- I. Antonova
- B. Bandreva
- P. Penkova
- L. Veleva
- D. Nikolova
- I. Canov
- P. Gavrilov
- I. Bochev

PGD Genetic consultants:

- I. Hadjiiska
- I. Dimova
- M. Savova
- B. Petkova
- N. Trpchevska
- M. Dimitrova
- R. Petkova
- M. Konovalova
- P. Penkova
- F. Fiorentino
- A. Biricik
- A. Nucitelli et al.

PGD team Genoma:

- T. Arabadgi
- L. Veleva
- D. Nikolova
- I. Canov
- P. Gavrilov
- I. Bochev