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Disclosure

Receipt of honoraria by Oxford Gene Technology (OGT). CEO medical director and share-holder of Reprogenetics Germany LLC.
K. R. Held: The role of reproductive Genetics in optimizing ART results
Declaration of interests

Medical director, CEO and shareholder of Reprogenetics Germany GmbH
<table>
<thead>
<tr>
<th>Problem</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low responders</td>
<td>IVF clinic, basic genetic tests: Chromosomes, <em>FMR1</em>, <em>FSHR</em>, <em>LHR</em></td>
</tr>
<tr>
<td>Low fertilization rates</td>
<td>IVF clinic, special genetic tests: DNA-fragmentation, sperm FISH</td>
</tr>
<tr>
<td>Repeated implantation failure</td>
<td>Basic and special genetic tests: Chromosomes, PGS, sperm FISH</td>
</tr>
<tr>
<td>Repeated abortes</td>
<td>Basic and special genetic tests: Chromosomes, PGS, sperm FISH</td>
</tr>
</tbody>
</table>
Chromosomal locations of plausible genes associated with POF

Figure 1 Schematic representation of chromosomal location of plausible genes associated with primary ovarian insufficiency.
<table>
<thead>
<tr>
<th>Type</th>
<th>OMIM</th>
<th>Genelocus</th>
</tr>
</thead>
<tbody>
<tr>
<td>POF1 **</td>
<td>311360</td>
<td>FMR1</td>
</tr>
<tr>
<td>POF 2A</td>
<td>300511</td>
<td>DIAPH2</td>
</tr>
<tr>
<td>POF 2B</td>
<td>300604</td>
<td>POF1B</td>
</tr>
<tr>
<td>POF 3</td>
<td>608996</td>
<td>FOXL2</td>
</tr>
<tr>
<td>POF 4</td>
<td>300510</td>
<td>BMP15</td>
</tr>
<tr>
<td>POF 5</td>
<td>611548</td>
<td>NOBOX</td>
</tr>
<tr>
<td>POF 6</td>
<td>612310</td>
<td>FIGLA</td>
</tr>
<tr>
<td>POF 7*</td>
<td>612964(SF1)</td>
<td>9q33</td>
</tr>
<tr>
<td>POF(N)*</td>
<td>233300</td>
<td>FSHR</td>
</tr>
<tr>
<td>POF(N)</td>
<td>152790</td>
<td>LHR</td>
</tr>
<tr>
<td>POF(N)</td>
<td>147380</td>
<td>INHA</td>
</tr>
</tbody>
</table>
Gene polymorphism and ovarian response
Genotypes for LHR gene rs13405728 and FSHR p.Asn680Ser and p.Thr307Ala polymorphism

La Marca A, Sighinolfi G, Argento C, Grisendi V, Casarini L, Volpe A, Simoni M.
Polymorphisms in gonadotropin and gonadotropin receptor genes as markers of ovarian reserve and response in in vitro fertilization.

Mohiyiddeen L, Newman WG, McBurney H, Mulugeta B, Roberts SA, Nardo LG.
Follicle-stimulating hormone receptor gene polymorphisms are not associated with ovarian reserve markers.
**FSHR** gene polymorphism p.Asn680Ser and ovarian response

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asn/Asn (n = 22)</th>
<th>Asn/Ser (n = 71)</th>
<th>Ser/Ser (n = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor responder</td>
<td>0</td>
<td>15 (21.1)</td>
<td>7 (46.7)</td>
<td>0.008a</td>
</tr>
<tr>
<td>Normal responder</td>
<td>22 (100)</td>
<td>46 (64.8)</td>
<td>0</td>
<td>0.004a</td>
</tr>
<tr>
<td>Hyper responder</td>
<td>0</td>
<td>10 (14.1)</td>
<td>8 (53.3)</td>
<td>0.037a</td>
</tr>
</tbody>
</table>

*aUsing Chi-square test, FSHR - Follicle-stimulating hormone receptor; Asn - Asparagines, Ser - Serine; Figures in parenthesis are in percentage*

Sheikhha et al., 2011
del(X)    PBD

Portnoi M F et al., 2006

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;35</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>&gt;42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>39173</td>
<td>20066</td>
<td>20475</td>
<td>9549</td>
<td>5503</td>
</tr>
<tr>
<td>PR/cycle</td>
<td>47,7%</td>
<td>38,7%</td>
<td>29,8%</td>
<td>20,0%</td>
<td>8,8%</td>
</tr>
<tr>
<td>LB/cycle</td>
<td>41,6%</td>
<td>31,9%</td>
<td>22,0%</td>
<td>12,4%</td>
<td>4,1%</td>
</tr>
<tr>
<td>IR</td>
<td>36,8%</td>
<td>27,0%</td>
<td>17,7%</td>
<td>9,6%</td>
<td>3,7%</td>
</tr>
</tbody>
</table>

PR: pregnancy rate; LB: live birth; IR: implantation rate
Euploidy rate in oocytes (%) (aCGH N=4.628)

- ≤30Y: 52.46%
- 31-35Y: 48.48%
- 36-37Y: 40.00%
- 38-40Y: 28.88%
- ≥41Y: 14.74%

Legend:
- ▲ euploid
- □ balanced oocytes
Required number of fertilized oocytes to transfer one (■) or two (□) „euploid“ embryos: (N=1776; 2011- Sept. 2014)

- ≤30y: Average number of fertilized oocytes per cycle
- 31-35y: Average number of fertilized oocytes per cycle
- 36-37y: Average number of fertilized oocytes per cycle
- 38-40y: Average number of fertilized oocytes per cycle
- ≥41y: Average number of fertilized oocytes per cycle
Polar body biopsy
Embryo biopsy
Blastozyst (TE) biopsy

Verlinsky & Kuliev, 2005
Meiosis

McKinlay Gardener et al., Chromosome abnormalities and genetic counseling, 2012 (modified)

Alberts et al., Molecular Biology of th cell, Garland Science 2002
Chromosome und chromatid malsegregation in meiosis I
Pellestor et al., 2004
PB-D Sequential Hybridisation with 2 probes system:

FISH: MultiVysion PB™ (Abbott) chromosomes 13, 16, 18, 21 and 22 + 4-Color-Mix chromosomen X, 15, 16 (α-Satellit), 17 (Cellay)
Meiosis - Predivision

McKinlay Gardener et al., Chromosome abnormalities and genetic counseling, Oxford 2012 (modified)
Meiosis - Predivision

McKinlay Gardener et al., Chromosome abnormalities and genetic counseling, Oxford 2012 (modified)
PB1
t(4;17)(q22;q23)

NGS

arrayCGH V3
Blastocysts biopsy and aCGH eliminates the maternal age effect on implantation

Reprogenetics data to 10/10/2011
Cycles with no euploid embryos do increase with maternal age.

Age related pregnancy rates/transfer using aCGH (N=569) vs controls

- 31-35Y
- 36-37Y
- 38-40Y
- ≥ 41Y

**aCGH** vs **controls**
AMA: increased pregnancy and birth rates (%) after aCGH diagnostics in polarbodies (pat. 38 – 40y, n= 155   controls        aCGH      )
All age groups: transfer rates (%) after aCGH
### Patients with good prognosis with PGD

<table>
<thead>
<tr>
<th># day 3 embryos</th>
<th>egg donors</th>
<th>&lt;35 years old</th>
<th>35-39 years old</th>
<th>40-42 years old</th>
<th>&gt;42 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>n/a</td>
<td>69% 37%</td>
<td>57% 31%</td>
<td>n/a 37%</td>
<td>n/a 16%</td>
</tr>
<tr>
<td>5-7</td>
<td>100% 38%</td>
<td>93% 37%</td>
<td>85% 26%</td>
<td>60% 16%</td>
<td>53% 8%</td>
</tr>
<tr>
<td>8-10</td>
<td>100% 54%</td>
<td>96% 38%</td>
<td>93% 29%</td>
<td>72% 17%</td>
<td>53% 8%</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>100% 43%</td>
<td>100% 44%</td>
<td>97% 32%</td>
<td>84% 13%</td>
<td>91% 13%</td>
</tr>
</tbody>
</table>

768 cycles, 6404 embryos. Linear regression analysis: Euploidy significantly decreased with age (p<0.001) but cohort size.
age group 36-40y: transfer rate ( ), pregnancy/transfer ( ), pregnancy/cycle ( )

No. Oocytes

- 1-3
- 4-6
- 7-9
- ≥ 10

0 20 40 60 80 100 120

0 1 2 3 4
All age groups: pregnancy/cycle rates (%) after aCGH

<table>
<thead>
<tr>
<th>No. Oocytes</th>
<th>1-3</th>
<th>4-6</th>
<th>7-9</th>
<th>&gt; 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>≤ 35 y</td>
<td>36-40 y</td>
<td>≥ 41 y</td>
<td></td>
</tr>
</tbody>
</table>

- No. Oocytes: 1-3, 4-6, 7-9, > 10
- Age group: ≤ 35 y, 36-40 y, ≥ 41 y

Graph showing the relationship between the number of oocytes and pregnancy/cycle rates (%) for different age groups.
The clinical consequences of balanced translocations

McKinlay Gardener et al., Chromosome abnormalities and genetic counseling, Oxford 2012 (modified)
Complex chromosome aberrations

McKinlay Gardener et al., Chromosome abnormalities and genetic counseling, Oxford 2012 (modified)
Unbalanced chromosome 1;10 oocyte due to whole chromosome malsegregation in MI, but balanced for chromosome 15

R12-067-PA  t(1;10)(p32.1;q24.1)
8;21 translocation

R14-196-ZK  2a

OGT

illumin
Required number of fertilized oocytes to anticipate one (■) or two (□) „euploid“ embryo(s) for transfer (2011 - II. Q 2016, a-CGH)

Anzahl befruchtete Eizellen

PGS

PGD

- durchschn. Zahl PNs/FP
- für 1 Embryo
- für 2 Embryos

Reprogenetics
### Why Polar Body Diagnostic

The major application of PBD is the detection of maternally derived chromosomal aneuploidies and translocations in oocytes.

- Pregnancy rates and birth rates can be increased per transfer and per cycle by polar body diagnostics.
- Prerequisite is the analysis of both polar bodies and a sufficient number of fertilized oocytes (5 – 8).
- Numerical and structural aberrations of ≈ 5 Mb can be safely diagnosed by either BAC or oligoarrays.
- In countries non permitting PGD or PGS via blastomere or TE biopsy, PBD may offer a viable alternative.
# Genetic causes and prevalence of male infertility

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Unselected patients n=12945 (%)</th>
<th>Azoospermic patients N=1446 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>47,XXY</td>
<td>2.8</td>
<td>15.0</td>
</tr>
<tr>
<td>XX male</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Translocations</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Other chromosomal aberrations</td>
<td>&lt;0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>CF/CBAVD</td>
<td>0.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Hypogonadotr. hypogonadism</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Y chrom. AZF deletions</td>
<td>0.3</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>4.3</strong></td>
<td><strong>20.6</strong></td>
</tr>
</tbody>
</table>

Tüttelmann, 2011
sperm-FISH results
(N=386, n. a. N=34)

Translocation carrier (N=12)
unbalanced 11,4% - 58%

OAT patients
Aneuploidy rate
(13, 18, 21, X, Y)

normal 69,1%
moderate increase 22,1%
high increase 8,8%
Pericentric inversion

Gardener & Sutherland, 2004
Morel et al., 2007
Array-CGH in Trophektoderm

Robertson Translokation 13;14 – hier: Monosomie 13; Trisomie 14 / Trisomie 13; Monosomie 14
Biopsy stage: before arrays

- 30% postmeiotic abnormalities undetected
- Triple amount of cells to analyze vs. blastocysts
- More embryo damage
- PGD can compensate the damage but does not reach its potential
- Centers not proficient in blastocyst culture until recently
- Applied clinically in 2005 *
- First positive results in 2010**

* McArthur et al. (2005), ** Schoolcraft et al. (2010)
Blastocysts mosaicism

Conclusions

• Numerous monogenic defects are associated with fertility disorders, they may be either syndromic or non-syndromic.

• While syndromic forms are usually recognised by associated clinical symptoms, non-syndromic forms have to be detected by genetic tests (molecular tests or chromosome analysis).

• Genetic testing is indicated in all non-syndromic cases with RIF, RA, POR and POI as well as severe oligo-/azoospermia.

• With few exceptions the detection of a genetic defect does not alter the treatment strategy but rather helps to explain a treatment failure.

• Structural chromosome aberrations and advanced maternal age are the most important factors influencing ART. Depending on indication comprehensive chromosome analysis in either oocytes or blastocysts improves greatly ART outcome.
Thank you for your attention

S. Knebel, PhD
A. Drenckhan, PhD
A. Radtke
B. Bertram
J. Haffner
I. Gerdt
E. Petanova