Health of children born after ART including PGD

Florence Belva
Medical Genetics, UZ Brussel
Introduction: IVF, ICSI and PGD

Neonatal outcome after ART

PGD: Medical follow-up in childhood

PGD: Psychological follow-up in childhood

Concerns and Future perspectives

Conclusion
Introduction: IVF, ICSI and PGD
Introduction
IVF and ICSI

- ICSI ≠ IVF

- More invasive technique (°1991, UZ Brussel)
- Main indication: Male factor infertility
- ICSI ‘in progress’

- Worldwide > 5 million children born after ART
- UZ Brussel: ± 20 000 children conceived via ART
Introduction

PGD (2)

● Pre-implantation Genetic Diagnosis

= ICSI + Embryo biopsy

→ More invasive than ICSI

→ Indication: couples at high risk for transmitting a genetic disease

→ Early form of prenatal diagnosis but requires in vitro fertilisation
Assisted Reproductive Technologies (ART)

1. egg production stimulated by hormone therapy
2. eggs retrieved from ovary
3. sperm sample provided
4. eggs and sperm combined to allow fertilization
5. fertilized eggs introduced into uterus

Embryo biopsy

ICSI / IVF
• Introduction: IVF, ICSI and PGD
• Neonatal outcome after ART
Neonatal outcome after ART IVF and ICSI

- All singletons after IVF/ICSI have compared to the general population:

  **increased risk**
  - Low birth weight (< 2 500 g) RR 1.6 – 1.8
  - Preterm birth (< 37 weeks) RR 1.5 – 2.0
  - Small for gestational age RR 1.4 – 1.6
  - Perinatal mortality RR 1.7 – 2.2

Neonatal outcome after ART IVF and ICSI

Congenital malformations: 30 – 40 % excess risk

- Risk not different according to origin of sperm
  Bonduelle 2002, Kallen 2010

- Risk not different according to fresh or frozen ET

- Risk not different according to mode of conception

- Risk after vitrified embryo transfer?
- Risk after embryo biopsy?
Neonatal outcome after ART PGD (1)

- ESHRE PGD Consortium
  - Established in 1997
  - Aims
    - To survey the availability of PGD
    - To produce guidelines and protocols
    - To formulate a consensus on the use of PGD
    - To educate in the science of genetics & reproduction
    - To collect data
    - To initiate follow-up studies
  - Data: indications, pregnancies, children
    - 60 centres worldwide
    - unique!
Neonatal outcome after ART PGD (2)

- ESHRE PGD Consortium
  - Data collection 1997-2007
  - > 5,000 clinical pregnancies
  - > 5,000 newborns

The ESHRE PGD Consortium: 10 years of data collection

J.C. Harper 1,2,*, L. Wilton 3, J. Traeger-Synodinos 4, V. Goossens 5, C. Moutou 6, S.B. SenGupta 1, T. Pehlivan Budak 7, P. Renwick 8, M. De Rycke 9, J.P.M. Geraedts 10, and G. Harton 11
Neonatal outcome after ART PGD (3)

ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010+

C. Moutou1, V. Goossens2, E. Coonen3, M. De Rycke4, G. Kokkali5, P. Renwick6, S.B. SenGupta7, K. Vesela8, and J. Traeger-Synodinos9,*

- 1,600 pregnancies and
- 1,238 children
- + cumulative data I-XI
Neonatal outcome after ART PGD (4)

### Table IXb Method of delivery and gestational age, data XII.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Singleton</th>
<th>Twin</th>
<th>Triplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. deliveries</td>
<td>1062</td>
<td>844</td>
<td>211</td>
<td>7</td>
</tr>
<tr>
<td>Term at delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm</td>
<td>253</td>
<td>108</td>
<td>140</td>
<td>5</td>
</tr>
<tr>
<td>Term</td>
<td>761</td>
<td>697</td>
<td>63</td>
<td>1</td>
</tr>
<tr>
<td>Post term</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>45</td>
<td>36</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table Xlb Data on children born, data collection XII.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total children born</td>
<td>1238</td>
</tr>
<tr>
<td>Mean birthweight (g)</td>
<td></td>
</tr>
<tr>
<td>Singletons</td>
<td>3223</td>
</tr>
<tr>
<td>Twins</td>
<td>2317</td>
</tr>
<tr>
<td>Triplets</td>
<td>1752</td>
</tr>
</tbody>
</table>

\( (759/812)^a \)
\( (376/408)^a \)
\( (18/18)^a \)
Supplementary Table XIIb: Congenital malformations and neonatal complications at birth, data collection XII.

<table>
<thead>
<tr>
<th>Category</th>
<th>Count/Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No data available</td>
<td>159/1022</td>
<td>15.6%</td>
</tr>
<tr>
<td>No malformation</td>
<td>841/863(^a)</td>
<td>97.4%</td>
</tr>
<tr>
<td>Singletons</td>
<td>658/678(^a)</td>
<td>97.0%</td>
</tr>
<tr>
<td>Twins</td>
<td>178/180(^a)</td>
<td>98.9%</td>
</tr>
<tr>
<td>Triplet</td>
<td>5/5(^a)</td>
<td>100%</td>
</tr>
<tr>
<td>Babies with malformation</td>
<td>20 singletons, 2 twins(^b)</td>
<td></td>
</tr>
<tr>
<td>No neonatal data available</td>
<td>264/1022</td>
<td>25.8%</td>
</tr>
<tr>
<td>No neonatal complications</td>
<td>681/758</td>
<td>89.8%</td>
</tr>
<tr>
<td>Singletons</td>
<td>558/599</td>
<td>93.1%</td>
</tr>
</tbody>
</table>
Neonatal outcome after ART PGD (6)

- UZ Brussel PGD cohort study

- Safety concern!

- Prospective data collection focussing on health of ART children
  → Since 1991/1993
  → Questionnaires
  → Clinical examination by staff of Dpt Medical Genetics

Liebaers 2010, Desmyttere 2009, 2010
Neonatal outcome after ART PGD (7)

- UZ Brussel PGD cohort study
  - 1993-2008
  - Largest cohort worldwide
  - Neonatal outcome; visit at 3 months

<table>
<thead>
<tr>
<th></th>
<th>PGD N = 995</th>
<th>ICSI N = 1507</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singletons</td>
<td>670 (67%)</td>
<td>1059 (70%)</td>
</tr>
<tr>
<td>Twins</td>
<td>308 (31%)</td>
<td>433 (29%)</td>
</tr>
<tr>
<td>Triplets</td>
<td>17</td>
<td>15</td>
</tr>
</tbody>
</table>
|                | PGD  
N = 995 | ICSI  
N = 1507 |   P  |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Singletons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean birth weight (g)</td>
<td>3263 ± 544</td>
<td>3237 ± 583</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Twins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean birth weight (g)</td>
<td>2346 ± 552</td>
<td>2265 ± 582</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Singletons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (w)</td>
<td>38.7 ± 2.3</td>
<td>38.7 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Birth &lt; 37w (%)</td>
<td>11</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Birth &lt; 32w (%)</td>
<td>0.6</td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Twins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (w)</td>
<td>35.3 ± 1.6</td>
<td>35.0 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Birth &lt; 37w (%)</td>
<td>0.6</td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Birth &lt; 32w (%)</td>
<td>9</td>
<td>14</td>
<td>NS</td>
</tr>
</tbody>
</table>

Desmyttere 2012
<table>
<thead>
<tr>
<th></th>
<th>PGD</th>
<th>ICSI</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Singletons</strong></td>
<td>N=670</td>
<td>N=1059</td>
<td></td>
</tr>
<tr>
<td>N° children with major malformation # (%)</td>
<td>14 (2.1%)</td>
<td>25 (2.4%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Twins</strong></td>
<td>N=308</td>
<td>N=433</td>
<td></td>
</tr>
<tr>
<td>N° children with major malformation # (%)</td>
<td>7 (2.3%)</td>
<td>15 (3.5%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>N=995</td>
<td>N=1507</td>
<td></td>
</tr>
<tr>
<td>N° children with major malformation # (%)</td>
<td>23 (2.3%)</td>
<td>40 (2.7%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PGD</th>
<th>ICSI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Singletons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillborn</td>
<td>8</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Neonatal ≤ 7d</td>
<td>0</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>8/678 (1.2%)</td>
<td>21/1078 (1.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>Twins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillborns</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Neonatal ≤ 7d</td>
<td>8</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>26/326 (8.0%)</td>
<td>24/449 (5.3%)</td>
<td></td>
</tr>
</tbody>
</table>

*Desmyttere 2012*
Neonatal outcome after ART PGD (8)

- **UZ Brussel PGD cohort study** Desmyttere 2012
  - Comparable outcome for PGD and ICSI
  - **Strengths**
    - Matched comparison group
    - Proper clinical examination
    - Strict definition of a major congenital malformation

- **Literature: limited!**

<table>
<thead>
<tr>
<th></th>
<th># infants</th>
<th>Major malformations</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strom et al (2000)</td>
<td>109</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Tur-Kapsa et al (2005)</td>
<td>480</td>
<td>8</td>
<td>1.7</td>
</tr>
<tr>
<td>Harper et al (2012) PGD consortium I-X</td>
<td>4 021</td>
<td>84</td>
<td>2.0</td>
</tr>
<tr>
<td>Desmyttere et al (2012) UZ Brussel</td>
<td>995</td>
<td>23</td>
<td>2.3</td>
</tr>
</tbody>
</table>
● Introduction: IVF, ICSI and PGD
● Neonatal outcome after ART
● PGD: Medical follow-up in childhood
PGD follow-up studies

- Data collection focusing on medical and psychological outcome
  - Questionnaires
  - Clinical examination at 2 years of age

- 70 singletons born after PGD
- 70 singletons born after ICSI
- 70 singletons born after Spontaneous Conception (SC)
PGD: childhood Medical follow-up

- **UZ Brussel** Desmyttere 2009

- **Biometrics**
  - Weight SDS, height SDS: comparable findings
  - Lower BMI SDS in PGD offspring

- **Anthropometry**
  - Comparable findings: waist, arm circumference

- **Hospital admission and Surgical interventions**
  - Comparable between the groups
PGD: childhood Medical follow-up (2)

- **Literature: limited!**
  - **Growth** Banerjee 2008
    - Up to 4 years (average: 18 months)
    - in 49 PGD/PGS and 66 Spontaneously conceived children
  - Comparable findings between the groups

- **Upcoming: Body composition data** UZ Brussel
  - At 5-6 years
  - Preliminary data: 50 PGD and 50 ICSI singletons
● Introduction: IVF, ICSI and PGD
● Neonatal outcome after ART
● PGD: Medical follow-up in childhood
● PGD: Psychological follow-up in childhood
PGD: childhood
Psychological follow-up

- **UZ Brussel** Nekkebroeck 2008
  - Mental and psychomotor development
  - Socio-emotional & language development
    - No differences between the groups

- **Upcoming: data on cognitive and psychomotor development** UZ Brussel
  - At 5-6 years
  - In 50 PGD, 50 ICSI and 50 control singletons
PGD: childhood Psychological follow-up (2)

● Literature: limited!
  → Neurodevelopment up to 4 years in PGD/PGS offspring Banerjee 2008
    ● Lower score for locomotor development in PGD/PGS
  → Neurological, cognitive and behavioural development at 4 years in PGS offspring
    Schendelaar 2013
    ● 49 PGS children (31 singletons)
    ● 64 ART children (42 singletons)
    ● Comparable findings for singletons
PGD follow-up studies

- PGD couples
  - Mostly have no infertility problem
    ≠ IVF and ICSI couples
  - But need to undergo all ‘in-vitro procedures’ (hormonal stimulation, sperm injection, embryo culture, embryo transfer) applied in patients with infertility problems
    ~ IVF and ICSI
  - And require embryo biopsy
    ~ PGS

⇒ Particular setting
IVF and ICSI: Medical follow-up studies

Beyond childhood up to puberty

- Body composition
- Metabolic profile
- Blood pressure
IVF and ICSI
Body composition

→ Age 8-18 years (IVF)
  ● Peripheral body fat mass was significantly **higher** in IVF compared with SC
  ● Total body fat was **higher** compared with SC (not-statistically significant) Ceelen 2007

→ Age 14 years (ICSI)
  ● In *girls*: More peripheral, central and total adiposity
  ● In *boys*: More peripheral and total adiposity in boys with advanced pubertal stage Belva 2012
→ Age 7 years (IVF)  Miles 2007
  ● Higher IGF-I and IGF-II levels
  ● Comparable glucose and insulin
  ● more favorable lipid profile!

→ Age 4-18 years (IVF)  Ceelen 2008, Sakka 2010
  ● Higher fasting glucose levels (still in normal range)
  ● Comparable insulin levels
  ● Higher levels of triglycerides
  ● no difference in markers of insulin resistance and inflammation
IVF and ICSI
Blood pressure

- **IVF children**
  - Age 4-18 years
    - Higher BP compared with SC  Ceelen 2008, Sakka 2010

- **ICSI children**
  - Age 8 years
    - Higher BP compared with SC  Belva 2007
  - Age 14 years
    - No difference in BP compared with SC peers  Belva 2012
● Introduction: IVF, ICSI and PGD
● Neonatal outcome after ART
● PGD: Medical follow-up in childhood
● PGD: Psychological follow-up in childhood
● Concerns and Future perspectives
Concerns and Future perspectives

- Epigenetic modifications?
- Cancer risk?
Why might ART-children have epigenetic modifications?

- Direct: changing of epigenetic patterns due to insults from the technique itself:
  - ovarian stimulation
  - freezing and thawing of gametes and embryos
  - ex-vivo manipulation and maturation of gametes and embryos

- Indirect: allowance of propagation of abnormal epigenetic patterns associated with underlying infertility

Potential impact on:
- embryo development
- child/adult health
- transgenerational phenotypes

Figure 4 Assisted reproduction treatment and potential alterations of the phenotypical fetal/adult programme.
Concerns Epigenetics (2)

● Why might ART-children have epigenetic modifications?

→ Subfertility background Doornbos 2007, Kobayashi 2009
  ● Imprinting errors in sperm from infertile men
  ● IVF-women have higher methylation indices

→ Hormonal stimulation Sato 2007
  ● Δ DNA methylation in oocytes from mice & humans

  ● Animals: Large Offspring Syndrome & Alterations in methylation and expression levels of genes involved in organogenesis
Concerns
Epigenetics

- Current data suggest a possible association between ART and imprinting disorders
  → Beckwith-Wiedeman Syndrome
  → Angelman Syndrome


Risk remains low!

- Low-level epigenetic abnormalities among ‘normal’ IVF/ICSI offspring?
  → Yes Katari 2009, Turan 2009, Gomes 2009, Katagiri 2010
Evidence for epigenetic alterations in normal ART offspring? YES

<table>
<thead>
<tr>
<th>Author</th>
<th>N° samples</th>
<th>Tissue</th>
<th>Gene(s)</th>
<th>Results in ART vs control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katari 2009</td>
<td>10 IVF 10 SC</td>
<td>Cord blood Placenta</td>
<td>&gt;700</td>
<td>Δ methylation~Δgene expression several of the genes with Δ expression: implicated in chronic metabolic disorders</td>
</tr>
<tr>
<td>Turan 2009</td>
<td>98 IVF 160 SC</td>
<td>Cord blood Placenta</td>
<td>IGF2/H19 IGF2R</td>
<td>Δ methylation and ↑ variation no correlation with low birth weight</td>
</tr>
<tr>
<td>Gomes 2009</td>
<td>18 ART 30 SC 3 BWS</td>
<td>Cord blood Placenta Periph blood</td>
<td>KvDMRI</td>
<td>Δ methylation in 3/18 ART, in 0/30 SC, in 3/3 BWS</td>
</tr>
<tr>
<td>Katagiri 2010</td>
<td>65 ART 924 SC</td>
<td>Placenta</td>
<td>IGF2 H19</td>
<td>No difference between groups Low birth weight babies: ↓H19 expression</td>
</tr>
</tbody>
</table>
Evidence for epigenetic alterations in normal ART offspring? NO

<table>
<thead>
<tr>
<th>Author</th>
<th>Nº samples</th>
<th>Tissue</th>
<th>Gene(s)</th>
<th>Results in ART vs control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanber 2009</td>
<td>19 SGA ICSI 29 AGA SC</td>
<td>Buccal smear</td>
<td>10, IGF2 H19</td>
<td>1 ICSI child: Δ methylation in 2 genes Imprinting defects are rare in SGA ICSI</td>
</tr>
<tr>
<td>Tierling 2010</td>
<td>112 ART 73 SC</td>
<td>Cord blood Placenta</td>
<td>10, IGF2 H19</td>
<td>No difference</td>
</tr>
<tr>
<td>Cheng 2011</td>
<td>60 ART 60 SC</td>
<td>Cord blood Periph blood</td>
<td>&gt;70 imprinted genes</td>
<td>Global imprinting profile is similar except for 3 differentially expressed imprinted genes</td>
</tr>
<tr>
<td>Oliver 2012</td>
<td>66 ART 69 SC</td>
<td>Periph blood</td>
<td>IGF2 H19</td>
<td>No difference</td>
</tr>
</tbody>
</table>
Concerns  
Cancer risk

- **Meta-analysis including 25 studies** Hargreave 2013
  - Children born after fertility treatment were at risk for all cancers (RR 1.33); leukemia (RR 1.65)
    - Due to factors related to subfertility?
    - Due to procedure itself?

- **Study from UK** Williams 2013
  - No increased risk of cancer among children < 15y born after ART (108 cancers identified, 109.7 expected)

- Long term follow-up of large cohorts is warranted!
Future perspectives

- Public health obligation to assess the health implications of ART conception
  - Hypertension and obesity
  - Risk factors for cardiovascular disease

- Epigenetics and ART
  - Aberrant methylation patterns in ART offspring?
  - What is the consequence of subtle alterations in gene expression on the phenotype of ART children?

- Attention for embryo culture properties including duration of embryo culture time
Introduction: IVF, ICSI and PGD

Neonatal outcome after ART

PGD: Medical follow-up in childhood

PGD: Psychological follow-up in childhood

Concerns and Future perspectives

Conclusion
Conclusion

- **Up to date: in PGD: overall reassuring data**
  - growth/overall health up to 2 years
  - Psychological well-being

- **Special attention to long term health outcome**
  - Cardiometabolic outcome
    - Body composition: altered?
    - Blood pressure: higher?
    - Metabolism: unfavorable lipid and glucose profile?

- **Epigenetics and Cancer**

- **Continued follow-up is highly warranted!**
Thanks to

- **Follow-up team**
  - Maryse Bonduelle
  - Inge Liebaers
  - Felix De Schrijver
  - Sonja Desmyttere
  - Julie Nekkebroeck
  - Christiane Winter
  - Andrea Buysse
  - Leen Ausloos
  - Ellen Van Moer

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  - FWO Vlaanderen
  - WF Willy Gepts
  - OZR VUB
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  - Schering-Plough
  - Ferring International Center