Recent Advances in Assisted Reproductive Technologies

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IVF: The Success of Technology

3 million IVF children
1 / 100 - IVF baby
~ 500,000 IVF cycles/year

IVF Success Rates:
Fresh Cycle – Live Birth/Transfer

UCSF 2005 Live Birth/Transfer
UCSF 2006 Ongoing Pregnancy/Transfer
US National Live Birth/Transfer
Overview

IVF

Cryopreservation

Fertility Preservation

ICSI

eSET

IVM

PGD

Intracytoplasmic Sperm Injection (ICSI)

ICSI

IVF

eSET

IVM

PGD

Indication for ICSI

- Ejaculated spermatozoa
  - Oligozoospermia
  - Ashthenoospermia
  - Teratozoospermia
  - Antisperm antibodies
  - Autoconserved frozen sperm from ca patients
  - Ejaculatory disorders

- Epididymal spermatozoa
  - Failure of epididymal sperm recovery
  - Azoospermia caused by testicular failure (maturation arrest, germ cell aplasia)
  - Obstruction of both ejaculatory ducts

- Testicular spermatozoa

- Other
  - Fertilization failure from conventional IVF
  - Non-male infertility
  - PGD

Male Factor
Indication for ICSI

**Ejaculated spermatozoa**
- Oligozoospermia
- Asthenozoospermia
- Teratozoospermia
- Anti-sperm antibodies
- Autoconserved frozen sperm from ca patients
- Ejaculatory disorders

**Epididymal spermatozoa**
- Congenital bilateral absence of the vas deference
- Failed vasoepididymostomy
- Failed vaso-vasostomy
- Obstruction of both ejaculatory ducts

**Testicular spermatozoa**
- Failure of epididymal sperm recovery
- Azoospermia caused by testicular failure
  (maturation arrest, germ cell aplasia)

**Other**
- Fertilization failure from conventional IVF
- Non-male infertility
- PGD

Incidence of ICSI

<table>
<thead>
<tr>
<th></th>
<th>ESTRIDE 2006</th>
<th># of Cycles</th>
<th>% ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>69,000</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>46,800</td>
<td>54%</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>27,700</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>15,400</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>10,700</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>8,900</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>9,000</td>
<td>46%</td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>4,400</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>122,900</td>
<td>56%</td>
<td></td>
</tr>
</tbody>
</table>

ICSI Outcome

**Perinatal risks**
- Twins and singleton after IVF and ICSI have 2-fold increased risk for perinatal mortality, preterm delivery and low birth weight; 3-fold risk for very low birth weight;
- Vanishing twins could be one of the causes
- Infertility itself can play a causative role
- IVF = ICSI


**Congenital Abnormalities**
- A higher rate of inherited chromosomal anomalies;
- Mainly due to paternal structural chromosome anomalies;
- Malformation IVF = ICSI; higher than general population;

Belva et al. 2007

<table>
<thead>
<tr>
<th></th>
<th>ICSI</th>
<th>Matched control</th>
</tr>
</thead>
<tbody>
<tr>
<td># Children</td>
<td>150</td>
<td>147</td>
</tr>
<tr>
<td>By age 8, major congenital malformations</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

ESHRE 2007
ICSI Outcome

Long-term follow-up

- 5-year-olds: Growth, cognitive and emotional development, psychological well being of the families - comparable;
- 8-year-olds: pubertal staging, neurological status, need for more remedial therapy, surgery or hospitalization – comparable;
- ICSI children need special attention – puberty and future fertility

Oocyte Cryopreservation

Challenges of Oocyte Cryopreservation

- Spindle and microtubule damage due to low temperature
- Microfilaments may be absent following freezing
- Inability of mitochondria to form normal aggregates
- High polyploidy rate
- Parthenogenesis
- Altered gene/protein expression

Why Oocyte Cryopreservation

- Problem with embryo freezing – Religious/ethical/legal
- Fertility preservation – in single women (cancer or non-cancer)
- Oocyte donation – simplify the procedure
- Stem cell research

Gook et al Hum Reprod 2007
Methods of Oocyte Cryopreservation

<table>
<thead>
<tr>
<th>Slow freezing</th>
<th>Vitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low concentration</td>
<td>High concentration</td>
</tr>
<tr>
<td>Seeding</td>
<td>No intracellular ice</td>
</tr>
<tr>
<td>Cooling - 0.3°C/min</td>
<td>Glass-like solidification (~700,000°C/min)</td>
</tr>
<tr>
<td>Easy to learn</td>
<td>Difficult to do</td>
</tr>
<tr>
<td>Damage – intracellular ice</td>
<td>Damage – chemical toxicity</td>
</tr>
</tbody>
</table>

Success of Oocyte Cryopreservation

<table>
<thead>
<tr>
<th>All reports up to 2006</th>
<th>Slow Freezing</th>
<th>Vitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oktay et al. Fertil Steril 2006</td>
<td>33.7</td>
<td>32.3</td>
</tr>
<tr>
<td>Patient Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilization Rate</td>
<td>64.9% (2,478/3,818)</td>
<td>74.2% (637/859)</td>
</tr>
<tr>
<td>Clinical Preg / Transfer</td>
<td>20.6% (153/742)</td>
<td>45.5% (61/134)</td>
</tr>
<tr>
<td>Live Birth / Transfer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>10.1% (185/1,828)</td>
<td>17.1% (81/473)</td>
</tr>
</tbody>
</table>

Efficiency of Oocyte Cryopreservation

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Patient Age</td>
<td>31.1</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Clinical Pregnancy per Transfer</td>
<td>37.8 (28/74)</td>
<td>54.5</td>
<td>.004</td>
</tr>
<tr>
<td></td>
<td>(2,317/4,250)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live Birth per Transfer</td>
<td>32.4 (24/74)</td>
<td>47.8</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>(2,031/4,250)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Improve Embryo Cryopreservation

- ICSI
- IVF
- eSET
- IVM
- PGD

Cryopreservation
**Improve Embryo Cryopreservation**

<table>
<thead>
<tr>
<th></th>
<th>Slow Freezing</th>
<th>Vitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived</td>
<td>76% (32/42)</td>
<td>87% (33/38)</td>
</tr>
<tr>
<td>D2 cleavage</td>
<td>67% (28/42)</td>
<td>76% (29/38)</td>
</tr>
<tr>
<td>Survived</td>
<td>72% (38/53)</td>
<td>85% (39/46)</td>
</tr>
<tr>
<td>100% intact</td>
<td>30% (16/53)</td>
<td>48% (22/46)</td>
</tr>
<tr>
<td>Survived</td>
<td>64% (23/36)</td>
<td>93% (38/41)</td>
</tr>
<tr>
<td>100% intact</td>
<td>22% (8/36)</td>
<td>71% (29/41)</td>
</tr>
</tbody>
</table>

**Improve Embryo Cryopreservation - Vitrification**

- Zygotes 5881
- Day3 Embryos 236
- Blastocysts 6328

- Patient age 34.1±4.5
- Survival Rate 100%
- Survival 85%
- Survival Rate 90%
- Cleave Rate 93%
- Clinical Preg 44%
- Clinical Preg 53%
- Blast Formation 52%
- Implantation 20%
- Live Birth 45%

**Ovarian Tissue Freezing and Transplantation**

- Cryopreservation
- ICSI
- IVF
- Fertility Preservation
- eSET
- IVM

**Why Ovarian Tissue Freezing and Transplantation**

- Over 80,000 cancer patients are potentially exposed to sterilizing therapy
- High breast cancer survival rates
- Fertility preservation in children – 6,500 new cases
- Chemotherapy for non-cancer diseases
- Oophorectomy for benign ovarian conditions
**Ovarian Tissue Freezing and Transplantation**

- Slow freeze strips of cortex or whole ovary
- Screening tissue before freezing and after thawing (light microscopy and molecular markers)
- 5% primordial follicles lost during freezing and thawing
- 65% primordial follicles lost during re-vascularization
- Orthotopic transplant (pelvic) – resumption of ovarian function – spontaneous conception
- Heterotopic transplant (subcutaneous) – IVF – Embryo transfer

**Success of Ovarian Tissue Freezing and Transplantation**

<table>
<thead>
<tr>
<th>Orthotopic</th>
<th>Heterotopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>2005</td>
</tr>
<tr>
<td>Donnez et al, Lancet</td>
<td>Oktay et al, Hum Reprod</td>
</tr>
</tbody>
</table>

**In Vitro Maturation (IVM)**

Completion of first meiotic division from GV to MII

- **Nuclear maturation**
  - **Cytoplasmic maturation:** Preparation for fertilization and embryo development
    - RNA molecules
    - Proteins
    - Imprinted genes
- **Membrane maturation:** Receptors and function
Why In Vitro Maturation (IVM)

Eliminating gonadotropin administration

- Eliminates OHSS in PCOS patients
- Eliminates the potential of long-term side effects of gonadotropin stimulation
- Results in lower cost and convenience

Culture Conditions: IVM Media

- Growth factors
- Energy substrates and nutrients
- Hyaluronic Acid
- Steroids
- Antioxidants

IVM Clinical Experience

PCOS and Natural Cycle Patients

- 746 PCOS patient cycles; 561 natural cycles
- Maturation rates 55% - 85%
- Fertilization rates 70% - 90%
- Pregnancy rate per transfer 25% - 35%
- Implantation Rates 10 – 15%

Obstetric Outcomes

- 379 deliveries
- Early, preliminary results reassuring

Jurema et al, 2006, Ferti Steril
Preimplantation Genetic Diagnosis (PGD)

- IVF
- ICSI
- eSET
- IVM
- PGD

Indications for PGD

- Autosomal dominant
  - Huntington disease
  - Marfan syndrome
  - Myotonic dystrophy
  - Neurolipomatosis
  - ...

- Autosomal recessive
  - Sickle cell
  - Cystic fibrosis
  - Spinal muscular atrophy
  - Tay-Sachs disease
  - ...

- Sex-linked recessive
  - Duchenne's muscular dystrophy
  - Hemophilia
  - ...

- Sex-linked dominant
  - Fragile X syndrome
  - Alport syndrome
  - ...

- Structural chromosomal aberrations
  - Reciprocal translocation
  - Robertsonian translocation
  - Inversion
  - Deletion

Why Preimplantation Genetic Screen (PGS)

Aneuploidy screening (AS)

- Advanced maternal age
- History of aneuploidy pregnancy
- Recurrent miscarriage
- Repetitive IVF failure
Preimplantation Genetic Screen (PGS) RCT

<table>
<thead>
<tr>
<th>Mastenbroek et al. NEJM 2007</th>
<th>PGS</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No.</td>
<td>206</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>Clinical Pregnancy</td>
<td>30%</td>
<td>44%</td>
<td>.003</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>18%</td>
<td>18%</td>
<td>.97</td>
</tr>
<tr>
<td>Ongoing Pregnancy</td>
<td>25%</td>
<td>37%</td>
<td>.01</td>
</tr>
<tr>
<td>Live Birth</td>
<td>24%</td>
<td>35%</td>
<td>.01</td>
</tr>
</tbody>
</table>

PGD for Cancer Predisposition Syndromes

- Mutation in particular genes cause adult-onset cancer (APC, NF2, BRCA1, etc.)
- Prenatal diagnosis and selective pregnancy termination is emotionally difficult
- PGD ensures the pregnancy free of mutation
- PGD procedure is invasive, needs IVF and limited success
- Controversial – where to draw the line?

Elective Single Embryo Transfer (eSET)

- ICSI
- Cryopreservation
- Fertility Preservation
- IVF
- eSET

IVF Success Rates: Implantation Rates

- US National 2005 Implantation Rates
- UCSF 2005 Implantation Rates
Why Elective Single Embryo Transfer (eSET)

<table>
<thead>
<tr>
<th>2005 CDC Report</th>
<th>Average # Transferred</th>
<th>% Multiples /Live Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>2.4</td>
<td>35.6%</td>
</tr>
<tr>
<td>35-37</td>
<td>2.6</td>
<td>30.9%</td>
</tr>
<tr>
<td>38-40</td>
<td>3.0</td>
<td>25.1%</td>
</tr>
<tr>
<td>41-42</td>
<td>3.2</td>
<td>16.8%</td>
</tr>
</tbody>
</table>

RCT Studies for Elective Single Embryo Transfer (eSET)

<table>
<thead>
<tr>
<th>RCT Studies</th>
<th>eSET</th>
<th>DET</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Selected Patient Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy Rate</td>
<td>30% (154/516)</td>
<td>46% (232/506)</td>
</tr>
<tr>
<td>Twin Rate</td>
<td>2% (3/154)</td>
<td>35% (232/506)</td>
</tr>
<tr>
<td>In Unselected Patient Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy Rate</td>
<td>21% (33/154)</td>
<td>40% (62/154)</td>
</tr>
<tr>
<td>Twin Rate</td>
<td>0% (0/33)</td>
<td>21% (13/62)</td>
</tr>
</tbody>
</table>

UCSF Experience for Elective Single Embryo Transfer (eSET)

<table>
<thead>
<tr>
<th>UCSF Experience</th>
<th>Day3 eSET</th>
<th>Day5 eSET</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Selected Patient Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Age</td>
<td>29.8 ± 5.5</td>
<td>32.2 ± 4.4</td>
</tr>
<tr>
<td>Pregnancy per ET</td>
<td>73% (16/22)</td>
<td>63% (10/16)</td>
</tr>
<tr>
<td>Ongoing per ET</td>
<td>55% (12/22)</td>
<td>50% (8/16)</td>
</tr>
<tr>
<td>Twining Rate</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Summary

- IVF
- Cryopreservation
- ICSI
- eSET
- IVM
- PGD

Fertility Preservation

Future Advancements in ART

- Minimal stimulation and natural cycle
- IVM (*in vitro* maturation) and IVG (*in vitro* growth)
- Molecular Methods to select ideal oocyte and embryo
- Non-invasive quality assessment of oocyte and embryo
- Dynamic microfluidic embryo culture
- Improve Fertility preservation

Thank you