Comparison of the methods of Nuclear Transfer (GVT, SNT, PNT)

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I have nothing to disclose…
Before fertilization:

- Germinal vesicle transfer (GVT)
- Spindle chromosome complex transfer (MST)
- PB1 transfer (PB1GT)

After fertilization:

- PB2 transfer (PB2GT)
- Pronuclear transfer (PNT)

NT methods:
One “bad” oocyte may result in four “good” embryos!

“It's a kind of magic!”
Set-up

Medium

Pre-IVM and IVM secret medium
HVJ-E Cell Fusion Kit
Cytochalasin b
global® total® w/ HEPES
global® total
PVP
Mineral Oil

Hardware

Micromanipulators
Infrared laser
Polarization microscopy
Time-lapse incubators
Skillful embryologist
GV oocyte

Germinal vesicle (GV)

Donor GV

Enucleation

GVT reconstitution

In vitro maturation

Reconstituted GV oocyte

MII oocyte

Fertilization/activation

Zygote

Two cell embryo

Blastocyst
**GVT**

**Advantages**
- Before spindle formation
- Nuclear envelope
- Extremely small cytoplasm drop
- Pre-IVM gives as much time as needed

**Disadvantages**
- Proper stimulation
- Not all cells are competent
- Cumulus cells
- IVM needed
- Extremely careful manipulations
- Unknown efficiency

*Ab ovo*
Double NT

Donor fresh GV oocyte → GVT → Reconstituted GV oocyte

Germinal vesicle (GV) → In vitro maturation

Thawed GV oocyte

In vitro matured MII oocyte

Zygote

Blastocyst

PNT reconstitution

Thawed MII oocyte (same donor)
Double NT

1: -13, mos-22, XX
9: mos+8, mos+10, +15, XX
SNT

**Advantages**

- It works!
- Quite simple
- Easy way to get rid of excess cytoplasm
- Before first/second meiosis
- Condensed chromatin
- “Fertilize and forget”

**Disadvantages**

- Polarization microscopy needed
- Spindle fragility/instability
- Proper timings!
- Abnormal cells shapes are PAIN
- A lot of cells!
- Low efficiency?
Abnormal pronuclear formation and spindle morphology in human ST zygotes

Tachibana et al., 2013
SNT
MI SNT

For the very first time patient has at least one embryo!
**Pronuclear transfer**

1. **Zygote**
2. **Pronuclei**
3. **Donor zygote**
4. **Enucleation**
5. **PNT (Pronuclear transfer)**
6. **Reconstitution**
7. **Reconstituted zygote**
8. **Two cell embryo**
9. **Blastocyst**
<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• It works!</td>
<td>• Proper timings</td>
</tr>
<tr>
<td>• Only fertilized cells</td>
<td>• After meiosis</td>
</tr>
<tr>
<td>• Nuclear envelope</td>
<td>• DNA replication</td>
</tr>
<tr>
<td>• Take any PN whatever you need</td>
<td>• Centrosome</td>
</tr>
<tr>
<td>• 1PN\3PN may be used</td>
<td>• PNs are much more fragile than GV</td>
</tr>
<tr>
<td></td>
<td>• Highly recommended to get rid of PB1</td>
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None of the variants of the biological mother and all mitochondrial variants of the egg cell donor were detected in the fetus. Nuclear DNA analysis is consistent with the biological parents and the combined results with a successful mitochondrial donation.

Interpretation
The analysis of the mitochondrial genome revealed that all mitochondrial variants detected in the fetus are shared with the mitochondrial donor and not with its biological mother. STR marker analysis of the fetal DNA showed that the fetal nuclear DNA is inherited from the biological mother and father. Genetic counselling is recommended.
PNT
Functional Human Oocytes Generated by Transfer of Polar Body Genomes

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Polar Body Genome Transfer for Preventing the Transmission of Inherited Mitochondrial Diseases

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http://dx.doi.org/10.10385.cell.2014.04.042

Review of the safety and efficacy of polar body transfer to avoid mitochondrial disease

Addendum to ‘Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2014 update’

Report provided to the Human Fertilisation and Embryology Authority (HFEA), October 2014

Review panel chair: Dr Andy Greenfield, Medical Research Council Harwell and HFEA member
PB1T

Donor oocyte

Isolation of PB1

Enucleation of recipient oocyte

PB1T

Isolation of spindle

SNT

Blastocyst
PB1GT

**Advantages**

- Unlike spindle, PB has its solid membrane
- Hard to damage
- Easy to perform
- Low number of mitochondria
- Additional set of patient’s chromosomes

**Disadvantages**

- SNT needed (+ almost all its disadvantages)
- Only intact PBs are suitable for the procedure
- Thus no vitrification recommended
- High risk of apoptosis
Isolation of PB2

Partial enucleation of recipient zygote

PB2T

Isolation of pronuclei

Enucleation of recipient zygote

Donor zygote

PNT

Blastocyst
PB2GT

Advantages

• Low number of mitochondria
• Additional set of patient’s chromosomes

Disadvantages

• Strict timings!
• No direct indications
• Centrosome
• Only intact PBs are suitable for the procedure
• Thus no vitrification recommended
• High risk of apoptosis
Risk of apoptosis
• Human beings is not so unique
• All types of NTs are suitable for human
• There is no perfect\absolute technique!
• **Strict indications are required!**
• Time-lapse along with polarization microscopy are indispensable tools
• We know very little about Nature, but it seems that she wants to tell us something important…
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