Ovarian rejuvenation by Autologous stem cell ovarian transplant (ASCOT).

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Disclosure information: Nothing to declare
Depletion of the ovarian reserve: follicles and AMH

Impairment of ovarian function

- Ovarian Aging

Therapeutic alternatives

- Acute premature ovarian insufficiency (POI) due to chemotherapy (ChT)

Visser et al., *Nature Reviews Endocrinology* 2012.
In vitro activation (IVA) of dormant follicles

- Follicular rescue and live birth after IVA and ovarian fragmentation in POI patients.

- Residual follicles are able to grow and be ovulated in an adequate ovarian environment.

Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment

Kazuhiro Kawamura, Yuan Cheng, Nao Suzuki, Masashi Deguchi, Yorino Sato, Seido Takae, Chi-hong Ho, Nanami Kawamura, Midori Tamura, Shu Hashimoto, Yodo Sugishita, Yoshiharu Morimoto, Yoshihiko Hosoi, Nobuhiro Yoshioka, Bunpei Ishizuka, and Aaron J. Hsueh

PNAS
Follicular rescue of remaining follicles by:

- Ovarian fragmentation for follicle activation (OFFA).
- Autologous Stem cell ovarian transplantation (ASCOT)
OUR EXPERIENCE

DO IT AGAIN

Repeat

RCT
✓ Avoid IVA drugs.

✓ Minimize cryodamage/ischemia.

✓ Look for the best vascularized site.

✓ Allow for natural pregnancy
Ovarian fragmentation for follicular activation (OFFA)

PI: César Díaz-García

**ClinicalTrials.gov**
A service of the U.S. National Institutes of Health

Now Available: Final Rule for FDAAA 801 and NIH Policy on Clinical Trial Reporting

**Follicular Activation in Poor Responders (FAPPOR)**

This study is currently recruiting participants. (see Contacts and Locations)

Verified September 2016 by Instituto de Investigacion Sanitaria La Fe

Sponsor:
Instituto de Investigacion Sanitaria La Fe

Information provided by (Responsible Party):
Instituto de Investigacion Sanitaria La Fe

ClinicalTrials.gov identifier:
NCT02354963

First received: January 22, 2015
Last updated: September 21, 2016
Last verified: September 2016

**IVA ➔ OFFA**

- Drug free
- Unique surgery
- No tissue cryopreservation
- On site transplant
Innovation

<table>
<thead>
<tr>
<th>OFFA in POI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PATIENTS</td>
<td>14</td>
</tr>
<tr>
<td>OOCYTES</td>
<td>21</td>
</tr>
<tr>
<td>PREGNANCIES</td>
<td>5</td>
</tr>
<tr>
<td>LIVE BIRTHS</td>
<td>3</td>
</tr>
</tbody>
</table>
Follicular rescue of remaining follicles by:

- Ovarian fragmentation for follicle activation (OFFA).
- Autologous Stem cell ovarian transplantation (ASCOT).


Sanders JE, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood.* 1996.

BMDSC represent a heterogeneous group of mononuclear cells, including several hematopoietic and stromal stem/progenitor cells.

Objectives and End points questions

1. Do Bone Marrow Derived Stem Cells (BMDSC) arrive into the ovarian tissue?

2. To assess if BMDSC could induce ovarian rescue in mice ovaries mimicking Primary Ovarian Insufficiency (POI) and Diminished Ovarian Reserve (DOR) conditions.

3. To analyze BMDSC in human cortex from Poor Responder (PR) patients xenografted into SCID mice.

4. To optimize ovarian reserve in PR patients by autologous stem cell ovarian transplant (ASCOT).
Experimental design 1

**Cell labeling with Molday Ion Rhodamine B (MIRB)**

- MIRB nanoparticles
- MIRB labeled-human cells

**POI model**
- 18 NOD/SCID mice
- Standard regimen: 120 mg/kg Cy, 12 mg/kg Bu

**Control Group**
- 100 μl of saline

**PBMNC Group**
- 1x10^6 human PBMNC

**BMDSC Group**
- 1x10^6 human BMDSC

**PR model**
- 18 NOD/SCID mice
- Reduced regimen: 12 mg/kg Cy, 1.2 mg/kg Bu

**Ovarian hyperstimulation and mating**

- Estrous cycle, ovaries, oocytes/embryos

**Short-term effects**

**Long-term effects**

Consecutive matings for 3 months

Cy: Cyclophosphamide  
Bu: Busulphan

**Experimental treatment**

- Standard regimen: 120 mg/kg Cy, 12 mg/kg Bu
- Reduced regimen: 12 mg/kg Cy, 1.2 mg/kg Bu

**Cell therapy**

- BMDSC treatment

**Control and treated groups**

- Control Group (saline)
- PBMNC Group (human PBMNC)
- BMDSC Group (human BMDSC)
Estrous cycle

Innovation

BMDSCs infusion increased the number of preovulatory follicles, MII-ovulated oocytes and embryos.

Restored long term fertility
Ovarian stroma regeneration

**BMDSC infusion:**

- Increase ovarian vascularization
- Improve cell proliferation
- Reduce apoptosis in stroma and follicles even in the high chemotherapy dose.

Do BMDSC arrive into the human ovaries?

Do these cells stimulate follicular growth in human ovarian tissue?

Is whole BMDSC active or only isolated CD133+ cells?
Innovation

**Experimental design 2: human ovarian tissue**

### Xenograft

- Human ovarian cortex (OC) from Poor Responder (PR) women
- Ovariectomized NOD-SCID mice

### Cell infusion

- BMDSC
  - 100 μl of saline
  - 1x10⁶ human BMDSC
- CD133+
  - 3x10⁵ human CD133

### Tissue sampling and analysis

- Cell engraftment
- Follicular density and growth
- Vascularization and proliferation of ovarian stroma
- Estradiol secretion
- Cell tracking
- Other organs

### Timeline

- D-7
- D0
- D+1 / D+7 / D+14
**BMDSC: human ovarian tissue**

- Injected cells localized near blood vessels and close to follicles, in contact with GC.

- Secondary follicles were only observed in OC grafts receiving cell injection.

**Stem cell infusion promoted growth of human follicles**

*Herraiz et al. Fertil Steril 2018;109:908–18*
BMDSC: human ovarian tissue

- Improved ovarian vascularization
- Increased cell proliferation
- Promoted estradiol secretion

✓ Human BMDSCs rescued the already existing follicles to growth and to produce healthy offspring.

✓ Follicular rescue could be promoted by the regeneration of the ovarian niche as suggested by the increased vascularization and proliferation, and suppressed apoptosis.

BMDSC-based therapies could be a suitable alternative to increase the reproductive potential of patients with impaired ovarian function.
Provided that BMDSC transfer works in an animal model of human ovarian grafting:

✓ Do we increase follicular recruitment in PR patients?
✓ If so, how does it work?
✓ Do we improve oocyte quantity and quality in PR patients?
Pilot study with 20 PR patients based on the ESHRE criteria.

Patients are **considered as their own control** as only one ovary received intervention.

**Autologous Stem Cell Ovarian Transplantation (ASCOT).**

**STUDY GROUP**

- Maternal age ≤40 years.
- Two episodes of PR after COS (≤3 oocytes obtained).
- AFC> 2 follicles with at least 1 follicle in the ovary that will be perfused.
- AMH > 0,5 - ≤3pmol/L.
- Serum FSH levels ≤20IU/l.
- Regular menses.

Clinical Trial Nº: NCT02240342
1. **Mobilization** of BM derived stem cells by a 5-day treatment with Granulocyte colony stimulating factor (G-CSF, 10 ug/kg/day).

2. **Isolation** of BM derived stem cells from peripheral blood by **apheresis**.

3. **Infusion** of stem cells (volume: 40.4±12.3 ml) into the ovarian artery by **catheterism** (contralateral ovary served as control).
### PRIMARY OBJECTIVE

**Patient follow-up (6 months)**

**Serum AMH**
- Every 2 days during 15 days.
- Weekly up to 2 months.
- Monthly up to 6 months.

**Antral follicle count (AFC)**
- Weekly up to 2 months.
- Monthly up to 6 months.

### SECONDARY OBJECTIVES

**IVF**

- PGT-A
- Pick-up & ICSI

**ASCOT**

Previously reported for 12 PR (ESHRE, SRI 2016-2017)
High variability among patients AMH response:

Total AFC significantly increased after 8 to 22 days (on day 15 p=0.004).

A positive response was observed in 81% of patients:

- Increase in AFC ≥3 of
- Increase in AMH (± SD) in 2 consecutive determinations
- High FGF-2 and THROMBOSPONDIN-1 (THSP-1) values in aphae and serum AMH.

No difference was detected between the control and infused ovary.
In these firsts attempts:

- 15 PR completed the IVF cycles, 2 were excluded and 3 are still ongoing.
- COS protocol after ASCOT was the same than the used in the previous cycle.
Results: Cycle comparison and retrieved oocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Previous (n=24)</th>
<th>Post-ASCOT (All, n=28)</th>
<th>Post-ASCOT optimum (n=4)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (yr)</td>
<td>36 [34-37]</td>
<td>38 [36-38]</td>
<td>34 [33-37]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Partner age (yr)</td>
<td>36 [35-40]</td>
<td>37 [36-44]</td>
<td>35 [34-38]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Years of infertility</td>
<td>3[2-4]</td>
<td>4 [3-5]</td>
<td>3[2-3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AMH (pM)</td>
<td>1.0 [0.9-3.0]</td>
<td>2.8 [0.7-3.7]</td>
<td>2.9[2.3-4.1]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AFC total</td>
<td>3[0-5]</td>
<td>5[4-6]</td>
<td>8[7-9]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- Control ovary</td>
<td>2[2-3]</td>
<td>2[1-3]</td>
<td>NS</td>
<td>0.02</td>
</tr>
<tr>
<td>- Infused ovary</td>
<td>0[0-1]</td>
<td>0[0-1]</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Punctured follicles</td>
<td>3[1-6]</td>
<td>3[1-4]</td>
<td>3[2-4]</td>
<td>0.035</td>
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<tr>
<td>- Control ovary</td>
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<td>2[2-2]</td>
<td></td>
</tr>
<tr>
<td>- Infused ovary</td>
<td>0[0-1]</td>
<td>1[0-2]</td>
<td>1[0-2]</td>
<td></td>
</tr>
<tr>
<td>MII oocytes</td>
<td>2[0-3]</td>
<td>2[1-3]</td>
<td>2.5[1-4]</td>
<td>NS</td>
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<tr>
<td>- Control ovary</td>
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<td>1[1-2]</td>
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</tr>
<tr>
<td>- Infused ovary</td>
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<td>0[0-1]</td>
<td>0.5[0-1]</td>
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<tr>
<td>- Control ovary</td>
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<td>0[0-1]</td>
<td>0.5[0-2]</td>
<td>NS</td>
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<tr>
<td>- Infused ovary</td>
<td>0[0-0]</td>
<td>0[0-1]</td>
<td>0.5[0-1]</td>
<td>NS</td>
</tr>
<tr>
<td>Cancellation</td>
<td>7/24(29.2%)</td>
<td>4/28 (16.6%)</td>
<td>0/4(0%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

- **Optimum COS** were considered when started 15 days after ASCOT or following an AFC increase.
- Values are shown as Median [Inter quartile range]
- Bonferroni adjustment was applied for multiple test correction

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Herraiz et al. Fertil Steril 2018;110(3):496-505
## Results: Individual outcomes

<table>
<thead>
<tr>
<th>PR</th>
<th>Age</th>
<th>Cycles</th>
<th>MII</th>
<th>MII iO</th>
<th>MII cO</th>
<th>Embryos (E)</th>
<th>Euploid E</th>
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<tbody>
<tr>
<td>1</td>
<td>38</td>
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<td>2</td>
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<tr>
<td>3</td>
<td>38</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>2</td>
<td>3</td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
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<td>0</td>
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<td>8</td>
<td>37</td>
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<td>6</td>
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<td>4</td>
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<td>35</td>
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<td>8</td>
<td>3</td>
<td>5</td>
<td>6</td>
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</tr>
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<td>38</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>38</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>33</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>3</td>
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<td>3</td>
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<tr>
<td>16</td>
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<tr>
<td>17</td>
<td>36</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>
OVERALL OUTCOMES

IVF outcomes (n=28 cycles/15PR)

- Oocyte pick-up: 85.7% Positive, 14.3% Negative
- MII: 82.1% Positive, 17.9% Negative
- Embryo: 67.8% Positive, 32.2% Negative

<table>
<thead>
<tr>
<th></th>
<th>PREVIOUS CYCLES</th>
<th>ASCOT CYCLES</th>
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</thead>
<tbody>
<tr>
<td>IVF Cycles</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>MII oocytes</td>
<td>35</td>
<td>51</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>69%</td>
<td>75%</td>
</tr>
<tr>
<td>Day 3 embryos</td>
<td>24</td>
<td>36 (31*)</td>
</tr>
<tr>
<td>Euploid embryos</td>
<td>--</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>Pregnancies</td>
<td>0</td>
<td>2 **</td>
</tr>
<tr>
<td>Live birth</td>
<td>0</td>
<td>1 **</td>
</tr>
</tbody>
</table>

* Blastocysts screened aCGH
** + 3 spontaneous pregnancies

Herraiz et al. Fertil Steril 2018;110(3):496-505
**TOTAL PREGNANCY RATE**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Years of infertility</th>
<th>IVF Cycle before ASCOT</th>
<th>IVF Pregnancies</th>
<th>Spontaneous pregnancies</th>
<th>Miscarriages</th>
<th>Live Births</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>38</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1 + 1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1 + 1</td>
</tr>
</tbody>
</table>

**ASCOT PREGNANCY RATE:**

- **Per patient:** 3/15 (20%)
- **TOTAL:** 5 pregnancies in 15 patients (33.3%)

Last PR included have its embryos vitrified because they are still undergoing IVF attempts.
✓ ASCOT enhances ovarian reserve in 81% PR patients
✓ ASCOT increases AFC 15 days after procedure. 1st COS attempt should be 2 weeks after ASCOT.
✓ IVF cycle after ASCOT resulted in more follicles developed
✓ The IVF cycle was not specially successful after ASCOT: 16% euploidy, 1 LB
✓ But, a total of 5 pregnancies and 3 live births were achieved after ASCOT, 2 women having 2 pregnancies each.

FUTURE DIRECTIONS:
Redefine inclusion criteria: Age<37, target population POI? Improve ASCOT by using less invasive approaches.
SUMMARY: Ovarian niche rejuvenation by bone marrow derived stem cells

Chemotherapy-induced POI and PR mouse models

Xenograft mouse model: Ovarian cortex (OC) from PR women

ASCOT (Autologous Stem Cell Ovarian Transplantation): Poor Responder patients

Fertility rescue and ovarian follicle growth promotion by bone marrow stem cell infusion

Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders
Acknowledgements

Prof. Antonio Pellicer
Dr. César Díaz-García
Prof. Carlos Simón

Anna Buigues

Dr. Mónica Romeu
Dr. Susana Martínez
Dr. Nuria Pellicer
Dr. Loida Pamplona
Dr. Inés Gómez
Dr. Pilar Solves
Dr. José Martínez
Dr. Lourdes Cordón
8th International IVIRMA Congress
Palma de Mallorca (Spain)
April, 4-6 2019

International IVI Awards

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• Basic Research Award
• Clinical Research Award

Journalistic Awards (3rd Edition)
• Reproductive Medicine
• Maternal-Fetal Health
Thank you