FEMALE FERTILITY PRESERVATION FOR MEDICAL REASONS

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Title of Presentation
Female fertility preservation for medical reasons

Disclosure of Interest: Nothing to Disclose
OUTLINE

- Epidemiology
- Indications
- Physiology
- Prevention
- Ovarian stimulation
  - Ovarian reserve
  - Ovarian response
  - Oocyte and embryo cryopreservation
- Ovarian cortex cryopreservation
- Duration
- Safety
- Algorithms
% Survival in Common Pediatric Cancers

Data* from Parker SL et al, Ca - Cancer J Clin, 46:5-25, 1996
*except retinoblastoma
Spectrum of late Effects: the cost of cure

Life-Threatening → Life-Altering

Life-Threatening: Cardiomyopathy, Pulmonary fibrosis, High grade second cancers

Life-Altering: Obesity, Immunodeficiency, Chronic hepatitis, Endocrinopathy, Asplenia

Infertility, Neurocognitive deficits, Seizure disorder, Low grade second cancers, Hearing/vision loss, Amputation, Chronic pain, Short stature

Adapted from Hudson M.
Impact of Treating Childhood Cancer:
years of life saved
(adapted from Cromer J)
1. Non-oncologic patients.

2. Oncologic patients.

3. Patients who wish to postpone their fertility for social reasons.
NON-MALIGNANT PATHOLOGIES WITH RISK OF POF

Bone-marrow transplantation
- Sickle cell anemia
- Thalassemia major
- Aplastic anemia
- Autoimmune diseases unresponsive to immunosuppressive therapy

Autoimmune diseases requiring chemotherapy
- Systemic lupus erythematosus
- Rheumatoid arthritis
- Behcet’s disease
- Wegener’s disease
- Multiple sclerosis

Ovarian pathologies
- Recurrent ovarian cysts
- Ovarian torsion

Endocrine or genetic diseases
- Turner syndrome
- Galactosemia
- Family history of premature ovarian failure

Sex reassignment surgery in transsexuals

Modified from P. JADOUL et al (2010)
Human Reproduction Update 16-6, 617-630
1. Non-oncologic patients.

2. Oncologic patients.

3. Patients who wish to postpone their fertility for social reasons.
<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Conditions</th>
</tr>
</thead>
</table>
| Low risk <20% | Acute lymphoblastic leukemia  
Wilm’s tumor  
Soft-tissue sarcoma: stage I  
Germ-cell tumors (with gonadal preservation and no radiotherapy)  
Retinoblastoma  
Brain tumor: surgery only, cranial irradiation <24 Gy |
| Medium risk | Acute myeloblastic leukemia  
Hepatoblastoma  
Osteosarcoma  
Ewing’s sarcoma stage II or III  
Neuroblastoma  
Non-Hodgkin lymphoma  
Hodgkin’s disease: alternating treatment  
Brain tumor: craniospinal radiotherapy, cranial irradiation >24 Gy |
| High risk >80% | Whole-body irradiation  
Localized pelvic radiotherapy  
Chemotherapy conditioning for bone-marrow transplantation  
Hodgkin’s disease: treatment with alkylating-drugs  
Soft-tissue sarcoma: stage IV  
Metastatic Ewing’s sarcoma |
THE HUMAN OVARY AND POTENTIAL TARGETS OF CHEMOTHERAPEUTIC AGENTS
Consulting young female cancer patients - Risk assessment

Ovarian reserve

Toxicity risk

Pelvic Rx.
Alkylating agents
platinum agents
Taxanes
Plant alkaloids
Anthracyclines
Anti metabolites

Assessment of an individual Patient sterilization risk

Meirow, Anderson, Wallace 2010
EFFECTS OF CHEMOTHERAPY

Correlation of age with baseline primordial follicle counts is depicted. Primordial follicle counts inversely correlated with age in both control (●) and chemotherapy (▲) groups.

Figure 2. Chemotherapy-induced decline in follicle reserve. Our quantitative histological analysis revealed that the primordial ovarian follicle density of chemotherapy-exposed females with a mean age of 26.7 years was similar to that of females of 36.5 years of age with no prior exposure. This suggests that chemotherapy exposure may advance "ovarian age" by nearly a decade. Adapted from Oktay O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer 2007;110:2222-2229, with permission.

Rodríguez Wallberg et al, 2012
uterine characteristics after irradiation in childhood and adolescence:

<table>
<thead>
<tr>
<th></th>
<th>Irradiation</th>
<th>Chemotherapy and control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine volume</td>
<td>6.8 (1.7-12.7)</td>
<td>17.3 (7.0-25.9)</td>
</tr>
<tr>
<td>Endometrium (mm)</td>
<td>0 (0-2.6)</td>
<td>5.4 (3.5-7.5)</td>
</tr>
</tbody>
</table>
Gonadal damage is age and dose dependent.
STRATEGIES FOR FEMALE FERTILITY PRESERVATION

- Patient’s age
- Basal ovarian reserve
- Type and stage of the oncologic pathology
- Proposed therapeutic plan:
  - Chemotherapy: type, dose and duration
  - Radiotherapy: field, dose and duration
  - Surgery
- Forseeable long term effets
- Possibility of delaying start of chemotherapy
- Possibility of receiving ovulatory agents
- Social situation
- Tumour biology and possibility of ovarian metastases
FERTILITY PRESERVATION FOR PATIENTS WITH CANCER: AMERICAN SOCIETY OF CLINICAL ONCOLOGY CLINICAL PRACTICE GUIDELINE UPDATE

Key Recommendations
- Discuss fertility preservation with all patients of reproductive age (and with parents or guardians of children and adolescents) if infertility is a potential risk of therapy
- Refer patients who express an interest in fertility preservation (and patients who are ambivalent) to reproductive specialists
- Address fertility preservation as early as possible, before treatment starts
- Document fertility preservation discussions in the medical record
- Answer basic questions about whether fertility preservation may have an impact on successful cancer treatment
- Refer patients to psychosocial providers if they experience distress about potential infertility
- Encourage patients to participate in registries and clinical studies

Adult Males
- Present sperm cryopreservation (sperm banking) as the only established fertility preservation method
- Do not recommend hormonal therapy in men; it is not successful in preserving fertility
- Inform patients that other methods (e.g., testicular tissue cryopreservation, which does not require sexual maturity, for the purpose of future reimplantation or grafting of human testicular tissue) are experimental
- Advise men of a potentially higher risk of genetic damage in sperm collected after initiation of chemotherapy

Adult Females
- Present both embryo and oocyte cryopreservation as established fertility preservation methods
- Discuss the option of ovarian transposition (oophoropexy) when pelvic radiation therapy is performed as cancer treatment
- Inform patients of conservative gynecologic surgery and radiation therapy options
- Inform patients that there is insufficient evidence regarding the effectiveness of ovarian suppression (gonadotropin-releasing hormone analogs) as a fertility preservation method, and these agents should not be relied on to preserve fertility
- Inform patients that other methods (e.g., ovarian tissue cryopreservation, which does not require sexual maturity, for the purpose of future transplantation) are still experimental

Children
- Use established methods of fertility preservation (sperm cryopreservation and oocyte cryopreservation) for postpubertal minor children, with patient assent, if appropriate, and parent or guardian consent
- Present information on additional methods that are available for children but are still investigational
- Refer for experimental protocols when available
PREVENTION OF GONADAL DAMAGE
STRATEGIES FOR FERTILITY PRESERVATION

Reducing gonadotoxicity

Pelvic RT

- Ovarian Transposition

ChemoT

- GnRH-Agonists
- Less toxic ChemoT
Objective:
To move the ovaries from the irradiation field before starting RT

Indication:
Tumours requiring pelvic irradiation

Technique:
Laparoscopic / laparotomic

Medial

Lateral

Important:
- Preserving ovarian vascularization
- Easy access for possible future OPU
- Cryopreserving ovarian fragments
PREMATURE OVARIAN FAILURE (POF) IN ONCOLOGIC PATIENTS UNDER GnRH-a OR ORAL CONTRACEPTIVES COTREATMENT WITH CHEMOTHERAPY

<table>
<thead>
<tr>
<th></th>
<th>Study groups</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH-a</td>
<td>25/225 (11.1%)</td>
<td>105/189 (55.5%)</td>
</tr>
<tr>
<td>Oral Contraceptives</td>
<td>14/106 (13.2%)</td>
<td>82/275 (29.8%)</td>
</tr>
</tbody>
</table>

BLUMENFELD and VON WOLF HUM. REPROD. UPDATE (2008)
Cryopreservation Strategies

1. EMBRYO CRYOPRESERVATION

2. OOCYTE CRYOPRESERVATION

3. OVARIAN TISSUE CRYOPRESERVATION
## EMBRYO FREEZING

### Results according to frozen time (years)

Embryo freezing programme **I.U.DEXEUS n=2547 transfers**

<table>
<thead>
<tr>
<th>Frozen Time</th>
<th>Pregnancy rate/transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>≤ 5 years</strong></td>
<td>835/2479 (33.68%)</td>
</tr>
<tr>
<td>5-7 years</td>
<td>16/44 36.4%</td>
</tr>
<tr>
<td>7-9 years</td>
<td>5/16 31.3%</td>
</tr>
<tr>
<td>≥9 years</td>
<td>5/8 62.5%</td>
</tr>
<tr>
<td><strong>&gt; 5 years</strong></td>
<td>26/68 (38.2%)</td>
</tr>
</tbody>
</table>

1 baby born after **14 years** of cryopreservation (emb-DON)
1 baby born after **10 years** of cryopreservation

*d.n.s*
OOCYTE CRYOPRESERVATION

**Requisites:**
- Postpubertal or even premenarche (Reichman et al 2012)
- No contraindication for ovarian stimulation
- Time

**Advantages:**
- No need for partner or for accepting donor semen

**Drawbacks:**
- > 10 oocytes cryopreserved

- Slow freezing: Pregnancy rate per cryopreserved oocyte: 2%
- Vitrification: Pregnancy rate per vitrified oocyte: 7%
Live birth using vitrified–warmed oocytes in invasive ovarian cancer: case report and literature review

Manuel Álvarez, Miquel Solé, Marta Devesa, Rafael Fábregas,Montserrat Boada, Rosa Tur, Buenaventura Coroleu, Anna Veiga, Pedro N. Barri

Service of Reproductive Medicine, Department of Obstetrics, Gynaecology and Reproduction, University Hospital Quirón,Deus, Barcelona, Spain. *Service of Oncological Gynaecology, Department of Obstetrics, Gynaecology and Reproduction, University Hospital Quirón Deus, Barcelona, Spain. †Center of Regenerative Medicine in Barcelona, Spain.

Dr Manuel Álvarez graduated from the Autonoma University of Madrid (Faculty of Medicine) in 1992. He became a specialist in gynaecology and obstetrics at the Institut Universitary Dexeus, Barcelona where he is developing his professional career. He is an active member of the Service of Reproductive Medicine as senior MD and he is mainly focused on monitoring, IVF purchase and embryo transfer. He also holds the post of Secretary of the Teaching Committee at University Hospital Quiron Deus, cooperating with programmes for improving the quality and efficiency of future specialists in obstetrics and gynaecology.

Abstract This article reports the live birth of a healthy newborn using vitrified–warmed oocytes in a young patient with invasive mucinous ovarian carcinoma (stage IIc). Diagnosis was performed after a laparoscopic left adnexectomy. She underwent two cycles of ovarian stimulation, and 14 oocytes were vitrified before fertility-sparing surgery with bilateral preservational omentectomy. One year later, a transfer of two embryos was performed after liquefaction of warmed oocytes. Eighteen days after the transfer, she underwent a laparotomy because of abdominal pain, vaginal bleeding and haemoptysis. A right caesarian section was performed at week 38 of gestation, resulting in the birth of a healthy boy weighing 2500 g. As far as we know, this is the first live birth reported through vitrified–warmed oocytes in a patient with invasive ovarian cancer. Although oocyte vitrification is an alternative to be considered for fertility preservation in highly selected cases of ovarian cancer, controversial issues are discussed.
Mature oocyte cryopreservation: a guideline

IT’S NO LONGER EXPERIMENTAL!!

There is good evidence that cryopreserved mature oocytes when vitrified/warmed oocytes are used as part of IVF, 
result in better outcomes in terms of live birth rates, a decrease in chromosomal abnormalities, birth defects, and 
developmental deficits. The evidence indicates that oocyte vitrification and warming should no longer be considered 
OVARIAN STIMULATION IN H-D CANCER PATIENTS

Dexeus Protocol

Menses
Kaplan-Meier analysis of recurrence-free survival analysis did not show a difference between in vitro fertilization (IVF; dotted line, n=29) and control (solid line, n=31) patients.

Oktay et al (2005)
J Clin Oncol 23:4347-4353
Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic

**Objective:** To evaluate the current available data regarding ovarian performance of patients diagnosed with malignant disease undergoing controlled ovarian hyperstimulation (COH) for fertility preservation, before radio/chemotherapy, compared with age-matched, healthy patients undergoing COH for in vitro fertilization/intracytoplasmic sperm injection (IVF-ICSI).

**Design:** Meta-analysis of the data available from a systematic review of the literature.

**Setting:** Academic centers of infertility and IVF.

**Patient(s):** Patients with malignant disease, before radio/chemotherapy, undergoing COH for fertility preservation within comparative studies with healthy, age-matched controls.

**Intervention(s):** None.

**Main Outcome Measure(s):** Peak estradiol levels on day of human chorionic gonadotropin administration, number of oocytes retrieved, fertilization rate, incidence of low ovarian response, and cycle cancellation.

**Result(s):** Only seven retrospective, case-controlled studies were found to match our objective. Overall, the results of the meta-analysis indicate that the number of retrieved oocytes rate was statistically significantly lower compared with age-matched healthy IVF patients. The incidence of poor ovarian performance and risk of cycle cancellation as well as the calculated number of two pronuclei zygotes achieved among patients with cancer were comparable with their age-matched controls.

**Conclusion(s):** Women with malignant disease should expect a lower number of oocytes retrieved after COH for fertility preservation, compared with healthy, age-matched patients. Presently, there is paucity of evidence to assess the effect of a specific malignant disease on ovarian response to COH before IVF for fertility preservation. Multicentric studies should be conducted to resolve these important issues. (Fertil Steril® 2012;97:125-33. ©2012 by American Society for Reproductive Medicine.)

**Key Words:** Cancer, fertility preservation, fertilization rate, in vitro fertilization, retrieved oocyte numbers
### Patient Demographics According to the Type of Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Non-HD, antagonist FSH (n=66)</th>
<th>HD, letrozole FSH (n=142)</th>
<th>Control (n=97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.6 ± 5.7</td>
<td>33.2 ± 4.3</td>
<td>31.9 ± 5.3</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>8.7 ± 1.7</td>
<td>9.6 ± 2.4</td>
<td>9.9 ± 1.6</td>
</tr>
<tr>
<td>Total FSH, IU</td>
<td>1,803 ± 889</td>
<td>1,755 ± 1,114</td>
<td>1,947 ± 808</td>
</tr>
<tr>
<td>Peak serum E₂, pg/ml</td>
<td>1,744 ± 1,242</td>
<td>381 ± 191</td>
<td>2,109 ± 1,260</td>
</tr>
<tr>
<td>Retrieved oocytes</td>
<td>12.2 ± 6.5</td>
<td>9.8 ± 7.1</td>
<td>12.4 ± 5.4</td>
</tr>
<tr>
<td>% MII oocytes</td>
<td>75.3 ± 18.5</td>
<td>74.4 ± 22.1</td>
<td>74.2 ± 17.7</td>
</tr>
</tbody>
</table>

DOMINGO et al 2012
FERTILITY AND STERILITY 97-4:930-934
Meta-analysis of the number of oocytes retrieved from women with cancer and control women who underwent gonadotropin stimulation for fertility preservation: no significant difference between the two groups (P=.11; 95% confidence interval –0.21 to 0.02).

FERTILITY PRESERVATION

Ovarian response to controlled ovarian hyperstimulation in women with cancer is as expected according to an age-specific nomogram

Marta Devesa • Francisca Martínez • Buenaventura Coroleu • Ignacio Rodríguez • Clara González • Pedro Nolasco Barri
Table 2  Patients’ baseline characteristics and stimulation parameters

<table>
<thead>
<tr>
<th>Cancer patients</th>
<th>( N=48 )</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>32.81±4.07</td>
</tr>
<tr>
<td>AFC</td>
<td>12.89±5.6</td>
</tr>
<tr>
<td>Basal FSH (IU/l)</td>
<td>6.65±2</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>2.16±1.49</td>
</tr>
<tr>
<td>Total gonadotropin consumption (IU)</td>
<td>3350±964</td>
</tr>
<tr>
<td>Peak serum E2 levels (pg/ml)</td>
<td>1134±756</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>9.5±2.33</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>14.04±8.83</td>
</tr>
<tr>
<td>No. of MII oocytes</td>
<td>11.38±8.84</td>
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Values are expressed as mean ± standard deviation

\( AFC \) antral follicle count; \( E2 \) estradiol; \( MII \) metaphase II
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*AFC* antral follicle count; *E2* estradiol; *MII* metaphase II
Ovarian response in cancer patients (N=48)

Z-score 0.23; 95% CI [-0.13 – 0.60] (NS)
<table>
<thead>
<tr>
<th>Author</th>
<th>N</th>
<th>Comparison technique</th>
<th>Type of matching</th>
<th>No. of oocytes retrieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knopman et al. 2009</td>
<td>28 cases</td>
<td>Age-matched controls</td>
<td>Ratio 1:4</td>
<td>Differences NS</td>
</tr>
<tr>
<td></td>
<td>135 controls</td>
<td>Male factor infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noyes et al. 2010</td>
<td>50 cases</td>
<td>Not matched</td>
<td></td>
<td>Differences NS</td>
</tr>
<tr>
<td></td>
<td>32 controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quintero et al. 2010</td>
<td>50 cases</td>
<td>Age-matched</td>
<td>Not specified</td>
<td>Differences NS</td>
</tr>
<tr>
<td></td>
<td>50 controls</td>
<td>Male factor/oocyte cryopreservation/ oocyte donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Das et al. 2011</td>
<td>41 cases</td>
<td>Age-matched</td>
<td>Not specified</td>
<td>Differences NS</td>
</tr>
<tr>
<td></td>
<td>48 controls</td>
<td>Male factor infertility, 1st IVF cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domingo et al. 2012</td>
<td>223 cases</td>
<td>Age-matched</td>
<td>Not specified</td>
<td>Significant differences</td>
</tr>
<tr>
<td></td>
<td>97 controls</td>
<td>Male factor infertility</td>
<td></td>
<td>(lower in cancer patients)</td>
</tr>
<tr>
<td>Almog et al. 2012</td>
<td>81 cases</td>
<td>Matched by age and closest date of stimulation</td>
<td>1:1</td>
<td>Differences NS</td>
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<tr>
<td></td>
<td>81 controls</td>
<td>Male factor infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-Velasco et al. 2013</td>
<td>355 oncological cases</td>
<td>Not matched</td>
<td></td>
<td>Differences NS</td>
</tr>
<tr>
<td></td>
<td>560 non oncological cases</td>
<td>Matched by age, race, IVF cycle No., date of stimulation and fertilization method</td>
<td>1:1</td>
<td>Differences NS</td>
</tr>
<tr>
<td>Johnson et al. 2013</td>
<td>50 cases</td>
<td>Male or tubal factor infertility and oocyte donnors</td>
<td></td>
<td>Differences NS</td>
</tr>
<tr>
<td>Devesa et al. (submitted)</td>
<td>48 cases</td>
<td>Z-score</td>
<td></td>
<td>Differences NS</td>
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<tr>
<td></td>
<td>(1536 cycles)</td>
<td>Age-specific nomogram 1st IVF cycles due to male factor exclusively/oocyte donnors/ age-related fertility preservation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant
Effective method for emergency fertility preservation: random-start controlled ovarian stimulation

Hakan Cakmak, M.D., Audra Katz, R.N., Marcelle I. Cedars, M.D., and Mitchell P. Rosen, M.D.

Fertility and Sterility® Vol. 100, No. 6, December 2013
Comparison of outcomes of conventional-and random-start controlled ovarian stimulation cycles.

<table>
<thead>
<tr>
<th></th>
<th>Conventional start</th>
<th>Random start</th>
<th>P value</th>
<th>Late follicular phase start</th>
<th>Luteal phase start</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 88; 103 cycles)</td>
<td>(n = 35; 35 cycles)</td>
<td></td>
<td>(n = 13; 13 cycles)</td>
<td>(n = 22; 22 cycles)</td>
<td></td>
</tr>
<tr>
<td>Antral follicle count (AFC)</td>
<td>13.0 (11.7–14.5)</td>
<td>11.5 (9.6–13.8)</td>
<td>NS</td>
<td>10.5 (7.8–14.2)</td>
<td>12.1 (9.6–15.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Days of ovarian stimulation</td>
<td>9.3 (9.0–9.5)</td>
<td>10.9 (10.4–11.5)</td>
<td>&lt;.001</td>
<td>10.5 (9.6–11.4)</td>
<td>11.2 (10.5–12.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total dose of gonadotropins (IU)</td>
<td>3,404 (3,180–3,628)</td>
<td>4,158 (3,774–4,542)</td>
<td>.001</td>
<td>3,842 (3,213–4,472)</td>
<td>4,344 (3,860–4,827)</td>
<td>.005</td>
</tr>
<tr>
<td>Gonadotropin daily dose (IU/d)</td>
<td>361 (345–378)</td>
<td>372 (343–400)</td>
<td>NS</td>
<td>371 (324–418)</td>
<td>373 (337–409)</td>
<td>NS</td>
</tr>
<tr>
<td>Follicles ≥ 13 mm</td>
<td>10.5 (9.3–11.9)</td>
<td>11.8 (9.6–14.5)</td>
<td>NS</td>
<td>10.9 (7.8–15.4)</td>
<td>12.3 (9.5–16.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>14.4 (12.8–16.2)</td>
<td>14.5 (11.8–17.8)</td>
<td>NS</td>
<td>13.0 (9.3–18.2)</td>
<td>15.5 (11.9–20.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Mature oocytes (MII) retrieved</td>
<td>9.7 (8.4–11.2)</td>
<td>9.9 (7.7–12.7)</td>
<td>NS</td>
<td>9.1 (6.0–13.7)</td>
<td>10.3 (7.5–14.2)</td>
<td>NS</td>
</tr>
<tr>
<td>MII oocytes/total oocytes ratio</td>
<td>0.66 (0.62–0.71)</td>
<td>0.67 (0.59–0.76)</td>
<td>NS</td>
<td>0.68 (0.56–0.82)</td>
<td>0.67 (0.58–0.78)</td>
<td>NS</td>
</tr>
<tr>
<td>Oocytes/AFC ratio</td>
<td>1.09 (0.99–1.19)</td>
<td>1.26 (1.07–1.49)</td>
<td>NS</td>
<td>1.24 (0.95–1.62)</td>
<td>1.28 (1.04–1.57)</td>
<td>NS</td>
</tr>
<tr>
<td>Mature oocytes/AFC</td>
<td>0.73 (0.65–0.82)</td>
<td>0.85 (0.70–1.04)</td>
<td>NS</td>
<td>0.84 (0.61–1.17)</td>
<td>0.86 (0.67–1.10)</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate after ICSI (2PN/MII)</td>
<td>0.72 (0.65–0.80)</td>
<td>0.87 (0.72–1.00)</td>
<td>NS</td>
<td>0.85 (0.67–1.00)</td>
<td>0.88 (0.70–1.10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Data were presented as geometric mean (95% confidence interval) unless otherwise indicated. All comparisons were adjusted for age and BMI with linear regression models. 2PN = two pronuclei; ICSI = intracytoplasmic sperm injection; MII = metaphase II.

* P value obtained after comparison of conventional- vs. late follicular– vs. luteal phase–start groups.
* P = .01 vs. conventional start.
* P = .008 vs. conventional start.
* Arithmetic mean (95% confidence interval).
* P = .001 vs. conventional start.

### Comparison of outcomes of conventional-and random-start controlled ovarian stimulation cycles.

| Source                                      | Conventional start (n = 88; 103 cycles) | Random start (n = 35; 35 cycles) | P value | Late follicular phase start (n = 13; 13 cycles) | Luteal phase start (n = 22; 22 cycles) | P value
|------------------------------------------------|---------------------------------------|----------------------------------|---------|-----------------------------------------------|----------------------------------------|---------
| Antral follicle count (AFC)                   | 13.0 (11.7–14.5)                      | 11.5 (9.6–13.8)                  | NS      | 10.5 (7.8–14.2)                              | 12.1 (9.6–15.2)                        | NS      
| Days of ovarian stimulation                   | 9.3 (9.0–9.5)                         | 10.9 (10.4–11.5)                 | < .001  | 10.5 (9.6–11.4)                              | 11.2 (10.5–12.0)                        | < .001  
| Total dose of gonadotropins (IU)              | 3,404 (3,180–3,628)                    | 4,158 (3,774–4,542)              | .001    | 3,842 (3,213–4,472)                          | 4,344 (3,860–4,827)                     | .005    
| Gonadotropin daily dose (IU/d)                | 361 (345–378)                         | 372 (343–400)                    | NS      | 371 (324–418)                               | 373 (337–409)                          | NS      
| Follicles ≥ 13 mm                            | 10.5 (9.3–11.9)                       | 11.8 (9.6–14.5)                  | NS      | 10.9 (7.8–15.4)                              | 12.3 (9.5–16.0)                        | NS      
| Oocytes retrieved                             | 14.4 (12.8–16.2)                      | 14.5 (11.8–17.8)                 | NS      | 13.0 (9.3–18.2)                              | 15.5 (11.9–20.1)                       | NS      
| Mature oocytes (MI) retrieved                 | 9.7 (8.4–11.2)                        | 9.9 (7.7–12.7)                   | NS      | 9.1 (6.0–13.7)                               | 10.3 (7.5–14.2)                        | NS      
| MI oocytes/total oocytes ratio                | 0.66 (0.62–0.71)                      | 0.67 (0.59–0.76)                 | NS      | 0.68 (0.56–0.82)                             | 0.67 (0.58–0.78)                       | NS      
| Oocytes/AFC ratio                             | 1.09 (0.99–1.19)                      | 1.26 (1.07–1.49)                 | NS      | 1.24 (0.95–1.62)                             | 1.28 (1.04–1.57)                       | NS      
| Mature oocytes/AFC                            | 0.73 (0.65–0.82)                      | 0.85 (0.70–1.04)                 | NS      | 0.84 (0.61–1.17)                             | 0.86 (0.67–1.10)                       | NS      
| Fertilization rate after ICSI (2PN/MI)        | 0.72 (0.65–0.80)                      | 0.87 (0.72–1.00)                 | NS      | 0.85 (0.67–1.00)                             | 0.88 (0.70–1.00)                       | NS      

Note: Data were presented as geometric mean (95% confidence interval) unless otherwise indicated. All comparisons were adjusted for age and BMI with linear regression models. 2PN = two pronuclei; ICSI = intracytoplasmic sperm injection; MI = metaphase II.

P value obtained after comparison of conventional vs. late follicular vs. luteal phase–start groups.

* P = .01 vs. conventional start.
* P = .008 vs. conventional start.
* P = .001 vs. conventional start.

Arithmetic mean (95% confidence interval).

POTENCIAL EVOLUTIVO DE EMBRIONES OBTENIDOS TRAS LA ESTIMULACIÓN EN FASE LÚTEA.

Resultados 2014

F Martínez, E Clua, M Devesa, G Arroyo, C González, M Soler, R Tur, B Coroleu

“Merck Serono Ayudas a la Investigación 2012”  NCT 01645241

✓ 9 oocyte donors
✓ Each donor undergoes 1 cycle of FF stimulation and 1 cycle of LF stimulation
✓ Comparison of:
  Both cycles
  Pregnancy rates in recipients receiving FF oocytes vs. LF oocytes
<table>
<thead>
<tr>
<th>DONORS</th>
<th>LF</th>
<th>FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>26.78 ± 2.95</td>
<td></td>
</tr>
<tr>
<td>AFC</td>
<td>22.11 ± 12.53</td>
<td></td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>3.56 ± 2.16</td>
<td></td>
</tr>
<tr>
<td>Dose of Gns</td>
<td>2147 ± 535</td>
<td>2261 ± 940</td>
</tr>
<tr>
<td>Duration of stimulation</td>
<td>9.89 ± 1.27</td>
<td>10.44 ± 1.74</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>22.56 ± 10.56</td>
<td>16.67 ± 6.65</td>
</tr>
<tr>
<td>MII</td>
<td>16.88 ± 7.52</td>
<td>14.00 ± 6.96</td>
</tr>
</tbody>
</table>

p=NS
<table>
<thead>
<tr>
<th>RECIPIENTS CYCLES</th>
<th>LF (N=12)</th>
<th>FF (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors’ age</td>
<td>26.83±2.98</td>
<td>26.88±3.27</td>
</tr>
<tr>
<td>Recipients’ age</td>
<td>43.92±4.48</td>
<td>44.13±2.47</td>
</tr>
<tr>
<td>Fresh Oocytes donated</td>
<td>8.75±1.96</td>
<td>8.38±2.67</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>76.47%</td>
<td>73.33%</td>
</tr>
<tr>
<td>Embryos replaced</td>
<td>1.67±0.65</td>
<td>1.50±0.53</td>
</tr>
<tr>
<td>Embryo quality</td>
<td>8.50±1.00</td>
<td>8.50±1.49</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>58.3%</td>
<td>62.5%</td>
</tr>
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</table>

p=NS
<table>
<thead>
<tr>
<th>CRYOPRESERVATION</th>
<th>CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Embryos</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>9.5 ± 2.33 days</strong></td>
</tr>
<tr>
<td></td>
<td>HUQD, 2013</td>
</tr>
<tr>
<td><strong>Oocytes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ovarian tissue</strong></td>
<td>Minimum a surgical intervention</td>
</tr>
<tr>
<td></td>
<td>No need for ovarian stimulation . No time limitation</td>
</tr>
<tr>
<td></td>
<td>Risk of reimplanting malignant cells (mínimal in breast cancer)</td>
</tr>
<tr>
<td></td>
<td>Graft survival?</td>
</tr>
<tr>
<td>CRYOPRESERVATION</td>
<td>CHARACTERISTICS</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Embryos</td>
<td>LBR 27%</td>
</tr>
<tr>
<td>Oocytes</td>
<td>LBR 7% per oocyte</td>
</tr>
</tbody>
</table>
From current data it does not seem that in certain cancers ovarian stimulation techniques increases the risk of recurrence.

Provide that the oncologist thinks that it is possible to delay the start of oncology treatment for 15-20 days.

Treatment must be started as soon as possible. If it is not in follicular phase, antagonists can be administered and stimulation started once oestradiol levels of less than 50 pg/ml have been reached.

- Administration of letrozole, 5 mg for 5 days. Administration can be extended until low oestradiol levels have been reached. It can be administered throughout the stimulation phase.
- Gonadotrophins (dose 150 IU FSH) starting on the day following letrozole.
- Antagonists when the follicle ≥ 14 mm. diameter
- Ovulatory discharge with GnRH agonists
- Post-aspiration: administer letrozole or antagonists
Indications
1. Hormone-sensitive tumours
2. Patients reluctant to undergo ovarian stimulation.
3. No time for IVF before starting oncological therapy
Ovarian tissue cryopreservation: Results

Livebirth after orthotopic transplantation of cryopreserved ovarian tissue

J Donnez, M M Dolmans, D Demyssize, P Jadoul, C Pirard, J Squifflet, B Martinez-Madrid, A Van Langendonckt

OPTIONS FOR CRYOPRESERVATION OF OVARIAN TISSUE AND REIMPLANTATION

Jacques Donnez and Marie-Madeleine Dolmans
The risk of ovarian metastasis according to cancer types

Cancers with low risk of ovarian involvement
- Wilm’s tumour
- Ewing’s sarcoma
- Breast cancer
  - Stage I-III
  - Infiltrative ductal histological subtype
- Non-Hodgkin’s lymphoma
- Hodgkin’s lymphoma
- Non-genital rhabdomyosarcoma
- Osteogenic sarcoma
- Squamous cell carcinoma of the cervix

Cancers with moderate risk of ovarian involvement
- Adenocarcinoma/adenosquamous carcinoma of the cervix
- Colon cancer
- Breast cancer
  - Stage IV
  - Infiltrative lobular histological subtype

Cancers with high risk of ovarian involvement
- Leukaemia
- Neuroblastoma
- Burkitt lymphoma

M. Sonmezer and K. Oktay 2004
Human Reproduction Update, 10-3: 251-266
L. Bastings et al.

**Methods:** A systematic review of literature

**Results:** A total of 289 studies were included

**Conclusions:** It is advisable to refrain from ovarian tissue autotransplantation in survivors of leukaemia. With survivors of all other malignancies, current knowledge regarding the safety of autotransplantation should be discussed. The most reassuring data regarding autotransplantation safety were found for lymphoma patients.
Previous unilateral oophorectomy for freezing

- 143 Patients
  - ≤ 35 years
  - > 35% Risk of POF

- Follow-up (X) 58 months

- Global risk of POF (22%)

- Pregnancy rate 72% (41/57)
  - Spontaneous 94%
  - Miscarriage 25%

- Time X 6 months

↑ Leukemia-BMT
↓ Breast cancer

RBOline 26-3:272-9
These three case reports describe the long-term duration of function of ovarian cortical tissue grafts among patients in a university fertility preservation programme in Europe and in a private practice programme in the USA. One woman underwent sterilizing cancer treatment and had frozen ovarian tissue transplanted, and two women underwent fresh ovarian tissue transplants. The function of ovarian cortical strips has continued for more than 7 years in these three women, with the birth of eight healthy babies following a single graft per patient.
**OVARIAN TISSUE CRYOPRESERVATION:**

**Options:**

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Whole ovary with vascular pedicle</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fragments of ovarian cortex</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cryopreservation</th>
<th>Slow freezing</th>
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<tbody>
<tr>
<td></td>
<td>Vitrification</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Transplant</th>
<th>Orthotopic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterotopic</td>
</tr>
</tbody>
</table>
LIVE BIRTH IN A WOMAN WITHOUT OVARIES AFTER AUTOGRAPH OF FROZEN-THAWED OVARIAN TISSUE COMBINED WITH GROWTH FACTORS

Justo Callejo et al.

Journal of Ovarian Research 2013, 6:33
Improving ovarian tissue cryopreservation for oncologic patients: slow freezing versus vitrification, effect of different procedures and devices

Sonia Herraiz, Ph.D., Edurne Novella-Maestre, Ph.D., Beatriz Rodríguez, B.Sc., César Díaz, M.D., María Sánchez-Serrano, Ph.D., M.D., Vicente Mirabet, Ph.D., and Antonio Pellicer, Ph.D., M.D.

Fertility and Sterility® Vol. 101, No. 3, March 2014
CONCLUSION(S): The present study is the first to show survival and growth of isolated murine ovarian follicles 1 week after autotransplantation of isolated OCs in a fibrin scaffold.
1. Reducing toxicity

2. Cryopreservation

3. Conservative surgery
Fertility sparing surgery

1. Ovarian cancer:
   - Borderline tumours
   - Invasive carcinoma stage Ia G1

2. Endometrial cancer: stage Ia G1,

3. Cervical cancer: < 2 cms, stages Ia – Ib1,

Querleu D et al, Bull Cancer 2008
Gurgan T et al, Placenta 2008
Fujiwara H et al, Hum Rep 2009
Koskas et al. Fertil Steril 2012
In women:

- If ovarian stimulation is possible
  - Embryo cryopreservation was the method with the greatest chance of success (only possible if there is a partner or they accept banked semen)
  - Oocyte cryopreservation now has a promising future thanks to vitrification
- If ovarian stimulation is not possible, there are various techniques:
  - Cryopreservation of ovarian tissue and later orthotopic autotransplant
  - Aspiration of antral follicles and in-vitro vitrification of mature oocytes
- GnRH analogues for gonadal protection, though the results published to date are not conclusive.
- In cases of abdominal radiotherapy: ovarian transposition
- Conservative gynaecological surgery whenever possible
FERTILITY PRESERVATION METHODS IN WOMEN AT RISK OF PREMATURE OVARIAN FAILURE

Medical therapy (GnRH agonists, still controversial)

- Ovarian transposition (In case of pelvic irradiation)

- Aspiration of immature oocytes with or without stimulation

- Aspiration of mature oocytes with stimulation (2 weeks)

- Cryopreservation of ovarian tissue (Immediate therapy required or in prepubertal girls)

  - Orthotopic transplantation
  - Isolation of primordial follicles

  - Aspiration of immature oocytes, in vitro maturation and fertilization

  - Vitrification of oocytes

  - Spontaneous function or stimulated and/or IVF

  - In vitro maturation

  - Artificial ovary
FERTILITY PRESERVATION: MOVING AHEAD FASTER THAN EXPECTED!

Pedro Barri – Antonio Pellicer

J. Assist. Reprod. Genet
Published online: 18 January 2014
Gracias por su atención
Thank you for your attention

pbarri@dexeus.com