

The International Society for Fertility Preservation

# THE 2nd WORLD CONGRESS ON FERTILITY PRESERVATION

December 8–10, 2011  
Miami Beach, Florida

## FINAL PROGRAM

Speaker presentations are available on-line  
after completing a brief evaluation at:  
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[www.isfp-fertility.org](http://www.isfp-fertility.org)

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Fertility Preservation and University of Kansas  
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**ISFP**

Message from the Congress Chair:

On behalf of the International Society of Fertility Preservation, I would like to extend my warm welcome to you all.

The International Society of Fertility Preservation (ISFP) was founded in 2007 to promote progress in fertility preservation through international networking, sharing information and new discoveries. The Society is growing fast, both in quality and quantity, and has established itself as a leading international organization for fertility preservation.

The World Congress is one of the most important functions of the ISFP, and I am delighted and honored to serve as Organizing Chair of the 2<sup>nd</sup> ISFP World Congress in Miami Beach, FL, USA. As you can see, the scientific program is filled with current and important topics that will be presented by world-renowned experts. In addition, we are offering a hands-on workshop on vitrification as a pre-Congress course. I am also very pleased with the number and quality of abstracts submitted this year. All of these reflect the significance of the World Congress.

I would like to thank each of you for participating in the 2<sup>nd</sup> ISFP World Congress on Fertility Preservation. It would not be possible to have a successful meeting without the support of sponsors, exhibitors, the planning committee, CME staff, speakers, and most of all, the attendees. I hope all of you enjoy the 2<sup>nd</sup> ISFP World Congress in Miami Beach.

Sincerely,

A handwritten signature in black ink, appearing to read 'S. Samuel Kim', written in a cursive style.

S. Samuel Kim, M.D.  
President, ISFP  
Congress Chair, The 2<sup>nd</sup> ISFP World Congress on Fertility Preservation

# **The Second ISFP World Congress on Fertility Preservation**

## **Contributors**

**These organizations have generously supported this congress  
and we acknowledge them with sincerest appreciation.**

### **Platinum Level: Ferring Pharmaceuticals**

### **Bronze Level: Walgreens**

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**Lance Armstrong Foundation  
Cornell Center for Reproductive Medicine (Zev Rosenwaks)  
KU Department of OB/GYN (Carl Weiner)  
Cleveland Clinic Department of OB/GYN (Tommaso Falcone)**

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**Cook Medical  
Catholic University of Louvain (Jacques Donnez)  
University of Kansas Reproductive Endocrinology (Samuel Kim)**

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**Xytex Cryo International (Michael Tucker)  
Yale Fertility Center (Pasquale Patrizio)  
IVI (Antonio Pellicer)  
Dexeus University (Pedro Barri)  
ReproTech, Ltd.  
Reproductive Biology Associates (Zsolt Peter Nagy)  
California Cryobank**

# **The Second ISFP World Congress on Fertility Preservation**

## **Exhibitors**

We wish to acknowledge and sincerely thank  
these organizations for exhibiting at this congress.

**California Cryobank**

**Ferring Pharmaceuticals**

**Freedom Pharmacy**

**Merck**

**Origio**

**Vitrolife**

**Walgreens**

# Thursday Agenda

December 8, 2011

07:30 – 09:00 Registration (North Lobby)  
06:30 – 07:30 Continental Breakfast (Shimmer)

## **Hands-On Workshop for Fertility Preservation:** (Flash)

08:50 – 09:00 **Welcome**  
Ri-Cheng Chian, MSc, PhD

09:00 – 09:30 **Methodology of Ovarian Tissue Cryopreservation**  
Debra Gook, MD

09:30 – 10:00 **Video Presentation for Ovarian Tissue Banking**  
S. Samuel Kim, MD; Sherman Silber, MD

10:00 – 10:30 **Cryobiology of Oocyte Preservation**  
Ri-Cheng Chian, MSc, PhD

10:30 – 11:00 **Update of Success Rate with Oocyte Vitrification**  
Zsolt Peter Nagy, MD, PhD

11:00 – 12:30 **Hands-On Practice for Oocyte Vitrification with Mouse Oocytes**

12:30 – 12:50 **Question and Answer Session**

12:50 – 13:00 **Closing Remarks**  
Ri-Cheng Chian, MSc, PhD

13:00 – 14:00 Lunch (Shimmer)

## **Evening Events:**

17:00 – 19:00 **Welcome Reception and Poster Presentation** (Fleur de Lis & Fontaine)

**There will be a cash bar and light food served at the reception.**

# Friday Morning Agenda

December 9, 2011

07:30 – 18:00 Registration (North Lobby)

07:30 – 18:00 Exhibits (Fleur de Lis)

06:30 – 08:00 Continental Breakfast (Gotham)

## **Scientific Sessions, 2<sup>nd</sup> ISFP World Congress: (Fontaine)**

08:00 – 08:15 **Welcome**

S. Samuel Kim, MD

08:15 – 08:30 **Lance Armstrong Foundation**

Emily Eargle, MSSW

08:30 – 08:45 **Presentation by Patient Advocates**

Alice Crisci

**Session I** – Moderators: Jacques Donnez, MD; Samuel Kim, MD; Hamish Wallace, MD

09:00 – 09:30 **Ethical and Legal Dilemmas in Fertility Preservation**

Nannette Elster, JD

09:30 – 10:00 **Quality of Life and Reproductive Health in Cancer Survivors**

Leslie R. Schover, PhD

10:00 – 10:30 **Database to Enhance Fertility Preservation**

Hillary Klonoff-Cohen, PhD

10:30 – 11:00 **Effects of Chemotherapy in the Pediatric Population**

Hamish Wallace, MD

11:00 – 11:30 Refreshments, Break and Exhibits (Fleur de Lis)

11:30 – 12:00 **Breast Cancer and Fertility in the Surgeon's Perspective**

Carol Connor, MD

12:00 – 12:30 **Fertility Preservation for GYN Cancer Patients**

Giuseppe DelPriore, MD, MPH

12:30 – 13:30 Luncheon (Gotham)

# Friday Afternoon Agenda

December 9, 2011

**Session II** – Moderators: Debra Gook, PhD; Lynn Westphal, MD

13:30 – 14:00 **Recent Clinical and Research Progress in Vitrification of Reproductive Cells**  
Ri-Cheng Chian, MSc, PhD

14:00 – 14:30 **Management and Results of Cryo-Oocyte Bank**  
Zsolt Peter Nagy, MD, PhD

14:30 – 15:00 **Next Generation of Cryopreservation Technology**  
Amir Arav, DVM, PhD

15:00 – 16:00 **4 Selected Oral Communication Abstract Presentations**  
15:00 – Nicole Noyes  
15:15 – Serena Dovey  
15:30 – Kenny Rodriguez-Wallberg  
15:45 – Nathalie Rives

16:00 – 16:30 Refreshments, Break and Exhibits (Fleur de Lis)

**Session III** – Moderators: Zsolt Peter Nagy, MD, PhD; Claus Yding Andersen, MSc, DMSc

16:30 – 17:00 **Oocyte Cryopreservation: Postnatal Outcome**  
Antonio Pellicer, MD

17:00 – 17:30 **Advantages and Limitations of Immature Oocyte Cryopreservation**  
Debra Gook, PhD

17:30 – 18:00 **Trend of Fertility Preservation Strategies in Europe**  
Pedro Barri, MD, PhD

18:00 – 18:30 **Fertility Preservation in Breast Cancer Patients: Harvard Experience**  
Elizabeth Ginsburg, MD

18:30 Adjournment

# Saturday Morning Agenda

December 10, 2011

07:30 – 10:30 Registration (North Lobby)

07:30 – 18:00 Exhibits (Fleur de Lis)

06:30 – 08:30 Continental Breakfast (Gotham)

## **Scientific Sessions, 2<sup>nd</sup> ISFP World Congress:** (Fontaine)

**Session IV** – Moderators: Bruno Salle, MD, PhD; Antonio Pellicer, MD

08:00 – 08:30 **The Future of Translational Research in Fertility Preservation**

Mary Zelinski, PhD

08:30 – 09:00 **DNA Damage / Repair with Ovarian Tissue Cryopreservation**

S. Samuel Kim, MD

09:00 – 09:30 **Ovarian Tissue Transplantation: Danish Model and Outcome**

Claus Yding Andersen, MSc, DMSc

09:30 – 10:00 **Strategies to Improve Post-Transplant Survival of Ovarian Tissue**

Jacques Donnez, MD, PhD

10:00 – 10:30 Refreshment, Break and Exhibits (Fleur de Lis)

**Session V** – Moderators: Pasquale Patrizio, MD; Jehoshua Dor, MD

10:30 – 11:00 **Medical Options for Fertility Preservation**

Dror Meirow, MD

11:00 – 11:30 **Risks of Cancer Cell Reintroduction after Ovarian Transplantation**

Marie-Madeleine Dolmans, MD, PhD

11:30 – 12:00 **Potential Challenges of Whole Ovary Transplant**

Pasquale Patrizio, MD, MBE; Bruno Salle, MD, PhD

12:00 – 12:30 **The Future Role of Social Egg Banking**

Dror Meirow, MD; Pasquale Patrizio, MD

12:30 – 13:30 Luncheon (Gotham)



# Saturday Afternoon Agenda

December 10, 2011

**Session VI** – Moderators: Zev Rosenwaks, MD; Dror Meirow, MD; Chii-Ruey Tzeng, MD, PhD

13:30 – 14:30 **4 Selected Oral Communication Abstract Presentations**

13:30 – Deepa Bhartiya

13:45 – Yoni Cohen

14:00 – Mahmoud Salama

14:15 – Michael Grynberg

14:30 – 15:00 **ART for Fertility Preservation**

Glenn Schattman, MD

15:00 – 15:30 **From Pluripotent Stem Cells to Germ Cells**

Outi Hovatta, MD

15:30 – 16:00 Refreshment, Break and Exhibits (Fleur de Lis)

16:00 – 16:30 **Current Status of In Vitro Growth and Maturation**

Evelyn Telfer, PhD

16:30 – 17:00 **IVM: An Old Technology in a New Era?**

Johan Smitz, MD, PhD

17:00 – 17:30 **Mechanism of DNA Damage with Chemotherapy / Radiotherapy**

David Albertini, PhD

17:30 – 18:00 **Non-Traditional Animal Models for Advancing Fertility Preservation Studies in Humans**

Pierre Comizzoli, PhD

18:00 – 18:30 **Prize Presentation and Closing Ceremony**

Presentations by ISFP Officers:

Pedro Barri, MD

Jacques Donnez, MD

S. Samuel Kim, MD

Pasquale Patrizio, MD

Antonio Pellicer, MD

## Friday Morning Abstracts

09:00 – 09:30 **Ethical and Legal Dilemmas in Fertility Preservation**  
Nannette Elster, JD

09:30 – 10:00 **Quality of Life and Reproductive Health in Cancer Survivors**  
Leslie R. Schover, PhD

10:00 – 10:30 **Database to Enhance Fertility Preservation**  
Hillary Klonoff-Cohen, PhD

10:30 – 11:00 **Effects of Chemotherapy in the Pediatric Population**  
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Carol Connor, MD

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Giuseppe DelPriore, MD, MPH

## **Ethical and Legal Dilemmas in Fertility Preservation**

**Nanette Elster, JD, MPH**

Recent scientific advances have made the once remote possibility of conception following cancer treatment more feasible, however, these advances are not without risks including legal and ethical risks. This presentation will discuss some of the legal and ethical challenges inherent in fertility preservation including informed consent for both children and adults, disposition of gametes and embryos after death as well as accessibility of fertility preservation services.



# Quality of Life and Reproductive Health in Cancer Survivors

**Leslie R. Schover, PhD**

Cancer treatment may interfere with becoming a parent because of direct damage to gametogenesis in males and females, damage to uterine capacity to carry a pregnancy, damage to the ability to have functional sexual intercourse, or lessening of physical attractiveness to a potential mate. Young cancer survivors may also forego having children out of anxieties such as fear that fertility preservation would cause a dangerous delay in starting cancer treatment or that fertility-sparing modifications of cancer treatment would be less successful in controlling cancer. Women have exaggerated fears about a pregnancy causing a cancer recurrence and survivors of both genders worry about birth defects and lifetime cancer risk in any child born after a parent's cancer treatment. Other worries are passing on a genetic risk for inherited cancer syndromes or dying prematurely and being unable to protect a young child. Survivors who are infertile but want to be social parents face barriers to adopting a child after cancer and often know little about options of donated gametes or embryos. The majority of young survivors do want biological children, however, particularly those childless at the time of cancer diagnosis. Our own interview data show that as long as an average of 10 years after cancer treatment, women diagnosed before age 40 remain very distressed about unfulfilled desires to have a child. Despite the efforts to standardize communications from oncologists about fertility risk and options for fertility preservation, at least half of patients still are not getting the information they need to make a rational decision. Patients who get more information feel more comfortable with their decisions about fertility preservation, even though a majority still do not choose to cryopreserve gametes. For men, both teenagers and men over 40 may be good candidates to bank sperm and should not be ruled out because of age. Teens may need to be counseled separately from their parents so that everyone's opinions and needs can be heard and a family decision made. However, we need to focus more attention on the needs of the thousands of young survivors who did not preserve fertility and need to make decisions on their options for parenthood after cancer.



## Database to Enhance Fertility Preservation

**Hillary Klonoff-Cohen, PhD**

**Objective:** Currently, no comprehensive research database exists to evaluate the different fertility preservation strategies. The largest national data sets (e.g., NCHS, CDC, and SEER) contain no questions pertaining to infertility caused by cancer or other fertility-compromising treatments. Moreover, the Society of Assisted Reproductive Technology (SART) database does not address fertility preservation procedures. Creation of a Fertility Preservation Database will provide information on the prevalence, demographic characteristics of patients, procedural types, efficacy, safety and long-term effects, and success rates (e.g., pregnancy and birth outcomes) of fertility preservation.

**Methods:** We conducted two feasibility studies. The first study ascertained the prevalence of fertility preservation options for cancer patients at 16 clinics within 100 miles of UC San Diego. The purpose of the second feasibility study was to determine whether we could acquire an adequate sample of cancer and non-cancer patients who choose to undergo fertility preservation by contacting every SART-affiliated fertility clinic in California. Clinic Directors provided the total number of fertility preservation procedures performed in 2009.

**Results:** In the first feasibility study, of the 11 clinics that responded, 8 (73%) reported performing fertility preservation; 1 (9%) reported having had an inquiry about it, while 2 (18%) reported not having done any. Of the 54 patients seen at these fertility clinics, the vast majority (> 40 patients, 75%) had a cancer diagnosis, and of these, most (> 26, 65%) had breast cancer. All patients underwent embryo cryopreservation (except one case where the patient died). Forty women (74%) still had embryos in storage and three (5.5%) had attempted pregnancy using frozen embryo transfers, resulting in one successful pregnancy. In our second study, out of 44 fertility clinics, 21 responded; seven of which did not perform fertility preservation. In 2009, a total of 77 cancer patients were treated with fertility preservation procedures at the responding clinics in California. To be maximally conservative, we assumed that all the non-responding sites treated zero cancer patients. This would imply that the 44 clinics in California (rather than 21 responders) treated the 77 cancer patients with fertility preservation. Based on a total of 395 fertility clinics within the US, using the same ratio would translate to 691 cancer patients per year in 2009.

**Conclusions:** From this feasibility study, we conclude that there will be ample eligible cancer patients available for participation in a registry to elucidate medical, social, ethical and legal issues of fertility preservation for physicians and patients.





# **Fertility Preservation Options for Children with Cancer**

**Hamish Wallace, MD**

With increasing numbers of survivors from cancer at a young age the issue of fertility preservation has assumed greater importance. This lecture will describe normal ovarian function and summarises what is known about the effect of chemotherapy and radiotherapy on the ovary and uterus. The value of an assessment of ovarian reserve for the individual patient using AMH will be discussed. Recent prospective studies on AMH during chemotherapy in children will be reviewed.

To date, there have been at least 17 pregnancies worldwide after othotopic reimplantation of frozen-thawed ovarian cortex. The success rate is unclear as the denominator (the number of women in whom frozen-thawed ovarian tissue has been reimplanted) is unknown. There have been no pregnancies reported following the reimplantation of ovarian tissue harvested pre-pubertally, but with the accepted age related decline from birth in the number of non-growing follicles, young children are potentially ideal candidates for this procedure

All young patients with cancer or leukaemia should have their fertility prognosis discussed before treatment begins. Sperm and embryo cryopreservation should be considered standard practice and be widely available for those at significant risk of infertility. The Edinburgh experience of ovarian cryopreservation will be presented. Risks and benefits from reimplantation of frozen/thawed ovarian cortical strips will be discussed. For pre-pubertal girls ovarian tissue cryopreservation should be considered if the risk of a premature menopause is high, but for the pre-pubertal boy there are no established techniques in current practice.



## **Breast Cancer and Fertility in the Surgeon's Perspective**

**Carol S. Connor, MD**

**Objective:** Review the surgeon's role in the evaluation and treatment of newly diagnosed young breast cancer patients and the challenges to integration of fertility preservation into surgical therapy.

**Methods/Results:** Fertility preservation is an important option for young women with newly diagnosed breast cancer. The surgeon is often the first specialist to see a newly diagnosed breast cancer patient, perform the initial evaluation, provide appropriate referrals to other specialists, and coordinate the treatment plan. The principles of surgical management of young breast cancer patients will be reviewed, with special attention to the management of young patients receiving neoadjuvant therapy. Clinical and biologic factors that contribute to treatment decisions in young breast cancer patients will be presented, including a summary of fertility preservation options based upon these factors. A case study will be presented that demonstrates the need for involvement of the surgeon in a multidisciplinary plan for fertility preservation.

**Conclusion:** Education of breast surgeons regarding the options for fertility preservation in young patients with breast cancer could potentially improve patient referral to reproductive specialists and facilitate the coordination of care in these often complex clinical scenarios.



## **Fertility Preservation for GYN Cancer Patients**

**Giuseppe DelPriore, MD, MPH**

Fertility preservation for all cancer patients has become an important quality-of-life outcome for oncologist and infertility specialists. Several overseeing and regulatory entities have similarly weighed in encouraging consideration of fertility preservation as part of the treatment planning for cancer patients. And finally, the public and the courts have adopted fertility preservation as standard of care in certain circumstances.

Gynecologic cancers including breast cancer may have special population issues similar to those affecting pediatric patients. In other cancers the interaction with fertility between the disease and its treatment, may be less apparent although not less important. For gynecologic cancers the impact on fertility is inescapable. Participants should know the latest oncologic principles related to this field.

Cervical cancer is the most common gynecologic cancer seen in many areas of the world. In developing countries it surpasses all other cancers including breast. Unlike breast, the peak age of incidence is relatively young, corresponding to the reproductive age period. Fortunately the benefits of chemotherapy have been demonstrated in almost all settings of cervical cancer care but especially in fertility preservation when used for neoadjuvant or adjuvant therapy.

Ovarian cancer which is thought of as the most lethal gynecologic cancer nevertheless still allows fertility preservation to be considered during its treatment. Coordination with surgeon, chemotherapist, and infertility specialists is especially important in this cancer.

Endometrial cancer is a common cancer in Western cultures. It is often thought of as an indolent cancer allowing for fertility preservation to be considered. However risks do exist and must be recognized and dealt with.

Although the intersection of fertility in cancer care is complex, this presentation will be a practical guide limited to the most relevant clinical updates.



## **Friday Afternoon Abstracts**

- 13:30 – 14:00 **Recent Clinical and Research Progress in Vitrification of Reproductive Cells**  
Ri-Cheng Chian, MSc, PhD
- 14:00 – 14:30 **Management and Results of Cryo-Oocyte Bank**  
Zsolt Peter Nagy, MD, PhD
- 14:30 – 15:00 **Next Generation of Cryopreservation Technology**  
Amir Arav, DVM, PhD
- 16:30 – 17:00 **Oocyte Cryopreservation: Postnatal Outcome**  
Antonio Pellicer, MD
- 17:00 – 17:30 **Advantages and Limitations of Immature Oocyte Cryopreservation**  
Debra Gook, PhD
- 17:30 – 18:00 **Trend of Fertility Preservation Strategies in Europe**  
Pedro Barri, MD, PhD
- 18:00 – 18:30 **Fertility Preservation in Breast Cancer Patients: Harvard Experience**  
Elizabeth Ginsburg, MD





# **Recent Clinical and Research Progress in Vitrification of Reproductive Cells**

**Ri-Cheng Chian, MSc, PhD**

Cryobiology is the branch of biology involving the study of the effects of low temperatures on organisms, in which most often for the purpose of achieving cryopreservation. The cryobiology is core of fertility cryopreservation. The principal application for human fertility cryopreservation was begun with sperm freezing, and then with embryo and oocyte as well as gonadal cryopreservation. Although the advanced knowledge and medical achievements have been obtained in the field of fertility cryopreservation, especially with recent development of oocyte and ovarian tissue cryopreservation, the field of cryobiology can be considered as relatively new branch of biology.

Many factors affect the successful cryopreservation of the cells. The first, it may depend on the cell type, cell size, cell growth phase, cell water content, cell lipid content and the composition of the cells as well as cell density. The second, it may depend on the composition of freezing or vitrification medium, cooling rate, storage temperature and duration of storage, warming rate and recovery medium. The third, it may be the most important to supplemented cryoprotectant into aqueous solution.

The mechanism of cryoprotectants action can be considered as lowering the freezing point and preventing ice crystal formation of intracellular and extracellular solutes. It has been considered that there may be minor or server toxicity of cryoprotectants. This toxicity of cryoprotectants is related directly to its concentration to be used, and the cell exposure temperature and time. Although some theories of cryobiology developed, it may not be applied to all types of cells. Theoretical considerations are needed for further development in the field of cryobiology.

The development of an effective oocyte cryopreservation system has a significant impact on clinical practice of assisted reproduction. With modified slow freezing method, particularly increased sucrose concentration in suspending solution, the improved survival and pregnancy rates have been obtained. However, super-rapid cooling of human oocytes has resulted in relatively higher survival rate. Pregnancies achieved with cryopreservation of oocytes regardless of slow freezing or vitrification do not appear to be associated with adverse pregnancy outcomes, indicating that cryopreservation of oocytes represent a novel option and efficient method for female fertility preservation. It seems that cryopreservation of mature stage oocytes has better results than freezing immature stage oocytes, because oocyte maturation rate will be significantly reduced when the oocytes were cryopreserved at immature stage followed by IVM. Although a couple of thousands live births obtained from the cryopreserved oocytes and appeared no difference in congenital anomalies compared with naturally conceived infants, more live birth data and long-term monitoring are required to assure the safe and expeditious development of oocyte cryopreservation technology.

In addition, the updated information for cryopreservation of ovarian tissues followed by transplantation will also be discussed in this presentation.



## **Management and Results of Cryo-Oocyte Bank**

**Zsolt Peter Nagy, MD, PhD**

The efficiency of oocyte cryopreservation has dramatically increased in recent years, mainly due to the employment of the vitrification technique. There are several potential benefits of oocyte cryopreservation, including fertility preservation for medical and social reasons; ethical/moral consideration or legislative restrictions. However, one of the first applications of egg freezing relates to oocyte donation. Oocyte donation has been a well-established practice for decades to treat IVF patients with advanced reproductive age or other conditions. Since its introduction, it was always the practice to perform it through fresh donation, however this is associated with many disadvantages, including long waiting times, limited donor choice, and difficulty of synchronization. A donor egg cryo-bank, on the other hand, can eliminate all these difficulties. Recent publications and our own experience with over 500 recipient cycles demonstrate that using the vitrification technique, high survival rates (90% or more) of donor eggs can be achieved followed by high fertilization (>70%) and satisfactory embryo development (>50% good quality embryos). Clinical pregnancy rates are consistently above 50% (70% when two embryos and 50% when a single embryo is transferred). Evaluation of children born after the use of vitrified eggs does not show higher rates of birth defects which helps to reassure the safety aspects of the technique. Thus our experience with cryo-egg donation is highly positive. It provides similar outcomes to fresh donation, without the difficulty of synchronization, and with several benefits including immediate access to a large variety of donors, increased safety by possible quarantining eggs, as well as economic benefits to recipient making the treatment more affordable.



## Next Generation of Cryopreservation Technology

**Amir Arav, DVM, PhD**

Imagine a world without liquid nitrogen storage. What unifies the tissue and cell cryobanking is that they store the samples in liquid nitrogen. Storage of cryopreserved samples, under liquid nitrogen, is very demanding in terms of maintenance, storage space, storage equipment and costs. An alternative that would minimize costs, storage and maintenance has been gaining a foothold in the field of cell preservation in recent years - the dry storage. In nature, many plants and animals can enter the state of anhydrobiosis by accumulating disaccharides such as trehalose in their cells to as much as 50% of their dry weight. Following Nature's lead, trehalose is being used these days during the process of freeze-drying *in vitro* as well. Drying of cells can be achieved by either convective-drying or freeze-drying. Freeze-drying (lyophilisation), the more commonly used technique, was known for hundreds of years as a method for meat and vegetable preservation among the people who lived in very high altitudes, like in the Andes mountain dwellers of South America. In more recent times, freeze-drying is used for preparation of food products such as instant coffee, tea and soup, fish food and even ice cream for NASA astronauts. It is also widely used to prepare pharmaceuticals, viral, bacterial, fungal or yeast products in a dry and convenient form for handling, transporting and long-term storage. Freeze-drying is achieved by sublimation of the ice after freezing the sample to subzero temperatures. The process is damaging to the cellular membrane and some degree of chromosomal damage may also take place due to endogenous nucleases.

To date, embryonic development after intracytoplasmic sperm injection (ICSI) with freeze-dried sperm heads has been reported in humans and hamster, cattle, pigs, rhesus macaque and cats, and live offspring were reported in mice, rabbits, rat and fish . We have recently demonstrated the use of sheep freeze-dried somatic cells for somatic cell nuclear transfer. We utilized the directional freezing technology to freeze-dry somatic cells which were kept at room temperature for 3 years. These cells were rehydrated and then used to direct embryonic development following nuclear transfer into *in vitro* matured enucleated oocytes. Finally, human hematopoietic stem cells that were lyophilized and rehydrated with water were viable and have maintained their clonogenic capacity showing that they were able to develop into all blood lineages. This was the first report to show cells that have undergone complete lyophilization and following rehydration have maintained not only their viability but also their functionality.



# Oocyte Cryopreservation: Postnatal Outcome

**Antonio Pellicer, MD**

**OBJECTIVE:** The number of vitrified live births has augmented lately in parallel with the increasing use of this technique. There are few published data on perinatal outcome of these pregnancies. The aim of this study was to compare the obstetric and perinatal outcome in pregnancies and children conceived after oocyte vitrification and standard IVF treatments conducted with fresh oocytes.

**DESIGN:** Retrospective cohort study.

**PATIENTS AND METHODS:** Maternal and neonatal data were recorded for all patients who had delivered babies achieved after undergoing an IVF treatment carried out with vitrified and fresh oocytes. A cohort of 395 pregnancies (516 live births) following oocyte vitrification was compared with another cohort of 390 pregnancies (500 live births) achieved using fresh oocytes (control group). Cases and controls were matched according to the number of fetuses (singleton vs. twins) and the source of oocytes (own vs donated oocytes). A live birth was defined as any pregnancy with at least one live born delivered beyond 25 weeks' gestation. We assessed the incidence of gestational diabetes (GD), gestational hypertension (GH), preterm premature rupture of membranes (PRM) and preterm delivery (PD) in both groups. Birth outcomes were also studied. Comparisons between groups were performed by Student's t-test.

**RESULTS:** Gestational age at delivery was  $38.2 \pm 2.5$  weeks and  $38.0 \pm 2.3$  weeks for vitrified and fresh oocytes groups, respectively ( $p=NS$ ). The mean birth weight was  $2.811 \pm 0.664$  g and  $2.810 \pm 0.661$ g for vitrified and fresh oocytes groups, respectively ( $p=NS$ ). There were 4 major birth defects in the vitrification group (0.7%) and 2 in the control group (0.8%) ( $p=NS$ ). In the ovum donation group singleton pregnancies, the relative risk of suffering PRM was **RR=0.725 (CI95%0.291-1.822 GD: RR=0.998 (CI95%0.561-0.739)**, GH: RR=0.830 (CI95%0.493-1.425) and PD: RR=1.388 (CI95%0.651-2.958 between fresh and vitrified groups (NS). The obstetric outcome for multiple pregnancies derived from ovum donation was as follows: PRM was RR=1.072(CI95%0.480-2.395) GD: RR=0.741 (CI95%0.304-1.807), GH: RR=0.007 (CI95%0.350-0.160) and PD: RR=0.007 (CI95%0.814-3.141) respectively (NS). In the group of singleton pregnancies developed from own oocytes no any patient presented premature delivery risk and none of the patients suffered from gestational diabetes though the relative risk were not calculated. The relative risks of suffering other obstetric outcomes were as follows GH: RR=0.925 (CI95%0.248-3.455) and PD: RR=0.279 (CI95%0.068-1.143) for vitrified and fresh oocytes (NS). In the group of multiple pregnancies developed from own oocytes cycles the obstetric risks were: PRM RR=0.944(CI95%0.055-16.32 GD: RR=0.488 (CI95%0.098-2.433, GH: RR=0.588 (CI95%0.0862- 4.009) and PD: RR=0.375 (CI95%0.078-1.812) respectively (NS). In all cases RR CI95% included 1 and in consequence were not significant. No differences were found ( $p=NS$ ) when analysing separately data for singletons and twins, and ovum donation vs own oocytes cycles.

**CONCLUSIONS:** Obstetric and perinatal outcomes in oocyte vitrification are similar to those achieved using fresh oocytes. These findings are reassuring regarding the safety of the cryopreservation procedure.





# **Advantages and Limitations of Immature Oocyte Cryopreservation**

**Debra Gook, PhD**

For many young women with malignant disease the urgency to commence cytotoxic treatments prohibits the option to undergo ovarian stimulation and collection of mature oocytes for cryopreservation. For these women collection of immature oocytes, whether in the form of ovarian cortex containing preantral follicles or following aspiration of spontaneous antral follicles, provides the only option available at present to preserve fertility. The advantage of collection at any time in the cycle and/or during other operative procedures, together with the reduced cost compared to a stimulation cycle, may seem attractive. However, what is the clinical efficiency of these procedures? The question is whether these technologies, as applied today, provide the patient with a realistic opportunity to achieve a pregnancy or are they just providing hope?

The benchmark for comparison is mature oocyte cryopreservation. Recent advances in mature oocyte cryopreservation have seen equivalent rates of fertilization, embryo development and implantation rates to those of fresh oocytes. Vitrification of mature oocytes in an open system using the method reported first by Katayama [1] results in extremely high survival of oocytes from young women (donated oocytes) and also infertile women. Although survival is lower with controlled rate freezing, under optimal conditions it can produce equivalent outcomes to fresh oocytes.

In-vitro maturation of oocytes from hCG primed ovaries has been practised with little change for over 10 years, primarily for women with ovaries of polycystic morphology but also, more recently, for fertility preservation patients in conjunction with vitrification. Maturation prior to vitrification gives an indication that at least some mature oocytes will be available for clinical use, but subsequent embryo development and implantation rates appear reduced compared to outcomes with mature oocytes from stimulated cycles. Therefore, is it more beneficial for fertility preservation patients to vitrify oocyte cumulus complexes pending improvements in IVM technology?

The potential contained within a small area of ovarian tissue, due to the number of follicles/gametes present, is obviously higher than using other approaches. However, the attrition which occurs due to cryopreservation and post graft ischemia or through follicle culture is extremely high. As with in-vitro maturation, pregnancies have been achieved with ovarian tissue cryopreservation and grafting but embryo quality from aspirated follicles within this tissue also appears to be compromised.

So, should we be more persuasive with oncologists in influencing a delay in therapy to collect mature oocytes for cryopreservation in order to achieve a realistic prospect of fertility preservation?



# **Trend of Fertility Preservation Strategies in Europe**

**Pedro Barri, MD, PhD**

In Europe there is no single network for fertility preservation and the approach has so far been at national level. However all the strategies should be under the guidelines of the European Tissue directives 2004/23/EG and 2006/86/EC.

We will present different situations of different countries, from those who have set up their own national networks to those that work on the basis of individual Centres.

In order to compile this information we have used documents from the ESHRE Special Interest Group on Fertility Preservation, which covers practically all information about Europe, and also from the Spanish Fertility Society, which presents the strategy followed in this country.

We started by analysing the situation of legal cover and access to the techniques in several European countries and then gathered the information through a questionnaire sent to 28 countries. The response from the 23 countries that answered showed that in 6 countries (Germany, Denmark, Bulgaria, Sweden, the Netherlands and Norway) there are national fertility preservation programmes, while in 3 countries (Finland, France, Switzerland) programmes are in preparation. Three countries (Germany, Denmark and Norway) have centralised activity registers.

In Spain, 64 hospitals offer this service but only 22% of the centres offer all of the cryopreservation strategies for semen, oocytes, embryos and ovarian tissue. The remaining centres outsource some activity.

This year in Spain there have been 59 cases of oocyte vitrification, 54 cases of embryo freezing, 23 cases of freezing of ovarian tissue and 649 cases of semen being frozen in order to preserve the fertility of oncology patients.

Probably in this year (2011), a number of national registers will be consolidated in Europe and global figures for activity and results will become available.



# **Fertility Preservation in Breast Cancer Patients: Harvard Experience**

**Elizabeth Ginsburg, MD**

**Objective:** To review the experience with fertility preservation in breast cancer patients at Brigham & Women's Hospital, Harvard Medical School

**Materials and Methods:** A review of the past 2 year experience in our institution and Dana Farber Cancer Institute was undertaken.

**Results:** Women undergoing fertility preservation prior to chemotherapy are very likely to be successful at banking eggs and/ or embryos, however after chemotherapy cancellation rates are high. National data show that fertility counseling does not appear to be occurring adequately in oncology practice.

**Conclusions:** Discussion of impact of cancer treatment is not performed as often as it should be, and referrals for fertility preservation likely lag behind need. Patients with breast cancer who present for fertility preservation prior to chemotherapy have excellent responses to ovulation induction and are highly likely to bank eggs and/ or embryos successfully.

**Support:** None



## **Saturday Morning Abstracts**

- 08:00 – 08:30 **The Future of Translational Research in Fertility Preservation**  
Mary Zelinski, PhD
- 08:30 – 09:00 **DNA Damage / Repair with Ovarian Tissue Cryopreservation**  
S. Samuel Kim, MD
- 09:00 – 09:30 **Ovarian Tissue Transplantation: Danish Model and Outcome**  
Claus Yding Andersen, MSc, DMSc
- 09:30 – 10:00 **Strategies to Improve Post-Transplant Survival of Ovarian Tissue**  
Jacques Donnez, MD, PhD
- 10:30 – 11:00 **Medical Options for Fertility Preservation**  
Dror Meirou, MD
- 11:00 – 11:30 **Risks of Cancer Cell Reintroduction after Ovarian Transplantation**  
Marie-Madeleine Dolmans, MD, PhD
- 11:30 – 12:00 **Potential Challenges of Whole Ovary Transplant**  
Pasquale Patrizio, MD, MBE; Bruno Salle, MD, PhD
- 12:00 – 12:30 **The Future Role of Social Egg Banking**  
Dror Meirou, MD; Pasquale Patrizio, MD





# **The Future of Translational Research in Fertility Preservation**

**Mary B. Zelinski, PhD**

Two theoretical possibilities for preserving female fertility from side-effect damage caused by chemo- or radiotherapy are currently under investigation using the nonhuman primate model for translational research in fertility preservation. First, reducing or eliminating the gametotoxic effects of cancer therapies on the ovary *in vivo* remains challenging. Recently, intraovarian infusion of an agonist of sphingosine-1-phosphate, FTY720, prior to ovarian X-irradiation was shown to protect a cohort of preantral follicles from radiation damage in macaques, allowing birth of live, healthy offspring. Similarly, exposure of human ovarian cortex to anti-apoptotic agents *in vitro* prior to, or *in vivo* during xenotransplantation preserved primordial follicles, thus supporting the feasibility of *in vivo* protection. Other agents, such as tamoxifen and imatinib, inhibit preantral follicle and/or oocyte destruction caused by chemotherapy drugs in rodents. Further translational research will be necessary to identify the most potent fertoprotective agents along with their safety, and to develop clinically acceptable delivery targeted to the ovary. Second, preventing exposure to gametotoxic effects by removing the gametes or ovary prior to therapy, and returning gametes or embryos for fertility after eradicating the cancer is being investigated in the nonhuman primate using *in vitro* follicle culture as well as ovarian cortex cryopreservation/transplantation. It is now possible to grow primate primary and secondary follicles to the antral stage in the encapsulated 3D culture system. Future studies will optimize the protocol to yield more mature oocytes capable of fertilization and embryonic development, determine whether cultured follicles are equivalent in structure and function to those developed *in vivo*, and to understand the basic biology of the primate follicle. Systematic study of vitrification methods using macaque ovarian cortex revealed cryoprotectants that preserve primordial, primary as well as secondary follicles. Macaque follicles isolated from vitrified ovarian tissue can survive, grow, form an antrum and produce steroids in 3D culture, indicating functional preservation of cryopreserved follicles, and the utility of 3D follicle culture as a bioassay to screen cryopreservation methods prior to *in vivo* studies involving tissue transplantation. Future directions include development of novel vitrification protocols compatible with closed system storage, vitrification of individual follicles, improving heterotopic transplantation of ovarian cortex and achieving live offspring from vitrified- thawed tissue via transplantation and/or 3D follicle culture. Research in nonhuman primates will continue to provide an evidence-based foundation for safely producing meiotically and developmentally competent oocytes from fresh or cryopreserved tissue to enhance clinical fertility preservation options for female cancer patients.



# **DNA Damage / Repair with Ovarian Tissue Cryopreservation**

**S. Samuel Kim, MD**

The cancer survival rate has increased dramatically during last two decades. Currently, more than 11 million cancer survivors are living in the US. Unfortunately, aggressive cancer treatment can result in infertility. Loss of fertility after cancer therapy can profoundly impact on quality of life and cause significant distress to young cancer patients. Of note, four percent of the newly diagnosed cancer patients in the US are under age 35.

There are a few options to preserve fertility in young female cancer patients including embryo cryopreservation, oocyte cryopreservation, and ovarian tissue cryopreservation. Ovarian tissue cryopreservation is the only option for pre-pubertal girls and for those who cannot delay cancer treatment. As of October 2011, seventeen babies have been born after transplantation of cryopreserved ovarian tissue.

Cryopreservation of human ovarian tissue by slow freezing technique has been successful since 1994 and is currently considered as a standard method. Although over 70% of primordial follicles survive (morphologically) after slow freezing and rapid thawing, significant ultra-structural damage can be detected by TEM. Many investigators have explored strategies to optimize follicle survival after ovarian tissue cryopreservation. Theoretically, vitrification can be an ideal method as it can eliminate ice formation that is the main cause of cryoinjury. However, high concentration of cryoprotectants and devitrification can be problematic. For the successful pregnancy outcome, the genomic integrity of oocytes subjected to ovarian tissue cryopreservation should be thoroughly investigated.

Therefore, we investigated DNA damage, apoptosis, autophagy after slow freezing and vitrification of bovine ovarian tissue using four biomarkers, H2AX, RAD 51, cPARP, and LC3B. In addition, we assessed DNA damage and apoptosis in ovarian tissue after treatment with four different vitrification solutions (40% DMSO, 40% PROH, 40% EG, VP11) for 10 min and 60 min. We noticed that oocytes within primordial and primary follicles generate an acute DNA repair process in response to DNA damage induced by tissue cryopreservation. Apoptosis of follicles was more severe after vitrification compared to slow freezing. Interestingly, there was no sign of DNA damage and repair even after prolonged exposure to cryoprotectants with high concentration (up to 60 min). However, exposure to these cryoprotectants induced chromatin condensation.

Although benefits and efficacy of vitrification of ovarian tissue should be further investigated, our results of this study show that cellular and biochemical damage to follicles appears to be more severe with vitrification compared to slow freezing technology.



## **Ovarian Tissue Transplantation: Danish Model and Outcome**

**Claus Yding Andersen, MSc, DMSc**

Girls and women suffering from disease that require treatment with gonadotoxic drugs may as a side effect lose the ovarian function. When the ovaries are depleted of follicles many women experience profound effects on the physical and psychological status. Menstrual cycles cease and pregnancies will be unobtainable. To young girls it may further imply that a normal pubertal development fails.

Cryopreservation of ovarian tissue is a new method, which has been developed in an attempt to circumvent the long-term ablative effect on reproductive performance by gonadotoxic treatment. Removing one whole ovary or part of an ovary from women in their reproductive years prior to treatment and cryopreserving the tissue can retain a viable pool of follicles. When the women have been cured and is considered fit, the thawed ovarian tissue may be transplanted to women who entered menopause.

Laboratory of Reproductive Biology at University Hospital of Copenhagen is the only center in Denmark offering cryopreservation of ovarian tissue as a treatment in close collaboration with three fertility clinics round the country. Totally, more than 500 girls and women have had ovarian tissue cryopreserved in Denmark. The youngest girl was half a year old and the oldest 38 years. We have currently cryopreserved ovarian tissue from around 100 girls younger than 18 years of age. The ovarian tissue is excised at the local hospital and transported on ice to our laboratory, where cryopreservation and storage is performed. In case of transplantation the frozen tissue will be transported to the local hospital for the operation. This transport model has been validated and has now been used for more than 250 cases.

In Denmark, a total of 19 women (13 having their tissue transported prior to cryopreservation) have experienced transplantation of frozen/thawed ovarian tissue a total of 26 times (7 women having tissue transplanted twice). All women regained ovarian function and none have experienced relapse as a consequence of the transplantation. Over a period of 20 – 25 weeks levels of FSH gradually return to pre-menopausal levels and menstrual cycles are regained. The longevity of the tissue depends on the age of the woman at tissue retrieval and the amount of tissue transplanted. Most women experience return of ovarian function for several years with just a fraction of tissue from one ovary being replaced. Recently, one child has had ovarian tissue transplanted for natural induction of puberty; this case will be presented in detail.

Seven women have been pregnant; in most cases following natural conception. Two women have delivered three healthy babies as a result of transplanted frozen/thawed ovarian tissue. In the latter two cases the tissue was transported 4–5 hours prior to cryopreservation. The presentation will review our experiences and results with transplantation of cryopreserved ovarian tissue.



# Strategies to Improve Post-Transplant Survival of Ovarian Tissue

**Jacques Donnez, MD, PhD**

The different cryopreservation options available for fertility preservation in cancer patients are embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation.

The only established method of fertility preservation is embryo cryopreservation, but this requires the patient to be of pubertal age, have a partner, and be able to undergo a cycle of ovarian stimulation.

Cryopreservation of ovarian tissue is the only option available for prepubertal girls, and for woman who cannot delay the start of chemotherapy.

More or less 50 cases of orthotopic reimplantation of cryopreserved ovarian tissue have so far been reported and 17 live births have been achieved, yielding a pregnancy rate of more than 25%. In our department, eight women have undergone orthotopic reimplantation of cryopreserved tissue either once or twice. Restoration of ovarian function, proved by follicular development and estradiol secretion, occurred in all cases. A time interval of 3.5 to 5 months was observed.

In order to improve the survival of the graft, angiogenic factors and/or antioxidants could be delivered. Furthermore, antioapoptotic factors could be also administered to improve follicle survival. In animal models, angiogenic factors and antioxidants have been exogenously delivered in the animal or directly in the ovarian tissue prior grafting. Additionally, they can be host-delivered through the induction of granulation tissue. This option has been tested both in humans and animals.

Several antioapoptotic factors, such as kit ligand, vitamin C, growth differentiation factor-9 have been shown to improve survival rate and development of preantral follicles in *in vitro* culture experiments. Such factors could be locally applied in the ovarian tissue before its transplantation.









# **Risks of Cancer Cell Reintroduction after Ovarian Transplantation**

**Marie-Madeleine Dolmans, MD, PhD**

Reversing treatment-related premature ovarian failure using auto-transplantation of frozen-thawed ovarian tissue harvested before chemo-radiotherapy is becoming an increasingly realistic prospect for clinical application, since more than 15 live births have already been reported with this technique. Our objective is to offer young patients at risk of premature ovarian failure after treatment, safe fertility preservation options.

One major concern raised by the transplantation of ovarian cortical fragments in cancer patients is the potential risk that the cryopreserved ovarian tissue might harbor malignant cells that could induce a recurrence of the disease after re-implantation.

Hematological malignancies and breast cancer are the most frequent indications for ovarian tissue cryopreservation. Both carry the risk of ovarian metastasis.

We therefore decided to conduct a study to evaluate the presence of breast cancer cells and leukemic cells in human cryopreserved ovarian tissue from patients with advanced breast cancer disease and chronic myeloid leukemia or acute lymphoblastic leukemia.

In each case, histology, polymerase chain reaction for disease-specific markers and xenografting were used to test the frozen-thawed ovarian tissue. Results show that malignant cells may be present in ovarian tissue from leukemic patients and give rise to tumor development in mice after xenografting (n=5/12, acute leukemia). For the mice grafted with ovarian tissue from patients with advanced breast cancer, PCR and MGB2-gene sequencing were positive on the ovarian tissue of 5 out of 10 patients, but none of the xenografted mice developed tumor masses during the 6-month grafting period.

Although the malignant potential of these cells is not yet known, the current study demonstrates that conventional histology and IHC need to be associated with more sensitive screening methods, like PCR and sequencing, before ovarian tissue transplantation can be contemplated.

Research in this field has to continue, in order to develop different possibilities for fertility preservation that will allow us to propose the most appropriate option to patients, according to disease.



## Potential Challenges of Whole Ovary Transplant

**Pasquale Patrizio, MD, MBE; Bruno Salle, MD, PhD**

Patients with cancer who desire to preserve their future reproductive potential but require immediate gonadotoxic treatments (chemo and/or radiotherapy), are left with few options, all experimental, for fertility preservation. These options include: a) cryopreservation of ovarian tissue as cortical strips; b) dual cryopreservation of both ovarian cortical tissue and preservation, after *in vitro* maturation (IVM), of immature oocytes extracted from small antral follicles visible within the ovarian cortex at the time of the harvest; c) cryopreservation of one whole ovary; d) *in vitro* folliculogenesis.

Ovarian tissue cryopreservation and transplantation as orthotopic allografts has shown reproductive success. Typically, it takes about 4 to 5 months for resumption of endocrine function as evidenced by return of menses or by normalization of FSH and estradiol. However, the re-transplanted cortical pieces only retain ovarian function for short time. One reason is that the amount of cryopreserved/thawed cortical tissue re-transplanted during a graft is limited. Another reason is that the cortical tissue is grafted without a vascular anastomosis and is, therefore, completely dependent for its survival on the development of a new vasculature; a process which requires at least a week. By the time neo-vascularization occurs, the grafts will have already sustained significant ischemic damage resulting in massive loss of primordial follicles, ultimately responsible for the limited functional lifespan of the graft.

The strategy of whole human ovary cryopreservation has a major potential advantage over the cortical strips: it allows for immediate perfusion of the transplanted organ thereby reducing the ischemic damage, thus theoretically resulting in long-term resumption of both ovarian and endocrine function. However, whole ovary cryopreservation may not be a realistic option for many patients due to inherent technical challenges and difficulties. The re-transplantation process requires a very experienced microvascular surgeon due to the small diameter of the ovarian artery (about 0.4 mm). This difficulty is further exacerbated by inadequate length of the vascular pedicle (preferable to be 3 or more centimeters). If the microvascular anastomosis fails, then the whole organ survival is irreversibly compromised, thus preventing a second attempt at transplantation. This is in contrast to failure of transplanted cortical strips, when another attempt can be performed with the remaining, additional frozen cortical strips.

Should attempts at whole ovary cryopreservation be abandoned? A short answer is no, since there are still potential benefits with whole ovary preservation.

An issue that remains unresolved is the handling of ovarian tissue containing metastasis from systemic cancers such as leukemia. In this setting, whole ovary cryopreservation has an advantage over cortical strips. Patients with malignancies at high risk of ovarian metastasis could have an ovary removed, perfused *in vitro* to stimulate folliculogenesis and then frozen for future *in vitro* use. Whole ovary cryopreservation could be a valuable research tool for perfecting *in vitro* folliculogenesis by excising small amounts of cortical tissue, over time, for the *in vitro* experiments.



## **The Future Role of Social Egg Banking**

**Dror Meiorow, MD; Pasquale Patrizio, MD, MBE**

In the last three decades, an increasing trend for women from western countries in delaying child-bearing to a later age has been reported. Demographic studies from both Europe and the United States have shown that the age at first pregnancy as well as the number of pregnancies in women over age 35 has been rising since 1980. The confluence of these two epidemiologic trends has led to the need for better and more widely available strategies for postponing fertility.

Oocytes cryopreservation has recently been proposed for women that wish to postpone their reproductive plans at later age for career or social reasons. The utilization of oocyte preservation in this setting is appealing also from an ethical perspective, allowing maintenance of reproductive autonomy and rights, thus avoiding the stigma of childlessness or resorting to oocytes donation to fulfill the desire of motherhood at a later age.

Our experience on oocyte cryopreservation for social reasons include 27 cycles (in 21 patients) for a total of 231 oocytes (average oocytes per patient 8.5), of which 134 cryopreserved by slow freezing and 97 by vitrification. The median age was 37 (age range 31-42). According to job classification the patients that cryostored oocytes were: 8 businesswomen; 4 M.D.; 2 psychologists; 3 teachers; 1 lawyer; 1 minister; 1 student; and 1 chemist. However, despite increasing reports about the safety (hundreds of documented births and reassuring data on obstetrical and neonatal safety), the 2009 practice committee opinion of the American Society for Reproductive Medicine (ASRM) still considers oocyte cryopreservation an experimental procedure requiring an investigational IRB-approved protocol. However, since experimental procedures cannot be advertised and are not covered by insurance plans, patients are not properly informed of this option and cannot benefit from it.

As laboratory techniques for oocyte cryopreservation continue to improve and evidence of high survival and pregnancy rates comparable to those obtained with fresh oocytes continue to accumulate, it is anticipated that soon ASRM will remove the label of experimental.





## **Saturday Afternoon Abstracts**

15:00 – 15:30 **From Pluripotent Stem Cells to Germ Cells**  
Outi Hovatta, MD

16:00 – 16:30 **Current Status of In Vitro Growth and Maturation**  
Evelyn Telfer, PhD

16:30 – 17:00 **IVM: An Old Technology in a New Era?**  
Johan Smitz, MD, PhD

17:00 – 17:30 **Mechanism of DNA Damage with Chemotherapy / Radiotherapy**  
David Albertini, PhD

17:30 – 18:00 **Non-Traditional Animal Models for Advancing Fertility Preservation  
Studies in Humans**  
Pierre Comizzoli, PhD



## **From Pluripotent Stem Cells to Germ Cells**

**Outi Hovatta, MD**

Obtaining gametes using pluripotent stem cells as a source would be highly desired. The process would give us a lot of new information regarding gametogenesis, particularly in human where the early stages of this process can only be reached in vitro. There is a lack of human oocytes for research, for somatic cell nuclear transfer experiment, and for clinical purposes. Sperm from stem cell would add our knowledge on human spermatogenesis and if successful from autologous induced pluripotent stem cells in a clinically safe manner, possibly in treatment of couples with an azoospermic male partner.

Oocytes able to parthenogenetic activation and blastocyst formation in vitro have been obtained from mouse embryonic stem cells (mESC). Oocyte-like cells from other cells types, such as stem cells in skin have been reported, but no functionality of such morphologically oocyte-like cells has been proven. Fertilization would be the first real functional proof. Postmeiotic male germ cells have been obtained in embryoid bodies from mouse and human embryonic stem cells. These cell have at highest been spermatid-like, with no functionality by fertilization proven.

Improvements in these cultures are underway. We are testing three-dimensional cultures in which we get several testicular cell types from human embryonic stem cell lines in media used for testicular cells.

Induced pluripotent stem cells (iPSC) can be obtained from several human adult cell types. We have established them from skin fibroblasts using non-replicating lentiviral vectors with the Cre-Lox recombination that allows their removal from the cells. We also get these cells using non-integrating Sendai viruses. Together with our collaborative partners in Stanford University we differentiated iPSC starting in embryoid bodies. We selected VASA-positive cells, and continued differentiating up to postmeiotic germ cells. Synaptonemal complex 3 positive cells were obtained, showing that skin-derived iPSC can differentiate to germ cell lineage and undergo meiosis. More research is needed to get mature germ cell. Possible clinical use of iPSC-differentiated cells is a long way ahead.



## Current Status of In Vitro Growth and Maturation

**Evelyn E. Telfer, PhD**

The ability to develop human oocytes from the earliest follicular stages through to maturation and fertilisation *in vitro* would revolutionise fertility preservation practice. This has been achieved in mouse where *in vitro* grown (IVG) oocytes from primordial follicles have resulted in the production of live offspring. However, developing IVG systems to support complete development of human oocytes has been more difficult because of differences in scale of timing and size. Successes in growing human *oocytes in vitro* are being made in a step wise manner and the challenge now is to obtain complete oocyte development *in vitro*.

Our lab has been working on a multi-step culture system to support growth and development of bovine and human oocytes from primordial through to fully grown using fresh and cryopreserved ovarian cortical tissue. Our recent work has shown that human and bovine primordial follicles can be activated *in vitro* within ovarian cortical pieces and grow to multilaminar preantral (secondary) stages within 6 days (Step 1). These preantral follicles can be isolated and have the potential to grow to the antral stage (Step 2) within a total culture period of 10 days. A further step that involves growing oocyte-granulosa cell complexes on collagen membranes (step 3) results in fully grown oocytes which can be placed into maturation medium for IVM. This multi-step approach makes the complete *in vitro* development of oocytes from human tissue a practical and viable prospect. This presentation will focus on the approaches being taken to optimise IVG of human oocytes and strategies for assessment of subsequent growth rate and competence of IVG oocytes will be discussed.



## **IVM: An Old Technology in a New Era?**

**Johan Smitz, MD, PhD**

Women with a normal antral follicle count can yield immature oocytes after a short-course HP-hMG stimulation with maximally 450 IU as a total dose. Improvements of the clinical and laboratory aspects of IVM treatment should increase the implantation potential of IVM-derived embryos as these are still suboptimal. Outcomes between different centers are still very variable, which is due to differences in IVM cycle treatment (stimulation with gonadotrophines or not, hCG triggering or not, variable lab procedures, mixed transfers).

According to some authors, Human chorionic gonadotrophin (hCG) priming before immature oocyte retrieval in patients with PCOS would lead to an increased maturation rate of the collected oocytes, nonetheless prospective studies on the effect of hCG-priming in IVM treated women with PCOS are still insufficient to conclude. Giving hCG-priming induces also recovery of in vivo matured oocytes, which are than confounding the real result from the IVM procedure. HCG triggers meiotic reinitiation and leads to the interruption of communication within the oocyte-cumulus complex (OCC) and might therefore compromise subsequent oocyte and embryonic developmental potential. The heterogeneity of oocyte maturation stages at oocyte retrieval after an HCG bolus results in differential fertilization schedules interfering with regular working schemes in the IVF laboratory leading to dyssynchronous developmental stages complicating the embryo transfer and cryopreservation procedures.

High survival rates of vitrified IVM-derived embryos have been reported enhancing the outcomes after IVM treatment. The presentation will discuss the IVM results derived exclusively from GV oocytes with a compacted cumulus cell mass, retrieved from very small antral follicles <10 mm without hCG trigger.





# **Mechanism of DNA Damage with Chemotherapy / Radiotherapy**

**David Albertini, PhD**

Non-cancerous somatic cells deploy cell cycle checkpoints to correct abnormalities in chromosome balance or DNA structure. While oocytes draw upon elements of a DNA damage repair response (DDR) in resolving homologous recombination events during meiotic prophase, their ability to sustain the DDR throughout the course of oogenesis and into embryogenesis has not been thoroughly investigated. This lecture will review new findings on the expression and localization of DDR components during the growth and maturative stages of oogenesis in a variety of mammals under both normal conditions of follicular development and in response to DNA damage induced by gamma radiation or chemotherapeutic agents. Our results suggest that a constitutive DDR pathway exists in mammalian oocytes that can be upregulated under conditions of extreme genotoxic stress. The implications of these findings with respect to maternal aging and cancer survivorship will be discussed.



# **Non-Traditional Animal Models for Advancing Fertility Preservation Studies in Humans**

**Pierre Comizzoli, PhD**

Sustaining viable populations of any wildlife species requires a combination of adequate habitat protection as well as a good understanding of environmental and biological factors (including reproductive mechanisms) that ensure species survival. Thousands of species are under threat of extinction due to habitat loss/degradation, over-exploitation, pollution, disease, alien species invasions and urban sprawl. This has served as incentive for intensive management of animal populations, both ex situ (in captivity) and in situ (living in their natural habitat). Assisted reproductive technologies developed for addressing human infertility and enhancing livestock production have shown encouraging promise for wildlife species. However, species-specific physiological variations and a lack of fundamental knowledge have limited how these tools can be used to help rapidly re-build sustainable populations of endangered species. Despite limitations, there is enormous potential in applying human-related fertility preservation strategies to wild animals, especially approaches that could assist managing or 'rescuing' the genomes of genetically valuable individuals. Indeed, one of the highest priorities in wildlife ex situ management is sustaining all existing genetic diversity to (1) preserve heterozygosity to avoid inbreeding depression and (2) ensure species integrity and the persistence of genomic adaptability to environmental changes. There are specific components of the rapidly emerging field of fertility preservation in men and women that are highly compatible with preserving valuable genomes of individuals or populations of threatened wildlife. Besides the more 'classical' approaches focusing on sperm and oocyte freezing, strategies associated with gonadal tissue cryopreservation and in vitro culture are especially attractive for better protecting and extending fertility for rare and endangered individuals. Likewise, lessons learned over the last decades in wildlife reproductive biology (either from wild or captive populations) are highly relevant to the advancement of human health and fertility. Additionally, studies conducted at the molecular or cellular level always are related to physiological investigations in wild individuals or entire populations and take into account the interactions with the environment. The substantial amount of scholarly knowledge generated by multispecies and comparative approaches therefore is critical to better understand and mitigate complex issues affecting human beings (fertility, contraception, impact of the environmental changes). Comparative approaches in fertility preservation could benefit to the intensive and practical management of gene diversity in endangered species and lead to translational tools for human reproductive medicine.



## **Overview:**

Fertility preservation is a substantial quality of life issue for young cancer survivors. As a consequence, the demand for fertility preservation has dramatically increased. The aim of the Congress is to update current scientific and clinical development of fertility preservation strategies. The Congress will not only propagate the current knowledge but also provide an opportunity for networking.

## **Target Audience:**

This congress is designed for reproductive endocrinologists, hematology-oncologists, gynecologic oncologists, psychologists, basic scientists, clinical researchers, oncology nurses, REI nurses, oncology social workers and others interested in fertility preservation.

## **Certificates:**

- Certificates of Attendance are in your registration packets.\*
- The top white copy is your official "Certificate of Attendance" to keep for your records.
- You must turn in the yellow copy of your certificate to the registration desk.
- You will not be sent any other type of certificate after the program.

\* On-site or A/R Registrations: Certificates will not be issued to those participants whose registration fees are paid on site, or to those whose registration fees have not yet been paid. A "Verification of Attendance" form will be in your registration packet instead. Complete this form and turn it in to the registration desk. A certificate will be sent to you once payment is received and verified at the KUMC Continuing Education office.

## **Objectives:**

**At the completion of this symposium, participants should be able to:**

- Educate medical professionals to facilitate FP referrals.
- Define the ethical and legal issues related to FP.
- Explain the impact of cancer treatment on fertility and reproduction.
- Evaluate the current status of gamete cryopreservation.
- Assess the strategies and values of emerging technology in ovarian transplantation.
- Recognize the basic physiology and effects of cryopreservation.
- Analyze physiology and current techniques of folliculogenesis and IVM.
- Identify new fertility preservation technologies.

## **Accreditation:**

All participants are required to sign attendance rosters at the beginning of each day. A certificate of completion will be provided to all activity participants based on documentation of actual attendance time, meeting minimum attendance requirements specific to the activity and payment in full. If you are not paid in full, your certificate will be mailed to you upon receipt of payment.

### ***Nurses:***

Up to 15.5 contact hours will be awarded to all individuals based on documentation of actual attendance time, meeting minimum attendance requirements specific to the activity, and payment in full.

University of Kansas School of Nursing is accredited as a provider of continuing nursing education by the American Nurses Credentialing Center's Commission on Accreditation.

Accredited status does not imply endorsement by the provider or ANCC of any commercial products displayed in conjunction with this activity.

### ***Physicians:***

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the KU Medical Center Office of Continuing Medical Education and International Society for Fertility Preservation. The KU Medical Center Office of Continuing Medical Education is accredited by the ACCME to provide continuing medical education for physicians.

The KU Medical Center Office of Continuing Medical Education designates this live activity for a maximum of 15.5 *AMA PRA Category 1 Credits*<sup>™</sup>. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

## **Scientific Chair:**

**S. Samuel Kim**, MD, President, International Society for Fertility Preservation; Director, Reproductive Endocrinology, University of Kansas Medical Center, Kansas City, Kan., USA

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## Faculty and Planning Committee Disclosure Information

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Marie-Madeleine Dolmans, MD, PhD  
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**Amir Aray**, DVM, PhD –He has received salary from Coredynamics for his role as CEO.  
**Giuseppe DelPriore**, MD, MPH- He has received honorarium from Precision Therapeutic for his role as a speaker, and from Covidien and Boehringer Ingelheim for his participation on the advisory council.  
**Leslie Schover**, MD, PhD- She stands to receive potential royalties from NCI for her role as Co-Principle Investigator.



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Tomasso Falcone, MD

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**S. Samuel Kim, MD-** He has received royalties from Cambridge University Press for his role as an editor.

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**Pasquale Patrizio, MD, MBE-** He has received honorarium from EMD Serono for his role on the Speakers Bureau.

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Nathalie Rives, PhD

**A disclosure announcement will either be placed by the sign in sheets at the registration table and/or the moderator will make an announcement from the podium regarding her disclosure status.**

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- Clinical content was validated by a review of the activity for fair balance and bias, appropriate patient treatment recommendations, and whether scientific studies cited in the activity conform to standards accepted by the scientific community.
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