FERTILITY PRESERVATION UPDATE: CONSENSUS MEETING

June, 6th-7th, 2011

Museu Colet
c/ Buenos Aires, 56
Barcelona

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Introduction

Chemotherapy and radiotherapy intended for oncologic and autoimmune disease treatments have often dramatic consequences for fertility, both in men and women.

Fertility preservation is an emerging field in assisted reproduction as it provides possible solutions for cancer survivors and those affected by diseases whose treatment impairs fertility. Fertility preservation for social reasons is also becoming an option for women that want to postpone motherhood.

Fertility preservation has to be provided to young individuals at risk of threatened fertility. This includes the preservation of ovarian and testicular tissue, oocytes and sperm as well as embryos, depending on the age and the status of the affected individual. Reproductive medicine and oncology specialists must share their knowledge and expertise in order to provide the best option for the patients.

The goal of the meeting is to offer an updated review of fertility preservation techniques that are currently available and those that are still experimental. The program involves a panel of international experts in the field that will discuss about the state of the art of Fertility Preservation and establish the current recommendations for efficient treatment.

The final aim of this meeting is to draw a consensus document on Fertility Preservation including an update on medical and social indications, techniques available and results obtained. A set of recommendations will be put in place for fertility and oncology specialists.

Program

Monday, June 6th

9:15 **Welcome**

SESSION 1

Chairperson: M. Boada (Barcelona, Spain)

9:30 Cancer prevalence in children and young adults
O. Cruz (Barcelona, Spain)

10:00 Effects of chemotherapy and radiotherapy on the ovary
D. Meirow (Tel Hashomer, Israel)

10:30 Effect of chemotherapy and radiotherapy on the testis
C. Ortega (Brussels, Belgium)

11:00 Discussion & Conclusions

11:30 Coffee break

SESSION 2

Chairperson: J. A. García Velasco (Madrid, Spain)

12:00 Fertility preservation options in children and adolescents
H. Wallace (Edinburgh, United Kingdom)

12:30 Oncologic and medical fertility preservation in adult women
P. N. Barri (Barcelona, Spain)

13:00 The use of GnRH agonists and oral contraceptives for protection against chemotherapy
Y. Englert (Brussels, Belgium)

13:30 Discussion & Conclusions

14:00 Lunch

SESSION 3

Chairperson: M. Devesa (Barcelona, Spain)

15:00 Ovarian tissue obtention and transport prior to cryopreservation
C. Y. Andersen (Copenhagen, Denmark)

15:30 Whole ovary and ovarian tissue cryopreservation
B. Martínez (Madrid, Spain)

16:00 Heterotopic vs orthotopic ovarian autotransplantation
J. Donnez (Brussels, Belgium)

16:30 Discussion & Conclusions

17:15 Bus to PRBB and CRM[B], visit and cocktail

Tuesday, June 7th

SESSION 4

Chairperson: F. Martinez (Barcelona, Spain)

09:00 In vivo and in vitro folliculogenesis
H. Picton (Leeds, United Kingdom)

09:30 Ovarian stimulation for fertility preservation
J. A. García Velasco (Madrid, Spain)

10:00 Use of in vitro maturation for fertility preservation
G. Arroyo (Barcelona, Spain)

10:30 Discussion & Conclusions

11:00 Coffee break

SESSION 5

Chairperson: A. Veiga (Barcelona, Spain)

11:30 Oocyte cryopreservation: slow freezing and vitrification
L. Rienzi (Rome, Italy)

12:00 Sperm and testicular tissue cryopreservation
J. A. Castilla (Granada, Spain)

12:30 Potential for stem cell renewal of function in ovaries and testes
R. Vassena (Barcelona, Spain)

13:00 Discussion & Conclusions

13:30 Lunch

SESSION 6

Chairperson: P.N. Barri (Barcelona, Spain)

15:00 Psychological aspects of fertility preservation
D. Guerra (Barcelona, Spain)

15:30 Ethical aspects of fertility preservation in patients affected by severe disease
F. Shenfield (London, UK)

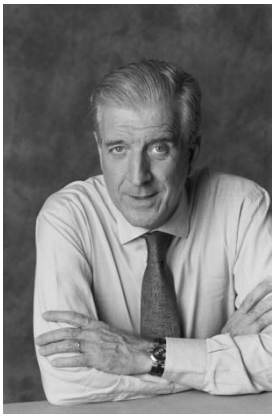
16:00 Is there a place for ovarian tissue and oocyte cryopreservation for social reasons?
G. Pennings (Gent, Belgium)

16:30 Discussion & Conclusions

Program

Monday, 6th		Tuesday 7th	
9:00	Registration		
9:15	Welcome	9:00	Helen Picton
9:30	Ofelia Cruz	9:30	Juan Antonio García
10:00	Dror Meirow	10:00	Gemma Arroyo
10:30	Carolina Ortega	10:30	Discussion & Conclusions
11:00	Discussion & Conclusions	11:00	Coffee break
11:30	Coffee break	11:30	Laura Rienzi
12:00	Hamish Wallace	12:00	José Antonio Castilla
12:30	Pedro N. Barri	12:30	Rita Vassena
13:00	Yvon Englert	13:00	Discussion & Conclusions
13:30	Discussion & Conclusions	13:30	Lunch
14:00	Lunch		
15:00	Claus Yding Andersen	15:00	Diana Guerra
15:30	Belen Martínez	15:30	Françoise Shenfield
16:00	Jacques Donnez	16:00	Guido Pennings
16:30	Discussion & Conclusions	16:30	Discussion & Conclusions
17:15	Bus to PRBB	17:00	End of the meeting
18:00	Presentation and visit to PRBB		
19:00	Presentation and visit to CMR[B]		
19:30	Cocktail at PRBB terrace		
20:30	Bus to IRLA Hotel		

Scientific organizers



Pedro N. Barri PhD He graduated from the Faculty of Medicine in Barcelona in 1971. He had his training in Obstetrics and Gynecology was in Barcelona and in France and England. He received his doctorate in 1993 writing the thesis "Respuesta Anómala a la Estimulación de la Maduración Folicular en Fecundación In Vitro" with qualification Cum Laude. In December, 2003 he is nominated A Corresponding Academician of the Reial Acadèmia of Medicine of Catalonia. He is an Active Founding Member of the Faculty Staff of the Institut Universitari Dexeus in Barcelona. At present, he is the Director of the Department of Obstetrics, Gynaecology and Reproduction of the same institution. From January, 2011 he is The Director of the Chair of Investigation in Obstetrics and Gynaecology of the Institut Universitari Dexeus of the Universitat Autònoma de Barcelona. Dr. Pedro N. Barri is Honorary President of the Spanish Fertility Society, Honorary member of the Argentina Society of Sterility and Fertility, has been a member of the Executive Committee of the International Society of Gynecological Endocrinology since 1998, he is also member of the French Society of Sterility and member of the American Society of Reproductive Medicine. He is a member of the Editorial Board of different journals. Dr. Barri has been Ad Hoc Reviewer of the journals “Human Reproduction”, “Fertility and Sterility” and “Gynecological Endocrinology” during the period of 1990-1992-1994-1996-1998-2000-2005.He has published 102 articles and he has presented 283 lectures and 172 scientific conferences and has participated as guest professor in many national and international congresses.

Anna Veiga PhD. Graduated in Biology (1974-1979). Ph.D. in Biology (Cum Laude). Universitat Autònoma de Barcelona 1991. IVF laboratory Director (1982-2004), Scientific Director of Servei de Medicina de la Reproducción, Dpto Obstetricia y Ginecología, Institut Universitari Dexeus (2005). Coordinator of the Msc. Master course on Reproductive Biology and ART, Universitat Autónoma de Barcelona since 1998. Associate professor of Departament de Ciències Experimentals i de la Vida, Universitat Pompeu Fabra since 2002. Director of the Barcelona Stem Cell Bank. Centre for Regenerative Medicine in Barcelona. since 2005. Chairman Elect of the European Society for Human Reproduction and Embryology (ESHRE) since 2009. Founder and President of the Spanish Embryologist Society (ASEBIR) (1993 – 2003) Member of the Alpha Scientists in Reproductive Medicine Executive committee (1998-2002). Member of the Executive committee of the European Society for Human Reproduction and Embryology (ESHRE) (2001- 2005). Chairman of the Special Interest Group in Stem Cells. Her main areas of interest are Clinical Embryology, Reproductive Genetics, Embryonic Stem Cell research and Bioethics.

Buenaventura Coroleu PhD. Graduate in Medicine in 1982 at Universitat de Barcelona. Especialist in Gynaecology and Obstetrics. He became a Doctor of Medicine in 2003 with the thesis "Ecography as a method of optimazation in embryonic transfer after in vitro fertilization".Active member of the Medical Corp of the Dexeus University Institute. At present he is Head of the Reproductive Service at the Department of Obstetrics, Gynaecology and Reproduction at the Dexeus University Institute.Dr. Buenaventura Coroleu is member of the scientific societies: European Society of Human Reproduction and Embryology (ESHRE); Spanish Society of Gynaecology and Obstetrics (SEGO); Spanish Society of Fertility (SEF) and Catalan Society of Obstretics and Gynaecology (SCOG). Chairman of the Spanish Society of Fertility from 2006 to 2010. He was also a member of the National Comission of Assisted Human Reproduction from the Department of Health from 2006 to 2010. Currently honorary chairman of the Spanish Society of Fertility. He has participated as a guest professor at more than 60 Symposiums, Meetings and National Congresses and at more than 20 International Symposiums, Congresses and has participated in the publication of more than 60 articles in national magazines and more than 60 articles in international high impact magazines.

Rosa Tur PhD Graduated in Medicine from Hospital Clinic University in Barcelona in 1977. Specialized in Gynaecology and Obstetrics in 1980. She got her PhD in 1994 with a thesis on “Multiple pregnancies in assisted reproduction: analysis of possible risk factors”, with an A mark Cum Laude and Distinction Chief of Reproduction Endocrinology Area of Obstetrics, Gynaecology and Reproduction Department of Institut Universitari Dexeus. At present she holds the office of Coordinator of Reproductive Endocrinology in the Spanish fertility Society. Her areas of interest are: reproductive endocrinology, multiple pregnancy prevention, fertility preservation, age and reproduction.

Montserrat Boada BSc in Fundamental Biology (1984) and PhD cum laude in Cell Biology (1992) at Universidad Autónoma de Barcelona ART laboratories director. I.U. Dexeus of Barcelona, since 2004. Member of the Advisory Commission for ART of the Catalan Government since 1993 and of the Spanish Government (1997- 2007). Member of the Advisory Committee on Bioethics of Catalonia since 2005. Member of the Advisory Committee of ESHRE (1998-2000 and since 2008). Areas of interest: PGD, fertility cryopreservation, ethics & law aspects of ART, Quality control.

Invited speakers

Monday, June 6th

Ofelia Cruz is assistant Professor of Pediatrics both at graduate and undergraduate level, at Hospital St Joan de Deu, Universitat de Barcelona since 1994. She received her degree in medicine from the University of Barcelona in 1984, and her doctorate (PhD) from the University of Barcelona in 1992. Her professional expertise is in the field of pediatric oncology and pediatric neuro-oncology. She is currently senior attendant physician in the Pediatric Oncology department at Hospital St Joan de Déu. She is the director of a master course in pediatric oncology at the Hospital St Joan de Deu/ University of Barcelona She has authored or co-authored journal or book publications in the field of pediatric oncology. She has co-directed a Medical dissertation on "Puberal development and gonadal damage in children after oncological treatment" (I. Martin PI, Universitat de Barcelona). She is member of the national and international pediatric oncology societies.

Cancer prevalence in children and young adults
Ofelia Cruz
Hospital St Joan de Déu, Universitat de Barcelona, Spain

Each year approximately 950 children are diagnosed with cancer in Spain, and 15,100 children and adolescents younger than 20 years in the United States. Approximately 1 to 2 children in every 10,000 children aged 14 years and younger diagnosed with cancer each year. These numbers match to an average annual incidence rate of 18.8 cases per 100,000 person-years for all cancers in patients younger than 20 years. The likelihood of a young person reaching adulthood and being diagnosed with cancer during childhood is approximately 1 in 300 for males and 1 in 333 for females. Childhood cancer comprises a group of uncommon diseases, different from their adult's counterparts. In order of frequency they are diagnosed with acute leukemias, CNS neoplasias, malignant lymphomas, neuroblastic tumors, soft tissue and bone sarcomas and Wilms tumor. Despite the rarity of childhood cancer, with a much less incidence than adult neoplasias, it has a tremendous familial and social impact on the affected, and is an important concern for public health, medical care and society. It is the main cause of disease related death among children 1 to 14 years of age.; on the other hand, survival rates have improved dramatically forma n estimation of 28% in the 1960s to current 5-year survival rates nearing 80% for children and adolescents. Owing to improvements in survival, increasing numbers of survivors are at risk of health problems. Childhood Cancer Survival Study investigations have played an important role in characterizing long-term morbidity and mortality associated with childhood cancer treatment. As a whole, chronic health conditions, functional impairment, activity limitations, and psychosocial dysfunction are reported more commonly by childhood cancer survivors compared with siblings or age-matched population controls. Sexual Development Issues of pubertal development, fertility, and pregnancy become significant concerns for survivors of childhood cancer as they mature. Alkylating chemotherapy and Radiotherapy to the gonads may cause gonadal dysfunction manifesting as delayed or arrested puberty, ovarian failure, or male/female infertility. The risk of injury is related to the cumulative dose of these modalities and combinations including alkylating agents and abdomino-pelvic radiation.

Dror Meirow Professor Dror Meirow is the head of fertility preservation research and clinical center at the Sheba medical center Israel. He is a Professor at the Sakler School of Medicine Tel-Aviv University. He received his MD from the Hebrew University Jerusalem Israel in 1983. He then specialized in Obstetrics and Gynecology at Hadassah University hospital Jerusalem. Since 2001 he is the head of fertility preservation program at the Haim-Sheba hospital Israel and the head of the Gynecology service for breast cancer patients. At 2008 Prof. Meirow established a research center specialized in ovarian research and molecular biology. His basic and clinical research is focused on the effects of chemotherapy on reproduction and modalities for fertility preservation in cancer patients; in particular ovarian tissue cryopreservation, IVF and medications that can prevent chemotherapy induced ovarian damage. In the field of fertility preservation he had contributed dozens of original articles, reviews and book chapters.

Chemotherapy effects on female reproduction and the success in preserving fertility using stored ovarian tissue

Dror Meirow
Fertility Preservation, Division of Obstetrics and Gynecology, Sheba Medical Center, Tel Hashomer, Israel

Chemotherapy and radiation treatment in young female cancer patients can often result in ovarian damage leading to premature ovarian failure and subsequent infertility. The extent of damage and future outcome are related to patient's age at the time of chemotherapy administration and previous exposure to chemotherapy protocols- both representing ovarian reserve at treatment. The acute damage caused by chemotherapy is related to the family of drugs administrated, drug doses and protocol of administration. The precise mechanism of ovarian damage is multifactorial, however in many cases a loss of the primordial follicle population has been implicated. This loss of follicular stockpiles has been shown to occur through several mechanisms, including: follicular apoptosis, blood vessel damage, and increased follicle activation that may result in the "burn-out" of primordial follicle reserves. In order to develop effective fertility preservation strategies it is essential to identify the exact mechanisms of chemotherapy-induced ovarian injury. This can enable in the future development of novel protective agents that could avoid this loss of primordial follicles. Currently we use a few methods in order to preserve fertility in order to prevent chemotherapy induced sterility and it is crucial that physicians will be familiar with all existing methods in order to advise patients individually about fertility preservation options.

Carolina Ortega Hrepich obtained her MD degree at the Universidad de la Sabana in Colombia (2001), graduated with honours her specialization in Obstetrics and Gynaecology at the Universidad de Los Andes in Chile (2006), and her subspecialisation in Reproductive Medicine (2008) at the Universidad del Desarrollo in Chile. She is author of different chapter books and publications in male infertility. Currently, she is working as Clinical visiting Fellow at the Universitair Ziekenhuis Brussel in Belgium.

Effect of chemotherapy and radiotherapy on the testis

Carolina Ortega
Centre for Reproductive Medicine, Universitair Ziekenhuis Brussels, Belgium

Currently, anti-cancer treatments are becoming highly effective to cure various cancers in men in their reproductive age. However, during cancer treatment different systems are affected and one of the mayor concerns is infertility related to either the malignancy itself or more frequently, the treatment. The testis is highly susceptible to the toxic effects of radiation and chemotherapy at all ages of life including the prepubertal period. Depending on the dose and type of drug used, chemo-radiotherapy can produce gonadal toxicity resulting in temporary oligozoospermia to permanent azoospermia. The testis has both an endocrine and exocrine function. The endocrine output depends on an adequate Leydig cell function. For the exocrine function, i.e; spermatogenesis, both a functional Sertoli cell and spermatogonial stem cell pool are necessary. Spermatogonial stem cells are undifferentiated cells, that give rise to the spermatogenic cells and, finally, the spermatozoa. Leydig cells are rather resistant to chemo- and radiotherapy. However, they seem more vulnerable to chemo- and radiotherapy in pre-pubertal life. While elevated LH indicates Leydig cell dysfunction, most men will show normal testosterone levels and will retain a normal bone density. Sertoli cells show a good resistance to chemo- and radiotherapy, except in the postnatal and pubertal period when their numbers are increasing through mitosis. Increases in FSH are due to indirect effects through loss of the germ cell pool. The extent of the gonadotoxicity on this germ cell is strongly related to the nature of the specific agents that are used and their dose or to the intensity of the radiation and the place of the body where it was administered. Transient reductions of sperm count can occur even after mild forms of chemotherapy or low doses of gonadal radiation, due to the destruction of the sensitive differentiating spermatogonia. Stronger chemotherapeutic regimes or higher doses of gonadal irradiation, however, lead to prolonged reduction in sperm count or complete azoospermia. Whether sperm production will eventually recover depends on the survival of the spermatogonial stem cells and the integrity of their ability to differentiate. High doses of radiation to the testis (>2.5 Gy) cause DNA damage and cell death. The most damaging chemotherapeutic agents in the adult man are the alkylating agents. Even though the gonadotoxic

effect of most anti-cancer treatments is known, it is difficult to predict the final effect on the fertility potential of the patient. There remain important interindividual differences in response to the treatment and even if a regimen with low gonadotoxicity is started, it is possible that eventually a more gonadotoxic treatment has to be administered because of earlier treatment failure. It has been reported that the prepubertal testis, assumed to be in a quiescent stage, is less sensitive to the gonadotoxic effects of chemo- and radiotherapy, recent findings have show that even in prepubertal life, the seminiferous tubules are mitotically active. Therefore even in prepubertal boys, fertility preservation is an important issue. Fertility preservation in prepubertal boys lies within the preservation of their spermatogonial stem cells. Three options for fertility preservation through the application of spermatogonial stem cells are currently under research: the spermatogonial stem cell transplantation, the grafting of testicular tissue pieces and the in vitro proliferation and/or maturation of spermatogonia. Each technique has of course its (dis)advantages, but each one of them might eventually find its way to the clinic. For post pubertal boys, sperm banking should be offered prior anti-cancer treatment starts. All the patients should have a psychological support. Patients undergoing anti-cancer treatment must be informed about their future fertility status and oncologist must discuss at diagnosis the different options available to preserve fertility. If the patient is a child, this information should be given to the parents or legal guardians.

W. Hamish Wallace Consultant Paediatric Oncologist, RHSC, Edinburgh since 1992. Reader in the Department of Reproductive and Developmental Sciences at the University of Edinburgh. St George's Hospital Medical School in 1980. Trained in paediatric oncology at Great Ormond Street, Birmingham, Edinburgh. MD.,FRCP., FRCPCH.,FRCS He became a Leukaemia Research Fund Research Fellow under the guidance of Professor Steve Shalet in Manchester in the late 1980s when he developed his research interest in the late endocrine effects of the treatment of childhood cancer and completed my MD thesis. His main research interest is in fertility prediction and preservation for cancer patients. Chair of the CCLG Hodgkin's Lymphoma Working Group (since 2002) and President of the European Network for Paediatric Hodgkin's Lymphoma (since 2007). Directs the HEBA Research Centre in Edinburgh which is committed to ongoing research into the effects of the treatment of childhood cancer on fertility, bone health and the vascular endothelium. Co-author of over 110 peer reviewed publications, and four books. Most recently co-edited with Prof Dan Green a multi-author book on "Late effects of the treatment of Childhood Cancer" and with Prof Chris Kelnar a book on "Endocrine complications of the treatment of childhood cancer". Co-founder and organiser for ESLCCC, a biannual conference on the late effects of the treatment of childhood cancer. Appointed Lead Clinician for the Children's and Young Peoples MCN for Cancer in Scotland "CATSCAN" in November 2007. Most recently he has agreed to chair a SIGN guideline on the late complications of the treatment of childhood cancer, which will build on the work of an earlier guideline SIGN 76, which he chaired and was published in 2004.

Fertility Preservation options in children and adolescents

W. Hamish B. Wallace
Department of Reproductive and Developmental Sciences at the University of Edinburgh

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. 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.

Pedro N. Barri *(CV in "Scientific Organizers" section)*

Oncologic and medical fertility preservation in adult women

Pedro N. Barri
Service of Reproductive Medicine. Department of Obstetrics, Gynecology and Reproduction. Institut Universitari Dexeus, Barcelona, Spain

Indications: the indications vary according to the characteristics of the patients, who fall into the following three types:

1) Non-oncological patients: Autoimmune diseases (lupus erythematosus, rheumatoid arthritis etc.); Genetic and/or chromosomal alterations (Turner syndrome, fragile X syndrome, triple X); Non-oncological blood diseases (aplastic anaemia, thalassaemia major, etc.); Patients who need surgical castration because of benign pathology or for prevention of future malignant pathology (Endometriosis, carriers of BRCA 1 and 2 mutations, etc.)
2) Oncological patients. The most frequent cancers in young women aged < 40 years are: Breast; Non-Hodgkin lymphoma; Melanoma; Cervical cancer; Leukaemia; Colorectal cancer
3) Patients who wish to postpone their fertility for social reasons. These are women, usually in their mid-thirties, who either wish to prioritise their professional careers or are not in a stable relationship and who desire to postpone their desire for children and preserve their fertility.

Options: the risk of permanent infertility in a woman after chemo/radiotherapy is variable and depends on the patient's age, her previous fertility and the type of treatment and the dose she receives. The options that can be offered are: Freezing of ovarian tissue; Vitrification of oocytes; Freezing of embryos; Pharmacological suppression of ovarian activity; Ovariopexy; Conservative surgery to preserve future fertility. Our therapeutic strategy must be individual and must be based on the medical and social characteristics of each case. We will present an overview of the various options that are currently available. These will later be presented in depth by the different speakers.

Yvon Englert Professor Yvon Englert is Gynaecologist/Obstetrician and PhD. from the Belgian "Université Libre de Bruxelles" as well as MBA from the French "Ecole des hautes etudes en santé publique" from Rennes. He is head of the Department Gynaecology/Obstetrics - Erasme Hospital and Head of the Laboratory for Research in Human Reproduction, Medicine faculty from the same university. He teaches reproductive medicine and medical Ethics. Trained in Clamart in France in IVF in the early 80', he has two post graduate diplomas from the UER Kremlin Bicetre (Paris) and performed his PhD thesis on the male aspects of IVF. In parallel, he is actively involved in ethical debates and had the honour to be the first chairman of the Belgian National Ethics Committee in 1995, to be member of the European Group of Ethics and of the Belgian Royal Academy for Medical Sciences. He is currently vice-dean of the medicine faculty. Author of more than 280 Abstracts and oral communications at scientific symposia, 200 Publications in scientific journals or books and published 6 scientific books as editor.

The use of GnRH agonists and oral contraceptives for protection against chemotherapy

Yvon Englert
Dept Obstre/Gyn Hospital Erasme and Laboratory for research on human reproduction, medicine faculty, Campus Erasme, Université Libre de Bruxelles, Belgium

Since the primary publication from Ataya et al (1985) describing a protective effect of GnRH against towards chemotherapy-induced gonad al damage in rats, various papers have confirmed the protective effect on ovarian function (and sometime on fertility) in animal models, including non-human primates against ovariotoxic chemotherapy, especially alkylant agents. The quality of design of these non-human data makes them of great interest and will be reviewed. Human literature is much weaker, especially because most publications compare study groups of patients to historical retrospective controls in case control settings. Nevertheless, more than 300 patients received Gnrh agonists in these studies and performed better than controls regarding to ovarian function. Much few prospective randomized studies were published but will be examined. An intermediary analysis of a large ongoing European multicentric prospective randomized study

coordinated by the authors will be commented in details and shows that long term follow up is essential to solve the question. All these studies, randomized as well as case controls, are focused up to now on ovarian function and it will be stressed that menstruating is a necessary but not sufficient criterion for fecundity, the ultimate goal of protection of the ovarian function in young women and children. If GnRH agonist strategy to protect ovarian function is still today a matter of debate, it is not only because of lack of definitive clinical evidence, but also because the classical knowledge of the Fsh independence of primordial follicles, the target to be protected against toxic effect of anti-mitotic drugs.

Claus Yding Andersen is professor in human reproductive physiology at University of Copenhagen, Denmark and has since 2009 been heading Laboratory of Reproductive Biology at the University Hosptial of Copenhagen, Denmark. Claus Yding Andersen qualified first as M.Sc. from the Danish Technical University (1979) and obtained his D.M,Sc from University of Copenhagen in 1997. Since 1986, he has worked at the Laboratory of Reproductive Biology at University Hospital of Copenhagen. His main interests include cryopreservation of gonadal tissue, ovarian endocrinology and human embryonic stem cells. He has published almost 200 scientific papers and has given a large number of international presentations.

Ovarian tissue obtention and transport prior to cryopreservation

Claus Yding Andersen
Laboratory of Reproductive Biology, University Hospital of Copenhagen, Denmark

Girls and women suffering from disease that require treatment with gonadotoxic drugs may as a side effect loose the ovarian function. The gonadotoxic effect is dependant on the specific treatment and is influenced by the dose of the therapeutical agent and the possible use of radiotherapy. Especially, alkylating substances and radiation to the abdomen in case of cancer diseases often cause irreversible damage to the ovaries. When the ovaries are depleted of follicles many women experience profound effects on the physical and psychological status. Menstrual cycles ceases 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 almost 500 girls and women have had ovarian tissue cryopreserved in Denmark. The youngest girl was 0,5 years 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 extracted 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 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 18 women (13 having their tissue transported prior to cryopreser-vation) have experienced transplantation of frozen/thawed ovarian tissue a total of 24 times (6 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 some 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. Six 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 cryopreservation. The presentation will review our experiences and results with transplantation of cryopreserved ovarian tissue.

Belen Martinez-Madrid (DVM, PhD) is currently a Professor in Animal Reproduction at the Department of Animal Medicine and Surgery, School of Veterinary Medicine, Universidad Complutense de Madrid (Spain). She followed her postdoctorate at the Department of Gynaecology, Université Catholique de Louvain (Brussels, Belgium) from 2002 to 2006, under the supervision of Professor Jacques Donnez. In 2008, she was a research visitor at the Department of Reproductive Sciences, Center for Species Survival, Smithsonian Institution (Washington DC, USA). Since 2002, her research work has focuses on fertility preservation by ovarian tissue cryopreservation (whole ovary, ovarian cortex and isolated follicles) mainly on humans, but also on domestic and endangered mammal species.

Whole ovary and ovarian tissue cryopreservation

Belen Martinez-Madrid
Animal Repdorudciton. Department of Animal Medicine and Surgery, School of Veterinary Medicine. Universidad Complutense de Madrid, Spain

Several options are currently available to preserve fertility in cancer patients and allow them to conceive when they have overcome their disease. Cryopreservation of ovarian tissue is the only option available for pre-pubertal girls and women who cannot delay the start of chemotherapy. Two approaches are currently being investigated: cryopreservation of ovarian cortical strips and cryopreservation of whole ovaries.

Cryopreservation of ovarian cortical tissue: the outer cortical layer of the ovary, containing primordial follicles, can be removed via laparoscopy and cut into thick strips to ensure adequate penetration of cryoprotectants. Cryopreservation can be performed using slow-rate freezing or vitrification. The standard method for human ovarian cryopreservation is slow-programmed freezing (1). However, vitrification technique seems to have advantages over slow freezing as it avoids ice crystal formation. Based on recent studies, vitrification is a rapid and simple technique that can be carried out using a clinical grade closed system, compatible with the European tissue directive (2). Ovarian transplantation procedures in humans have so far been exclusively limited to avascular cortical fragments. These small cortical pieces are grafted without vascular anastomosis and are completely dependent on the establishment of neovascularization after grafting. Consequently, the cells in the graft undergo significant ischemic and reperfusion damage, which can induce a high rate of follicular loss. Thus, longevity of the graft might be optimized both by using new vitrification techniques and by promoting rapid revascularization of the graft (3). Two different surgical approaches have been used in humans for transplantation of ovarian cortical tissue, orthotopic and heterotopic, which will be exposed by Professor Donnez. Since the first live birth after autotransplantation of cryopreserved ovarian tissue in humans was reported in 2004, orthotopic reimplantation has led to the birth of 13 healthy babies (4). Despite these very encouraging results, the procedure remains controversial as it does not appear in the official guidelines of the American Society for Reproductive Medicine (ASRM).

Cryopreservation of whole ovaries: the best way to reduce the ischemic interval between transplantation and revascularization is by transplantation of an intact ovary with vascular anastomosis. The challenge of whole ovary cryopreservation and transplantation are both the cryopreservation protocol for an entire organ and the surgical technique for vascular re-anastomosis of ovarian vessels. Recent studies on human whole ovary cryopreservation have shown that perfusion of the organ vasculature with cryoprotectants prior to freeze (5,6) and the use of a multi-gradient freezing device (7) improve tissue survival on ovarian vascular, follicular and stromal compartments, and led to similar rates of follicular viability compared to ovarian cortical strips. Concerning vascular re-transplantation of cryopreserved whole ovaries, in initial animal studies the success has been limited mostly due to thrombotic events. Removing a long segment of the infundibulo-pelvic ligament -approximately 5 cm- along with the ovary (8), careful cannulation of ovarian arteries (6), the choice of the ideal donor and recipient vessels and the use of end-to-end microvascular anastomosis (8) are crucial to the success of the procedure, avoiding damage to the blood vessels walls and thrombotic events. Notwithstanding, the main concern of this strategy is that there is only one chance for every step to go optimally. If dissection, cannulation, perfusion, cryopreservation, thawing or microvascular re-implantation is compromised at any step, the entire organ could be lost. For these reasons, whole ovary cryopreservation and transplantation should be view as an experimental strategy for the time being.

Jacques Donnez Professor Jacques Donnez studied medicine and completed his internship in gynecology and obstetrics at the Université Catholique de Louvain (UCL). His subsequent research career there has been devoted to identifying and understanding the molecular and pathophysiological mechanisms underlying female infertility and the development of new therapeutic options to restore or preserve women's health and fertility. In 1984 he defended his PhD thesis "The Fallopian tube: normal and pathological histophysiology" and then went on to found the Infertility Research Unit at UCL, becoming Head and Chairman of the Department of Gynaecology in 1986. His areas of interest are pelvic surgery, endometriosis and research on ovarian cryopreservation and transplantation. Prof. Jacques Donnez is Doctor Honoris Causa, Sun Yat-Sen University of Medical Sciences, Guangzhou, Canton, China (2000) and he was the President of International Society for Fertility Preservation (2007-2010).

Heterotopic vs orthotopic ovarian autotransplantation

Jacques Donnez
Department of Gynecology, Catholic University of Louvain, Cliniques Universitaires St. Luc, Brussels

Premature ovarian failure (POF) can occur naturally at an early age or be due to iatrogenic agents. Indeed, ovaries are very sensitive to cytotoxic treatment, especially to radiation and alkylating agents. Several options are currently available to preserve fertility in cancer patients and allow them to conceive when they have overcome their disease: embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation. Cryopreservation of ovarian tissue is the only option available for prepubertal girls and women who cannot delay the start of chemotherapy.

Orthotopic reimplantation: Since the first live birth after autotransplantation of cryopreserved ovarian tissue in humans was reported in 2004, orthotopic reimplantation has led to the birth of 15 healthy babies. Restoration of ovarian activity was always observed 4 to 5 ½ months after reimplantation and all pregnancies occurred after orthotopic reimplantation. A few cases of heterotopic transplantation have been described but, so far, only bad quality embryos have been obtained after IVF.

Heterotopic reimplantation: In case of heterotopic ovarian grafts, IVF is mandatory. Oktay et al. reported their results of OPU in 2 patients with implantation beneath the skin of the abdomen. In the first patient, 20 oocytes were retrieved during 8 percutaneous OPUs and only one 4-cell embryo developed but failed to implant (Oktay et al., 2004). The second patient underwent one OPU yielding one MII oocyte that did not fertilize (Oktay et al., 2008). Kim et al. (2008) reported a 27-month follow-up of 2 cancer patients after heterotopic transplantation between the rectus muscle and fascia. Six oocytes were retrieved from the grafts and 4 embryos developed, but we have no information about the total number of OPUs, the empty follicle rate or the quality of the oocytes or embryos. In a comparison of heterotopic and orthotopic sites, it should be pointed out that the rate of MII oocytes obtained after OPU from heterotopic grafts, is significantly lower than that from orthotopic transplants. We believe that ovarian cortex cryopreservation, associated or not with cryopreservation of immature oocytes, should be offered before gonadotoxic chemotherapy to all patients at high risk of POF when emergency IVF is not possible and that orthotopic reimplantation should be considered as the preferred option.

Tuesday, June 7th

Helen Picton Bsc, PhD. Professor. Chair of Reproduction and Early Development, University of Leeds, Prof. Helen Picton is an ovarian biologist by training. She obtained her Bsc and PhD from the University of Edinburgh and is currently based at the University of Leeds where she established and now heads the Division of Reproduction and Early Development within the Leeds Institute of Genetics, Health and Therapeutics. She is the Scientific Director of the Leeds Centre For Reproductive Medicine in the Leeds Teaching Hospitals NHS Trust and the Programme Director for an international MSc in Clinical Embryology run by the University of Leeds. The research portfolio of the Reproduction and Early Development Group includes fundamental investigations of the molecular and cellular biology of oogenesis and folliculogenesis in large animals and humans, the application of these fundamental investigations to studies of the aetiology of female infertility, and the conservation of female fertility. She was the Chair of the Society For Reproduction and Fertility from 2005-2009 and is the current coordinator of the ESHRE Task Force on fertility preservation in severe disease.

In vivo and *in vitro* Folliculogenesis

Helen Picton
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The development of technologies to grow and mature oocytes from the most abundant primordial follicles holds many attractions for research and fertility preservation in humans. Indeed, early staged follicles lend themselves to culture, however the complete in vitro growth (IVG) and in vitro maturation (IVM) of oocytes from primordial follicles can only be achieved on the basis of an in-depth understanding of the biology of follicle and oocyte growth in vivo. Strategies designed to support the IVG and maturation of oocytes and follicles from both fresh and cryopreserved tissues must mimic the sequence of developmental events and cellular checkpoints seen in vivo as oocyte development is reciprocally linked to follicle development.

Despite the demanding biology, several different follicle culture strategies have been developed and significant advances are being made. While the culture of isolated primordial follicles is possible, it is far from optimized and these follicles tend undergo abnormal activation and/or apoptosis as soon as they are put into culture so they rarely survive longer than a few days. In contrast, the growth of primordial follicles in situ either in whole ovaries (mice) or in fragments of cortical tissue (ruminants, primates and humans) has proven to be a relatively effective way of inducing and maintaining long-term primordial follicle growth in vitro. To date the in vitro production of mature, fertile metaphase II oocytes from primordial follicles has only been achieved in mice. In this species the normal follicular growth span from the primordial to Graafian follicle stages is relatively short, the follicles are small and the trophic requirements and the interactions between the follicular cells and oocytes are very well characterised. In contrast, recent progress with the in vitro folliculogenesis in large animals and humans has been marked by the in vitro production of multi-layer preantral follicles from primordial and primary follicles grown in situ within ovarian cortex, followed by antral cavity formation and somatic cell differentiation in isolated, in vitro-derived, preantral-staged follicles. In some, but by no means all culture systems preantral to antral development is supported by encapsulation of individual follicles within biological scaffolds such as alginate gels. The extensive IVG and IVM studies conducted in the Leeds laboratories have shown that three dimensional sheep and human antral follicles can consistently be produced in vitro in serum-free cultures, without the support of biological scaffolds or extracellular matrix and from both fresh and cryopreserved tissue with equal efficiency. These in vitro-derived somatic and germ cells have a similar morphology to oocytes and follicles grown in vivo, as assessed by electron microscopy, and the full sized sheep oocytes produced from sheep preantral follicles over 35-40 days can be induced to undergo nuclear maturation and progression to MII in response to the appropriate physiological stimuli. The stage is now set to incorporate the methodological advances derived from animal studies into a multistage IVG strategy for human follicles. Confirmation of the safety and efficacy of follicle culture strategies remain a priority before in vitro folliculogenesis can be used therapeutically for the restoration of fertility in young patients.

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Ovarian stimulation for fertility preservation
José Antonio García Velasco
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Oncological therapies, both radio and chemotherapy, and specially alkilating agents, are highly gonadotoxic. They will dramatically reduce follicular pool in women and, thus, their fertility potential. Today, there several available alternatives to preserve fertility in those women that requiere it; this will enable in the future achieve a pregnancy in case they are not successful spontaneously.

Learning points: chemotherapy, specially alkilating agents, and radiotherapy affect ovarian reserve, diminishing the chances of spontaneous pregnancy in cancer survivors. There is no way to precisely determine which patient will completely recover her gonadal function, who will be severely impaired, and who will lose her ovarian function. Spontaneous pregnancy after cancer are considered high risk pregnancies and should be referred to highly specialized units. From all fertility preservation options, egg vitrification offers the most efficient results today; it also minimizes both physical as well as moral compromises by avoiding creating embryos to freeze with her partner or unknown donor, and it offers realistic possibilities to achieve a pregnancy after surviving her disease. Up-to-date protocols combining aromatase inhibitors with recFSH and GnRH antagonists yield a reasonable number of oocytes in one cycle with similar serum estradiol levels that those reached in a natural cycle, requiring less than 3 weeks to finalize the egg retrieval.

Gemma Arroyo is licentiate in Biology, by Universitat de Barcelona, and embryologist since 1998 after a master of specialisation on human reproduction in Universitat Autònoma de Barcelona in collaboration with the Institut Universitari Dexeus where she starts working from 1999 until nowadays. Her main interest is focused on oocyte maturation in vitro, oocyte and early embryo morphology. She is member of the Association for the Study of the Reproductive Biology (ASEBIR) and Spanish Society of Fertility (SEF) where she participates actively on the groups of interest on embryo quality.

The use of *In vitro* maturation (IVM) for fertility preservation
Gemma Arroyo
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Cancer management of many common malignancies involves aggressive radiotherapy or chemotherapy. Unfortunately, the agents used for treatment of many types of cancer, even though successful in up to 95% of patients, carry a considerable risk for their fertility potential. Fertility preservation should be considered for all young people undergoing potentially gonadotoxic cancer treatment. Several strategies for fertility

preservation of these patients are available: freezing of oocytes (including cryopreservation of IVM oocytes), or embryos, and cryopreservation of ovarian tissue. Cryopreservation of oocytes or embryos after ovarian stimulation is not always possible in patients that demand fertility preservation. In certain cases, chemotherapy has to be started just after cancer diagnosis, with no time to allow for ovarian stimulation. Furthermore, it has to be taken into account that ovarian hormonal stimulation is contraindicated in patients with estrogen-sensitive tumours such as breast cancer. Collection of immature oocytes from unstimulated ovaries followed by IVM of the oocytes can be performed in the follicular phase for normal ovulating patients and in almost any given day for PCOS patients after priming with HCG. Another option would be to collect mature and immature oocytes after follicular stimulation under letrozole or tamoxifen plus low-dose FSH and triggering ovulation with HCG or GnRH agonist that decreases estradiol levels. IVM results show that after a mild stimulation with FSH and HCG, oocyte maturation rate is around 65%, the fertilization rate close to 65% while the pregnancy and implantation rates are 25% and 10-18% respectively. Miscarriage rate varies from 20-50% IVM oocytes have been successfully cryopreserved, with better results achieved with the use of vitrification. A combination of IVM of oocytes collected from biopsied pieces of ovarian tissue and subsequent cryopreservation of both matured oocytes and ovarian tissue may result in increased chances for future fertility in certain patients. The cryopreservation of IVM oocytes alone or combined with ovarian tissue cryopreservation is a realistic option for fertility preservation in patients where ovarian stimulation cannot be performed.

Laura Francesca Rienzi Biological degree at the University of Rome 'La Sapienza' (1993). Adjunct Professor of Clinical Embryology at the University of Perugia. Certification "Senior Clinical Embryologist" by the European Society of Human Reproduction and Embryology (ESHRE). II Level Master in "Reproductive Medicine" at the University of Padova. Research fellow at the Centre for Reproductive Medicine, Hôpital Necker in Paris, France. Laboratory Director at the Centre for Reproductive Medicine of the European Hospital in Rome, Italy. In 2008 she has created together with Dr. Filippo Maria Ubaldi the GENERA centres for Reproductive Medicine where she is the Laboratory Director of 3 different centres in Italy. Her current areas of interest include studies of gamete, zygote and embryo morphology in relation to their developmental ability, as well as the cryopreservation of embryos and oocytes. She is internationally recognized for her expertise in human clinical embryology and research as evidenced by invitations to speak at national (37) and international (54) scientific meetings.. Associate Editor of Human Reproduction Journal from 2008, Associate Editor of RBM online from 2010 and Member of the Editorial Committee of the magazine "Focus on Reproduction" from 2006 to 2009. She has been also Scientific Director of the Italian Journal for Reproductive Medicine "2PN" in 2004-2005.

Oocyte cryopreservation: slow freezing and vitrification

Laura Rienzi
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Cryopreservation of embryos at different stage of development is an indispensable part of assisted reproductive techniques. Frozen embryo transfer contributes already 25% of all births achieved by assisted reproduction worldwide, and with systematical application, it is estimated that up to 40% of implantations can be derived from frozen embryos. In many clinics, birth rates after transfer of cryopreserved embryos are close or identical to those achieved with their fresh counterparts, increasing considerably the overall success rate of ART procedures measured by delivery per oocyte aspiration rates. However, legal issues and moral concerns may restrict the application of embryo cryopreservation. Additionally, due to the lack of a partner it cannot be applied in many cases of fertility preservation with medical or social indications, and may create controversial issues in case of divorce or separation of partners. The most feasible solution for these problems is oocyte cryopreservation. Unfortunately, in spite of the relative early successes, widespread application of oocyte cryopreservation was hampered for a long time by inconsistent efficiency of the available cryopreservation methods. Stepwise adjustments of traditional slow freezing protocols as well as optimization of minimum volume vitrification methods have resulted in breakthroughs in this field. Recent prospective randomized studies in oocyte donation programs have found no significant differences between fresh and vitrified oocytes regarding the in vitro and in vivo developmental potential. To test the laboratory efficacy of oocyte vitrification as a routine application for the standard infertile population we have performed a prospective non-inferiority trial (Rienzi et la., 2010). We have found that oocyte vitrification procedure

followed by ICSI is not inferior to the fresh insemination procedure, with regard to fertilization and embryo developmental rates. We have then also estimated the cumulative reproductive outcome of a cohort of infertile couples undergoing ICSI and oocyte vitrification in restrictive legal conditions (limitation in the number of inseminated oocytes, embryo cryopreservation forbitten) (Ubaldi et al., 2010). In this prospective longitudinal cohort study, the cumulative ongoing pregnancy rates obtained by the insemination of fresh and vitrified oocytes from the same cohort were calculated as primary outcome measures. Moreover, the effect of basal and cycle characteristics on clinical outcomes were assessed. The overall ongoing pregnancy rates obtained in the fresh, and first and second warming cycles were 37.4, 25.0 and 27.3%, respectively. The overall cumulative ongoing clinical pregnancy rate observed per stimulation cycle was 53.3%. Maternal age was the only characteristic found to influence the reproductive outcome, with an inverse correlation between the age >40 and the ongoing pregnancy rates (P = 0.04, by multivariate regression analysis). We can conclude that high cumulative ongoing pregnancy rates can be obtained with transfers of embryos derived from fresh and cryopreserved oocytes in a typical infertile population. Female age significantly affects outcomes in this system. We believe that due to the latest advancements in the vitrification approach, cryopreservation of oocytes offers now new perspectives in Assisted Reproductive Technology.

José Antonio Castilla. Graduate in Medicine in 1985, PhD 1986 (Granada University) and specialist in Clinical Analyses. Senior clinical embryologist (ESHRE). Director of andrology and embryology laboratory in Hospital "Virgen de las Nieves", Granada (Spain) since 1991-. Founder of Sperm Bank CEIFER, Granada (Spain) since 1993. Scientific Director of Mas Vida Reproduction Center, Sevilla since 2010.Coordinator of Spanish External Quality Control Programme for Semen Analysis since 1999 and for Assisted Reproduction Laboratory since 2003. Founder member of Spanish Association of Clinical Embryologist (ASEBIR), in the executive committee since 1993 till 2000. Nowadays is Secretary of the executive committee of Spanish Fertility Society, Coordinator of Assisted Reproductive Technology Register of the Spanish Fertility Society, deputy coordinator of the Special Interest Group in Andrology of ESHRE, and member of the executive committee of European IVF Monitoring of ESHRE.

Sperm and testicular tissue cryopreservation

Jose A. Castilla
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In recent years, considerable advances have been made in the field of sperm and testicular tissue cryopreservation. Sperm cryopreservation is not influenced by the collection method (ejaculated semen, postejaculate urine, percutaneous or microsurgical epididymal sperm aspiration, percutaneous testicular sperm extraction (TESE), percutaneous biopsy, microsurgical or nonmicrosurgical TESE). However, testicular tissue cryopreservation protocol depend on the future use: autotransplantation of purified germ cell suspension, autotransplantation ot testicular parenchyma or in vitro maturation of immature germ cells for ICSI. Sperm Freezing media have evolved from components of animal origin, such as egg yolk, to others such as human albumin or lecithin. Classical vapour freezing techniques have gradually been replaced by programmable freezers that enable a record of freezing curves to be kept, together with greater reproducibility of the technique and homogeneity among the straws obtained from a donor. Moreover, these systems make it possible to conclude the freezing ramp at temperatures closer to -196ºC, thus avoiding possible recrystallization phenomena during the transition from the freezing system to the storage tank. At present, the addition of cryoprotectants to the semen and subsequent dilution is considered a key step in sperm cryosurvival. The risk of cross contamination among samples during storage in liquid nitrogen has led to the development of new systems for storage, such as high-security straws, and new tank technology (supercooled vapour or nitrogen). Reducing the risk of cross contamination has made it necessary to introduce strict protocols for the filling and handling of sample straws. Many of the new issues that have arisen in sperm cryoconservation are addressed in Royal Decree 1301/2006, on tissue and cell banks, such as the system employed for handling and identifying samples, and the need for quality control systems. A key element in maintaining quality levels in a sperm bank is that of internal and external quality.

Rita Vassena PhD Dr. Rita Vassena studied Veterinary Medicine at the University of Milan, graduating with a thesis aimed at the improvement of in vitro embryo culture by modulating the cAMP content of oocytes. From 2004 to 2006 Dr. Vassena worked in the laboratory of Keith Latham at Temple University in Philadelphia, where she focused on the problem of nuclear reprogramming in embryos cloned by somatic cell nuclear transfer and mechanisms of posttranslational regulation of the maternal pool of mRNAs in early embryos. She currently work at the Spanish National Stem Cell Bank, and the Center for Regenerative Medicine in Barcelona, where she carries two main lines of research: i) the study of preimplantation biology in human embryos and its impact on the pluripotency program initiation, and ii) the isolation, culture and characterization of human spermatogonial stem cells lines, as a potential source of differentiated tissues for therapeutic use.

Potential for stem cell renewal of function in ovaries and testes
Rita Vassena
Spanish National Stem Cell Bank, and the Center for Regenerative Medicine in Barcelona, Spain

During the last few years there has been a strong debate in the field of reproductive physiology over the possibility of de novo gamete production in the post natal female gonad. The long held belief, supported by decades of research observations, that female gametes in mammals are set in their number before birth, in the developing gonad, has been challenged by studies carried out in mice in J. Tilly laboratory in the mid 2000. Later, further studies highlighted the possibility of postnatal germline development in young mice. All in all, these results opened a debate with both scientific and ethical connotations. Another significant issue faced by reproductive physiology is the possibility to spontaneously reprogram male germline precursor to a more plastic, pluripotent state. This ability, robustly reported in the mouse by the Shinohara group since the mid 2000, has been investigated in human gonads as well, and led to the publication of a few promising reports a few years later. At the same time, this field of research has been the focus of criticism and even charges of non-reproducibility of the most striking data from a large part of the scientific community. This lecture will focus on the review of the current literature on these two topics, which will be put in a historical and scientific context. Unpublished data from the speaker own research will also be presented.

Diana Guerra PhD. Doctoral degree in the Universidad Autónoma de Barcelona (1988) With “Subjective expectancies in the alcohol and cannabis consumption”. Master in Clinical Psychology in the Universidad Autónoma de Barcelona. She has been working as Clinical Psychologist since 1981.Iinvolved in several subjects as a Psychologist: Drug addictions, Personality Disorders, Women Mental Health and Human Reproduction. Presently, she is working at IVI Barcelona and she is at the two Comities on Human Reproduction in Spain. President of the Patient Association “Asociación de Ayuda a la Fertilidad "Genera" from Catalunya (1998-2011). Member of the European Society of Human Reproduction and Embriology Paramedical Board. (1995-2003). Member of the Spanish Committee on Assisted Reproduction. Ministerio de Sanidad y Consumo (1998-2010). From 1998 to present Member of the Catalonian Committee on Assisted Reproduction of the Catalonian Government. First investigator on The European study of assisted reproduction families: family functioning and child development in Spain. European Community.

Psychological aspects of fertility preservation
Diana Guerra
IVI, Barcelona, Spain

Young individuals affected by cancer are confronted with a life crisis in two respects: the cancer diagnosis itself and the threat of impaired fertility. The rapidly growing field of fertility preservation has focused primarily on medical aspects of treatment for cancer survivors. Currently, less is understood about the psychological needs of men, women and children who would benefit from fertility preservation. Cancer is a life-threatening disease and can evoke fear of death; furthermore, infertility might compromise self-esteem, identity, sexuality and self-image. Besides decisions concerning treatment strategies, the patients concerned have to make an additional decision with regard to fertility preservation and this in a moment that is experienced as a life crisis and considerable challenge by most of them Nevertheless, the existing literature shows that fertility is an

important issue for cancer patients and that there is considerable concern regarding the fertility impairment due to cancer and its treatment. Recently, Tschudin and Bitzer reviewed the literature from 1998 to 2008. They identified only 24 studies that met their inclusion criteria on the psychological aspects of fertility preservation. Health professionals as well as patients and their parents consider fertility preservation an important option for young cancer patients, although for the patients themselves, the perceived relevance seems to depend on factors such as the stage of life at cancer diagnosis. All parties involved are shown to have knowledge and information deficits. Counseling regarding fertility issues is far from being offered globally to all patients at risk, and the provision of information by health professionals as well as patient and parental recall of having been informed seems to be selective. Not all physicians discuss fertility preservation with every cancer patient of childbearing age. This might be due to their partly insufficient knowledge, lack of accessible resources for fertility preservation and the particularly challenging counseling setting. Drs. Anderson and Yding said at Focus on Reproduction the last January that "Patient selection remains a challenge, to be confident in offering treatment to those who need it and reassuring to those who do not."
Affected patients and their families are interested in information about fertility issues. Many of them state that they are in favour of fertility preservation. At present, however, only some of them receive information prior to treatment for various reasons. Fertility preservation is far from being accessible to all, and not all health professionals have adequate knowledge and sufficient communication skills to counsel the concerned patients in a timely and supportive manner.

Françoise Shenfield PhD. LRCP, MRCS, MA (law and ethics). Fertility specialist at University College London Hospitals, UK, providing infertility treatments for over 25 years. Academic: qualified in medical Ethics and Law of ART with a master degree since 1992. Founder member of ESHRE Special Interest Group for Ethics and Law (now ex deputy) and its Taskforce for Ethics and law (now member).; member of FIGO' s ethics committee since 2003, co chairman since Dec 2009; Coordinator of the Cross border reproductive care (CBRC) Taskforce for ESHRE (collaboration of our Ethics and Law special interest group, and EIM); member of ESHRE executive committee (summer 2007-2011), she will become coordinator of all Special Interest Groups and Taskforces for ESHRE, remaining on executive committee for a further 2 years as a non-voting member. Publications: articles, books chapters and editor mostly in the field of ethics of ART.

Ethical aspects of fertility preservation in patients affected by severe disease

Françoise Shenfield
LRCP, MRCS, MA (Medical law and ethics), Clinical lecturer in fertility, UCLH, London, UK, member of ESHRE Ethics and law taskforce

Cryopreservation of gametes and/or reproductive tissues in order to preserve one's fertility is especially relevant in view of the increased longevity of patients (both females and males) whose reproductive potential is threatened by cancer, chronic illness and/or the iatrogenic complications of treatment. Therapeutic options have changed rapidly in the last few years, and range from storage of ovarian strips or biopsies in the pre pubertal female, to cryopreservation of immature or mature oocytes, or embryos in the post pubertal. For the male, testicular freezing if pre-pubertal and (mostly) ejaculated sperm (or if necessary testicular tissue) are the main options

General ethical principles and calculus. For all concerned, the ethical calculus must respect the patients' autonomy, with its legal corollary of consent for the planned cryo–preservation, obtained after appropriate information is given in understandable terms. This is complicated when the patient is an adolescent who may be mature enough to understand what is proposed and have different views to the parents and /or caring team, and whose legal rights vary between countries. For a child, "best interest" is the usual criterion, and consent given by the parents. The patients, or parent and child also need detailed information on the risks and benefits (the beneficence/non maleficence calculus) of the proposed intervention, further complicated by the possibility of "anticipated decision regret", especially from the parents for their child. Public interest is also engaged (a question of justice) as equitable access to (often expensive) techniques for all patients in similar circumstances have implications for policy decisions makers at national level.

Specific ethical aspects. Further questions to discuss entail the duration of cryopreservation of gametes or reproductive tissues (storage period), posthumous reproduction, and donation.

The most difficult question of all, in constant evolution: transition from research to "therapy". A difficult concern is when to decide that techniques which may be qualified as being "innovative" should be submitted to appropriate research or used for therapy. Pooling of data of similar techniques, and follow up of offspring are both recommended in order to increase the body of evidence which then informs patients and/or their surrogate decision makers in order to give proper consent. Decision making is facilitated in this field by the fact that preservation of cryopreserved material precedes use, where often time laps allow accumulation of experience. Consent for use will be given by a more mature, better informed person. At all stages, counseling is helpful as well as discussion of alternatives.

Guido Pennings Professor of ethics and bioethics at Ghent University (Belgium). He is also the director of the Bioethics Institute Ghent (BIG) at this university. He mainly publishes on ethical problems associated with medically assisted reproduction and genetics including sex selection, gamete donation, stem cell research, fertility preservation, and preimplantation genetic diagnosis. He is a member of the Task Force on Ethics and Law of the European Society of Human Reproduction and Embryology (ESHRE), the Belgian National Advisory Committee for Bioethics, the Federal Commission on research on embryos in vitro and the Ad Hoc Ethics Committee of the Centre for Reproductive Medicine of the University Hospital of the Free University Brussels.

Is there a place for ovarian tissue and oocyte cryopreservation for social reasons?

Guido Pennings
Bioethics Institute Ghent (BIG), Ghent University, Belgium

The breakthrough of the vitrification of oocytes opened the debate on the use of this technology for healthy women. Very soon, clinics all over the world offered fertility preservation to anyone who wanted it. All kinds of concerns were raised against these applications: it would encourage women to further postpone their pregnancies; it would be inappropriate use of medical technology, etc. We will look at these objections in more detail to see whether they stand scrutiny. The problem of the application of medical techniques for non-medical applications has a long history. The distinction between medical and non-medical applications has never been clear-cut. Moreover, analysis of the use of the label reveals that the concept 'medical' is used as a normative concept: it sanctions the use of a technique for certain problems and it justifies reimbursement from public funds. Simultaneously, the 'medical'-'non-medical' tandem frequently overlaps with the need-desire tandem. Desires, contrary to needs, are a matter of personal choice and autonomy. A key question is whether medical technology (fertility preservation) should be used to cure a health problem (infertility) that is caused by individual decisions (postponed parenthood) strongly determined by the social and cultural context (career, education). An analogy can be made with medical interventions (e.g., gastric bypasses) for morbidly obese persons. Some argue that one should alter the social and cultural context so that women can and want to have children earlier. Even if we believe that this is the way forward, this does not tell us what we should do in the meantime. Autonomy is a very strong argument to allow women to decide for themselves whether the burden of the intervention is worthwhile in order to preserve the chance of having genetically related children in the future. Moreover, postponement may well be in the best interest of the future children. An interesting exercise of prospective ethics is to work out the (unlikely?) scenario of a large scale uptake of this option. What will be done with the oocytes if they are not requested by the progenitors for their own reproduction? Can and/or should they be donated? Who is going to pay for the treatment? Fertility preservation for non-medical reasons is a highly complex issue that touches upon general views on the appropriate use of medicine, beliefs about motherhood and women's role in society, long-term consequences for society etc. The blunt rejection of these applications that dominate the present discussion in Europe is too simplistic. We need stronger arguments than those offered at the moment to made a convincing case for a prohibition of egg freezing for social reasons.

List of attendees

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Practical information

Venue of the meeting

Museu Colet
c/ Barcelona, 56. Barcelona, Spain

Speaker's hotel

Hotel AC IRLA
c/ Calvet, 40. Barcelona. Phone: + 34 93 241 62 10

Visit to Barcelona Biomedical Research Park and Center for Regenerative Medicine of Barcelona

Monday, June 6th
c/ Dr. Aiguader, 88. Barcelona, Spain

Barcelona Biomedical Research Park

CMR[B] Centre de Medicina Regenerativa de Barcelona
Centro de Medicina Regenerativa de Barcelona
Center of Regenerative Medicine in Barcelona

Contact person during the event

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Organizers

biocat International Center for Scientific Debate

Initiative fostered by: Welfare Projects "la Caixa" Foundation

The **International Center for Scientific Debate** (ICSD) is an initiative of Biocat, fostered by Welfare Projects "la Caixa" Foundation, which aims to drive top-notch international scientific meetings promoting dialogue, collaboration and open exchange of knowledge among experts of renowned prestige and the Catalan Scientific community. The meetings are global, integrative and multidisciplinary focused helping to tackle social needs in the field of life sciences and health, taking into consideration the complexity and constantly changing conditions of the world. The ISCD also aims to collaborate in the dissemination of knowledge, approaching science to society and contributing to position Barcelona and Catalonia as a city and a country of scientific excellence.

More information: www.biocat.cat/en/icsd

DexeusFoundation Woman's Health

The **Dexeus Foundation for Woman's Health**, a no-profit entity, aims to serve the health culture of the country, spreading all scientific, teaching and researching activities linked to the fields of Obstetrics, Gynaecology and Reproduction Medicine, promoting scientific and researching activities and organizing teaching activities. The **Dexeus Foundation for Woman's Health** awards financial assistance to the research, by granting Awards and Grants, and offers social oriented medical assistance services. It collaborates with the Chair of Research in Obstetrics and Gynaecology of UAB (Universidad Autónoma de Barcelona) and boosts the cooperation with social and scientific entities with common aims. Among the activities of medical assistance of social nature, the **Dexeus Foundation for Woman's Health**, through the professionals of Department of Obstetrics, Gynaecology and Reproduction USP Institut Universitari Dexeus, is realizing a program of cryopreservation of ovarian tissue, free of charge, for oncological patients in chemotherapy pre-treatment, in order to preserve their future fertility (> 5 years after the treatment). Our Fertility Preservation Unit of **Dexeus Foundation for Woman's Health** provides an effective and personalized service with a specific objective: preserving the fertility of those patients who, due to medical indication, need to postpone their reproductive project.

More information: www.fundaciondexeus.org

FERTILITY PRESERVATION UPDATE: CONSENSUS MEETING

Venue

Museu Colet
C/ Buenos Aires, 56
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