A window into the reproductive era research

Milan, Italy - September 30, October 1 - 2011
Venue
Congress Center Humanitas Clinical Institute
Via Manzoni 56
20156 Rozzano (Milan) – Italy

Language
The official language of this conference will be English

Location
Milan is to be considered Italy’s door to Europe. Financial capital of Italy with its stock exchange and large industrial sector, Milan is not just famous for its fashion. Historic sites such as the magnificent gothic Duomo Cathedral as well as Leonardo da Vinci’s Last Supper, housed at the Church of Santa Maria delle Grazie, are musts for every visitor. Not to mention La Scala, probably the world’s most illustrious Opera House. Splendid also is nearby Lake Como.
A window into the reproductive era research

Serono Symposia International Foundation conference on:

A window into the reproductive era research
Milan, Italy - September 30 - October 1, 2011

Aim of the conference
In the field of reproductive health, two needs are emerging that apparently conflict: research is providing continuous innovations in daily procedures, but the demand for infertility treatment is increasing all over the world. The key point of conflict between these two aspects is cost, because innovation demands investment, at least in its early phase, and the widening of ART to geographical areas with lower average incomes requires careful evaluation of the cost/benefit balance.

The aim of this conference, which follows the previous two successfully organized in 2003 and 2008 by Paolo Levi Setti and Serono Symposia International Foundation, is to provide participants with overviews of the most innovative procedures that will be introduced into ART in the near future, and with the background needed to offer the most up-to-date infertility treatment, according to different local conditions.

The conference is organized in four sessions with two keynote lectures. The Scientific Organizer intends this conference to place special emphasis on the contribution of young national and international scientists whose selected abstracts will be briefly presented orally. All the selected abstracts will be published as a special edition of the journal Placenta.

Learning objectives
Attending the conference, participants will acquire:
• Updates on innovative surgical procedure applied to reproductive health.
• Overviews of key factors of embryo development.
• Criteria to optimize ART outcomes, consistent with the reality in which they operate.

Target audience
Physicians and other healthcare professionals working in reproductive health centres.

Accreditation
“A window into the reproductive era research”, Milan, Italy, 30 September – 1 October, 2011 was granted 9 European CME credits (ECMEC) by the European Accreditation Council for Continuing Medical Education (EACCME). Serono Symposia International Foundation also submitted for accreditation this program by the Italian Ministry of Health.

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Scientific program
Friday - September 30, 2011

07.30 Registration
08.00 Scientific Organizer’s Welcome
Paolo Emanuele Levi Setti, Italy
08.10 SSIF Welcome
Robert Fischer, Germany

**Session I** Innovative surgical procedures in ART

Chairpersons: Elisabetta Coccia, Italy – Luigi Fedele, Italy

08.20 L1: Gynaecological fertility sparing surgery
Domenico Vitobello, Italy

08.40 L2: Robotic indications in reproductive surgery: pros and cons
Tommaso Falcone, USA

09.00 L3: Innovative approaches to endometriosis surgery
Edgardo Somigliana, Italy

09.20 L4: What’s left of reproductive surgery?
Togas Tulandi, Canada

09.40 L5: New ideas in myoma’s surgery
Carlo Bulletti, Italy

10.00 Discussion
10.30 Coffee Break

**Key note lecture**

Chairpersons: Paola Anserini, Italy - Filippo Ubbaldi, Italy

11.00 KNL1: Benefit and risk of application of European tissue management regulation in ART
Giulia Scaravelli, Italy

**Session II** Innovation in ART laboratories

Chairpersons: Elena Albani, Italy - Andrea Borini, Italy

11.30 L6: New culture devices in ART
Laura Rienzi, Italy

11.50 L7: Which scores are relevant for improving ART success?
Markus Montag, Germany

12.10 L8: A review of the promises and pitfalls of oocyte and embryo metabolomics
Zsolt Peter Nagy, USA

12.30 L9: Non invasive imaging of human embryos to predict competence
Renée Reijo Pera, USA

12.50 Discussion
13.30 Lunch

*Abstracts presentation order is not by ranking results but according to conference time availability of presenters

**Session III** Exploring the chaos: the early stages of embryo development

Chairpersons: Daniela Bettio, Italy
Hilde Van de Velde, Belgium

14.30 L10: Microarray CGH is the key to understand oocyte developmental competence
Dagan Wells, UK

14.50 L11: Is chromosome instability a common feature of human embryos? New array approaches to explore single cells genomes
Joris Vermeesch, Belgium

15.10 L12: Cell [pre]-destiny in the human preimplantation embryo and implications for IVF and PGD
Hilde Van de Velde, Belgium

15.30 L13: Is the polar body approach best for pre-implantation genetic screening
Joy Delhanty, UK

15.50 L14: Whole genome sequencing in a prenatal setting
Patrick Willems, Belgium

16.10 Discussion
16.40 Coffee Break

Abstracts selected for oral presentation

Chairpersons: Lucia De Santis, Italy - Guglielmo Ragusa, Italy

The Conference Scientific Committee has selected these papers as winners of the 3 Prizes, this session will also welcome the first 16 best-rated papers as oral presentations*:

17.00 High-resolution genome-wide array-CGH reveals copy number variations associated with premature ovarian failure
Paola Scaruffi, Italy (1st Prize Winner)

A novel optical microsurgery method for trophectoderm biopsy
Yulia Khramova, Russia (2nd Prize Winner, unable to attend the conference)

17.08 High anti-PLAC1 antibody levels in idiopathic infertile patients with repeated unexplained implantation failure
Maria Matteo, Italy (3rd Prize Winner)

17.16 Comparison between slow freezing and vitrification in blastocysts cryopreservation
Elena, Albani, Italy

17.24 Efficiency of one step dehydration protocol in day 3 human cleavage stage frozen embryos
Marzia Barberi, Italy

17.32 Validate cryopreservation procedure before storing human ovarian tissue in cancer patients
Graziaella Bracone, Italy

17.40 Oocyte vitrification: influence of operator and learning time on survival and development parameters
Federico Calzi, Italy

17.48 Focal Plane Array (FPA) Fourier Transform Infrared (FT-IR) Imaging Spectroscopy as a new technique to evaluate of human oocytes quality
Giorgia Gioacchini, Italy

17.56 Conclusions: Paolo Emanuele Levi Setti, Italy
Session IV: Present and future of ART

Chairpersons: Roberto Palermo, Italy - Guido Ragni, Italy

09.00 L15: Consenting for the “hot topics” in reproductive care
  Françoise Shenfield, UK

09.20 L16: The future of IVF: the next 5 years
  Pasquale Patrizio, USA

09.40 L17: Fertility preservation: a reappraisal
  Nicole Noyes, USA

10.00 L18: Lyophilization and rehydration of cells
  Amir Arav, Israel

10.20 L19: Work-up of repetitive implantation failures
  Michael Paidas, USA

10.40 Discussion

11.00 Coffee Break

Abstracts selected for oral presentation

Chairpersons: Ettore Cittadini, Italy - Antonio Palagiano, Italy

11.30 Annexin V magnetic-activated cell sorting versus swim-up for the selection of human sperm in ART: is the new approach better than the traditional one?
  Marco Nadalini, Italy

11.38 Does testicular sperm improve ICSI outcome in patients with necrozoospermia?
  Luciano Negri, Italy

11.46 Evaluation and Management of Recurrent Pregnancy Loss
  Marianna Pina Rambaldi, Italy

11.54 Cell-free DNA: a non-invasive test for assessing embryo quality
  Paola Scaralli, Italy

12.02 Robotic assisted approach for rectosigmoid resection in patients with deep infiltrating endometriosis
  Gabriele Sieto, Italy

  GnRH agonist protocol versus GnRH antagonist protocol in assisted reproduction
  Moshin Veaceslav, Russia [unable to attend the congress]

12.10 The concomitant presence of deep infiltrating endometriosis worsen the endometrioma-related negative impact on ovarian reserve and number of retrieved oocytes
  Jessica Ottolina, Italy

12.18 Laparoscopic management of infertility patients using 5-mm optic. The Humanitas Clinical Institute experience with 1406 consecutive cases
  Elena Zannoni, Italy

Key note lecture

Chairpersons: Irene Cetin, Italy - Enrico Ferrazzi, Italy

12.26 KLN 2: How endometrial secretomics can help in predicting implantation
  Carlos Simón, Spain

12.56 Conclusions
  Paolo Emanuele Levi Setti

13.06 End of the conference and lunch
Disclosure of faculty relationships

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Elena Albani  Declared no potential conflict of interest
Paola Anserini  Declared no potential conflict of interest
Amir Arav  Declared no potential conflict of interest
Daniela Bettio  Declared no potential conflict of interest
Andrea Borini  Declared no potential conflict of interest
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Irene Cetin  Declared no potential conflict of interest
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Lucia De Santis  Declared no potential conflict of interest
Joy Delhanty  Declared no potential conflict of interest
Tommaso Falcone  Declared no potential conflict of interest
Luigi Fedele  Declared no potential conflict of interest
Robert Fischer  Declared receiving honoraria or consultation fees by SSIF as being part of the Scientific Committee
Paolo Emanuele Levi Setti  Declared no potential conflict of interest
Markus Montag  Declared no potential conflict of interest
Zsolt Peter Nagy  Declared being a member of company advisory board (ORIGIO UNISENSE)
Nicole Noyes  Declared no potential conflict of interest
Michael Paidas  Declared receipt of grants and contracts by BioIncept, Inc.
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Laura Rienzi  Declared no potential conflict of interest
Giulia Scaravelli  Declared no potential conflict of interest
Carlos Simón  Declared no potential conflict of interest
Edgardo Somigliana  Declared no potential conflict of interest
Togas Tulandi  Declared receipt of honoraria or consultation fees by Ethicon Inc.
Hilde Van de Velde  Declared no potential conflict of interest
Dagan Wells  Declared to be stakeholder in a company: Reprogenetics. An independent company providing genetic testing services for IVF clinics
The following faculty has provided no information regarding significant relationship with commercial supporters and/or discussion of investigational or non-EMEA/FDA approved (off-label) uses of drugs as of September 5th, 2011

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Filippo Ubaldi  
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Abstracts
L1 - Gynaecological fertility sparing surgery

G. Bracone MSc, E. Albani MSc, D. Vitobello MD, S. Di Biccarì MSc, E. Gismano MSc, G. Siesto MD, P.E. Levi Setti MD
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According to the law in different countries regulating assisted reproductive technologies, approaches to fertility preservation before gonadotoxic treatments includes embryo or oocyte cryopreservation, and ovarian tissue banking followed by tissue transplantation or in vitro culture. Cryopreservation of ovarian tissue may be the only acceptable method to preserve fertility for prepubertal girls, for women who cannot delay the start of cancer treatment to undergo ovarian stimulation, and probably also for women with hormone-sensitive malignancies. Over the last twenty years, more and more fertility centres worldwide have attempted to perform ovarian tissue cryopreservation procedures. In our experience, it is suggested that cryopreservation protocols should be validated using donated human ovarian tissue before applying the technology in a clinical programme. Material and Methods: Small biopsies of ovarian cortical tissue, collected during laparoscopy, was donated from a 39-year-old woman for research purposes. The study was authorized by the Local Ethics Committee. In the laboratory, the tissue was cut and transferred to cryovials filled with cryopreservation media composed of 1.5M Propanediol (PROH), 0.2M Sucrose and 20% Human Serum Albumin (HSA) and slow frozen. After rapid thawing, samples were cultured for 48h at 37°C in 5% CO2 to allow protein expression. Fresh and frozen/thawed tissues were fixed, embedded in paraffin, and serially sectioned. Every third slide was stained with haematoxylin/eosin for histological evaluation to detect the presence of follicles. It was then possible to proceed with immunohistochemical (IIC) analysis of proliferation and anti-apoptotic markers on the same follicle in contiguous slices, to evaluate the functionality of the cells after the cryopreservation procedure compared to fresh ovarian tissue. Results: Histological and IIC analyses demonstrated that the amount of well preserved follicles and stroma cells was similar in the fresh and slow freezing/rapid thawing procedures. We present what we believe to be the first such triple evaluation of the same follicles and stroma cells.

Conclusions: Ovarian tissue cryopreservation is an important method for fertility preservation; however, before applying the method clinically to oncology patients, each laboratory should perform thorough validation of their procedures, because it could affect the future fertility of young women.
Minimally invasive surgical techniques are becoming increasing more common in gynecologic surgery. However, traditional laparoscopy can be challenging. A robotic surgical system gives several advantages over traditional laparoscopy and has been incorporated into reproductive gynecological surgeries. The objective of this lecture is to review recent publications on robotically assisted myomectomy and tubal reanastomosis. Recent clinical research supports robotic surgery as resulting in less postoperative pain, shorter hospital stays, faster return to normal activities, and decreased blood loss. Reproductive outcomes appear similar to alternative approaches. Drawbacks of robotic surgery include longer operating room times, the need for specialized training, and increased cost. Larger, prospective studies comparing robotic approaches with laparoscopy and conventional open surgery have been initiated and information regarding long-term outcomes after robotic surgery will be important in determining the ultimate utility of these procedures.
The precise relationship between endometriosis and infertility is debated. Surgery is considered to play a role within the framework of the therapeutic options to cure infertile women with the disease even though its effectiveness is generally modest. In fact, there is unquestionably the need to improve surgical techniques in this area. Specifically, two main aspects require optimization: 1) preventing the injury to the follicular reserve that follows surgical excision of ovarian endometriomas and 2) preventing post-surgical formation and re-formation of adhesions. The comparison between the excision/stripping and the vaporization/coagulation techniques represents the main point of debate on what is the best procedure to remove ovarian endometrioma. Randomized controlled trials showed that the excision technique is associated with a higher pregnancy rate and a lower rate of recurrence although it may determine severe injury to the ovarian reserve. Improvements to this latter aspect may be represented by a combined excisional-vaporization technique or by replacing diathermy coagulation with surgical ovarian suture. Barrier agents reduce but do not eliminate the post-surgical adhesion formation in women with endometriosis. Encouraging evidence has been reported with Interceed, Oxiplax/AP gel and Adept solution. However, available studies are mainly based on II look laparoscopies performed few weeks after the intervention and data on fertility is lacking. Clinical trials including pregnancy rate as a specific outcome are warranted.
The indications of reproductive surgery as a primary treatment for infertility have declined. The current use of reproductive surgery is to improve the live birth rate of ART treatment and for fertility preservation and it should be done with minimally invasive technique. The place of laparotomy in reproductive surgery has become historical.

Hysteroscopy is a valuable tool to evaluate the uterine cavity and to treat intrauterine pathology. In fact, in women who have failed at least one IVF cycle, hysteroscopy should be performed and intrauterine pathology including endometrial polyp, and submucous myoma should be removed. Randomized trials have shown that their removal is associated with increased live birth rate. A large intramural myoma with submucous component should be removed by laparoscopy. Other intrauterine pathologies that need to be corrected are intrauterine adhesions and uterine septum.

It is known that the presence of hydrosalpinx reduces the IVF pregnancy rate. Removal of the hydrosalpinx (salpingectomy) significantly improved the pregnancy and live birth rates. This has been demonstrated in a few randomized studies. Salpingectomy should be performed by laparoscopy and carefully to avoid compromising the ovarian blood flow. Another method to increase the IVF pregnancy rate in women with hydrosalpinx is proximal tubal occlusion by laparoscopy and recently by hysteroscopy. Ovarian endometrioma does not have to be removed before IVF treatment. However, endometrioma that interferes with oocyte retrieval needs removal and the best approach is by laparoscopic excision.

Treatments with chemotherapy or radiation often results in premature ovarian failure and sterility. In order to preserve fertility and ovarian function, cryopreservation of oocytes, embryo, or ovarian tissue has been performed. Ovarian tissue should be obtained by laparoscopy and currently pregnancy has been achieved by orthotopic transplantation of the thawed cryopreserved ovarian tissue. For those undergoing local pelvic radiation, laparoscopic ovarian suspension is associated with preservation of ovarian function in 70-80% of cases.
Uterine fibroids have been reported in 27% of infertile women and 50% of women with unexplained infertility become pregnant after myomectomy. In this review we evaluate the impact of myomas on pregnancy outcome before and after myomectomy with and without ART programs. Myomectomy was effective in improving the pregnancy outcome when compared with the pregnancy outcome before myomectomy. Pregnancy rate after myomectomy is significantly increased. There are no differences in approaching the surgical removal of myomas by laparoscopy versus laparotomy. The laparoscopic removal of myomas was effective on the pregnancy outcome as compared with no surgery at all. There is a beneficial effect of removing intramural, subserosal and submucosal myomata on the ongoing pregnancy, while there is not the same positive effect when the removal is regarding the only subserosal myomata. There is a positive impact of Assisted Reproductive Technologies in pregnancy outcome on women having myomas distorting the lumen cavity of the uterus, as well in those who did not have distortion of their cavity. That last analysis is apparently establishing that myomas did not influence pregnancy rate after ART in both cases distorting and not distorting the lumen cavity of the uterus. On the other hand Assisted Reproductive Technologies are effective in improving the pregnancy outcome in women who do not have myomas.

**Key words:** Myomas, Fibroids, Infertility, Sterility, In Vitro Fertilization, Surgical removal
One of the aims of the European Tissue Directive (EUTCD) 2004/23/EC, issued on 31 March 2004, was to achieve a common framework in terms of quality and safety standards in the field of organs, tissue and cell transplantation in all the European countries. The Directive objectives include the prevention of infectious diseases spread, by avoiding viral transmission and microbial contamination during transplantation procedures of human organs, tissues and cells. Under this Directive reproductive cells are also included, and therefore ART centres have had to conform to these requirements. The problem that arose after the issue of the Directive was that the criteria utilised to define the requested requirements were formulated by only considering scientific evidence based on the “events” that could take place in the transplantation “scenario”. The consequence of this oversight, is that professionals in the assisted reproductive medicine have had to comply with the Directive, with the knowledge that some of these requirements have little scientific basis in ART field. The implementation of the Directive in ART has evidently improved the safety and quality in the application of assisted reproductive technology, especially in those countries that have no specific legislation on assisted conception. However, the Directive still has some problematic areas in its application. Two of these include, the air quality in the IVF laboratories and the validity period of viral screening for couples undergoing ART procedures, the latter being dependent on the different country specific legislation that leads to different policies in this field being implemented. Furthermore, the application of the Directive, in the context of public hospitals in many European Countries such as Germany, Italy, France, and Spain, will depend and be financially supported by the different Local Health Authorities. This situation could result, if not carefully managed, in enormous differences in patient accessibility to treatments or inequity to treatment access, jeopardising the country’s inter-regional implementation. On the other hand, the full application of the EUTCD in all EU countries will result in a standardization of ART clinic activities and will enable a more reliable and precise comparison of the outcomes obtained in the different countries. To evaluate the cost-effectiveness of the application of the Directive it will be necessary to wait for its complete implementation by all the EU countries. Only then, will it be possible to comprehend the real cost burden that single couples and Governments will need to bear.
During the past decades, improvements in culture of pre-implantation embryos have contributed substantially in the success of human assisted reproductive techniques. However, most efforts were focused on optimization of media and gas components, while the established physical conditions and applied devices have remained essentially unchanged. Very recently, however, intensive research has been started to provide a more appropriate environment for the embryos and to replace the rather primitive and inappropriate devices with more sophisticated and practical instruments. Success has been reported with simple or sophisticated devices (microwells or microchannels) that allow accumulation of autocrine factors and establishment of a proper microenvironment for embryos cultured individually or in groups. The microchannel system may also offer certain level of automation and increased standardization of culture parameters. Continuous monitoring of individual embryos by optical or biochemical methods may help to determine the optimal day of transfer, and selection of the embryo with highest developmental competence for transfer. This advancement may eventually lead to adjustment of the culture environment to each individual embryo according to its actual needs. Connection of these techniques to additional radical approaches as automated ICSI or an ultimate assisted hatching with full removal of the zona pellucida after or even before fertilization may result in devices with high reliability and consistency, to increase the overall efficiency and decrease the work-intensity, and to eliminate the existing technological gap between laboratory embryology work and most other fields of biomedical sciences.
In the past several scoring systems were proposed for early human development aiming to assist in the identification of the best embryo. The most prominent scoring systems are oocyte morphology, pronuclear scoring and embryo morphology. These systems were and still are widely applied in the daily routine. Morphology assessment is based on the subjective judgement of the embryologist and sometimes even a variety of different scoring systems for one and the same parameter do exist. Therefore it is not surprising, that for almost every scoring system controversial results on its benefit can be found in the literature. However, the most important drawback of morphology based scoring systems is that the scoring criteria are usually assessed at static developmental time points by microscopy. The parameter “time” has been neglected as an additional and probably very important scoring factor.

With the introduction of time-lapse imaging systems which enable continuous embryo monitoring the relevance of certain scoring criteria like pronuclear morphology and embryo morphology must be critically revised. Single observations at a static time point may be misleading as time-lapse imaging reveals dynamic changes of morphological markers like the position of nucleolar precursor bodies in pronuclei or the fate of embryo morphology shortly after cleavage. Furthermore, early cleavage must be re-defined as time-lapse imaging allows for sub-categorizing this event into very early, medium and very late cleavage and each of these categories has a different prognostic value. There is growing evidence that morphology scoring systems which only rely on static observation have severe limitations. Proper scoring is even more important, if the ultimate aim is to transfer only one embryo. This can probably be best achieved using multiple scoring strategies based on morphological changes over time. Consequently the time has come to define new scoring criteria and new selection strategies based on the knowledge which we gain with time-lapse imaging.
Embryo viability assessment is one the most important and challenging tasks in IVF. Evaluation of embryo quality is critical when selecting the best embryo(s) to transfer or cryopreserve. Until recently, the only instrument used for embryo evaluation was the inverted light microscope, which provided information based on morphological characteristics. Developmental and morphological information gained from microscopic assessment have been positively associated with IVF outcomes, including pregnancy and implantation rates. However, based on general statistics, it is clear that IVF currently still results in relatively low pregnancy rates, while simultaneously being associated with relatively high multiple implantation rates. Only with novel embryo assessment and selection procedures would it be possible to improve these outcomes. Accordingly, it has been proposed that it is possible to test the culture environment of a developing embryo to gain valuable information regarding its viability. Different approaches have been used. These include the measurement of oxygen consumption by the embryo and testing of the soluble HLA-G in the environment, as it was proposed that secretion of HLA-G is associated with higher implantation rates. Amino-acid turnover, which appears to be correlated to blastocyst development, can be measured as an indication of embryo viability. Other approaches, such as time-lapse video observation or cumulus cell gene expression analysis, may be used in the future to gain a broader understanding of embryo viability. Proteomics and metabolomics are also useful tools for assessment of embryo developmental potential. Results from recent studies on predicting embryo viability by analyzing the metabolome of different stage embryos are promising, as increases in pregnancy and implantation rates were obtained using the metabolomic profile for embryo selection. Several novel approaches are currently being developed to aid in viability assessment. These need to be evaluated in prospective clinical trials, while considering their practicality in the clinical laboratory.
Little is known of the factors that predict success or failure in human embryo development. Consequently, in order to increase the chances of pregnancy through in vitro fertilization (IVF), multiple embryos are often transferred in spite of well-documented potential adverse outcomes. Recently, we demonstrated that time-lapse image analysis can be used to predict blastocyst fate prior to embryonic genome activation (EGA). Following time-lapse imaging, key developmental features were extracted to generate an algorithm that predicts success or failure in development to the blastocyst stage. By measuring three dynamic imaging parameters, (i) the duration of first cytokinesis, (ii) the time between the first and second mitosis and (iii) the time between the second and third mitosis, we determined mean values and standard deviations of embryos predicted to reach the blastocyst stage. In addition, the inclusion of other morphological characteristics, including fragmentation and blastomere size/shape together with our cell cycle parameters further assisted in predicting blastocyst fate. Results suggest that success or failure in human development is inherited, embryos that begin life with defective maternal, cell cycle and/or mitotic programs are likely to meet aberrant cytokinesis, embryo fragmentation, non-uniform blastomere size and/or shape as well as arrest of individual blastomeres or the entire embryo. The methods and algorithms presented here may provide a platform for improved and earlier diagnosis of embryo potential and allow the transfer of fewer embryos earlier in development.
There are many biological contributors to the competence of human oocytes and embryos, but perhaps the most clearly identified is chromosome abnormality. Multiple methods for the identification of viable oocytes/embryos have been reported, based upon morphological and, more recently, metabolomic criteria. These evaluations may provide a useful insight into developmental potential. However, some embryos with apparently poor morphological or metabolomic scores nevertheless succeed in producing healthy pregnancies, and consequently it is obvious that the data provided by such evaluations is not clear-cut. In contrast, a chromosomally abnormal oocyte will produce an embryo that has abnormalities in all of its cells and has little if any chance of producing a healthy child.

Although cytogenetic analysis appears to give a more straightforward indication of oocyte or embryo viability, clinical data obtained after screening of embryos for aneuploidy [i.e. preimplantation genetic screening- PGS] has often failed to yield the expected improvements in IVF treatment outcomes. It seems likely that the apparent failure of PGS in many studies was linked to both biological and technical issues. Biologically, the amount of genetic instability seen during the first few mitotic divisions may reduce the accuracy of testing performed at the cleavage stage. Technically, the standard method used for chromosome assessment, fluorescent in situ hybridisation (FISH), has several notable deficiencies, most importantly an inability to assess the full chromosome complement in each oocyte or embryo.

The technical limitations of FISH have been largely overcome with the introduction of microarray comparative genomic hybridisation (also called array-CGH or aCGH). This methodology permits the copy number of every chromosome to be determined in polar bodies, blastomeres and trophectoderm biopsies and shows great promise for the purpose of PGS. Screening using microarray-CGH may be particularly valuable if applied to polar bodies biopsied from oocytes, since biological issues that may affect diagnostic accuracy, such as chromosomal mosaicism, are not present at this time. This lecture will provide an overview of microarray-CGH and its use for PGS, paying particular attention to data from the screening of oocytes via polar body analysis.
Chromosomal abnormalities are common in human in vitro fertilized (IVF) early stage embryos. FISH studies of normally developing, good quality embryos from IVF patients have shown that 30–65% are aneuploid in at least one cell. The majority of couples are treated by IVF because of reduced fertility and hence, it was first thought that those imbalances were the cause of the reduced fertility. However, a similar proportion of aneuploidies were detected in embryos derived from normal fertile couples. Those early studies were performed using targeted FISH probes and did not screen the whole genome. When imbalances were detected with those locus specific probes, it was assumed that they represented chromosomal aneuploidies.

To enable aneuploidy detection of all chromosomes, genome wide analysis tools were required. It was with the development of single cell comparative genomic hybridization (CGH) (LeCaignec et al., 2006) that (1) the extent of chromosomal imbalances could be probed and (2) that for the first time also partial aneuploidies were reported in early human embryos. New high resolution array approaches for CGH revealed segmental rearrangements to be much more frequent in early human embryos than assumed with an occurrence of segmental aneuploidies in 10 to 38.5% of the embryos (Vanneste et al., 2009). The finding of a large number of chromosomal imbalances in cleavage stage embryos challenges the view that cleavage stage embryo selection against chromosomal imbalances could increase IVF success rate and increase the baby-take-home rate in patients treated by IVF for reduced fertility (Vanneste et al., 2009b). With the advent of genome wide SNP arrays it might also become feasible to use arrays as a tool to perform PGD in embryos to detect Mendelian disorders.

In this presentation, I will provide an overview of the different array techniques that have been developed both in my as well as in other laboratories over the last few years, discuss their possible applications to study human embryos and show how those techniques have established chromosome instability to be a common feature of early human embryogenesis.

References:
The fertilized human egg develops six days before implanting into the uterus. During this short period, major events occur that ensure the development into a human being. Many aspects of the human preimplantation development are unknown. The scarcity of the materials and the ethical objections regarding the use of human preimplantation embryos for research purposes in many countries has hampered the analysis of human preimplantation embryos. However, data from animal models cannot simply be extrapolated to the human.

Early mammalian blastomeres are thought to be flexible and totipotent allowing the embryo to overcome perturbations in its organization during preimplantation development. Totipotency is defined as the capacity of a single cell to generate an entire new fertile organism. The zygote is the ultimate totipotent cell, however it is unknown when and in which cells during the cleavage divisions totipotency is lost. Single cells (blastomeres) of 2-cell, 4-cell and 8-cell stage human embryos are thought but not proven to be totipotent. The first morphologically visible sign of differentiation becomes apparent at the fifth day of preimplantation development during blastulation when two lineages can be distinguished: the differentiated trophectoderm (TE), which ensures implantation into the uterus, and the undifferentiated inner cell mass (ICM), which develops into the foetus and which is also the source of embryonic stem cells (ESC). The undifferentiated status is sustained by the widely studied master regulator genes NANOG, POU5F1 and SOX2, but little is known about genes determining trophectoderm formation in the human embryo.

The overall aim of our research is to understand better the human preimplantation development. Major questions we want to answer are: (1) when does the cell lose its totipotency potential, (2) when do developmental decisions (TE or ICM) become irreversible, (3) is human preimplantation development regulative, (4) how many cells can an embryo lose without impairing the implantation capacity?

We showed for the first time that all four blastomeres at the 4-cell stage are able to develop individually into blastocysts with an ICM and a TE, indicating that the four blastomeres are plastic, not yet committed towards the ICM or TE and therefore potentially totipotent (Van de Velde et al., 2008). We also derived two distinct hESC lines from single blastomeres of two distinct 4-cell stage embryos, indicating that at least one blastomere at the 4-cell stage is pluripotent (Geens et al. 2009). At this moment, we investigate the capacity of outer cells of compacted embryos and blastocysts to develop into blastocysts with ICM. We also investigate regulative development by changing the position of the blastomeres at distinct stages.

Understanding human preimplantation development and totipotency is important for reproductive biology, more in particular to understand why some embryos implant and others do not. When an embryo loses cells by fragmentation, cryodamage or biopsy for preimplantation genetic diagnosis, it needs to be determined whether mass reduction and/or disturbed ICM/TE allocation form the basis of the reduced implantation capacity.
Pre-implantation genetic screening for aneuploidy is carried out with the aim of selecting oocytes or embryos that have the optimal chance of producing an ongoing pregnancy by eliminating those that have a detectable chromosomal anomaly. A variety of cells may be chosen for testing; the first polar body, with or without the corresponding second polar body, a single blastomere from a cleavage stage embryo or a group of cells from the trophectoderm at the blastocyst stage.

This presentation explains the different stages when aneuploidy may arise during oocyte development and the contribution made by post-zygotic aneuploidy to the overall burden as a basis for understanding the arguments for and against selecting polar bodies as the cells of choice for pre-implantation screening. The published outcomes of recent applications of testing polar bodies to detect aneuploid oocytes are discussed.
Worldwide there are huge differences between and within various countries with respect to accessibility, price and quality of molecular diagnostic testing. The bottlenecks in such testing include the vast number of genetic diseases (→ 2,000), the low number of samples per disease due to the low frequency of most genetic diseases (< 1 on 10,000), the nature of the disease mutation often being a private mutation, the genetic heterogeneity of many diseases, the high cost of testing and lack of reimbursement by governments and insurance companies, and the lack of an international organised network of diagnostic labs combining their portfolio of tests. All these bottlenecks impair a cost-effective and reliable diagnostic service, thereby holding molecular testing in many countries in a preclinical era. However, the quality, accessibility and cost-effectiveness of diagnostic tests for rare genetic disorders could be substantially improved by the creation of an international network of diagnostic labs combining their portfolio of tests and exchanging samples for rare genetic disorders. The first network of diagnostic labs offering genetic tests internationally was incorporated five years ago, and is called GENDIA (Genetic DiAgnostics). GENDIA consists of “referral labs” sending samples to GENDIA, “test labs” testing samples they receive from GENDIA, and a central GENDIA lab coordinating the network. Currently more than 2,000 different genetic tests including gene sequencing for almost 1,000 genes are available through GENDIA. Such international network of genetic diagnostic labs results in greater access to a large spectrum of genetic tests performed with higher quality at lower cost (Human Mutation 2008; 29: 772-775).

In the upcoming years technological advances will soon make whole genome/exome sequencing commonplace in the medical environment and spark an expansion of both basic and clinical research in which millions of patients with illnesses will have their complete (coding) DNA sequenced. It is therefore anticipated that a significant part of genetic testing will switch from testing of individual gene to whole genome/exome sequencing on next generation sequencers (NGS). As the latter NGS technology very soon will also incorporate quantitative testing for large deletions and duplications it will make microarray testing (CGHarray) obsolete. It is therefore anticipated that within a few years we will have a single technology (whole genome/exome NGS sequencing) available. At present it is still unclear which patients/individuals will be screened by NGS technology, but patients with unexplained (congenital) anomalies, screening of newborns, premarital screening, preconceptual screening and prenatal screening are possible target groups.

Premarital screening (screening of couples before they get married) is mainly performed in couples from Ashkenazi Jewish origin. Screening programs started in Israel, the US and certain European countries in the early 70s with carrier testing for Tay-Sachs disease by enzyme testing. Since then over one million people have now been screened, screening switched from enzyme testing to molecular testing, and the list of diseases expanded to include now 22 disease genes (Tay-Sachs Disease, Bloom Syndrome, Canavan Disease, Niemann-Pick A, Familial Dysautonomia, Torsion Dystonia, Mucolipidosis Type IV, Fanconi Anemia, Gaucher Disease, Factor XI Deficiency, Glycogen Storage Disease Type Ia, Maple Syrup Urine Disease, Non-Syndromic Sensorineural Hearing Loss, Familial Mediterranean Fever, Alpha 1-Antitrypsin Deficiency, Nemaline Myopathy, Usher Syndrome Type IF, Familial Hyperinsulinemia, Lipoamide Dehydrogenase Deficiency and Glycogen Storage Disease Type III, Familial Hypercholesterolemia, Cystic Fibrosis). In most cases the pathogenic variants common in the Ashkenazi Jewish population are screened for (in total 77 variants in 22 genes). In the orthodox community an organization called Dor Yeshorim carries out anonymous genetic screening of couples before marriage in order to reduce the risk of children with genetic diseases being born. Also prenatal testing for these genetic diseases is offered for Ashkenazi couples. These screening programs have been widely accepted by the Ashkenazi community, and have greatly reduced the frequency of the disorders.

Also in the US and Europe it has become main stream to screen for frequent disorders such as Down syndrome (maternal serum screening), cystic fibrosis/chromosome anomalies (preconceptual and prenatal), and neural tube defects (AFP in amniocentesis). Recently, a general genetic screening test for 416 relatively “common pathogenic variants” in 108 (mostly recessive) disorders has become available. The test is relatively cheap (~500 USD), and is offered in premarital (screening of couples before they get married), preconceptual (screening of couples before they get children), prenatal (CVS, AC) or fertility (IVF, sperm banks) settings. It is anticipated that whole exome NGS sequencing will find its way to premarital, preconceptual and prenatal settings in the years to come when cost will end up below 1,000 Euro per test, and the amount of false positives and false negatives will be better than that of conventional single gene Sanger sequencing.

Unfortunately, our ability to detect DNA variation has greatly outstripped our ability to interpret the impact of such variation on phenotype. Correlating variation in individual genomes with clinical phenotype remains extraordinarily challenging due to the lack of publically available, carefully curated information incorporating what is already known, and rapidly being discovered, about human disease variants and the
disease phenotypes to which these variants contribute. Most of the publically freely available databases of DNA variants associated with human diseases are boutique efforts from research laboratories that are fragmentary, difficult to access, and of uncertain accuracy. In contrast, a major source of information about variants and disease is currently not being exploited: the data generated through clinical testing of patients that are currently sequestered in clinical laboratories and rarely shared with each other or with the scientific community. To address this challenge, we initiated an international collaboration involving many clinical laboratories and curators of variant databases in order to enable the robust and systematic collection, curation and sharing of human genomic variants in an universal database called MutaDATABASE (www.MutaDATABASE.org). MutaDATABASE is a standardized, centralized, and free open access database with information on all human genes (close to 23,000), disease genes (close to 5,000), and disease-causing variants (Nature Biotechnology 2011; 29:117-118).
Whenever one reflects on the ethical questions raised by assisted reproductive technologies (ART) and fertility treatments in general, one may use an ethical calculus as a guide, including respect of patients’ autonomy, beneficence and non-maleficence (doing as much good and as little harm as possible), and justice. This simplified pragmatic approach helps practitioners, but with a caveat. No principle is supreme, and they all matter. Nevertheless, the legal equivalent or “key” to the respect of autonomy is necessary (although not sufficient) and essential to the ethical patient/doctor or researcher relationship: consent of all subjects is thus necessary to ethical practice.

However, the process of obtaining consent, “consenting”, may be more or less complex, depending on whose consent is sought, and their understanding; and the object of the consent.

Thus, the principles of consent, which may only be obtained after appropriate information is given in understandable terms, will be discussed. To know whether it is obtained for therapy or treatment is also essential, and finally two specific cases which render this difficult will be detailed.

Complex consent: for instance, cryo-preservation of reproductive cells or tissues 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. In case of parental consent, it is further complicated by the possibility of “anticipated decision regret”.

Second, arguably the most difficult question of all and in constant evolution is the transition from research to “therapy” and the different emphasis on consent in either case. Indeed, a difficult problem is when to decide that techniques which may be qualified as being “innovative” should be submitted to appropriate research or used for therapy. In such cases, 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.

At the same time, we can illustrate the varied approach to obtaining consent with some currently “hot topics”, such as preservation of cryo-preserved material, which actually precedes use, where often time laps allows accumulation of experience; this can be either in case of illness, or to postpone the ability to reproduce with one’s own genetic material. The case of “futile treatment” will also be addressed.

References:
Wallace WHB and Barr RD (2010). Fertility preservation for girls and young women with cancer: what are the remaining challenges?, Hum Reprod Update, 16:614-616
Over the last thirty-three years, the field of reproductive medicine and the use of Assisted Reproductive Techniques (ART's) for the treatment of infertility have rapidly evolved. As reproductive challenges have gradually been addressed and resolved, many still remain and new ones appear. In the following table I have listed topics which I consider representative of the reproductive challenges in ART for the next 5 years:

<table>
<thead>
<tr>
<th>a) Ovarian Stimulation Protocols</th>
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<tr>
<td>- Mild Stimulation</td>
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<tr>
<td>- Use of GnRH antagonist in luteal phase</td>
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<td>- In vitro Maturation (IVM)</td>
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<td>- Natural cycle and IVM</td>
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<th>b) To Reduce IVF inefficiency</th>
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<tr>
<td>- Selection of the competent Oocyte (create fewer embryos)</td>
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<td>- Selection of the competent Embryo (toward single embryo transfer by transferring only blastocyst; by assessing genome, proteome and metabolome)</td>
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<tr>
<td>- Innovations in the IVF-laboratory (automated embryo culture and imaging systems)</td>
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<tr>
<td>- New IVF media (specific for IVM supplemented with FSH, LH, SSS, EGF, Iron and Calcium, proper AA and carbohydrates, and other factors favoring oocyte meiotic entry)</td>
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<th>c) Fertility Preservation</th>
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<tr>
<td>- Oocyte freezing</td>
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<tr>
<td>- Ovarian cortical strips</td>
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<tr>
<td>- In vitro folliculogenesis (culture of ovarian strips and use of alginate scaffolds)</td>
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<tr>
<td>- Spermatogonial isolation, in vitro expansion and freezing</td>
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<tr>
<td>- Freeze/Dry (lyophylization)</td>
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<th>d) More Basic Science Studies to understand</th>
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<tr>
<td>- Folliculogenesis</td>
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<td>- Unexplained infertility</td>
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<td>- Genetics of Male infertility</td>
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<td>- Genetics of PCOS</td>
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<td>- Endometriosis</td>
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<tr>
<td>- The mechanism(s) responsible for Oocyte Aging</td>
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<th>e) Reappraisal</th>
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<tr>
<td>- Rate of congenital and genetic malformations</td>
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<tr>
<td>- Cross border reproductive care</td>
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<tr>
<td>- Guidelines for Ethical dilemmas (to pay oocyte donors and how much? Should the process be exclusively anonymous? Should all cases of oocyte donation be done with oocytes cryobanked and usable only after a quarantine period? Is Oocyte banking an effective tool to simplify the process of egg donation? How should the sale of oocytes be regulated? And how many oocytes should be given to a recipient for providing a reasonable chance of success?)</td>
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<tr>
<td>- Stem cell and Regenerative medicine (iPS, hESC, UCB, amniotic fluid MSC’s)</td>
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<td>- Preconception screening for recessive disease (saliva tests)</td>
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<th>f) Education</th>
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<tr>
<td>- Teaching Bioethics during Reproductive medicine fellowships</td>
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<tr>
<td>- E-learning courses and obligatory CME (including Epidemiology and Statistics)</td>
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Suggested reading:

Over the past 30 years, advancements in ART have revolutionized the opportunities available for the treatment of infertility. However, ART progress has not been limited to infertility; techniques have concurrently been designed to preserve reproductive potential in otherwise fertile people whose gonadal function may become compromised. Nowadays, the most utilized fertility preservation (FP) measures available to women are oocyte cryopreservation (OC), ovarian tissue cryopreservation (OTC) and in vitro maturation of immature oocytes (IVM). In addition, for female cancer patients electing FP, ovarian transplantation (pre-radiation) and co-administration of GnRH analogues during gonadotoxic chemotherapy also exist, albeit with limited usefulness.

OC is the FP method with the widest application; appropriate patients include women electing to delay childbearing (for a myriad of reasons), a newly-diagnosed malignancy, or a non-cancerous medical condition (sickle cell disease, scleroderma, systemic lupus erythematosus, etc.) whose treatment or disease progression places the afflicted patient at high risk for future ovarian stimulation. In the latter scenario, a gestational carrier may be required. Furthermore, OC has significantly advanced the field of oocyte donation by allowing fertility centers/”banks” to store the oocytes of young women for future recipient usage, even years later. To date, approximately 1200 babies have been born after OC, with no apparent increased incidence of congenital anomalies, suggesting the safety of this procedure.

OTC, another FP option, has gained notoriety in the past decade, particularly in young patients (ideally < 30 year old) diagnosed with malignancy, especially of hematologic origin. In fact, of the 10+ deliveries to date, Hodgkin’s Lymphoma is the cancer indication that has most often resulted in a live birth outcome. This technology holds great promise and will certainly play a role as FP advances forward. Although IVM has the potential to serve a broad group of women (newly-diagnosed cancer as well as healthy women, chiefly those with polycystic ovarian syndrome), currently its role in FP for patients diagnosed with malignancy is limited. Despite numerous reported successes (~1500 births worldwide), as yet, few have been achieved in women with cancer. Furthermore, a significant proportion of the births to date have come from oocytes already in metaphase II at time of extraction. However, one advantage of IVM is that it can be used in conjunction with OTC, the combination of which may provide women with the greatest chance for future pregnancy. An added benefit is that neither of these FP methods requires ovarian stimulation, making either (or both) ideal when a patient’s time is limited. However, IVM is more successful when preceded by ovarian priming. Therefore, if an adequate time-window between cancer diagnosis and treatment exists, OC should be the FP measure most strongly considered as it currently provides patients with the greatest chance for future liveborn outcome. Importantly, when applicable, novel combinations of FP methods should be entertained.

In summary, the above FP technologies have allowed reproductive endocrinologists to provide patients who desire or require FP, feasible options to achieve parenthood in the future. Such advancements parallel the global shift in age at first birth; thus, the combination has led to an explosion in the use of FP methods. In this presentation, strengths, weaknesses and challenges associated with all acceptable and available FP technologies will be reviewed.
Lyophilization also known as freeze drying is a method of preserving materials in a dry state for long periods of time. Using lyophilization at large scales was developed during WWII for the purpose of drying blood plasma and shortly after it was used for penicillin and bone and it has been recognized as an important technique for the preservation of biologicals. Although lyophilization of cells has numerous advantages over freezing such as: low cost storage, being lightweight, easily transportable, simplicity of handling and less prone to hazards, we rarely find cells preserved using this technique. Conventional freezing methods use cryoprotectants such as glycerol, DMSO, ethylene glycol and their likes. Using these cryoprotectants, which are liquid at room temperature and are toxic to the cells, makes the storage at high temperatures impossible. In addition, the drying process which needs to take place below the samples glass transition temperature (Tg) needs to be at very low temperatures usually below -80°C which cannot be achieved with current commercial lyophilizers.

Nevertheless, recent developments in the field show that most likely this will be the way to preserve live cells in the near future. Recently, it has been reported that freeze dried mice spermatozoa were able to generate normal offspring following injection (ICSI) into mature mice oocytes. We have recently reported for the first time that nucleated cells maintain genomic integrity following prolonged storage (4 years) in a dry state, and were able to direct early embryonic development following injection into enucleated sheep oocytes. Furthermore, we have reported and others have followed the ability of lyophilized hematopoietic stem cells derived from umbilical cord blood to proliferate and differentiate to different blood cells.

As more and more cells are being used for medical purposes the need for a better and easier preservation method arise and the obvious choice will be by lyophilization.
Recurrent pregnancy loss (RPL) is defined as the occurrence of 3 or more consecutive losses prior to 20 weeks of gestation. The overall risk of miscarriage is approximately 15% in unselected population and in women after first abortion but rises to 17-31% after two consecutive losses and to 25-46% after 3 or more. Hence, the high frequency of subsequent loss following two losses supports the decision of most clinicians to start a clinical evaluation after the second abortion. Common recognized causes of RPL are genetic abnormalities, endocrine dysfunction, immunologic disorders and uterine abnormalities. Aneuploidy is the most common cause of embryonic loss before 10 weeks of gestation. Genetic evaluation of products of conception is recommended after each abortion and abnormal results can be detected in 50-70% of cases. The parental karyotype is abnormal in 2-4% of all couples with RPL and higher frequencies up to 10% are found when other common causes are first excluded. Folate deficiency has been associated with aneuploidy and supplementation is recommended in all patients with RPL. Thyroid dysfunction has been implicated in pregnancy loss. Overt hypothyroidism or hyperthyroidism must be diagnosed and treated. Data on treatment of other minor thyroid dysfunction such as subclinical hypothyroidism or presence of antithyroid antibodies are insufficient to warrant uniform treatment in patients with RPL. While luteal phase deficiency has been associated with RPL, clinical trials have provided mixed results regarding efficacy of treatment with progesterone supplementation in women with unexplained RPL. Polycystic ovarian syndrome is a common condition frequently associated with RPL (36-56%). Some observational studies treating these patients with Metformin demonstrated a decrease of spontaneous abortion rate but data were not confirmed in a larger study. The presence of uterine abnormalities such as Mullerian fusion defects, submucous myomas endometrial polyps and Asherman syndrome have been described with RPL. Sonohysterography for evaluating uterine cavity followed by hysteroscopic resection of pathologic findings have been shown to improve pregnancy outcome. Antiphospholipid syndrome has been diagnosed in 15-40% of women with RPL and screening is recommended. Results of meta-analysis suggest that these patients could benefit from a combination treatment of heparin and low dose aspirin. Factor V Leiden is associated with a small increased risk of fetal loss, but other common mutations such as MTHFR and prothrombin gene mutation G20210A are not. Widespread screening for inherited thrombophilia appears to have limited value. After evaluation, RPL remains unexplained in approximately one half of couples. Following previous contrasting results, recent randomized trials demonstrate that treatment with low molecular weight heparin and/or aspirin do not improve pregnancy outcome in women with unexplained RPL. More intensive investigation of genetic etiologies of fetal loss are likely to reduce the proportion of unexplained recurrent fetal loss.
Endometrial secretomics describes the analysis of the molecular constituents of endometrial secretions. The relevance of this approach in the study of endometrial receptivity derives from the simple and minimally invasive means by which material for study can be obtained offering both researchers and clinicians a window on the intra-uterine environment.

The endometrial secretome is known to contain a number of mediators which are the reflection of the endometrial receptivity status which may be involved in the nurturing of the preimplantation embryo. The primary components are proteins, aminoacids, electrolytes, glucose, urea, cytokines, growth factors, metalloproteinases and their inhibitors, immunoglobulins, alpha-1 antitrypsin precursor, haptoglobin, and transferrin (Beier et al 1998, Parmar et al 2008). Recently Luminex technology has enabled the analysis of multiple mediators in a small sample of endometrial fluid. Two matched controlled studies have demonstrated that endometrial fluid aspiration can be performed immediately prior to embryo transfer in IVF cycles without negatively affecting implantation rates (Boomsma et al, 2009; van der Gaast et al, 2003).

Lipidomics is a fast developing technology that allows characterisation of a global lipid profile from a given cell or organism. The approach profits from the high sensitivity and resolution that results from the combination of high-performance liquid chromatography (HPLC) with mass spectrometry (Wenk, 2005). Most of these studies have concentrated on the role of prostaglandins (PGs) in endometrium; in this context defective endometrial PG synthesis in humans has been linked with repeated implantation failure in patients undergoing IVF (Achache et al., 2009).

Here based on our own research (Berlanga et al, 2011) we will discuss how lipidomics can help improve our current understanding of human endometrial receptivity both in basic and translational research.

References:


