Endometrium and Embryo Implantation: the Hidden Frontier
GENERAL INFORMATION

VENUE
Hilton Lyon
70 Quai Charles De Gaulle
69463 Lyon, France
Tel: +33-4-7817-5050

LANGUAGE
The official language of this Conference will be English.

LOCATION
Lyon, city of pairs with its 2 rivers, the Rhône and Saône, and its twin hilly districts, Fourvière “the hill of prayers” and Croix Rousse “the hill of workers”. A city proud of its monuments that cohabit marvelously with very contemporary buildings. Intimate and open, mysterious and influential, impetuous and nonchalant, the home of the famous bouchon bistros, a capital of gastronomy, surrounded by vineyards known the world over, listed by UNESCO as a World Heritage site, a city famous for the invention of the silk and the birthplace of the inventors of cinema.
Implantation is a complex process which requires the orchestration of a series of events involving both the embryo and the endometrium. Due to the complexity of the mechanisms involved and relative lack of information on some of them, it has been defined “black box” of assisted reproduction. Even with the transfer of high quality embryos, implantation rates still remain relatively low, thus failure of implantation is a major factor which influences negatively ART outcomes. Recently, thanks to the new research tools such as molecular biology, genetics and metabolomics, the implantation process becomes increasingly clear and this will soon provide solutions to improve outcomes. Aim of this event is therefore to review all these scientific attainments, starting from the physiology of embryo and endometrium to the mechanisms of implantation in stimulated cycles, till the diagnostic techniques for predicting implantation. An overview on present and future tools to improve implantation process will be provided.

LEARNING OBJECTIVES
This Conference will offer to participants the possibility to:

• Get an exhaustive overview of embryo-endometrium interaction
• Acquire the latest updates on mechanisms of endometrial receptivity in stimulated cycles
• Be updated on present and future solutions to improve implantation in ART

TARGET AUDIENCE
This program is targeted to clinicians and scientists working on Assisted Reproduction Techniques.

ACCREDITATION
Serono Symposia International Foundation will submit this program “Endometrium and Embryo Implantation: the Hidden Frontier” (Lyon, France - September 24-25, 2010) for accreditation by the European Accreditation Council for Continuing Medical Education (EACCME).
SCIENTIFIC SECRETARIAT

Serono Symposia International Foundation
Salita di San Nicola da Tolentino, 1/b
00187 Rome, Italy
Project Manager: Vanessa Spaziano
Phone: +39-06-420413 569
Fax: +39-06-420413 677
E-mail: info@seronosymposia.org
Serono Symposia International Foundation
is a Swiss Foundation with headquarters in
14, rue du Rhône, 1204 Geneva, Switzerland

ORGANIZING SECRETARIAT

Meridiano Congress International
Via Mentana, 2/B - 00185 Rome, Italy
Congress Coordinator: Sara Guglielmini
Phone: +39-06-88595 211
Fax: +39-06-88595 234
E-mail: s.guglielmini@meridiano.it
SCIENTIFIC ORGANIZER

Bruno Salle
Reproductive Medicine Service
Hospices Civils de Lyon
University Claude Bernard Lyon I
Unité INSERM 846, Cellules Souches & Cerveau
Lyon, France

SCIENTIFIC COMMITTEE

Paul Barriere
Reproductive Medicine and Biology Unit
Centre Hospitalier Universitaire de Nantes
Nantes, France

Eleonora Porcu
Infertility and IVF Center
University of Bologna
Bologna, Italy
LIST OF FACULTY MEMBERS

Aydın Arici
Department of Obstetrics, Gynecology & Reproductive Sciences
Yale University
School of Medicine
New Haven, CT, USA

Jean-Daniel Baki
Board of Directors
Serono Symposia International Foundation
Geneva, Switzerland

Paul Barriere
Reproductive Medicine and Biology Unit
Centre Hospitalier Universitaire de Nantes
Nantes, France

Mehdi Benchaib
Hôpital Femme Mère Enfant
Department of Medicine and Reproductive Biology
of Lyon Hospital
Bron Cedex, France

Carlo Bulletti
Physiopathology of Reproduction
University of Bologna, Polo Scientifico Didattico of Rimini
and ASL of Rimini
Cattolica, Italy

Ying Cheong
Division of Developmental Origins of Adult Diseases
University of Southampton School of Medicine
Southampton, UK

Francisco Dominguez
Embryomics
Research Department
Deio (Bizkaia), Spain

Robert Fischer
IVF Unit
Fertility Centre Hamburg
Hamburg, Germany

Thomas Freour
Reproductive Medicine and Biology Unit
Centre Hospitalier Universitaire de Nantes
Nantes, France

José A. Horcajadas
Fundación IVI-Instituto Universitario IVI
University of Valencia
iGenomix, Valencia, Spain

Jean Noël Hugues
Reproductive Medicine Unit
CHU Jean Verdier - University Paris XIII
Bondy, France

Efstratios M. Kolibianakis
1st Department of Obstetrics and Gynaecology,
Aristotle University of Thessaloniki
Thessaloniki, Greece

Nathalie Ledée
INSERM U-782
Endocrinologie et Génétique de la reproduction
et du developpement
Hôpital Antoine Béclère, Clamart, France

Santiago Munné
Reprogenetics
Livingston, New Jersey, USA

François Olivennes
IVF Centre
Eylau La Muette
Paris, France

Anna Ponnampalam
The Liggins Institute
The University of Auckland
Grafton, Auckland, New Zeland

Eleonora Porcu
Infertility and IVF Center
University of Bologna
Bologna, Italy

Ariel Revel
Hadassah Medical Organization
Obstetrics and Gynaecology Department
Jerusalem, Israel
Bruno Salle
Reproductive Medicine Service
Hospices Civils de Lyon
University Claude Bernard Lyon I
Unité INSERM 846, Cellules Souches & Cerveau
Lyon, France

Mourad W. Seif
Academic Department of Obstetrics and Gynaecology
University of Manchester
Manchester, UK

Emre Seli
Division of Reproductive Endocrinology and Infertility
Oocyte Donation and Gestational Surrogacy Program
Department of Obstetrics, Gynecology,
and Reproductive Sciences
Yale University School of Medicine
New Haven, CT, USA
SCIENTIFIC PROGRAM
FRIDAY - SEPTEMBER 24, 2010

08.00   Registration

09.00   Welcome on behalf of Serono Symposia International Foundation
        Jean-Daniel Baki, SSIF Board of Directors, Switzerland
        Robert Fischer, SSIF Scientific Committee, Germany

09.05   Introduction and Opening
        B. Salle, France

SESSION I

New Fundamental Basis

Chairmen: B. Salle, France - A. Arici, USA

09.15 Keynote Lecture: physiology of the embryo
        F. Dominguez, Spain

09.30 L1: Endometrium genomic and uterine receptivity
        A. Arici, USA

10.00 L2: Epigenetic regulation of endometrium receptivity
        A.P. Ponnampalam, New Zealand

10.30 L3: Endometrial functional genomics and proteomics during COS in ART
        J.A. Horcajadas, Spain

11.00 Coffee Break

11.15 L4: Immunology of the endometrium during ART
        N. Lédée, France

11.45 Discussion

12.30 Lunch
SESSION II
From Basis to Practice: Laboratory Tools

Chairmen: P. Barriere, France - E. Seli, USA

14.00  **L5:** Embryo and oocyte cryopreservation in the process of implantation
       E. Porcu, Italy

14.30  **L6:** DNA sperm fragmentation paternal effect on early implantation
       M. Benchaih, France

15.00  **L7:** Hatching in IVF and ICSI
       M.W. Seif, UK

15.30  **L8:** PGD and implantation
       S. Munné, USA

16.00  Coffee Break

16.15  **L9:** Is there an interest in addition of hyaluronan to improve implantation?
       E.M. Kolibianakis, Greece

16.45  **L10:** Embryo selection
       T. Freour, France

17.15  **L11:** How to improve culture medium to enhance implantation?
       E. Seli, USA

17.45  Discussion

18.15  End of the first conference day
SATURDAY - SEPTEMBER 25, 2010

SESSION III
From Basis to Practice: Clinical Approach

Chairmen: B. Salle, France - F. Olivennes, France

09.00  L12:  Impact of COS on human endometrial receptivity
            Y. Cheong, UK

09.30  L13:  Impact of uterine vascularization on receptivity and effects of medications aimed at improving it
            B. Salle, France

10.00  L14:  Uterine contractions and uterine receptivity
            C. Bulletti, Italy

10.30  Coffee Break

11.00  L15:  Thin endometrium in ART: what to do?
            A. Revel, Israel

11.30  L16:  hCG/LH supplementation during luteal phase may improve receptivity
            J.N. Hugues, France

12.00  Round Table: how to improve implantation
            A. Arici, USA - S. Munné, USA
            E. Seli, USA

12.45  Closing remarks
            B. Salle, France

13.00  Lunch

End of the conference
DISCLOSURE OF FACULTY RELATIONSHIPS

Serono Symposia International Foundation adheres to guidelines of the European Accreditation Council for Continuing Medical Education (EACCME) and all other professional organizations, as applicable, which state that programs awarding continuing education credits must be balanced, independent, objective, and scientifically rigorous. Investigative and other uses for pharmaceutical agents, medical devices, and other products (other than those uses indicated in approved product labeling/package insert for the product) may be presented in the program (which may reflect clinical experience, the professional literature or other clinical sources known to the presenter). We ask all presenters to provide participants with information about relationships with pharmaceutical or medical equipment companies that may have relevance to their lectures. This policy is not intended to exclude faculty who have relationships with such companies; it is only intended to inform participants of any potential conflicts so participants may form their own judgments, based on full disclosure of the facts. Further, all opinions and recommendations presented during the program and all program-related materials neither imply an endorsement, nor a recommendation, on the part of Serono Symposia International Foundation. All presentations solely represent the independent views of the presenters/authors.

The following faculty provided information regarding significant commercial relationships and/or discussions of investigational or non-EMEA/FDA approved (off-label) uses of drugs:

<table>
<thead>
<tr>
<th>Faculty Name</th>
<th>Relationship Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aydin Arici</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Jean-Daniel Baki</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Paul Barriere</td>
<td>Declared receipt of honoraria or consultation fee by Schering-Plough and Genevrier.</td>
</tr>
<tr>
<td>Mehdi Benchaib</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Carlo Bulletti</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Ying Cheong</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Francisco Dominguez</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Robert Fischer</td>
<td>Declared receipt of honoraria or consultation fee by SSIF.</td>
</tr>
<tr>
<td>Thomas Freour</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>José A. Horcajadas</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Jean Noël Hugues</td>
<td>Declared receipt of honoraria or consultation fees by Merck Serono.</td>
</tr>
<tr>
<td>Efstratios M. Kolibianakis</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Nathalie Lédée</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Santiago Munné</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>François Olivennes</td>
<td>Declared receipt of honoraria or consultation fee by Merck Serono and Genevrier.</td>
</tr>
<tr>
<td>Anna Ponnampalam</td>
<td>Declared no potential conflict of interest.</td>
</tr>
<tr>
<td>Eleonora Porcu</td>
<td>Declared no potential conflict of interest.</td>
</tr>
</tbody>
</table>
Ariel Revel  Declared no potential conflict of interest.
Mourad W. Seif  Declared no potential conflict of interest.
Emre Seli  Declared no potential conflict of interest.

The following faculty have 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 13, 2010:

Bruno Salle
ABSTRACTS
(L1 - L16)
Embryonic implantation, the process by which the human embryo orientates towards, attaches to and finally invades the underlying maternal endometrial tissue, requires a receptive endometrium, a functionally and chromosomically normal blastocyst and an adequate cross-communication between them. During apposition, human blastocysts find a location in which to implant, while they are guided to a specific area in the maternal endometrium. In the adhesion phase, which occurs 6 to 7 days after ovulation, within the so-called "implantation window", a direct contact occurs between the endometrial epithelium (EE) and the trophoectoderm (TE). Finally, in the invasion phase, the embryonic trophoblast traverses the basement membrane, passes the endometrial stroma and reaches the uterine vessels. Many molecules (hormones, cytokines, integrins, enzymes, etc) take part in the dialogue between the human blastocyst and the maternal endometrium to achieve implantation. But relatively little is known regarding the proteome/secretome of the human pre-implantation embryo, in particular the protein composition/secretion of the blastocyst just before implantation. Taking into account the legal and ethical guidelines, new approaches are needed to study the human embryo physiology prior to the implantation process.
Nowadays morphological assessment is the primary method used to determine embryo viability during IVF cycles, but technological advances in translational research have made possible the use of non-invasive methods to determine the proteomic and metabolic status of the human embryo.
Implantation is the process by which the blastocyst becomes intimately connected with the maternal endometrium. The independently developing blastocyst then becomes dependent on the maternal environment for its continued development. Although the factors involved in the regulation of blastocyst implantation are incompletely understood, recent studies have advanced our knowledge and understanding of the communication between maternal and fetal cells during this critical period.

A fundamental requirement for successful implantation is that there must be a synchrony between the development of the embryo and the uterus. Under the influence of ovarian hormones, the uterine lining reaches a transient receptive stage at which an embryo that has reached the blastocyst stage can attach. Overall, estrogen and progesterone control the cyclic changes that occur in the endometrium in preparation for pregnancy. Data suggest that the absolute amounts of both of these hormones may be of greater importance in inducing a normal secretory endometrium than the ratio between the two. Insufficient progesterone production in the luteal phase may result in imperfectly developed endometrium, failed implantation, or early pregnancy loss, suggesting that patients with these problems might respond to progesterone therapy. In the endometrium, growth factors (i.e., CSF, EGF, TGF-β) and cytokines (i.e., LIF, IL-1, IL-15) function to mediate the actions of steroids to promote endometrial receptivity, directly enhance embryo viability and growth, and induce decidual changes that control the trophoblastic invasion of the embryo. Several adhesive glycoproteins have been identified in the endometrium, including integrins, cadherins, and carbohydrate-rich membrane-bound glycoproteins. Currently, it is thought that each of these molecules has a role in the implantation cascade.

Leukocytes form a substantial proportion of the cells of human endometrium, accounting for approximately 7% of stromal cells in the proliferative endometrium and increasing to more than 30% in early pregnancy, characteristic of an immunologically active tissue. However, organization of the endometrial lymphoid system is atypical in several aspects. Large granular lymphocytes, resemble natural killer cells, form the most abundant lymphoid cell population in human endometrium in the late luteal phase and in early pregnancy. Their increase around the days of expected implantation, and their persistence in the first trimester of pregnancy followed by a rapid decline have suggested that these cells may play a role in implantation and placental development.

A range of proteins and peptides that are the products of homeobox genes have been found to be very important for early embryonic development. There are two Hox genes that are thought essential for implantation in mice because homozygous mutants of either of these genes are infertile as a result of unreceptive endometrium. The genes HoxA10 and HoxA11 are expressed in the endometrial glands and stroma of the uterus throughout the menstrual cycle in humans. The expression of both of these genes rises dramatically at the time of implantation during the mid-luteal phase, and thereafter remains elevated throughout the remainder of the cycle as well as in the decidua of pregnancy in humans. This pattern of expression in the adult suggests that Hox genes play a role in human implantation. As additional research is performed to understand the molecular aspects of endometrial receptivity and implantation, new markers may emerge which can be used to assess the implantation process.
Implantation is a highly controlled process, involving a dialogue between the endometrium and the implanting embryo which is crucial for the establishment and maintenance of pregnancy. There is limited information about the molecular regulation of implantation; however inadequate uterine receptivity is thought to be responsible for two thirds of implantation failures. Consequently, strict control of gene expression is crucial to ensure the endometrium undergoes regular implementation of cyclic periods of growth and differentiation. Aberrant expression of endometrial regulatory genes via inappropriate epigenetic modification may therefore provide a partial explanation for the failure of embryo implantation. Although it is not known to what extent epigenetic mechanisms are involved in the regulation of normal endometrium, there is increasing evidence that epigenetic mechanisms regulate many biological processes that are crucial for successful embryo implantation in the endometrium.

Methylation and histone acetylation have been directly correlated with the expression of implantation related genes, including LIF, HOXA10, glycodelin, MMPs and TIMPs, and E-Cadherin. Furthermore, the steroid hormone receptors themselves are susceptible to epigenetic modulation, which in turn are capable of inducing the modification to the chromatin structure of other genes. This is highlighted by the ability of the histone deacetylase inhibitor, trichostatin A (TSA), in the enhancement of oestradiol and progesterin-induced decidualisation of primary human endometrial stromal cells. Results from our group show that DNA methylation regulates both trophoblast invasiveness into the endometrium and uterine epithelial cell receptivity in vitro. Recent preliminary data from our group critically demonstrate that the expression and regulation of DNA methyltransferases (DMNTs) in human endometrium are dynamic, with the expression of DNMTs generally down-regulated at the time of implantation. We have also demonstrated that changes in global histone acetylation correlate well with expected transcription activity during the menstrual cycle. Furthermore, our data imply that there is an intrinsic interplay of two way communication between the epigenetic modulators and steroid hormone receptors.

Deregulation of implantation may have consequences beyond the failure of implantation and subsequent infertility. In mice, even a transient postponement of blastocyst attachment is sufficient to cause a detrimental ripple effect throughout the pregnancy, with aberrant spacing of embryos, defective placentation and retarded development of foetuses observed. Poor implantation and placentation are also associated with changes close to parturition such as intrauterine growth restriction, preeclampsia, and preterm birth in humans. The concept of small interruptions; such as inappropriate epigenetic regulation, in early embryo-uterine dialogue, leading to long reaching effects on the foetus throughout gestation, suggest a means by which developmental programming may be altered by early environmental interactions and maintained in this altered state by the offspring. The research findings to date in this field and long-term likelihood of clinical applications of this emerging research area will be discussed in the paper.
Controlled ovarian stimulation (COS) used in assisted reproduction techniques (ART) produces lower implantation rates per embryo transferred as compared to natural and ovum donation cycles, suggesting a suboptimal endometrial development. Endometrial alterations have been observed by histological and biochemical techniques during decades. The recent developments in functional genomics have provided objective tools to analyze the endometrium in natural cycles and evaluate the impact of COS protocols in endometrial development. COS cycles that use GnRH agonists and antagonists have been analyzed in detail during the window of implantation to establish the differences with respecting to the natural cycle. Even more, it has been demonstrated that endometrium from natural cycles follows different genomic patterns compared to COS cycles in the transition from the pre-receptive (days LH/hCG+1 until LH/hCG+5) to the receptive phase (day LH+7/hCG+7). Specifically, it has been demonstrated the existence of a two-day delay in the activation/repression of two clusters composed by 218 and 133 genes on day hCG+7 versus LH+7.

As differences in gene expression at RNA levels is not always reflected at protein levels, proteomics is also a new tool for the analysis of the protein expression during the window of implantation and to compare the changes induced by COS.

We have herein reviewed the results obtained in different studies from the molecular point of view to elucidate the changes induced by the different protocols used for ovarian stimulation in an attempt to evaluate their potential clinical implications.

The success of implantation depends on a receptive endometrium, a normal blastocyst and synchronized cross-talk at the maternal–fetal interface. Routinely, less than 5% of oocytes collected and only 20 to 25% of embryos transferred lead to a birth. Improving our result in ART relies on a better understanding of the pre- and peri-conception dialogue. A cascade of cytokines mediates this dialogue in the endometrium. Such cross-talk involving both the immune and endocrine systems is crucial to prevent implantation failure.

A mobilization of immune cells occurs in the middle luteal phase to induce the uterine receptivity and involves mainly uterine Natural Killer cells, T regulatory cells and dendritic cells. Such actors are clearly mandatory to establish a local, transitory and constructive environment suitable for pregnancy. Uterine Natural Killer cells (uNK), T regulatory cells and dendritic cells secrete an array of cytokines important for adequate local immune regulation, angiogenesis, placentation development, and establishment of ongoing pregnancy.

At the time of ovulation, some components in the seminal plasma and follicular fluids seem to be involved as triggering factors for such an adequate recruitment by a secretion of colony stimulating factors (GM-CSF and G-CSF). Recent studies using multiplexed bead-based assays explored the uterine luminal environment at the time of oocyte retrieval. They also highlight the importance of the pre-conception immune cross-talk. Human implantation is often described as a three-step process summarized as embryo apposition, adhesion and invasion. From an immunological point of view, apposition and adhesion require an initial pseudo-inflammatory expression from both parts to occur. While during trophoblastic invasion, the maternal role is dedicated to the control of the invasion by modulating angiogenesis and local cell death. Some authors recently clearly demonstrated that the endometrium behaves as a biosensor of the embryo both in human and animal models. Both deficient and excessive expression of cytokines and immune cell numbers and activation indeed play detrimental key roles in implantation, since these actors can have both positive and negative effects. For example, in human reproduction, a proper balance in the IL-12, IL-18 and IL-15 controls the local uNK (CD56+) recruitment and the sub-endometrial angiogenesis. The role of TWEAK appears important in implantation. TWEAK is described as acting in a Yin and Yang relationship with TNFα, because it counteracts its deleterious effects and has pro-angiogenic properties. Recent data suggest that a high IL-18/TWEAK mRNA expression might reflect a excessive and cytotoxic uNK cell recruitment whereas a low expression of IL-15/Fn-14 ratio might indicate uNK depletion. Such an approach may be helpful for revealing an imbalance of crucial cytotoxic/angiogenic/immunotrophic pathways.

New strategies are emerging to aid our understanding of the underlying rationale leading to implantation failures and to identify specific defective pathways prior to fertilization or implantation. Identification of new biomarkers testing to either adequate oocyte and sperm competence or adequate/inadequate uterine receptivity with the mechanism should help us to define appropriate treatment.


EMBRYO AND OOCYTE CRYOPRESERVATION IN THE PROCESS OF IMPLANTATION

Eleonora Porcu
Infertility and IVF Center, University of Bologna, Bologna, Italy

Embryo and oocyte cryopreservation increase ART flexibility and improve the cumulative pregnancy rate. In addition, postponing embryo transfer to a subsequent cycle is sometimes advisable or, at times, mandatory. Obviously, "fresh cycles" and "cryopreserved cycles" are different in both embryo and endometrium features. In my presentation, I will compare the efficiency of fresh embryo transfers in COS cycles with transfers of cryopreserved embryos and embryos deriving from cryopreserved oocytes in natural cycles and in hormonal replacement cycles. Furthermore, I will discuss and compare the role of different protocols of hormonal support of the endometrium in fresh and thawed cycles.
Currently, DNA fragmentation index (DFI) is calculated on the total sperm. The purpose of this study is to calculate the DFI but taking into account the morphology of the sperm head. The global DFI can be decomposed in two parts according to spermatozoa morphology: 1) DFI in spermatozoa with considered normal head, 2) DFI in spermatozoa with considered abnormal head. Thus we can determine if these partial DFIs enhance the prognostic power of DFI in the outcome in Assisted Reproductive Technique (ART).

A preliminary study was conducted retrospectively, and it concerned couples for whom a measure of DFIs was conducted within three months before attempting ART. These attempts have taken place at the Department of Medicine and Reproductive Biology of Lyon Hospital. Seventeen cycles were included, they are distributed as follows: 2 cycles of classical IVF (c-IVF), and 15 cycles of ICSI. The measurement of sperm DNA fragmentation was performed by the TUNEL technique.

The calculation of the DFIs was made taking into account the morphology of the spermatozoa head. Thus, three different DFIs were calculated: 1) 'global DFI': total spermatozoa with fragmented DNA / total spermatozoa, 2) 'normal morphology DFI': normal head spermatozoa with fragmented DNA / total normal head spermatozoa, and 3) 'abnormal morphology DFI': total abnormal head spermatozoa with fragmented DNA / total spermatozoa with abnormal head. A ROC curve allowed us to determine the optimal threshold values. This first study allowed us to validate the model of decomposition of DFI. A prospective study was then performed. This prospective study was conducted from November 2009 and December. 31 cycles were included distributed in 14 cycles c-IVF and 17 cycles of ICSI. The DFIs were measured from the semen used during ART procedure.

These studies have shown that the decomposition of global DFI in partials DFIs according to spermatozoa morphology increases the prognostic power of this parameter. Furthermore, we showed that even in the case of sub-normal sperm characteristics and/or normal value of global DFI, the decomposition of global DFI provides information on the outcome of the attempt.
HATCHING IN IVF AND ICSI

Mourad W. Seif  
Academic Department of Obstetrics and Gynaecology, University of Manchester, Manchester, UK

Artificial disruption of the Zona Pellucida (ZP) was first suggested in the 1980s and has since been known as Assisted Hatching. The ZP is a unique extracellular coat which is synthesised by, and surrounds mammalian oocytes. It is composed of three sulphated glycoproteins known as ZP1, ZP2, and ZP3 (huZP in humans) which are best characterised in mice and have both structural and biological functions. ZP is the site of primary interaction of sperms with oocytes, leading to species-specific binding of sperms and induction of acrosome reaction (by ZP3). Following fertilisation, huZP1 is not detected and huZP2 undergoes proteolysis leading to modification of ZP that is required to block polyspermy. Thereafter, Zona maintains the three-dimensional integrity of embryos and facilitates their passage through the fallopian tubes, in addition to providing protection against microorganisms and immune cells.

Implantation rate of embryos resulting from IVF is relatively low. While this can be partially explained by the poor quality of embryos and/or by defective endometrial receptivity, data suggest that cultured embryos hatch and implant at lower rates than occur naturally and that hardening of ZP, as a result of cross-linking of its constituent glycoproteins, may occur in the in-vitro environments. On the other hand, evidence suggests that embryos which have undergone Zona manipulation tend to implant a day earlier. Zona thickness (ZPT) may be influenced by smoking, women’s age and their hormonal profile. Recent data suggest that thickness variation (ZPTV) rather than ZPT correlates with embryo quality, thus higher ZPTV may be associated with improved embryo implantation.

Evidence from systematic review and meta-analysis, of randomised controlled trials, demonstrates significantly improved odds of clinical pregnancy rates after AH (OR 1.29, 95% CI 1.12 to 1.49). The significance was attenuated when the analysis was limited to more robust trials and, in fact, was eliminated when analysis was limited to trials reporting live birth rates. In women with poor prognosis and/or previous failed attempts, AH was associated with improved odds of 74% for frozen embryo transfer cycles and 25% for fresh ones.

Nevertheless, there is insufficient evidence to determine a positive effect of AH on live birth rates. While this may be explained by the under reporting of live birth outcomes, there remains a need to evaluate the cost implications, on the “take home baby” rate, for the inclusion of AH in ART-services.

AH is associated with a significant 67% increase in odds ratio of multiple pregnancy rates. This is indeed concerning since AH and multiple embryo transfer (ET) are often offered to older women, to improve their chances of pregnancy. These data support the case for single ET.

There is a need for more robust trials of high methodological quality to provide evidence on the full implications of AH on assisted conception, particularly with respect to live birth and adverse outcomes.
The majority of embryos produced in vitro are chromosomally abnormal when they are analyzed by array CGH, ranging from 46% normal embryos in women <35 years old to 18% in women >40 years old. If this data is compared to the IVF SART data of 2008, where 30%-6% of embryos implanted and reached term for those age groups, only 12-16% of ongoing implantation potential is not explained by chromosome abnormalities.

However, PGD using day 3 biopsy and FISH techniques has produced contradictory results, which have been attributed to mosaicism, the FISH technique or the biopsy stage. Our results clearly show that mosaicism, although rampant, it created only 7% of errors since most mosaics are a combination of only abnormal cells of different kinds. Instead the problem seem to have been high technical error rates in some studies (up to 50%), and high embryo damage caused by the biopsy (up to 59% in one study).

Recently, two developments can significantly improve PGD techniques. One is the advent of microarrays. The array CGH platform for instance can analyze all chromosomes dozens of times with >25% of the genome being directly assessed. With this technique we detected only a 2% error rate in day 3 biopsies and 0% in blastocyst biopsies. Implantation rates and miscarriage rates seem to be significantly improved, especially for patients with recurrent pregnancy loss (7% observed, 30% expected). The second recent development is vitrification. Survival rates for blastocyst vitrification of up to 99% allow performing blastocyst biopsy and having plenty of time for its analysis. Blastocyst biopsy seems less detrimental than day 3 embryo biopsy, and combined with a better uterine receptivity in thawed cycles yields implantation rates between 76-80%, and the decrease in implantation observed with increasing maternal age disappears.
IS THERE AN INTEREST IN ADDITION OF HYALURONAN TO IMPROVE IMPLANTATION?

Efstratios M. Kolibianakis
1st Department of Obstetrics and Gynaecology, Aristotle University of Thessaloniki, Thessaloniki, Greece

Hyaluronan is a linear polysaccharide naturally present in cumulus cells and in cervical mucus as well as in the oviductal, uterine and follicular fluid in mice, cattle, pig, and humans. Considering the presence of hyaluronan receptors both in the endometrium and the embryo, the involvement of hyaluronan in embryo implantation process appears likely and has been the subject of research for many years. Several studies have been performed up to date in order to assess whether the addition of hyaluronan in the human embryo culture system can improve pregnancy rates. However, their results are still inconclusive. The purpose of this systematic review and meta-analysis was to summarize the available evidence from randomized controlled trials regarding the effect of hyaluronan on the probability of pregnancy after IVF.

A literature search covering the period until December 2007 was performed independently by two reviewers aiming to identify studies that could answer the research question of interest: is clinical pregnancy rate increased with the use of hyaluronan in in-vitro culture after IVF? Reference lists of eligible studies and relevant review articles were also hand searched. By this process thirteen relevant randomized controlled trials were identified. The majority of these trials did not detect a statistically significant association between hyaluronan use and the probability of pregnancy. However, a significant difference in clinical pregnancy rates favouring the use of hyaluronan was observed by meta-analysis of the above studies. The use of hyaluronan increased clinical pregnancy rates by approximately 8%.

Based on the analysis of more than 4400 patients, it can be supported that the use of hyaluronan in in-vitro culture after IVF increases significantly clinical pregnancy rates.
Despite the huge improvement of knowledge in the field of early embryo metabolism, genomic, gamete competence and in vitro culture conditions, even with recent technical evolutions, the most accurate way of selecting embryos with the highest implantation potential in the IVF lab still remains to be identified and validated. Numerous studies are available in the literature, generally exploring promising strategies in opposition with traditional ones. But so far few randomised trials or meta-analysis, the most powerful studies related to evidence-based medicine criteria, have objectively compared the respective relevance of these strategies.

As some authors recently underlined, the safety of “traditional” non-invasive embryo quality markers is not as evident as one may think. Conversely, embryo micromanipulation, such as PGS and/or PGD, that non experts might fear to be detrimental for embryo viability, could yield high levels of information, without affecting embryo developmental competence. New tracks are currently being explored. On one hand, attention is paid to gamete competence, as it obviously affects embryo genomic content and energy reserves. Thus, oocyte-cumulus dialogue seems to be particularly critical for oocyte quality and subsequent embryo viability. On the other hand, embryo “surroundings”, rather than embryo itself, could give some information on its embryo developmental competence. Respirometry and metabolomics take a central place in this area.

Several issues will have to be addressed in the coming years, and clinical embryologists will certainly benefit routinely from these improvements, provided that they have accurately evaluated the respective limits and advantages of traditional versus modern approaches, objective vs subjective markers, invasive vs non-invasive methods, single parameter vs scores and multi-parameters analysis, high throughput analysis vs conventional assays.

The debate continues to determine if one single parameter will allow accurate embryo quality assessment in the near future or if a few criteria in combination will be necessary to make a decision in the IVF lab.

References:
Seli E, Robert C, Sirard MA. OMICS in assisted reproduction: possibilities and pitfalls. Mol Hum Reprod. 2010 under press
HOW TO IMPROVE CULTURE MEDIUM TO ENHANCE IMPLANTATION?

Emre Seli
Division of Reproductive Endocrinology and Infertility, Oocyte Donation and Gestational Surrogacy Program, Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA

Genetic program of the pre-implantation embryo contains the information that regulates progression through cleavage divisions and blastocysts formation, mediates embryo’s responses to environmental stimuli, and orchestrates its metabolic activity. Proper metabolic turnover is essential for a pre-implantation embryo to remain viable and develop an optimal phenotype for pregnancy potential.

A fundamental mechanism in pre-implantation embryo development is the switch from carboxylic acid to glucose metabolism, which occurs some time between pre- and post-compaction, and is among the best characterized phenomena of embryonic metabolic regulation. There are two main pathways to generate the ATP that is necessary for embryonic cellular metabolism: aerobic glycolysis (also called the tricarboxylic acid cycle or the Krebs cycle) and anaerobic glycolysis (Embden Meyerhof pathway). Carboxylic acid-based metabolism predominates in the early stages of development, where pyruvate and lactate are the embryo’s main sources of energy, and glucose uptake is minimal. As development progresses, glucose uptake steadily increases from the zygote stage to compaction, and glucose metabolism begins to predominate at the blastocyst stage. This increase in glucose consumption in late-stage pre-implantation embryos has been observed across a number of species, including in mouse, rat, human, cow, sheep, and pig embryos.

Pre-implantation development follows an organized series of critical events and requires that the metabolic needs of the embryo are met at the correct time. These changing nutrient requirements are reflected in the composition of the in vivo milieu of the embryo as it travels along the reproductive tract. Oviductal fluid, encountered by the embryo at pre-compaction stages, contains relatively high levels of pyruvate and lactate and low levels of glucose, while the reverse is seen in uterine fluid, with corresponding gradients of these molecules between the two locations.

Also essential to optimal embryonic development are amino acids, which serve a broad spectrum of functions including protein synthesis, metabolism, chelation, pH regulation, and acting as energy substrates.

Major metabolic components of the embryo culture environment have been investigated as potential biomarkers of embryo viability using animal models as well as human embryos. Indeed, utilization of nutrients including pyruvate, glucose, and amino acids, and generation of metabolites seem to correlate with viability of human embryos in culture. Studies of metabolic and metabolomic changes of the in vitro environment surrounding the embryo are likely to offer clues to how to improve culture medium to enhance implantation.
Despite ongoing development in embryo culture and selection technologies, IVF success rates have not increased significantly over the past few years. Hence the focus of research is now increasingly on the long neglected counterpart of human implantation - the endometrium. Whilst we already know that ovarian stimulation in IVF disturbs the development of the luteal phase endometrium, and that hormonal support during this phase supports implantation and early embryo development, there is still much to be learnt about the impact of ovarian stimulation on the mechanistic function of the endometrium.

Initial studies have shown that embryos transferred to oocyte donation recipients, in whom the endometrium has not been exposed to supra-physiological levels of sex steroid hormones, have higher implantation rates. The latter indicated that there was a problem with endometrial development in women who underwent COS. Later on, it was shown that there exists an implantation window whose duration depends on the level of oestrogen administered in the luteal phase. Mechanistically, COS has been shown to impact on endometrial expression of key implantation factors (integrins, selectins etc), but also recent studies have shown that extensive gene dysregulation occurs during the crucial window of implantation. Recently, Boomsma et al, 2010, using a non-invasive method of analyzing endometrial secretions at the time of embryo transfer, reported significant effect of ovarian stimulation on the expression of a number of cytokines, chemokines, growth factors and signaling factors in the secretions of the endometrium. The latter factors are vital for the embryo to begin the molecular dialogue and will ultimately determine whether the embryo will implant successfully or not.

The evidence for a detrimental effect of ovarian stimulation on endometrial receptivity is now clear, and raises questions as how best to ameliorate this effect. Milder stimulation regimens, increasing use of cryopreservation of embryos and transfer in a natural cycle can perhaps address these issues.

The question is: are there vascular parameters of the uterus which can be used to evaluate uterine receptivity? Many studies showed that the lower are the uterine vascular indexes the better are the pregnancy rates. Nevertheless many studies showed the opposite. Ultrasound examination of the endometrium is a commonly used non-invasive method to assess endometrial receptivity during in vitro fertilization (IVF) treatment. A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation and therefore assessment of endometrial blood flow in IVF treatment has attracted a lot of attention in recent years. Doppler study of uterine arteries does not reflect the actual blood flow to the endometrium. Endometrial and subendometrial blood flows can be more objectively and reliably measured with three-dimensional power Doppler ultrasound. However, conflicting results are reported with regard to their role in the prediction of pregnancy in IVF treatment. Relevant studies in the literature differed in patients’ characteristics, the day of ultrasound examination and the selection of the subendometrial region. As the degree of change in endometrial perfusion from the late follicular phase to the early luteal phase may be a more important determinant of endometrial receptivity, further studies should be conducted to determine the change in endometrial and subendometrial blood flows from late follicular phase to early luteal phase in order to delineate the role of endometrial and subendometrial blood flows in predicting IVF outcome.

Nevertheless according to the first publication by Goswamy and Steptoe 20 years ago we knew that an impaired vascularisation of the uterus (menopausal) is incompatible with any embryo implantation. May be many years after it is time to re evaluate one of the key parameters of implantation regarding uterine receptivity? And why not we may improve uterine vascularisation during IVF cycles to increase in these cases the pregnancy rate.
The importance of uterine contractility in the implantation of human embryos is becoming increasingly evident. In fact, recent findings show that the receptive phase of the endometrium seems to occur in close association with the appearance of morphological and biochemical dramatic changes possibly focused on embryo nidation. Throughout the menstrual cycle wavelike activity patterns of the uterus were identified with adequate wave patterns appearing to be related to successful implantation in spontaneous cycles and in assisted reproduction. Such patterns are controlled by steroid hormones.

Embryo attachment to the predecidualized endometrium and its invasion may be determined by the expression of proteolytic enzymes that require uterine quiescence for implantation. The uterine activity was detected both in vitro and in vivo by using invasive intrauterine pressure detection and noninvasive ultrasound approaches. Progesterone promotes local vasodilatation and uterine musculature quiescence by inducing nitric oxide synthesis in the decidua. At present, until new evidence emerges to demonstrate otherwise, the effects of progesterone are, directly or indirectly, the only determinant of endometrial preparation for embryo nidation, with the induction of uterine quiescence being one of these effects.

In summary, an adequate uterine contractility may provide for gamete/embryo transportation through the uterotubal cavities and successful embryo implantation in spontaneous or assisted reproduction. Inadequate uterine contractility may instead lead to ectopic pregnancies, miscarriages, retrograde bleeding with dysmenorrhea and endometriosis.
Embryo implantation is a complex process that is essential to obtain pregnancy. Though embryo quality is an important determinant of implantation, temporally and spatially coordinated differentiation of endometrial cells to attain uterine receptivity and a synchronized dialog between maternal and embryonic tissues are fundamental. Endometrial receptivity markers are being explored and could be of assistance in evaluating embryo implantation. Optimizing endometrial receptivity in fertility treatment will improve success rates as this step appears to be the bottleneck in IVF success. Treating underlying gynecological disease with medical or surgical interventions currently is the optimal therapy. Future therapies to improve implantation rates include altering the expression of key endometrial genes. The lecture will present data obtained from an in vitro model of implantation, genes involved in endometrial receptivity, and the mechanism involved in their expression as well as clinical data from patients with repeated implantation failure.
L16

HCG/LH SUPPLEMENTATION DURING LUTEAL PHASE MAY IMPROVE RECEPTIVITY

Jean Noël Hugues
Reproductive Medicine Unit - CHU Jean Verdier - University Paris XIII, France

Corpus luteum support is absolutely required in IVF / ICSI cycles because luteal phase is defective in almost all patients. Indeed, supra-physiological concentrations of steroids following controlled ovarian stimulation (COS) induce a negative feedback on LH pituitary release. Therefore, the strongest the stimulation, the highest the need for a luteal support and patients who require this support are paradoxically those who are more prone to OHSS. Therefore, the issue of the regimen for luteal support is critical.

Which luteal support should be prescribed? It has been well established that hCG is equally effective or superior to progesterone regarding the pregnancy rate but exposes to a higher risk of OHSS. In addition, the optimal estrogen exposure is still controversial.

How do LH and hCG work during luteal phase? Both hormones interact with the same receptor localized in the ovary but also in extra-gonadal reproductive tissues including the uterus.

The primary effect of LH and hCG during the early luteal phase is to ensure corpus luteum rescue following COH. The theoretical advantage of supplementation of hCG over progesterone is that the overall production of the corpus luteum is sustained with subsequent secretion of steroids but also of additional compounds (cytokines, growth factors, angiogenic substances) involved in the process of implantation.

Two clinical models have been proposed to separate the respective role of these ovarian factors.

- Efficacy of steroid supplementation has been assessed in oocyte donation model: a fair implantation rate is obtained in recipient women throughout the sequential administration of estrogens and progesterone alone.
- On the other hand, steroid supplementation does not seem sufficient in other situations. Animals with LH receptor knockout have an abnormal uterine phenotype which cannot be completely reversed by normalizing serum E2 and progesterone. Similarly, in humans, ovulation triggering with GnRH agonist in GnRH antagonist protocol is associated with a deeply suppressed LH secretion and a defective luteal phase which cannot be overcome by estrogen and progesterone alone.

Even if these clinical situations are not similar regarding the endocrine environment (previous amenorrhea, serum levels of endogenous LH and E2), these data attest that although E2 and progesterone play a critical role in the implantation process, steroid supply might not be sufficient to maintain normal uterine functions. Further clinical studies assessing the best effective dose of steroids and identifying the specific role of the other ovarian factors are needed to elucidate that issue.

The second target for LH and hCG is the endometrium itself. Indeed, functional LH/hCG receptors have been identified in the endometrium where full-length mRNAs are maximally expressed in the early luteal phase. HCG is one of the earliest embryonic signals secreted in high local concentrations by the blastocyst entering the uterine cavity and is likely involved in the process of implantation. Indeed, a direct role of locally applied hCG on endometrial growth factors and cytokines has been demonstrated using an intra-uterine microdialysis device. Administration of low dose hCG provoked a significant inhibition of intra-uterine IGFBP-1 and M-CSF known to restrict implantation. In contrast, secretion of LIF, VEGF and MMP-9 involved in endometrial angiogenesis and tissue invasion was significantly stimulated by hCG. In contrast, very few studies have assessed so far the effects of in vivo administration of hCG. To address this point, oocyte donation program offers a unique model system. In that situation, administration of hCG to recipients increases endometrial thickness and improves implantation rate specifically in ovulating recipients with low endogenous LH levels resulting from pituitary down-regulation with a GnRH agonist. These data suggest that LH/hCG might affect uterine receptivity independently of ovarian function.

In conclusion, LH and hCG interfere with uterine receptivity by acting on corpus luteum and on endometrium as well. Oocyte donation programs are actually the most appropriate model in humans to separate these effects. Additional studies are needed to assess the actual benefit of LH or hCG supplementation in the early luteal phase.