The multi-factorial equations of ART: defining and maximizing success in pregnancy rates

Sorrento, Italy, 18–19 May 2012

PARTICIPANT WORKBOOK

Biographies • Abstracts • Key slides • Key messages
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Serono Symposia International Foundation conference on:

The multi-factorial equations of ART: defining and maximizing success in pregnancy rates

Sorrento, Italy, 18–19 May 2012

Serono Symposia International Foundation (SSIF) welcomes you to Sorrento. We have gathered a team of leading international experts to discuss the factors that contribute to successful treatment of infertility with assisted reproductive technologies (ART). A particular focus of the talks will be how screening of the parameters that define the characteristics of infertility in couples can allow for personalization of treatment to optimize outcomes.

You will be able to claim CME credits for attending this event, which is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) and the Italian Ministry of Health.

It is hoped that during this conference, you will learn about:

- advanced methodologies to assess individual infertility profiles
- ovarian stimulation protocols and technologies to obtain the highest implantation rates
- advanced laboratory techniques
- current evidence-based recommendations to achieve the best outcomes from ART.

In addition to the above learning objectives for the overall conference, we have devised learning messages for each individual session and these are detailed in this workbook. A number of slides from each presentation are also included to support the learning messages.

We anticipate that the talks and case studies will inspire thought-provoking and informative debate on many of the challenges encountered in individualizing controlled ovarian stimulation and look forward to your active participation.
A varied and stimulating scientific programme has been planned, incorporating a range of different session types, including lectures, case studies, panel discussions and keypad voting. To assist you in orientation regarding each session type, please refer to the respective visual icon given alongside each session.

L = Lecture  CS = Case study  PD = Panel discussion  V = Voting

## Friday 18 May 2012

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### Session I
**Individualizing controlled ovarian stimulation**

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### Session II
**Customizing technologies to assess semen and oocytes**

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### Session I
**Case Studies**

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### Session II
**Case Studies**

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# Session I: Individualizing controlled ovarian stimulation

**Chairs:** R. Palermo, Italy; P. Patrizio, USA

**09.15 V1** Introduction to voting system and real-time survey  
F.M. Ubaldi, Italy

**09.20 L1** Biomarkers used to define stimulation protocols: AFC, AMH, FSH and inhibin-b  
A. La Marca, Italy

**09.45 L2** Interpretation of biomarkers and COS adjustment for patients with different demographic characteristics and pathologies  
S.M. Nelson, UK

**10.10 L3** LH supplementation in patients undergoing ART  
R. Orvieto, Israel

**10.35** Coffee break

# Session II: Customizing technologies to assess semen and oocytes

**Chairs:** D. Sakkas, USA; A. Sinisi, Italy

**13.30 V3** Real-time survey  
P.M. Nagy, USA

**13.40 L4** Other potential technologies to evaluate and select the best sperm for fertilization: IMSI, DNA fragmentation and hyaluronic acid test  
A. Ferlin, Italy

**14.05 L5** Other potential technologies to identify the most viable oocytes: polar body biopsy, spindle imaging and zona pellucida birefringence  
M. Montag, Germany

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# Session I: Case Studies

**Chairs:** F. Broekmans, the Netherlands; A. Revelli, Italy

**11.00 CS1** Clinical cases presentation on L1 and questions to audience  
A. La Marca, Italy

**11.15 CS2** Clinical cases presentation on L2 and questions to audience  
S. Nelson, UK

**11.30 CS3** Clinical cases presentation on L3 and questions to audience  
R. Orvieto, Israel

**11.45 PD1** "ART-TALK" panel discussion  
P. Devroye, Belgium

**12.15 V2** Revisiting real-time survey  
P. Devroye, Belgium

**12.30** Lunch break

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# Session II: Case Studies

**Chairs:** P.M. Nagy, USA; F.M. Ubaldi, Italy

**14.30 CS4** Clinical cases presentation on L4 and questions to audience  
A. Ferlin, Italy

**14.45 CS5** Clinical cases presentation on L5 and questions to audience  
M. Montag, Germany

**15.00** Coffee break

**15.20 PD2** "ART-TALK" panel discussion  
P. Devroye, Belgium

**15.50 V4** Revisiting real-time survey  
P. Devroye, Belgium

**16.30** Summary of the first working day’s results: take-home messages (Part II)  
P.M. Nagy, USA; F.M. Ubaldi, Italy

**16.30** End of the first day
Saturday 19 May 2012

Session III  Customizing the technologies to identify embryo competence

Chairs: P.E. Levi Setti, Italy; M. Montag, Germany

09.00 V5 Real-time survey
L. Rienzi, Italy

09.10 L6 Other technologies to identify embryos with the highest implantation potential: time lapse, respirometry, genomics and metabolomics
M. Meseguer, Spain

09.35 CS6 Clinical cases presentation on L6 and questions to audience
M. Meseguer, Spain

09.50 V6 Revisiting real-time survey
L. Rienzi, Italy

Session IV  Customizing the technologies to assess uterine environment

Chairs: C. Bulletti, Italy; R. Orvieto, Israel

10.00 V7 Real-time survey
C. Simón, Spain

10.10 L7 Other potential technologies to assess the most receptive endometrium: genomic profile and progesterone levels
A. Makrigiannakis, Greece

10.35 L8 Other potential non-invasive technologies to assess the most receptive endometrium: lipidomics
C. Simón, Spain

11.00 Coffee break

Session IV  Case Studies

Chairs: L. Rienzi, Italy; C. Simón, Spain

11.20 CS7 Clinical cases presentation on L7 and questions to audience
A. Makrigiannakis, Greece

11.35 CS8 Clinical cases presentation on L8 and questions to audience
C. Simón, Spain

11.50 PD3 "ART-TALK" panel discussion
P. Devroey, Belgium

12.20 V8 Revisiting real-time survey
P. Devroey, Belgium

12.30 Lunch break

Session V  Understanding the meaning of success in ART

Chairs: A. Borini, Italy; P. Devroey, Belgium

13.30 V9 Real-time survey
Y. Khalaf, UK

13.40 L9 Other potential definitions of success in ART: ITT, ET, cumulative pregnancy rates, one embryo–one baby and reduced multiples
F. Broekmans, the Netherlands

14.05 L10 Other potential approaches to maximize success in ART: increase the number of oocytes, decrease the number of oocytes and managing follicle population
S.K. Sunkara, UK

14.30 Coffee break

Session V  Case Studies

Chairs: A. Guglielmino, Italy; Y. Khalaf, UK

15.00 CS9 Clinical cases presentation on L9 and questions to audience
F. Broekmans, the Netherlands

15.15 CS10 Clinical cases presentation on L10 and questions to audience
S.K. Sunkara, UK

15.30 PD4 "ART-TALK" panel discussion
P. Patrizio, USA

16.00 V10 Revisiting real-time survey
P. Patrizio, USA

16.10 Closing panel discussion and take-home messages (Part II)
P.Z. Nagy, USA; P. Patrizio, USA; L. Rienzi, Italy; C. Simón, Spain; F.M. Ubaldi, Italy

16.30 Closing remarks

16.40 End of the conference
- Carlo Alviggi
  Local Scientific Committee, Federico II University, Naples, Italy

- Andrea Borini
  National Scientific Committee • Chairman, Tecnobios Procreazione, Centre for Reproductive Health, Bologna, Italy

- Frank J.M. Broekmans
  Chairman • Speaker, University Medical Centre Utrecht, Utrecht, the Netherlands

- Carlo Bulletti
  National Scientific Committee • Chairman, The Rimini’s General Hospital, University of Bologna, Rimini, Italy

- Ettore Cittadini
  National Scientific Committee, University of Palermo, Palermo, Italy

- Luigi Cobellis
  Local Scientific Committee, Federico II University, Naples, Italy

- Giuseppe de Placido
  Local Scientific Committee, Federico II University, Naples, Italy

- Paul Devroey
  Chairman, Centre for Reproductive Medicine, AZ Vrije Brussels University, Brussels, Belgium

- Alberto Ferlin
  Speaker, University of Padova, Padova, Italy

- Robert Fischer
  SSIF Scientific Committee Member, Fertility Centre Hamburg, Hamburg, Germany

- Antonino Guglielmino
  Chairman, Reproductive Medicine Unit, HERA Centre, Catania, Italy

- Yacoub Khalaf
  Chairman, Centre for Pre-implantation Genetic Diagnosis, London, UK

- Antonio La Marca
  Speaker, Mother-Infant Department, University of Modena and Reggio Emilia, Modena, Italy

- Paolo Emanuele Levi Setti
  National Scientific Committee • Chairman, Humanitas Clinical Institute, IRCCS, Milan, Italy
Antonis Makrigiannakis  
Speaker, Medical School, University of Crete, Heraklion, Greece

Marcos Meseguer  
Speaker, Infertility Institute of Valencia (IVI), Valencia, Spain

Markus Montag  
Speaker, University of Heidelberg, Heidelberg, Germany

Peter Zsolt Nagy  
Scientific Organizer • Chairman, Reproductive Biology Associates, Assisted Fertilization Technology, Atlanta, USA

Scott Nelson  
Speaker, University of Glasgow, Glasgow, UK

Raoul Orvieto  
Chairman • Speaker, Barzilai Medical Center, Ashkelon, and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

Antonio Palagiano  
Local Scientific Committee, Federico II University, Naples, Italy

Roberto Palermo  
National Scientific Committee • Chairman, Association of Doctors and Biologists for Assisted Reproduction, Palermo, Italy

Pasquale Patrizio  
Chairman, Yale University Fertility Center, New Haven, CT, USA

Alberto Revelli  
Chairman, St. Anna Hospital, University of Turin, Turin, Italy

Laura F. Rienzi  
Chairwoman, G.EN.E.R.A., Centre for Reproductive Medicine, Rome, Italy

Denny Sakkas  
Chairman, Boston IVF, Boston, MA, USA

Carlos Simón  
Chairman • Speaker, Valencia University, Infertility Institute of Valencia (IVI), Prince Felipe Research Centre [CIPF] & Valencia Stem Cell Bank [CIPF], Valencia, Spain
- **Antonio Sinisi**  
  Chairman, Federico II University, Naples, Italy

- **Sesh Kamal Sunkara**  
  Speaker, Guy’s and St Thomas’ Foundation Trust, King’s College London, London, UK

- **Filippo Maria Ubaldi**  
  Scientific Organizer • Chairman, G.EN.E.R.A., Centre for Reproductive Medicine, Rome, Italy

- **Fulvio Zullo**  
  Local Scientific Committee, Magna Graecia University of Catanzaro, Catanzaro, Italy
Biographies
Carlo Alviggi  
Local Scientific Committee  
Federico II University, Naples, Italy

Carlo Alviggi works as a Specialist in Reproductive Medicine at the Fertility Unit of the University of Naples “Federico II”. Since 2006, he has been working in the same unit as Assistant Professor. Dr Alviggi’s current research interests are the role of luteinizing hormone (LH) in folliculogenesis, the use of LH-containing drugs in patients undergoing controlled ovarian stimulation for in vitro fertilization, the pathogenesis of pelvic endometriosis, and the genetics of human reproduction. Dr Alviggi has published extensively and has been invited to lecture at over 40 international meetings dealing with reproductive medicine and gynaecological endocrinology. He has also served as ad hoc reviewer for international journals of these fields and has participated in several national and international [phase II-III] multicentre, prospective randomized trials.

Andrea Borini  
National Scientific Committee • Chairman  
Tecnobios Procreazione, Centre for Reproductive Health, Bologna, Italy

Andrea Borini is Clinical and Scientific Director at Tecnobios Procreazione, an Italian institution specializing in the diagnosis and treatment of sterility and assisted reproductive technologies (ART), being responsible for Tecnobios’ affiliated centres throughout Italy. Dr Borini graduated in Medicine and Surgery in 1986 and specialized in Obstetrics and Gynaecology at Bologna University, Italy, in 1991. From 1989 to 1991 he was a Research Fellow at California Irvine University, where he worked on couple infertility and methods of ART. Back in Italy, in 2007 he obtained a Master’s degree in Andrology at Padova University. He has worked as a reproductive medicine specialist since 1987; he joined Tecnobios as Sanitary Director in 1998, taking his present role in 2001. Dr Borini is President of the Italian Society for the Preservation of Fertility and has produced numerous publications in specialist journals.

Frank Broekmans  
Chairman • Speaker  
University Medical Centre Utrecht, Utrecht, the Netherlands

Frank Broekmans is Professor in Reproductive Endocrinology and Surgery, and Head of Reproductive Medicine at the University Medical Center, Utrecht, the Netherlands. He is also Chairman of the Dutch–Flemish Society for Fertility Studies. Graduating from the Faculty of Medicine at the VU Medical Center, Amsterdam, in 1983, Dr Broekmans later became Consultant OB/GYN in 1990, completed a Fellowship in Reproductive Medicine in 1993, and completed his PhD in 1995. Dr Broekmans’ scientific career has been devoted to the field of female reproductive ageing. His main research interests are ovarian ageing and dysfunction, as well as methods of testing ovarian reserve, including the use of anti-Müllerian hormone, inhibin B and antral follicle count. Dr Broekmans has held an Associate Editorship for Human Reproduction between 2006 and 2009 and has published over 110 peer-reviewed scientific papers, contributed to seven book chapters, and presented over 80 invited lectures at various international meetings.
Carlo Bulletti
National Scientific Committee • Chairman
The Rimini’s General Hospital, University of Bologna, Rimini, Italy

Carlo Bulletti is currently Director of the Unit of Physiopathology of Reproduction at The Cattolica’s General Hospital, Department of “Science of Quality of life” in the Polo Scientifico Didattico of Rimini, which is part of the University of Bologna in Rimini, Italy. He obtained his MD at the University of Bologna, Bologna, Italy, in 1978, specializing, in 1982, in obstetrics and gynaecology. He subsequently trained at the Mount Sinai Medical Center in New York, Ljubiana (in former Yugoslavia), Los Angeles and Paris. He is currently Professor of Physiopathology of Reproduction and Biotechnology of Reproduction at the University of Bologna. Professor Bulletti has been instrumental in the development of two models: the extracorporeal perfusion of the human uterus (with the first embryo implantation in an isolated uterus) and the first uterine pass effect (FUPE) of hormones delivered by the vaginal route. He has published 15 medical books, more than 180 articles and more than 130 chapters in medical books. His current research focuses on the endometrium, embryo implantation and endometriosis. He is a member of the American Society for Reproductive Medicine, the American Association of Gynecologic Laparoscopists, the New York Academy of Sciences, the European Society of Human Reproduction & Embryology Special Interest Group for Reproductive Surgery, and is Past President of the Società Italiana di Fertilità, Sterilità e Medicina della Riproduzione (SIFES-MR).

Ettore Cittadini
National Scientific Committee
University of Palermo, Palermo, Italy

Ettore Cittadini is a member of the National Scientific Committee, and currently President of the Fondazione per gli Studi sulla Riproduzione Umana, Honorary Associate of the Società Italiana di Ostetricia e Ginecologia, Scientific Director of the Centro d’Eccellenza Materno-Infantile [Sicilian region], President of the Istituto di Ricerca Biomedica del Mediterraneo and Honorary President of the Fondazione di Ricerche e Studi Ginecologici “Eva Candela – Onlus”. Since graduating in Medicine and Surgery at the University of Palermo in 1956, Professor Cittadini’s research has been devoted to the improvement of women’s health. This lifetime contribution has been recognized by his peers: in 1994 when he was conferred with a Certificate of Appreciation by the International Federation of Gynecology and Obstetrics and, most recently, in 2010, when he was presented with the gold medal of Merit for Public Health Service by the President of the Italian Republic.

Luigi Cobellis
Local Scientific Committee
Federico II University, Naples, Italy

Luigi Cobellis completed his MD (magna cum laude) at the Federico II University, Italy, in 1993, graduating in Obstetrics and Gynaecology in 1997, and obtaining a PhD in Gynaecological and Obstetrics Science in 2001. He has been a visiting scientist at the National Cancer Institute in Milan (1994–1999), the Department of Surgical Science at the University of Udine, where he held the Chair of Obstetrics and Gynaecology (1999–2000), the Department of Obstetrics and Gynaecology at the Nuffield Hospital, University of Oxford (2000–2001) and the University of Siena, where he held the Chair of Obstetrics and Gynaecology (2000–2003). Dr Cobellis is currently Senior Doctor in Obstetrics & Gynaecology at the Federico II University, and is author or co-author of 100 original papers that have been published in international journals.
Giuseppe de Placido  
Local Scientific Committee  
Federico II University, Naples, Italy

Giuseppe de Placido is Professor of Gynaecology and Obstetrics and Director of the Gynaecology and Obstetrics Specialty School at Naples University, Naples, Italy, as well as Director of the Department of Laparotomic and Endoscopic Gynaecological and Obstetrical Surgery and Centre for the Study and Treatment of Couple Sterility and Infertility at ‘Federico II’ hospital in Naples. His scientific activities focus on the physiopathology of human reproduction; he is President of the Italian Society of Gynaecological Endoscopy and has published over 70 articles in peer-reviewed journals as well as numerous book chapters.

Paul Devroey  
Chairman  
Centre for Reproductive Medicine, AZ Vrije Brussels University, Brussels, Belgium

Paul Devroey is currently Clinical Director of the Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel at the Vrije Brussels University, Brussels, Belgium. He obtained his PhD on oocyte donation at the Brussels Free University in 1989, and in 1991, while Professor of Reproductive Medicine at this institution, he was instrumental in pioneering the use of the intracytoplasmic sperm injection technique. He was a founding member and first President of the Belgian Society of Reproductive Medicine (1999–2001). Professor Devroey has received three national and four international research awards, has (co-)authored over 500 international peer-reviewed articles, and written four books. He is a member of the Editorial Board of Fertility and Sterility, and has been the Director of Medical Education of the International Federation of Fertility Societies (IFFS) since 2011.

Alberto Ferlin  
Speaker  
University of Padova, Padova, Italy

Alberto Ferlin is Assistant Professor of Clinical Pathology at the Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy. He completed his MD in 1995, and specialized in Endocrinology and Metabolism in 2000. In 2005, he earned his PhD in Endocrinological and Haematological Sciences. Dr Ferlin has authored more than 300 publications and has been an invited speaker in 30 international congresses and 60 national congresses. He has won 12 scientific awards in national and international congresses, and is a member of the scientific committee or president of more than 20 national and international congresses.

Robert Fischer  
SSIF Scientific Committee Member  
Fertility Centre Hamburg, Hamburg, Germany

Robert Fischer is Founder and Medical Director of the IVF unit at the Hamburg Fertility Center, one of the largest and leading German IVF centres. In July 1998 the Fertility Center of Hamburg was one of the first centres in Germany and worldwide to introduce certified quality management according to the ISO 9001. In 2002 the IVF laboratory was ISO 17025 certified. Prior to this he was Medical Director of the first outpatient IVF unit in Hamburg. Author of numerous publications in national and international scientific journals and books, as well as lectures at conferences worldwide, Dr Fischer is an active member of the American Society of Reproductive Medicine, founding member of the European Society of Human Reproduction and member of its advisory committee as well as founding member of the “ÄG Gynäkologische Endokrinologie und Fortpflanzungsmedizin” and “Berufsverband Reproduktionsmedizinischer Zentren”, both in Germany.
Antonino Guglielmino
Chairman
Reproductive Medicine Unit, HERA Centre, Catania, Italy

Antonino Guglielmino directs the Reproductive Medicine Unit (UMR) at the HERA Centre for the Study and Research of Fertility in Catania, Italy. After graduating in Medicine and Surgery in 1986 at Catania University and specializing in Gynaecology and Obstetrics in 1990, he was Clinical Assistant and Researcher at the Department of Gynaecology and Obstetrics of London University’s Guy’s Hospital and subsequently at the Department of Reproductive Medicine of the Free University of Brussels. He then became responsible for treatments at the Reproductive Medicine Unit at ‘Vittorio Emanuele’ hospital in Catania and was one of the founding members of the HERA Centre in 1995. He has been a member of the directing board of the Italian Society of Fertility and Sterility and Reproductive Medicine (SIFES and MR) and has contributed to the organization of several reproductive medicine meetings and conferences.

Yacoub Khalaf
Chairman
Centre for Pre-implantation Genetic Diagnosis, London, UK

Yacoub Khalaf was appointed Director of the Pre-implantation Genetic Diagnosis Programme at Guy’s and St Thomas’ Hospital NHS Foundation Trust, London, UK, in 2011. He has held the position of Medical Director and Human Fertilisation and Embryology Authority (HFEA) Person Responsible for the Assisted Conception Unit of this programme since 2004. He qualified in Egypt in 1984 (Assiut University) with Honours and completed his MD in Birmingham, UK, in 1994. Following research posts in Birmingham and London, Dr Khalaf became a Lecturer in Obstetrics and Gynaecology in 1996, a Subspecialty Fellow in Reproductive Medicine and Surgery in 2000, and was appointed Consultant in Reproductive Medicine and Surgery in 2002 at Guy’s and St Thomas’ Hospital NHS Foundation Trust. In 2003, he was appointed an Honorary Senior Lecturer in Reproductive Medicine and Surgery at King’s College London, a position he still holds. He is an advisor to the HFEA and a member of the HFEA Licensed Centres Panel. He is a member of the Training Subcommittee of the British Fertility Society and an Executive Board Member of the National Clinical Study Group in Reproductive Medicine. He has published and lectured widely on all aspects of assisted conception. His main research interests include infertility, uterine fibroids, assisted conception and recurrent miscarriage.

Antonio La Marca
Speaker
Mother-Infant Department, University of Modena and Reggio Emilia, Modena, Italy

Antonio La Marca is associate professor in Obstetrics and Gynecology at the University of Modena and Reggio Emilia. He graduated in Medicine and Surgery at the University of Siena in 1996, specializing in Obstetrics and Gynaecology at the same institution in 2001. In 2006 he obtained his PhD, again at the University of Siena. He has been working at the Institute of Gynaecology and Obstetrics of the University Hospital of Modena since 2003. Since 1998 he has published over 110 papers in the main peer-reviewed specialist journals in the field. Scientific interests include: ovarian reserve and pharmacological manipulation of ovarian activity.
Paolo Emanuele Levi Setti  
National Scientific Committee • Chairman  
Humanitas Clinical Institute, IRCCS, Milan, Italy

Paolo Emanuele Levi Setti graduated in Medicine and Surgery at the University of Milan School of Medicine in 1982 and was board certified in Obstetrics and Gynaecology at the same University in 1988. From 1988 to 1996 Professor Levi Setti chaired the Infertility and Assisted Reproduction Unit of the Department of Obstetrics and Gynaecology, San Paolo Biomedical Institute, Milan, operating in assisted reproduction. From 1989 to 1996 he coordinated the first European centre for assisted reproduction for HIV serodiscordant couples. Since 1994 he has been an invited lecturer at the first postgraduate School in Obstetrics and Gynecology and he is an invited lecturer in the graduate course of Obstetrics and Gynaecology, University of Milan School of Medicine. His research interest has, over the last few years, focused on the mechanisms of implantation, on the preservation of female and male fertility, and on the complications and outcome of assisted reproduction. He has been the scientific organizer of several international meetings and training courses in reproductive medicine. Professor Levi Setti is a member of many international institutions in the field of reproductive medicine (American Society for Reproductive Medicine, European Society of Human Reproduction and Embryology and the International Society for Fertility Preservation). He is currently running a second mandate (2011–2013) as President of the Italian Fertility and Sterility Society. He has authored more than 100 published articles, reviews and chapters in books. Since 2004 Professor Levi Setti has collaborated with the Italian Ministry of Health in revising data of the National Medically Assisted Reproduction Register. In December 2010 he was nominated Scientific Coordinator of an experimental project for an ART Network in Lombardy County. Since 1996 Professor Levi Setti has worked at the Humanitas Clinical and Scientific Institute in Milan and is currently Director of the Department of Gynaecology and heads the Operative Unit of Gynaecology and Reproductive Medicine. In May 2011 he was appointed, for the period July 2011 to June 2016, as Adjunct Professor at Yale University, School of Medicine.

Antonis Makrigiannakis  
Speaker  
Medical School, University of Crete, Heraklion, Greece

Antonis Makrigiannakis completed his PhD and post-doctoral studies on the molecular pharmacology of hormones and neuropeptides in Greece in a combined project with National Institutes of Health (NIH), USA. Further training and specialization in infertility and reproductive medicine was undertaken at the Division of Human Reproduction, University of Pennsylvania, PA, USA. Dr Makrigiannakis then moved to Imperial College, London, UK, where he worked at the Hammersmith Hospital as a Senior Scientist in the IVF Unit and the Centre of Reproductive Medicine. Dr Makrigiannakis now holds the positions of Professor and Director in Reproductive Medicine in the University of Crete. He has published more than 100 peer-reviewed papers, two books and chapters, and presented more than 100 invited lectures. Dr Makrigiannakis is a Founding Member of the Mediterranean Society of Reproductive Medicine, a member of the Executive Committee of the European Society of Reproductive Medicine, a member of the Executive Committee of the European Society for Clinical Investigation.
Marcos Meseguer

Speaker

Infertility Institute of Valencia (IVI), Valencia, Spain

Marcos Meseguer received his PhD (Obstetrics and Gynaecology) and MD from the University of Valencia, Spain, and a masters degree in Research Methods, Design and Statistics from Universidad Autónoma de Barcelona, Spain. From 2000 to 2004, Dr Meseguer served as Co-Director of the Andrology Laboratory at the Instituto Valenciano de Infertilidad (IVI) and is currently a Senior Embryologist in the IVF unit of IVI Valencia. Dr Meseguer is a member of various scientific societies and has received the prize paper of the Society of Reproduction and Infertility (American Society of Reproductive Medicine), three times the Lalor Foundation International Award from the American Society of Andrology, and twice the research award from the Spanish Society of Fertility. The primary areas of his research are embryology, male infertility and assisted reproduction in HIV/HVC serodiscordant couples. As Principal Investigator, his work has been funded through 10 projects sponsored by the Spanish Government and the Valencian Government, including two EUREKA projects (granted to high-quality technological projects) supported by the European Community. He is currently Statistics Assessor and Scientific Updater of IVI Valencia, and Associate Professor of the Master in Biotechnology from the University of Valencia. Dr Meseguer has published over 85 articles and 40 reviews or book chapters, and made more than 250 presentations at national and international congresses.

Markus Montag

Speaker

University of Heidelberg, Heidelberg, Germany

Markus Montag obtained his PhD at the German Cancer Research Centre and worked as a post-doc at NUH, Singapore with Professor Siu Choon Ng. Returning from Singapore he worked as a Laboratory Director in a private IVF unit before he became Director of the Reproductive Biology laboratory at the University Clinics of Bonn in 1995 and was appointed Associate Professor for Experimental Reproductive Medicine in 2009. He is also the Head of one of the largest cryobanks for ovarian tissue and a co-founder of the fertility preservation network FertiProtekt. He is actively involved in counselling at IVF centres all over the world and in education of young people in the field. His main research areas include the use of lasers in assisted reproduction, polar body biopsy, cryobanking of ovarian tissue, oocyte activation and polarization microscopy.

Peter Zsolt Nagy

Scientific Organizer • Chairman

Reproductive Biology Associates, Assisted Fertilization Technology, Atlanta, USA

Peter Zsolt Nagy qualified in Medicine (summa cum laude) from the Medical University of Budapest, Hungary, in 1986, and completed several postgraduate courses/degrees at the Free University of Brussels (VUB), Belgium, Medical University of Budapest and in the USA between 1991 and 2010. After holding the position of Scientific and Laboratory Director of the Clínica de Reproducción Humana in São Paulo, Brazil, from 1999 to 2002, he joined Reproductive Biology Associates in Atlanta, GA, USA, where he is currently Scientific and Laboratory Director. His current research interests include: patient treatment/management in obstetrics and gynaecology/infertility, and the cryopreservation of oocyte/donor-egg banking. Dr Nagy has been an invited/honorary speaker at over 100 international meetings on assisted reproduction, and he organized and/or chaired more than 60 congresses/symposia and workshops between 1991 and 2011. He is Associate Editor of Reproductive Biomedicine Online and Human Reproduction.
Scott Nelson
Speaker
University of Glasgow, Glasgow, UK
Scott Nelson obtained a BSc in Immunology from the University of Glasgow, Glasgow, UK, in 1994. After commencing clinical work in Obstetrics and Gynaecology, he furthered his education, gaining a PhD at the University of Dundee, Dundee, UK, graduating in 2003. In 2005 he was appointed as a Clinician Scientist by the University of Glasgow, with progression to the Muirhead Chair of Obstetrics and Gynaecology in August 2008. Professor Nelson’s research focuses on several key endocrine and metabolic pathways and their role in determining pregnancy and long-term maternal and offspring outcomes.

Raoul Orvieto
Chairman • Speaker
Barzilai Medical Center, Ashkelon, and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel
Raoul Orvieto is a full Professor at the Faculty of Health Sciences, Ben Gurion University of the Negev, and the Director of the Infertility and IVF Unit, and the Male Infertility center at the Barzilai Medical Center, Israel. He has been author and co-author of more than 150 publications in national and international journals. His scientific interests include: various aspects of controlled ovarian stimulation (COS), the role of gonadotrophin-releasing hormone (GnRH) analogues, and specifically GnRH agonist versus antagonist in COS for in vitro fertilization, and several aspects of ovarian hyperstimulation syndrome – pathophysiology, prediction, prevention and its relation to the inflammatory response.

Antonio Palagiano
Local Scientific Committee
Federico II University, Naples, Italy
Antonio Palagiano is a Professor at the School of Specialization in the Centre for Assisted Reproduction, Federico II University, Naples, Italy, and Vice President of the Italian Society of Fertility, Sterility and Reproductive Medicine. He obtained his degree (with Honors) at the Federico II University, before specializing in obstetrics and gynaecology, and going on to conduct research into receptors and 17-beta-oestradiol and progesterone in infertility at the Bridge Fertility Centre, London Hospital and Guy’s Hospital, University of London, UK. Having conducted the first successful gamete intra-fallopian tube transfer into a tiger at London Zoo, he then continued his studies on the mechanisms of embryo implantation in humans and felines. A speaker at over 70 national and international conferences, Professor Palagiano has authored more than 120 scientific publications in the field of medically assisted procreation and prenatal diagnosis.
Roberto Palermo

National Scientific Committee • Chairman

Association of Doctors and Biologists for Assisted Reproduction, Palermo, Italy

Roberto Palermo obtained his medical degree and specialized in Obstetrics and Gynaecology at the University of Palermo, Italy. In 1987 he completed a fellowship in Reproductive Medicine at the Jones Institute for Reproductive Medicine, Norfolk, VA, USA. In 2002 he obtained a Masters degree in Clinical Embryology from the University of Krems, Austria. His main clinical and research interests are in reproductive endocrinology and clinical embryology. He has served on the Board of the Italian Society for the Study of Fertility, Sterility and Reproductive Medicine and the Italian Society of Reproduction. He is currently the Scientific Director of the “Associazione Medici e Biologi per la Riproduzione Assistita – (AMBRA)”, Palermo, and he also directs a private in vitro fertilization programme in Palermo.

Pasquale Patrizio

Chairman

Yale University Fertility Center, New Haven, CT, USA

Pasquale Patrizio is currently Professor of Obstetrics and Gynaecology and Medical Director of the Yale Fertility Center and the Reproductive Endocrinology Clinical Practice, New Haven, CT, USA. Professor Patrizio received his medical training at the Faculty of Medicine and Surgery, University of Napoli II, Napoli, Italy, going on to complete a Residency in Obstetrics and Gynaecology at the same institution. Following another Residency in Andrology at the University of Pisa, Italy, he moved to the USA, where he completed a Residency in Obstetrics and Gynaecology (in 1993) and a Fellowship in Reproductive Endocrinology and Infertility (in 1995) at the University of California, USA. In 2003, he obtained his Masters in Bioethics at the University of Pennsylvania, USA. His clinical interests include infertility (female and male), in vitro fertilization, egg donation and gestational surrogacy, and the preservation of fertility in cancer patients. Among his many research interests are whole ovary cryopreservation, oocyte freezing, genetics of oocytes and cumulus cells, oocyte and embryo competence, isolation and freezing of male germ cells, and ethical issues in assisted reproduction. Professor Patrizio has published more than 300 scientific papers and an Atlas textbook on assisted reproduction. He is on the editorial board of many journals and has lectured worldwide on many areas of reproductive medicine.

Alberto Revelli

Chairman

St. Anna Hospital, University of Turin, Turin, Italy

Alberto Revelli is responsible for the Centre of Reproductive Medicine of the Department of Gynaecological and Obstetric Disciplines at the University of Turin–Sant’Anna Hospital, where he also coordinates research programmes in reproductive medicine. He graduated in Medicine and Surgery at the University of Turin in 1984, specializing in Obstetrics and Gynaecology in 1988 and obtaining a PhD in Reproductive Physiopathology in 1996. Prior to his present role, he was a Researcher and a Lecturer at Turin University, being responsible for several courses, including Physiopathology of Human Reproduction, Assisted Fertilization Techniques, and Gynaecology and Obstetrics, among others, and becoming Adjunct Professor in 2006. He is a member of the most important international Reproductive Medicine and Gynaecology societies, has organized numerous congresses and meetings, and has published books and over 50 articles in international peer-reviewed journals.
Laura Rienzi
Chairwoman
G.EN.E.R.A. Centre for Reproductive Medicine, Rome, Italy

Laura Francesca Rienzi is Laboratory Director of the G.EN.E.R.A. Centre for Reproductive Medicine in Rome, Italy; her main activities and responsibilities include the study of gamete, zygote and embryo morphology in relation to their developmental ability, and the cryopreservation of embryos and gametes. She received a degree in biology (magna cum laude) from the University of Rome ‘La Sapienza’ in 1993. After becoming a Research Fellow at the Centre for Reproductive Medicine, Hôpital Necker in Paris, France, she was appointed Laboratory Director of the Centre for Reproductive Medicine at the European Hospital in Rome, a position she held from 1996 to 2007. A speaker at 37 national and 54 international scientific meetings, Dr Rienzi is author of 67 original research papers, reviews and book chapters. She has been a member of the organizing committee of 12 educational events (six international), and is currently an Associate Editor of Human Reproduction.

Denny Sakkas
Chairman
Boston IVF, Boston, MA, USA

Dionisios (Denny) Sakkas is currently Scientific Director of Boston IVF Inc. in Waltham, MA, USA. He also holds the positions of Associate Professor (Adjunct) at the Department of Zoology, Melbourne University, Victoria, Australia, and Associate Professor (Adjunct) at the Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA. Professor Sakkas received his undergraduate training at the University of Melbourne, Australia, and obtained his PhD from Monash University, Melbourne in 1990. He has held the position of Laboratory Director of IVF units in Switzerland, the UK and the USA, and was Chief Scientific Officer with Molecular Biometrics Inc., New Haven, CT, USA, from 2008 to 2011 before joining Boston IVF Inc. He has organized conferences and workshops on reproduction-related topics, and is frequently invited as a speaker at major conferences worldwide. He has published more than 100 peer-reviewed manuscripts and 30 chapters in the field of fertilization, early embryo development and male infertility. He is an Editorial Board Member for Human Reproduction Update, Reproduction, Human Fertility and the Journal of Experimental Zoology.
Carlos Simón
Chairman • Speaker
Valencia University, Infertility Institute of Valencia (IVI), Prince Felipe Research Centre (CIPF) & Valencia Stem Cell Bank (CIPF), Valencia, Spain

Carlos Simón is a board-certified and full Professor of Obstetrics and Gynaecology at the University of Valencia, Valencia, Spain, and Scientific Director of both the Fundación IVI and the Prince Felipe Research Centre, Valencia. Since 1991, Dr Simón’s basic and clinical research has contributed to the advance of reproductive medicine, specifically in the understanding of human endometrial receptivity, embryo viability, embryonic implantation and endometriosis. Since 2001, Dr Simón has expanded his research into the field of stem cells, resulting in the derivation, characterization and registration in the Spanish National Stem Cell Bank (BNLC) of 10 human embryonic stem cell lines. As Principal Investigator, Dr Simón’s work has been funded through 10 projects sponsored by the Spanish Government, five by the Valencian Government, including a PROMETEO (granted to prestigious scientists), and 14 projects by international organizations, American universities and private companies. He has been the Director for 17 doctorates, all of whom qualified with cum laude, including four PhD awards of excellence and one European PhD. As inventor, his research has originated 11 patent applications, leading to the creation of three Biotechnology companies (iGenomix, Embryomics and Stemlifeline). Dr Simón has published a total of 264 papers in international peer-review journals and is an Editor of 14 books.

Antonio Sinisi
Chairman
Federico II University, Naples, Italy

Antonio Agostino Sinisi obtained his MD (cum laude) from the University of Napoli, Italy, in 1976, going on to complete an Internship and Residency in Endocrinology at the same university, followed by a Residency in Andrology at the University of Pisa, Italy. In 1997, he completed his Masters in Molecular Endocrinology at the University of Montpellier, Montpellier, France. Since 1996, he has been a serving MD at the Endocrinology Unit, University Hospital, Second University of Napoli and, in 2000, was appointed Associate Professor of Endocrinology at the same institution. Specializing in Endocrinology and Andrology, his current research activities include the role of hormones in the development and progression of prostate and testicular cancer, clinical and genetic aspects of hypogonadotropic hypogonadism, testicular tumour biology and iron metabolism in the testis. He is an Editorial Board member of the Journal of Endocrinological Investigation and l’Endocrinologo.
Sesh Kamal Sunkara
Speaker
Guy’s and St Thomas’ Foundation Trust, King’s College London, London, UK

Sesh Kamal Sunkara is an obstetrician and gynaecologist specializing in Reproductive Medicine and Surgery. She is presently working at Guy’s and St Thomas’ Foundation Trust Hospital, London, UK. Her field of research is ovarian stimulation protocols and interventions to improve outcome of poor responders undergoing in vitro fertilization treatment. She has published in this field and is currently undertaking translational research at King’s College London.

Filippo Maria Ubaldi
Scientific Organizers • Chairman
G.EN.E.R.A. Centre for Reproductive Medicine, Rome, Italy

Filippo Maria Ubaldi is Clinical Director of G.EN.E.R.A. Centre for Reproductive Medicine, in Rome, Italy. After obtaining a degree (cum laude) in medicine and surgery from the Università degli Studi di Roma “La Sapienza” in 1989, and specializing in Obstetrics and Gynaecology at the Università degli Studi di Chieti “Gabriele”, Professor Ubaldi spent some time as a medical visitor at the Center for Human Reproduction Medicine at the Brussels Free University, Belgium, before becoming a full-time researcher at the same institution from 1995 to 1996. In 1996, he was appointed Clinical Director of the Centre for Medicine and Biology of Human Reproduction at the European Hospital in Rome. He was also Professor of Endocrinology at the School of Specialization in Obstetrics and Gynecology at the Universita ‘degli Studi di Perugia, Perugia, Italy, from 2000 to 2003.

Fulvio Zullo
Local Scientific Committee
Magna Graecia University of Catanzaro, Cantazaro, Italy

Fulvio Zullo is Professor and Director of the Specialty School of Gynaecology and Obstetrics at Catanzaro University, Italy, as well as Director of the University Division of Gynaecology and Obstetrics at ‘Pugliese-Ciaccio’ hospital in Catanzaro. He graduated in Medicine and Surgery at the University of Naples in 1984, specializing in Obstetrics and Gynaecology in 1988. He obtained a PhD in Perinatal Medicine at Perugia University, following a post-doctoral fellowship at the Reproductive Medicine Laboratory of the Eastern Virginia Medical School and at the Jones Institute for Reproductive Medicine, he has been carrying out teaching and researching activities at Catanzaro University since 1993.
Abstracts

Key Slides
Markers of ovarian reserve are associated with ovarian ageing as they decline with chronological age and therefore may predict stages of reproductive ageing, including the menopause transition. Assessment of ovarian reserve includes measurement of serum follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH) and inhibin-b. Ultrasound determination of antral follicle count (AFC), ovarian vascularity and ovarian volume can have a role also.

AMH is set to dominate reproductive endocrinology because of its unique relationship with ovarian reserve. In infertile women, ovarian reserve markers can be used to predict low and high oocyte yield and treatment failure in women undergoing in vitro fertilization (IVF). However, the markers may have limitations when an in-depth analysis of their accuracy, cost, convenience and utility is performed. As ovarian reserve markers may permit the identification of both extremes of ovarian stimulation, a possible role for their measurement may be in the individualization of treatment strategies in order to reduce the clinical risk of assisted reproductive technology along with optimized treatment burden. It is fundamental to clarify the cost/benefit of its use in ovarian reserve testing before initiation of IVF and whether the ovarian reserve markers-determined strategy of ovarian stimulation for assisted conception may be associated with improved live birth rate.

There is now a rapidly increasing literature examining additional possibilities for AMH, all of which suggest that its reach extends far beyond the assisted conception clinic. The recognition that it is a significantly more accurate and reliable measure of ovarian reserve than AFC or FSH has led to its adoption by physicians to counsel women on their reproductive lifespan, the impact of gonadotoxic chemotherapy, radiotherapy and surgery on ovarian reserve and allow polycystic ovarian syndrome to be diagnosed by primary care physicians.
Notes
Biomarkers used to define stimulation protocols

Antonio La Marca, MD, PhD
Mother-Infant Department
University of Modena and Reggio Emilia, Modena, Italy

Ovarian response in IVF is dependent on

- Dose of FSH
- Pool of recruitable follicles
- FSH bioavailability
- Follicular response to FSH
- BMI
- Genetic polymorphisms

Could age be the only marker of ovarian response used in IVF?

- No good discrimination
- Low predictive performance

AMH is a good marker of ovarian reserve

Correlation between AMH and the number of oocytes

FSH and Inhibin B as markers of ovarian activity
Drawbacks and limitations

- Cannot be evaluated as single marker
- Need to measure a combination
- High intracycle variability
- Day 3-5 measurement only
- High intercycle variability

De Koning et al., 2008
Van Rooij et al., 2002
Seifer et al., 2002
Fertility declines with increasing age and this is reflected by changes in biomarkers such as antral follicle count (AFC), anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH) and inhibin-B. Age on its own has a low predictive performance. FSH levels are regulated by oestradiol and inhibin-B and begin to increase significantly at 39–40 years. AMH is produced by granulosa cells of the antral follicles and is the best indicator of ovarian pool decline. AMH and AFC have better diagnostic performances in the prediction of ovarian response to FSH.
L2: Interpretation of biomarkers and COS adjustment for patients with different demographic characteristics and pathologies

S.M. Nelson
University of Glasgow, Glasgow, UK

The personalization of controlled ovarian stimulation (COS) is now achievable by combining biomarkers with patient clinical characteristics. Prior to the first treatment cycle, we can predict the risk of ovarian hyperstimulation syndrome, the number of oocytes that will be retrieved, and the chance of live birth, thereby ensuring patients’ expectations are set appropriately. We can personalize ovarian stimulation strategies, number of embryos to be transferred and luteal support, with the overall effect of preventing complications. The participation of patients in all steps of this individualized treatment protocol is likely to minimize the psychological burden and ensure optimal treatment outcomes. This presentation will address how assisted reproductive technologies are leading the way in the application of 4P Medicine (Predict, Personalize, Prevent, Participate).
Notes
L2: Interpretation of biomarkers and COS adjustment for patients with different demographic characteristics and pathologies

S.M. Nelson
University of Glasgow, Glasgow, UK

Predict live birth

- Ovarian reserve
- Age
- Cause of infertility
- Smoking status
- Duration of infertility
- Obstetric history
- IVF history
- Pharmacodynamics
- BMI

Predict live birth with AMH

If you think of “predict” in conventional terms of low medium, or high risk

<table>
<thead>
<tr>
<th>AMH (ng/mL)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>=0.4</td>
<td>&lt;37</td>
</tr>
<tr>
<td>&gt;0.4 - 2.8</td>
<td>0.05 (0.01 to 0.16)</td>
</tr>
<tr>
<td>≥2.8</td>
<td>0.09 (0.02 to 0.24)</td>
</tr>
<tr>
<td>&lt;0.4</td>
<td>0.13 (0.04 to 0.36)</td>
</tr>
</tbody>
</table>

*Values in parenthesis are 95% confidence intervals

Predict complications

- Long course Agonist control
- Risk of OHSS increased
- Normal or lower Measurable Recruitment Advantageous?
- SAFER - BUT Reduced Recruitment Dis-advantageous?
- Excess ovarian follicles
- High Response
- Normal Response
- Reduced Response
- Negligible Response
- Risk of OHSS decreased
- Reduced burden
- Lower egg yield

Normal Response

- 1.0
- 5.0
- 15

Reduced Response

- 0.01
- 0.04
- 0.15

Negligible Response

- 0.0
- 0.02
- 0.12

■ L2: Interpretation of biomarkers and COS adjustment for patients with different demographic characteristics and pathologies

S.M. Nelson
University of Glasgow, Glasgow, UK
Key learning messages

- The 4Ps of assisted reproductive technologies are prediction of outcomes, personalization of treatment, participation by patients and prevention of complications.
- Before controlled ovarian stimulation (COS) treatment initiation, it is possible to predict the risk of ovarian hyperstimulation syndrome (OHSS), the number of oocytes retrieved and the chance of live birth.
- The biomarker for treatment personalization that shows least variability is anti-Müllerian hormone.
- The use of an agonist trigger in COS prevents early OHSS.
- In high-responders, the risk of OHSS can be reduced by about 50% with use of an antagonist-based COS protocol.
L3: LH supplementation in patients undergoing ART

R. Orvieto
Barzilai Medical Center, Ashkelon, and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

Aims: To present the available evidence that examine the role of luteinizing hormone (LH) supplementation during controlled ovarian stimulation (COS) to patients undergoing assisted reproductive technology (ART).

Methods: A literature review was conducted for all relevant articles assessing methods for identifying patients most likely to benefit from the addition of ‘LH activity’ supplementation to their COS protocol.

Results: The available studies on the role of LH supplementation in patients undergoing ART use different preparations, different daily doses and mode of administration. Moreover, variability exists between the commercial kits and the cut-off used when assessing the different biomarkers. Patients who may benefit from LH supplementation and who could be identified prior to COS are those: (1) with hypogonadotropic hypogonadism; (2) poor responder patients and those with advanced age; and (3) patients with high basal follicle-stimulating hormone (FSH)/LH ratio. Patients undergoing COS with the long gonadotrophin-releasing hormone (GnRH)-agonist suppression protocol may benefit from the addition of ‘LH activity’ supplementation, if: (1) they achieve an initial inadequate ovarian response to FSH alone; (2) their Day-8 LH levels are >1.99 IU/L; and (3) those with relative reduction in mid/late follicular LH concentrations. Moreover, patients who underwent COS with the GnRH-antagonist protocol and demonstrated raised progesterone levels on the day of human chorionic gonadotrophin administration may benefit from the addition of LH to their subsequent COS for in vitro fertilization (IVF).

Conclusions: The use of LH supplementation is associated with a tendency toward an improved IVF outcome in different subgroups of patients undergoing COS for IVF. While few of the selected indications for LH supplementation are based on evidence-based medicine, others are supported by observational studies. Therefore, further large studies are needed to investigate the true impact of LH supplementation on IVF outcome and to identify the selected groups of patients who are most likely to benefit from the addition of ‘LH activity’ supplementation to their COS protocol.
Notes
L3: LH supplementation in patients undergoing ART

R. Orvieto
Barzilai Medical Center, Ashkelon, and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

Overview
- Role of LH during the natural menstrual cycle
- Methods to identify patients who may benefit from LH supplementation?
  - Before COS
  - During COS
  - Type
  - Dose
  - Timing

Methods to identify patients who may benefit from LH supplementation?
Before COS

Hypogonadotropic hypogonadism
Follicular development can be achieved with both LH+FSH and FSH alone, BUT with FSH alone:
- Inadequate E2
- Reduced occurrence of ovulation
- Decreased endometrial thickness
- Lower oocyte fertilization rates

LH
(The European Recombinant Human LH Study Group, 1996)

Methods to identify patients who may benefit from LH supplementation?
Patients with high basal FSH/LH ratio

Day 3 LH value <3 IU/L
Day 3 FSH/LH ratio >3.6
Predictive of a poor response to the long GnRH-agonist suppressive COS protocol.
(Mülchert 1996)

Methods to identify patients who may benefit from LH supplementation?
Before COS

Poor responders & patients >35yrs

Methods to identify patients who may benefit from LH supplementation?
Before COS

GnRH-antagonist
GnRH-agonist

Pre-treatment cycle
Treatment cycle
In patients with hypogonadotrophic hypogonadism, administration of recombinant human luteinizing hormone (r-hLH) or LH activity allows follicles to become successfully luteinized when exposed to human chorionic gonadotrophin (hCG).

Administration of r-hLH to patients receiving follicle-stimulating hormone (FSH) and gonadotrophin-releasing hormone (GnRH) analogues for in vitro fertilization (IVF) is particularly beneficial in women aged >35 years and in hyporesponders to FSH.

A FSH/LH ratio >3.6 is predictive of a poor response to the long-GnRH-agonist protocol.

Women undergoing controlled ovarian stimulation (COS) with a GnRH antagonist protocol who have raised progesterone levels on the day of hCG administration may benefit from LH supplementation.

The use of LH supplementation is associated with a tendency towards an improved IVF outcome in some subgroups of patients undergoing COS.
At least two reasons exist for the need of new potential technologies to evaluate and select the ideal sperm in addition to standard semen analysis in couples undergoing assisted reproductive technology: repeated in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) failure (in idiopathic and male-factor infertile couples) and infertile males with normal routine semen analysis. Both conditions have been suggested to be related to a paternal effect on early embryo development.

Sperm selection for ICSI, based on standard morphology and motility, can fail to select normal cells, and most methods to evaluate their physiological status do not allow their later use. Therefore, different methodologies to select sperm have been described in recent years in the hope of selecting a viable sperm with the best prognosis for fertilization and pregnancy outcome. One such technique is the motile sperm organelle morphology examination (MSOME), which enables the evaluation of the fine nuclear morphology of motile spermatozoa in real time at high magnification (×6000). The most predictive factor of sperm quality is the incidence of vacuoles in the sperm head, and vacuoles appear to be related to sperm DNA damage. By studying single sperm selected by high-magnification microscopy, we also showed significantly better mitochondrial function, chromatin status and aneuploidy rate than that observed in unselected cells, and these parameters were further improved when nuclear vacuoles were lacking. On this basis, a new microinjection procedure called intracytoplasmic morphologically selected sperm injection (IMSI) has been developed. The pooled data of IMSI cycles demonstrate both a significant improvement in implantation and pregnancy rates and a significant reduction in miscarriage rates. However, more randomized controlled trials are needed to confirm these results, and clear indications for IMSI are still lacking or have not completely reached an agreement.

A number of studies have highlighted the significance of sperm DNA integrity as an important factor that affects functional competence of the sperm, and different assays have been developed to assess sperm DNA damage. The aetiology of sperm DNA damage is multifactorial, resulting from aberrant chromatin packaging during spermiogenesis, defective apoptosis, excessive reactive oxygen species production, decreased seminal antioxidants, or from external factors such as drugs, pollution, cigarette smoking, fever, xenobiotics, high testicular temperature, varicocele and advanced age. Chromatin restructuring during spermiogenesis makes the sperm chromatin transcriptionally and translationally inert, as a result of which the DNA repair capacity of the sperm is limited. The breaks in the DNA that may have escaped repair prior to compaction or damage occurring after chromatin remodelling has been completed are delivered to the oocyte. Consequently, the biological effect of abnormal sperm chromatin structure depends on both the magnitude of sperm chromatin damage and the capacity of the oocyte to repair it after fertilization. A variety of assays have been developed to measure sperm DNA damage, including those in which DNA fragmentation is measured directly (such as the TUNEL and comet assay) or indirectly (such as the sperm chromatin structure assay [SCSA] and acridine orange staining). Although some studies showed negative effects of high DNA fragmentation on IVF and ICSI results, there are still some problems with the use of these tests: different tests measure different kinds of DNA damage, tests are not standardized, intra-individual variability is high, normal values are not yet well defined, and overall studies have been performed on limited number of subjects.

Finally, the hyaluronic acid (HA) assay has been proposed as a method to select a healthy sperm for use with ICSI. HA is a linear polysaccharide present in the extracellular matrix of cumulus oophorus around the oocyte that seems to play an important role in natural human fertilization. Therefore, this polysaccharide may play a critical role in the selection of mature, functionally competent spermatozoa during in vivo fertilization. Some studies, in fact, suggested that HA-binding ability might be a tool to select the more mature spermatozoa having reached their final nuclear and cytoplasmic maturation, even if controversies exist. It seems that spermatozoa bound to HA have a significant reduction in DNA fragmentation and nuclear anomalies and their injection determines improvement of embryo quality after ICSI. However, additional studies are needed to show whether the HA test improves also implantation rate and pregnancies.

L4: Other potential technologies to evaluate and select the best sperm for fertilization: IMSI, DNA fragmentation and hyaluronic acid test

A. Ferlin
University of Padova, Padova, Italy
L4: Other potential technologies to evaluate and select the best sperm for fertilization: IMSI, DNA fragmentation and hyaluronic acid test

A. Ferlin
University of Padova, Padova, Italy

Sperm function and sperm aneuploidies evaluated on single cells selected with high magnification technique from patients with severe testicular damage

<table>
<thead>
<tr>
<th>Single sperm cells</th>
<th>Group A (n=100)</th>
<th>Group B (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nuclear vacuole)</td>
<td>(normal sperm)</td>
</tr>
<tr>
<td>Mitochondria (%)</td>
<td>52 ± 14.7</td>
<td>15.3 ± 4.9</td>
</tr>
<tr>
<td>Acridine Orange (%)</td>
<td>71 ± 11.1</td>
<td>5.5 ± 3.0</td>
</tr>
<tr>
<td>TUNEL (%)</td>
<td>40 ± 11.6</td>
<td>0.3 ± 4.8</td>
</tr>
<tr>
<td>Aneuploidies (%)</td>
<td>5.1 ± 3.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Garolla et al. High-power microscopy for selecting spermatozoa for ICSI by physiological status. RBM Online, 2008

Key learning messages

- Standard morphology and motility tests for sperm can fail to select normal cells
- Motile sperm organelle morphology examination (MSOME) allows real-time evaluation of fine nuclear morphology at high magnification (> x6000)
- Vacuoles appear to be related to DNA damage and are a predictive factor of sperm quality
- Assays that have been developed to measure sperm DNA damage include those in which DNA fragmentation is measured directly (such as the TUNEL and comet assay) or indirectly (such as the sperm chromatin structure assay and acridine orange staining), but normal values are not yet well defined
- Spermatозоа bound to hyaluronic acid have a significant reduction in DNA fragmentation and nuclear anomalies

Influence of sperm DNA damage on IVF and ICSI

- Fertilization rate
  - Negative effect
    - Tomlinson, 2000
    - Tomlinson, 2002
    - Morris, 2002
    - Payne, 2005
    - Huang, 2005
    - Velez de la Calle, 2008
  - No effect
    - Larson, 2003
    - Tomsu, 2002
    - Tomsu, 2002
    - Seli, 2004
    - Bungum, 2007
    - Lin, 2008
    - Nucopoullos, 2008
    - Velez de la Calle, 2008
    - Tarozzi, 2009 (IVF)
  - Positive effect
    - Payne, 2005
  - No effect
    - Lopes, 1998
    - Host, 2000 (IVF)

- Pregnancy rate
  - Negative effect
    - Tomlinson, 2000
    - Tomlinson, 2002
    - Morris, 2002
    - Benchaib, 2003
    - Payne, 2005
    - Huang, 2005
    - Velez de la Calle, 2008
  - No effect
    - Larson, 2003
    - Tomsu, 2002
    - Tomsu, 2002
    - Seli, 2004
    - Bungum, 2007
    - Lin, 2008
    - Nucopoullos, 2008
    - Velez de la Calle, 2008
    - Tarozzi, 2009 (IVF)
  - Positive effect
    - Payne, 2005
  - No effect
    - Tomlinson, 2000
    - Tomlinson, 2002
    - Morris, 2002
    - Benchaib, 2003
    - Payne, 2005
    - Huang, 2005
    - Velez de la Calle, 2008

New development
Array CGH on single sperm

HA-ICSI significantly improves embryo quality and development... but not implantation rate and pregnancy.
L5: Other potential technologies to identify the most viable oocytes: polar body biopsy, spindle imaging and zona pellucida birefringence

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Polar body biopsy (PBB), spindle imaging and zona imaging focus on very distinct and different aspects of the evaluation of oocyte viability. PBB identifies the chromosomal complement of the oocyte and helps in detecting numerical and structural chromosomal aberrations that may compromise the developmental potential of an oocyte. It is an invasive technique; however, as the polar bodies are not necessary for embryo development, their removal is not expected to have a negative impact on the embryo. Recent studies have shown that PBB in combination with array-CGH (comparative genomic hybridization) is a powerful diagnostic tool as it enables the investigation of all 24 chromosomes. Nevertheless, the long-term benefit of PBB in combination with array-CGH regarding increased live birth rate has not been proven yet.

Spindle imaging is accomplished by polarization microscopy and provides the diagnostic measure to map the nuclear cycle of an oocyte based on the presence and fate of the spindle. The technique allows the characterization of the meiotic progression from the germinal vesicle stage to the fertilization-competent metaphase-II oocyte. Visualizing the transition phase during the meiotic cycle may help to optimize and individualize the time of intracytoplasmic sperm injection (ICSI). According to spindle imaging the most viable oocyte is the one that has a clearly detectable metaphase-II spindle at the proper time after human chorionic gonadotrophin triggering. A meta-analysis on spindle imaging favoured spindle imaging in ICSI treatment cycles, but the benefit of the technique depends on proper laboratory settings as the integrity of the spindle in the oocyte is influenced by external parameters.

Zona imaging can be viewed as the complementary technique to spindle imaging. It is also based on polarization microscopy and visualizes birefringent compartments within the zona pellucida which are otherwise invisible and cannot be distinguished by standard light microscopy. Although the underlying physiology of zona imaging is not fully understood, zona imaging somehow reflects the cytoplasmic competence of an oocyte for embryo development and implantation and thus is another add-on in identifying viable oocytes.

All of the techniques above can be combined and such a strategy may have an additional beneficial effect. However, none of these techniques can be replaced by one or the other. It also needs to be mentioned that no randomized controlled trials were undertaken to verify the potential of these techniques in view of an increase in the take-home baby rate, although one such trial is underway for PBB and array-CGH.
Other potential technologies to identify the most viable oocytes: polar body biopsy, spindle imaging, and zona pellucida birefringence

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Other potential technologies to identify the most viable oocytes: polar body biopsy, spindle imaging, and zona pellucida birefringence

Polar body biopsy and array-CGH: the impact of chromosomal disorders

Maternal age and aneuploidy rates

Spindle imaging by polarization microscopy: visualizing the invisible

Spindle dynamics is physiological

Zona imaging by polarization microscopy: a parameter of cytoplasmic maturity


Key learning messages

- Oocyte morphology is not a reliable predictor of oocyte viability
- Polar body biopsy is a viable alternative for pre-implantation genetic screening if it is combined with array-comparative genomic hybridization
- Spindle imaging is used to assess meiotic maturity
- Zona imaging can be used to assess cytoplasmic maturity and developmental competence
- Spindle imaging and zona imaging are existing technologies and so can have immediate benefit
- Cumulus gene expression is an evolving technology with huge potential for the future
L6: Other technologies to identify embryos with the highest implantation potential: time lapse, respirometry, genomics and metabolomics

M. Meseguer

Infertility Institute of Valencia (IVI), Valencia, Spain

Since the birth of Louise Brown, conceived using in vitro fertilization pioneered by Professor Robert G. Edwards – for which, in 2010, he won the Nobel Prize – the evolution in assisted reproductive technologies (ART) has been remarkable. Success rates have dramatically increased and complications, especially multiple births and ovarian hyperstimulation syndrome, will soon have disappeared from our terminology. Nonetheless, ART is still evolving and many efforts, such as embryo selection procedures, are being made to standardize the different steps of ART. Among the problems still to be resolved is the large variability in success rates, not only with different types of patients, but also at different centres.

This presentation will evaluate the new technologies that could be applied to improve embryo selection. The use of these technologies is currently under discussion: it could be that some of the techniques in question do not help to improve results or are even detrimental, but also some studies have shown some technical shortcomings, suggesting that outcomes would be different if the techniques were properly implemented.

Similarly, it is becoming ever more apparent that single embryo transfer using a healthy embryo with the highest chance of implanting to result in a single healthy baby at term is the ultimate goal of ART. This implies the ability to screen the embryos in the laboratory in order to ascertain which embryo has the highest chance of implantation. For this purpose, preimplantation genetic screening has been employed. However, non-invasive methods of embryo screening, such as time-lapse imaging, oxygen consumption, proteomics or metabolomics, may be preferred in the future with the ultimate goal of identifying a high-quality embryo.

Some of the efforts made to identify the best embryos are summarized in this presentation. Oxygen consumption measurements from oocytes and embryos could be applied routinely in the clinical embryology laboratory in order to assess quality, complementing the classic microscope-based methods. Oxygen consumption has been used and evaluated as a tool for the selection of viable embryos based on the exact timing of embryo development events together with morphological pattern assessment by using an automatic time-lapse system to monitor embryo development. Embryo metabolism results in variations in the spent culture medium as the embryos use up glucose, pyruvate, amino acids, sugars and oxygen; they also produce a large variety of substances such as lactate, ammonia, enzymes and hormones.

A non-invasive metabolomic profile of the culture medium surrounding the embryo with an analysis of the components produced or depleted can be achieved by different technologies, such as proton magnetic resonance or liquid chromatography followed by mass spectrometry, to correlate profiles with reproductive potential. Multiple protein analysis of human embryo secretions offers the chance to expand our perspective of a non-invasive quantification of human embryonic viability and even chromosomal normality, whilst avoiding any type of embryo manipulation.
Notes
L6: Other technologies to identify embryos with the highest implantation potential: time lapse, respirometry, genomics and metabolomics

M. Meseguer
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Time-lapse technology: evaluation of embryo quality and new markers for embryo selection

Current methods of embryo evaluation rely mainly on static observations of the embryo morphology. These observations are intrinsically restricted to specific times and, concluding that the developmental stage of the embryo is predictable. The imprecision present in embryo assessment can lead to flawed assumptions.

Time-lapse technology represents an approach to overcome the limitations of conventional methods. Time-lapse systems use automated and programmed techniques to capture images at a defined interval over a period of time as the embryo grows. The ability to observe and analyze the behavior of an embryo, including the timing of cleavages, is a valuable tool for evaluating the quality of embryos.

Table: Precise definition of exact timings (n=9530)

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<th>Upper Limit</th>
<th>Minimum</th>
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<td>58.86</td>
<td>59.35</td>
<td>33.37</td>
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</tr>
</tbody>
</table>

Morula
86.63
Blastocyst
92.18

Success rates:
- Exclusion Criteria
- Direct Cleavage
- Uneven Blastomere
- T4: 35-40h
- T5: 48-56h

Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging

María Cruz - Blanca Galán - Marisol García - Andrés Peña Fatou - Mar Montes
Uma Pirela-Casas - Manuel Martos - Marisol Nicolás

Received: 4 October 2010; Accepted: 12 February 2011
C. Springer Science+Business Media, LLC 2011

Abstract
Purpose: The aim of the current study was to determine whether time-lapse monitoring could improve selected outcome parameters of IVF cycles performed with fresh embryos. We evaluated the time-lapse monitoring of embryo morphology and development and pregnancy rates between the Embryoscope® (EO) and a standard incubator (SI). We assessed if the time-lapse analysis of embryo kinetics and implantation potential predicted embryo implantation success.

Results: A total of 115 cycles were retrieved from two centers supplemented with time-lapse monitoring. The inclusion criteria were patients from the EO group were monitored by time-lapse technology, whereas embryos from the SI group were analyzed by conventional methods.

Oxygen consumption is a quality marker for human oocyte competence conditioned by ovarian stimulation regimes

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03/05/2012   16:33
**Key learning messages**

- Time-lapse imaging with the EmbryoScope® allows the undisturbed assessment of embryos through multiple planes and at multiple timepoints throughout the entire culture period.
- Timing and duration of events on time-lapse imaging can be predictive of outcome.
- The EmbryoScope® can also be used to measure oxygen respiration rates.
- Higher oxygen consumption rates in oocytes have been associated with higher fertilization rates and higher implantation rates of the resulting embryos.
- Metabolomic profiling of the culture medium may prove useful for assessing embryo quality.
- Protein analysis of embryo secretions may provide another non-invasive means of embryo assessment.
L7: Other potential technologies to assess the most receptive endometrium: genomic profile and progesterone levels

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The complex developmental process of blastocyst implantation involves a series of steps leading to an effective ‘crosstalk’ between invasive trophoblast cells and the maternal endometrium. This dynamic process involves the spatiotemporally coordinated effects of numerous endocrine, paracrine and autocrine factors. These communications are required for the synchronized interaction between a blastocyst competent for implantation and an adhesive endometrium. Thus, the success of implantation depends on achieving the appropriate embryo development to the blastocyst stage and its invasion into the decidualized endometrial stroma. In assisted reproductive technologies, where high-quality embryos are transferred, impaired uterine receptivity is believed to be one of the major reasons behind failure of the establishment of pregnancy.

In recent years, global gene expression analysis has been applied in several studies to identify genes and their networks associated with receptivity of the human endometrium. These studies have generated a large amount of information about the regulation and dysregulation of the window of implantation genes in fertile, subfertile and refractory conditions. However, none of the molecular markers has been applied successfully in practice, and this lack of clinical application was also demonstrated with the highly promising molecule, leukaemia inhibitory factor, and so the hunt for potentially informative markers of uterine receptivity continues.

Among the diverse factors affecting embryo implantation, elevation of circulating progesterone levels at the end of the follicular phase in controlled ovarian stimulation for in vitro fertilization embryo transfer seems to have a negative impact on embryo implantation and, therefore, on cycle outcome. Although the mechanism by which increased serum progesterone concentrations affect cycle outcome is still unclear, some data suggest that it impairs endometrial receptivity rather than oocyte quality. The aetiology of premature progesterone rise seems to be multifactorial. To prevent this rise, future studies should focus on individualizing treatment protocols, proper monitoring of endocrinological profile during stimulation and, subsequently, timing of the trigger according to the patient’s response.
Notes
Other potential technologies to assess the most receptive endometrium:
genomic profile, progesterone levels

A. Makrigiannakis
Medical School, University of Crete, Heraklion, Greece

Endometrial Receptivity

- Approximately 80-90% of IVF/ICSI patients undergo embryo transfer into the uterus following successful fertilization

- However, only about 25% of them achieve pregnancy

- Impaired uterine receptivity is one of the major reasons for the failure of assisted reproductive techniques

- The phenomena of implantation and trophoblast invasion is currently considered as the major limiting factor for the establishment of pregnancy

Receptive endometrium features

Morphological markers
Biochemical markers
Gene expression pattern

Gene expression profiles of the peri-implantation endometrium in natural and gonadotropin-stimulated cycles

Differential gene expression between infertile and fertile women

CONCLUSIONS

- Analysis of the molecular relationships between differently expressed genes in infertile versus fertile women showed a complex network where the majority of signals are mediated through eutrogen-activated protein kinase (ERKs, P38MAPK, Jnk) complexes, where different genes such as those for ADRBs, metalloproteinases, IGFBP1 and others seem to play important roles.

- No marker has been identified to date that is specific and sensitive in identification of receptive endometrium and predicting implantation.
Key learning messages

- Impaired uterine receptivity is considered one of the main reasons for failure to establish pregnancy
- Gene-expression analysis has not yet identified a molecular marker that can be used in clinical practice for uterine receptivity assessment
- Elevated circulating progesterone at the end of the follicular phase in controlled ovarian stimulation for in vitro fertilization embryo transfer seems to have a negative impact on embryo implantation
- When progesterone levels are elevated at the end of the follicular phase, vitrification of embryos is recommended with embryo transfer in a subsequent natural cycle
- Future studies should focus on individualizing treatment protocols and preventing this rise in progesterone levels

Endometrial receptivity is affected in women with high circulating levels at the end of the follicular phase: a functional genomics analysis

RESULTS

- A total of 140 and 370 genes, using parametric and non-parametric methods, respectively, were found to be dysregulated by more than 2-fold in women with high serum P levels.
- Comparing the list of dysregulated genes in women with high P levels with the 25 WOI genes, 13 WOI showed a dysregulation (7 up-regulated and 6 down-regulated) in women with high P levels.
- 8 of these 13 genes were shown to have putative progesterone response elements in their regulatory sequences.
- Apart from the FOLR1 gene, the other 12 genes are the gene targets included in the endometrial receptivity array, a genomic tool recently published and used for endometrial receptivity evaluation

Labarta et al, Mol Hum Reprod 2011

Potential modifying factors for P rise

- Take into consideration the patient’s response to a certain treatment protocol: earlier trigger in high responders
  - Kyrou et al, Fertil Steril 2013
- Mild stimulation protocol: prevention of high E2 concentrations associated with P rise in the follicular phase
  - Kyrou et al, Hum Reprod 2009
- Vitrification of all embryos and embryo transfer in natural cycle once P concentration has breached that compatible with successful outcome
  - Kyrou et al, Fertil Steril 2013

Significantly lower PR in the presence of P elevation in patients treated with GnRH antagonists and gonadotrophins: a systematic review and meta-analysis

P elevation on the day of hCG administration was associated with a significantly decreased probability of clinical pregnancy per cycle (99%, 95% CI -17 to -2)

Koliou et al, Curr Pharmaceut Biotechnol 2012

Elevated P levels on the day of rCG administration can induce significant alterations in the gene expression profile of the endometrium

In the event of increased values, cryopreservation of all the obtained embryos and their transfer in a natural cycle is recommended as the clinical guideline

Labarta et al, Mol Hum Reprod 2011

Fatemi et al, Fertil Steril 2010

Kyrou et al, Fertil Steril 2011

Kyrou et al, Hum Reprod 2009
L8: Other potential non-invasive technologies to assess the most receptive endometrium: lipidomics

C. Simón

Valencia University, Infertility Institute of Valencia (IVI), Prince Felipe Research Centre (CIPF) & Valencia Stem Cell Bank (CIPF), Valencia, Spain

After more than 40 years of improvements in assisted reproductive technologies, the diagnosis of the endometrial factor and the assessment of endometrial receptivity in infertile couples are still lacking. The endometrium contains and secretes lipids whose relevance in reproduction has been identified in animal models. In humans, the majority of studies have investigated the lipid content in endometrial tissue but not in endometrial fluid (EF). Therefore, lipidomics, the study of the presence and concentration of lipid species, can serve to unravel the specific lipid composition of EF through the menstrual cycle and to predict the endometrial receptive state.

The analysis of endometrial secretions is a new, non-disruptive possibility for the investigation of endometrial receptivity, as the aspiration of EF does not affect pregnancy rates. This approach provides reliable read-outs of individual molecules correlating with the day of cycle and has proven to be effective in combined molecule readouts using a luminex system, thus opening the field for a timely synchronized, non-invasive application.

Prostaglandins (PGs) are lipid mediators with important roles in reproductive processes, including ovulation, implantation and menstruation. PG biosynthesis is started by phospholipase A₂ (PLA₂) enzymes, which release arachidonic acid (AA) from membrane phospholipids. Sequential oxidation of AA by cyclooxygenases (COX-1 and COX-2), and the action of terminal PG synthases (PGSs), leads to the generation of individual PGs, namely PGD₂, PGE₂, PGF₁-alpha and PGI₂. cPLA₂, COX-1 and -2 act as rate-limiting factors in the production of PGs, and their role in the endometrium is well established in mouse and human models. These proteins represent upstream, common factors in the signalling pathway that leads to PG production. Manipulating their levels or activity has an effect on the synthesis of all PGs, and thus is not informative regarding the production mechanisms affecting individual PGs. PGSs, on the other hand, represent the downstream, terminal mediators in that pathway, and mediate the catalysis from inactive PGs to the terminal, active PGs. There is one PGS for each PG: i.e., PGES catalyzes PGE production; PGFS mediates PGF synthesis, etc. In addition, some PGs can be converted from one to another through the action of specific enzymes. In particular, PGF is the result not only of PGFS, but can also be produced from PGD in a reaction catalysed by 11-ketoreductase, or from PGE₂ through the action of 9-ketoreductase (CBR1).

The aims of this presentation were first to demonstrate the specificity of the lipidomic profile in endometrial secretions in optimal, suboptimal and refractory endometrial cycles from the same patient to identify specific lipidomic biomarkers of endometrial receptivity. Second, to understand the production mechanism(s) of these lipids by human endometrial epithelial cells and their putative effects in blastocysts. Finally, to prove the diagnostic sensibility and sensitivity of PGE₂ and PGF₁-alpha levels in EF obtained 24 hours before Day 3 and Day 5 embryo transfer, by correlating these biomarker levels with cycle outcome.
L8: Other potential non-invasive technologies to assess the most receptive endometrium: lipidomics

C. Simón

Valencia University, Infertility Institute of Valencia (IVI), Prince Felipe Research Centre (CIPF) & Valencia Stem Cell Bank (CIPF), Valencia, Spain

Non invasive technologies to assess the most receptive endometrium: LIPIDOMICS

Prof. Carlos Simón MD, PhD
Professor Obst/Gyn, University of Valencia.
Scientific Director, Fundación IVI.

The lipidomics can be defined as large-scale study of lipid species present in a cell or biological system and their intersecting pathways and metabolic networks.

LIPIDOMICS

Lipids from EF extracts are identified by liquid chromatography combined with tandem mass-spectrometry.

OBJECTIVES

a) To demonstrate the specificity of the lipidomic profile in endometrial secretions in optimal, suboptimal and refractory endometrial cycles.

b) To understand the production mechanism/s of these lipids by human endometrial epithelial cells and their putative effects in blastocysts.

c) To prove the diagnostic accuracy of PGE2, PGF2α levels in EF obtained 24 hours before embryo transfer by correlating them with cycle outcome.

SECRETOMICS OF ENDOMETRIAL FLUID (EF): NON-INVASIVE DIAGNOSIS

- Aspiration of endometrial secretion does not affect pregnancy rates
  - Van der Gaast et al. (2002) RBmOnline

- Glycolitin levels correlate with menstrual cycle phase on endometrial aspirations
  - Van der Gaast et al. (2009) BJOG

- The profile of cytokines can be determined in endometrial secretions
  - Simón et al. (1996) J Reprod Immunol
  - Boomsma et al. (2009) RBmOnline

SECRETOMICS OF ENDOMETRIAL FLUID (EF): NON-INVASIVE DIAGNOSIS

SAMPLE ANALYSIS

Lipids from EF extracts are identified by liquid chromatography combined with tandem mass-spectrometry.

LIPIDOMIC PROFILE DURING WOI

- Aspiration of endometrial secretion does not affect pregnancy rates
  - Van der Gaast et al. (2002) RBmOnline

- Glycolitin levels correlate with menstrual cycle phase on endometrial aspirations
  - Van der Gaast et al. (2009) BJOG

- The profile of cytokines can be determined in endometrial secretions
  - Simón et al. (1996) J Reprod Immunol
  - Boomsma et al. (2009) RBmOnline
Key learning messages

- Lipidomics is the large-scale study of lipids in a cell or biological system and their interacting pathways and metabolic networks.
- Aspiration of endometrial fluid and the analysis of endometrial secretions represents a new methodology for investigating endometrial receptivity.
- Lipidomic profiles in endometrial secretions are specific in optimal, suboptimal and refractory endometrial cycles.

- In patients who achieve pregnancy, prostaglandin levels are elevated in endometrial fluid 24 hours before transfer.
- Endometrial prostaglandin synthases are responsible for the production and secretion of prostaglandins in the endometrial fluid.
The classical method of reporting on success in assisted reproductive technologies (ART) across scientific papers is the probability of pregnancy per cycle. Recent years have shown a trend towards replacing pregnancy by live birth as a unit of expression. This reporting mode still presents information that is highly insufficient if we take the patient perspective as the starting point. Infertile couples indicated for in vitro fertilization/intracytoplasmic sperm injection should be informed on the prospects of healthy live birth to be achieved within a reasonable period of time. The calculations of such a prospect need to account for several pitfalls that may easily be overlooked. Mentioned here are the couples who agreed to start treatment but who never really did and the couples who started treatment but dropped out somewhere during or after the first treatment cycle, without having achieved a live birth. The way these dropouts are handled in the statistics may considerably affect the prospects you will use for patient counselling.

Taking both the patient perspective and the costs for society into account, live birth after ART is preferred to be a singleton. The multiple pregnancy epidemic induced by ART has created enormous costs for society owing to the high rate of severely damaged children in need of chronic health or institutional care, let alone the life-time burden for the parents of these children. Claiming success to be defined as a singleton live birth achieved within a year of treatment implies a rigorous application of single embryo transfer, wide application of cryo-preservation and subsequent single cryo-embryo replacement, but also efficient repeat cycle planning in order to maximize the 1 year result. Increasing evidence has demonstrated that such a safety policy will not affect the overall prospects for the outcome of treatment, although a more intensified investment for both the treatment team as well as the couple is demanded.
Other potential definitions of success in ART: ITT, ET, cumulative pregnancy rates, one embryo–one baby and reduced multiples

F. Broekmans
University Medical Centre Utrecht, Utrecht, the Netherlands

Agenda

- Looking at Success of IVF/ICSI treatment
- Success according to who??
- Changes in approach
  - Patient selection
  - Stimulation
  - Laboratory
  - Embryo transfer
  - Follow up
- Conclusions

Risk of ovarian hyperstimulation syndrome (OHSS)

- Characterized by a sudden increase in vascular permeability and accumulation of fluid in the abdomen
- Vasoconstriction with risk of venous and arterial thrombosis
- OHSS is potentially fatal, but can be avoided by cautious use of gonadotropins and careful monitoring of stimulation regimens
- Prevalence of OHSS in Europe: ~1.2%
- Estimate of death due to OHSS in stimulated cycles: 1:400,000–1:500,000

The patient’s success

IVF/ICSI can be
- Complex
- Time-consuming
- Expensive
- Stressful
- Associated with risks such as OHSS

What can we do to improve the patient’s experience?

"Recognition of increased fetal risk significantly reduces desire for multiples"

"Important to involve affected staff, patients saw no need for a trial and found randomization unacceptable"

"Essential to develop a single ET strategy that does not negatively affect pregnancy rates"

The patient’s wish – information, information, information...

Singleton: $9,845
Twins: $37,947
Tri-Quadruplets: $109,765

Costs...can be reduced

Proportion associated with ART

- Singleton: 2% $9,845
- Twins: 35% $37,947
- Tri-Quadruplets: 77% $109,765


Assessment of success

Out of every 100 couples starting IVF...

...only 50 will achieve an ongoing pregnancy within a 1-year treatment period...

Lintsen, HR 2007

Key learning messages

- The success of in vitro fertilization (IVF) can be viewed from the perspective of doctors, patients and health insurers and according to the time frame, singleton versus multiple births and laboratory outcomes, such as fertilization and embryo usage rate.

- The prevalence of ovarian hyperstimulation syndrome in Europe is about 1.2%.

- Patients like to be informed; recognition of the increased risk with multiple births reduces the desire for multiple pregnancies.

- IVF costs can be reduced by reducing the number of multiple births.

- Higher doses of recombinant human follicle-stimulating hormone do not necessarily translate into greater clinical pregnancy rates.

- Birth rates are similar for single and double embryo transfer but single embryo transfer avoids multiple births.
L10: Other potential approaches to maximize success in ART: increase the number of oocytes, decrease the number of oocytes and managing follicle population

S.K. Sunkara
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Since its inception, in vitro fertilization (IVF) treatment has much improved, providing, in 2009, a 32.3% live birth rate for women aged <35 years in the UK. A vital clinical milestone to optimize the success of IVF treatment was the introduction of controlled ovarian stimulation (COS) regimens. The paradigm shift from a natural unifollicular to a multifollicular stimulated IVF cycle has been an important contributing factor and there is a multitude of ovulation-inducing drugs that are currently used. Another aspect of COS is better cycle control and the avoidance of a premature luteinizing hormone (LH) surge. The introduction of gonadotrophin-releasing hormone (GnRH) agonists and subsequently the GnRH antagonists into IVF regimens played a significant role by reducing the incidence of a premature LH surge and allowing cycle programming.

More couples today are seeking IVF treatment. However, with its widespread use and acceptance have come challenges. Given the biological heterogeneity of women seeking IVF treatment, there is a need to tailor stimulation regimens for individual women in order to maximize IVF outcome whilst minimizing side effects. Two major challenges of COS are women with polycystic ovary syndrome (PCOS) and women with poor ovarian response.

At present, long GnRH agonist pituitary downregulation combined with exogenous gonadotrophins is the most frequently used COS regimen in IVF treatment. In women with PCOS and in hyper-responders, it is of utmost importance to use a safe COS regimen, with negligible risk of ovarian hyperstimulation syndrome (OHSS) and the best chance of desired live birth rates (LBRs). The GnRH antagonist regimen, which has been reported to be associated with a lower incidence of OHSS and comparable LBRs to the GnRH agonist regimen, is likely to be beneficial for women with PCOS. Women with poor ovarian response have a low LBR and COS regimens aim to augment the response to stimulation, oocytes retrieved and subsequent IVF outcomes. Several interventions and adjuvants such as androgen, LH and growth hormone supplementation have been proposed to improve the IVF outcome of poor responders. However, available data need to be interpreted with caution until robust evidence is available. Given that the number of oocytes retrieved is an important prognostic variable and there is a non-linear association between the number of oocytes retrieved and live birth following IVF, clinicians should aim to optimize ovarian response by individualizing COS regimens.

The characteristics of each individual woman play a significant role in the success of IVF treatment. It is important to establish a woman’s phenotype, aided by tests of ovarian reserve such as the antral follicle count and anti-Müllerian hormone, and facilitate personalized COS to achieve the desired response and final outcome.
Notes
L10: Other potential approaches to maximize success in ART: increase the number of oocytes, decrease the number of oocytes and managing follicle population

S.K. Sunkara
Guy’s and St Thomas’ Foundation Trust, King’s College London, London, UK

Background
- Number of oocytes retrieved following controlled ovarian stimulation (COS) is an important prognostic variable in IVF (Sunkara et al., 2011)
- There is a strong association between the number of oocytes retrieved and live birth (LB) following IVF
- The number of oocytes is a robust surrogate outcome for clinical success

Discussion
- The primary aim of IVF treatment is to achieve a healthy live birth
- Two particular challenges to COS are women with poor ovarian response and women likely to hyper-responder
- Whilst poor responders have lower success rates compared to normal responders (Ulug et al., 2003), hyper-responders have an increased risk of OHSS

Background
- The relationship between the number of oocytes retrieved and LB following IVF is non-linear

Discussion
- Moreover, it has been shown that the chance of LB is reduced with very high number of oocytes following a fresh IVF cycle (Sunkara et al., 2011)
- The GnRH agonist long regimen is the most widely used in COS (Maklon et al., 2006)
Key learning messages

- Currently, the most frequently used regimen for controlled ovarian stimulation (COS) is long gonadotrophin-releasing hormone agonist pituitary downregulation combined with exogenous gonadotrophin administration
- There is a strong, non-linear relationship between the number of oocytes retrieved and live birth
- The gonadotrophin-releasing hormone antagonist regimen is likely to be beneficial for women with polycystic ovary syndrome owing to the low reported risk of ovarian hyperstimulation syndrome

- Adjuvant therapy with androgen, luteinizing hormone and growth hormone supplementation has been proposed to improve outcomes from in vitro fertilization in poor responders
- It is important to personalize COS treatment according to a woman’s phenotype and ovarian reserve testing to achieve the desired response and outcome
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